Association between Th-17 cytokine profile and clinical features in patients with spondyloarthritis

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Abstract

Objective

In recent years, a substantial amount of information has become available on the relationship between cytokines associated with the Th-17 profile and the development of spondyloarthritis (SpA). The purpose of this study was to evaluate inflammation markers in serum and synovial fluid (SF) and levels of cytokines related to the Th-17 profile in patients with different subtypes of SpA and healthy subjects.

Methods

We evaluated this cytokine profile in light of the clinical activity of the disease in 62 patients. Serum cytokine levels (IL-17, IL-6, IL-1 alpha, TNF alpha, IFN-gamma) were measured by flow cytometry. IL-23, serum amyloid (SAA) and metalloproteinase 3 (MMP-3) were measured with ELISA. In all patients, clinical evaluation was performed using the activity and function indexes of the disease.

Results

A comparison showed that IL-17, IL-23, IL-1 alpha, IL-6, and TNF-alpha levels were significantly higher in the serum of SpA patients than healthy subjects (HS), and there were no differences among SpA subtypes. In SF we found higher concentrations of cytokines, but only IL-23 showed significant differences (p<0.05). We found a relationship between enthesitis and peripheral involvement and serum IL-17 levels (9 to 63 pg / ml). There was a correlation between levels above 63 pg/ml and a history of infection. Higher levels of IL-23 in synovial fluid could suggest local amplification of the Th-17 cytokine profile.

Conclusion

These results suggest a possible relationship between IL-17 and enthesis involvement in SpA.

Key words

lymphocyte Th-17, IL-17, IL-23, spondyloarthritis, enthesis

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Introduction

Spondyloarthritis (SpA) is a group of diseases with different clinical manifestations and a common genetic predisposition. Regardless of the SpA subtype, the principal clinical manifestation is inflammatory back pain, followed by peripheral arthritis and enthesitis (1, 2). An association with HLA-B27 is widely recognised. Recently, papers related to the genome in patients with ankylosing spondylitis (AS) have identified and validated loci other than HLA-B27 involved in the pathogenesis of the disease. These loci include aminopeptidase (ERAP-1), interleukin (IL) 23 receptor (IL23R), the IL-1 receptor (IL-1RII), and two loci coding for unknown genes (3-5).

In AS, histopathology has confirmed inflammation in the sacroiliac joints, enthesis and vertebral bodies adjacent to the intervertebral disks, peripheral joints, gastrointestinal tract and the eye. The cell type in these lesions is unknown because of limited access to tissue (6), T cells (CD4>CD8), CD68 macrophages, fibroblast proliferation, neovascularisation and over expression of TNF-alpha mRNA and TGF-beta (7). In peripheral synovitis, studies have found increased vascularity and endothelial cell activation with increased expression of adhesion molecules and chemoattractant factors. CD4 T cells, natural killer (NK) cells, B lymphocytes and CD68 macrophages have also been observed (8).

Recent information on cytokines, animal models and genome association studies suggest mechanisms by which the IL-23/IL-17 axis is involved in the generation of this disease (3, 5). In SpA, IL-17 levels and cytokines are increased. Moreover, a recent study found a strong association between the presence of SpA and receptor polymorphisms of IL-23R (5). In murine models, Th-17 cells are related to joint a process similar to those of AS; in HLA-B27/B2mHu transgenic mice, the IL-17/IL-23 cytokine profile was activated. This suggest a new paradigm that relates this family of cytokines to stress-response protein folding in the endoplasmic reticulum (UPR) and overproduction of IL-23, which leads to the CD4 Th-17 memory production of inflammatory cytokines, such as IL-17 (9). Also, studies have described a percentage of CD4 T cells positive for IL-17 in peripheral blood cells of patients with SpA and RA compared with healthy controls, resulting in a higher secretion of IL-17 after stimulation with PMA. Particularly, this increase occurs with excess IL-10 production (10, 11). Another study showed that levels of IL-23 protein and CCL20, the protein chemoattractant for Th-17 cells, have increased expression in joint tissue but no correlation with disease activity in SpA (11).

In the present study, we evaluated the inflammation markers, serum levels and synovial fluid of cytokines related to the Th-17 profile in patients with different subtypes of SpA, AR patients and healthy subjects. These levels correlated with clinical parameters of disease activity.

Patients

Serum samples were obtained from 62 patients with SpA who were diagnosed according to the European Group for the Study of the Spondyloarthropathies (ESSG) criteria (12). Forty-three men and 19 women attending the SpA clinic at the Central Military Hospital in Bogota, Colombia, were enrolled by convenience between January 2008 and May 2009 (Table I). Thirteen patients were classified as having reactive arthritis (ReA), 19 patients as having AS [based on the modified New York criteria (13) and 30 patients as having undifferentiated spondyloarthritis (uSpA). ReA patients had a history of diarrhoea. Clinical evaluations included records of infection associated with uveitis, enthesitis, buttock pain and arthritis as initial symptoms. Serum samples from HS were obtained from the serum bank of the Central Military Hospital from subjects without autoimmune or infectious disease, taking gender and age into account. The project was approved by the institutional ethics committee; all subjects signed informed consent.

The disease activity state was measured by Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) (14), which considers the presence of inflammatory back pain as well as the number

of painful entheses and swollen joints. The functional status was assessed with the Bath Ankylosing Spondylitis Functional Index (BASFI) (15). Patients were classified as having peripheral involvement if they had lower limb oligoarthritis, dactylitis or enthesitis of the elbows, plantar fascia or Achilles tendon. They were classified as having axial involvement if they had enthesitis in the costochondral junction and iliac crest. At the time of the study, all patients received NSAIDs and sulfasalazine; none were receiving biologic therapy or intra-auricular or systemic corticosteroids.

We identified 16 patients susceptible to puncture of the knee (6 with uSpA and 10 with ReA), and we simultaneously obtained their serum and SF. All these patients were receiving NSAIDs and sulfasalazine (1.5 to 2 g/day). This result was compared with a matched group of control patients with RA, classified according to the American College of Rheumatology criteria (16). The SF and serum samples were taken simultaneously for the assessment of clinical activity parameters and stored at -80°C.

Methods
Flow cytometry
Cytokine concentrations in serum were determined using the Becton Dickinson cytometric bead array flow cytometry system. The capture beads, phycoerythrin-conjugated antibodies, recombinant standards and serum samples were processed according to the manufacturer's recommendations. Samples were acquired using a FACS CANTO II flow cytometer. The data were acquired with the FACS DIVA software, and the results were generated in graphical and tabular format using BD FCAP software, allowing the analysis of 1800 events. Cytokine levels were expressed as mean ± standard deviation in pg/mL.

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SF and serum levels of IL-6, IL-8 and TNF-alpha (BD Biosciences), IL-1-alpha, IL-17, IL-23, MMP-3 (R&D Systems), IFN-alpha, and IFN-beta (PBL Biomedical Laboratories) were measured simultaneously according to manufacturer's recommendations. The samples were analysed in duplicate.

Table I. Demographic data in spondyloarthritis and healthy subjects.

	SpA (n=62)	AS (n=20)	uSpA (n=29)	ReA (n=13)
Age* (years)	31.9 ± 9.9	32.3 ± 9.1	33.0 ± 10.6	28.2 ± 8.2
Age of onset of symptoms*(months)	26.9 ± 7.3	26.3 ± 5.6	28.0 ± 8.4	26.0 ± 7.7
Evolution *(years)	5.01 ± 5,7	6.0 ± 5.8	5.4 ± 5.9	2.5 ± 4.6
Sex M**	43 (69.4)	15 (75.0)	17 (58.6)	11 (84.6)
Arthritis**	23 (37.1)	4 (20.0)	8 (27.6)	11 (84.6)
Enthesitis**	57 (91.9)	18 (90.0)	28 (96.6)	11 (84.6)
Peripheral involvement	38 (61.3)	15 (75.0)	21 (72.4)	2 (15.4)
Axial involvement	7 (11.3)	4 (20.0)	0 (0.0)	3 (23.1)
HLA-B27 positive	26 (41.9)	14 (70.0)	5 (17.2)	7 (53.8)
Background				
Inflammatory back pain **	42 (67.7)	16 (80.0)	21 (72.4)	5 (41.7)
Arthritis**	44 (71.0)	13 (65.0)	19 (65.5)	12 (92.3)
Enthesitis**	34 (54.8)	10 (50.0)	16 (55.2)	7 (65.5)
Infection**	18 (29)	5 (25.5)	0 (0.0)	13 (100)
Uveitis**	8 (12.9)	4 (20.0)	0 (0.0)	4 (30.8)
Dactylitis**	12 (19.4)	2 (10.0)	5 (17.2)	5 (38.5)
Buttock pain**	11 (17.7)	3 (15.0)	7 (24.1)	1 (7.7)
Activity				
BASDAI	6.1 ± 2.0	6.4 ± 2.0	6.2 ± 1.6	5.7 ± 2.7
BASFI	5.8 ± 2.3	5.4 ± 2.3	5.9 ± 2.1	5.0 ± 2.7
ESR	13.5 ± 13.8	13.4 ± 12.8	15.2 ± 10.4	26.7 ± 18.1
RCP HS	9.4 ± 16.5	7.9 ± 16.4	4.5 ± 9.9	22.4 ± 22.2
LBP	7.5 ± 3.7	0.53 ± 2.4	6.6 ± 3.5	10.0 ± 4.8
SAA	853.8 ± 946.2	752.5 ± 871.8	652.0 ± 820.2	1459.7 ± 1124.7

*Mean ± SD, **Frequency, n (%).
SpA: Spondyloarthritis; AS: ankylosing spondylitis; uSpA: undifferentiated spondyloarthritis; ReA: reactive arthritis; CRP-Hs: C reactive protein high sensitivity – mg/L; ESR: erythro-sedimentation rate – mm^3/h; LBP: lipopolysaccharide binding protein – ng/mL; SAA: serum A amyloid – mg/mL.

The values for each cytokine were expressed as mean ± standard deviation in pg/mL. CRP ultrasensitive levels were measured with chemoluminescence. Comparisons were made between samples processed the same day.

Statistical analysis
Comparisons between groups were made with a Kruskal Wallis, Mann-Whitney U and Wilcoxon test, given the previously confirmed nonparametric nature of the data. The correlation coefficients were calculated with the Spearman statistic. These analyses were performed using STATA SE 9.0 software for Windows. To assess the relationship between clinical parameters of disease activity, disease history and levels of biomarkers, a canonical correlation analysis was performed using the Stat Graphics 5.1 for Windows. To represent the association between categories (StatAdvisor) of variables and the frequency response of these categories, multiple correspondence analyses were performed using STAAD software 4.5.

Results
1. SpA patients had higher serum levels of cytokines clinical characteristics and serum analysis of 62 individuals with SpA and 46 HS were evaluated for the following cytokines associated with the Th-17 profile: IL-1 alpha, IL-6, IL-17, IL-23, TNF-alpha, IFN-gamma and MMP-3. Between–groups comparisons showed that levels of IL-17, IL-23, IL-1 alpha, IL-6 and TNF-alpha were significantly higher in the serum of SpA patients than in HS ($p<0.05$ Kruskal Wallis test). IL-6 levels were significantly higher in AS patients compared to uSpA patients ($p<0.05$ U-test). We found higher levels of IL-1 alpha and IL-6 in patients with ReA. The serum concentrations of cytokines are shown in Table II. Beside of cytokines levels in SF were higher than serum levels in patients with SpA, only significant differences were shown for IL-23 ($p<0.05$ Wilcoxon test). IL-23 concentrations were significantly higher in patients with SpA SF rather than in patients with RA ($p<0.05$ U-test).

Table II. Concentration of biomarkers associated to Th-17 profile.

Diagnosis	n.	IL-17	IL-23	TNF-α	IL-6	IFN-γ	IL-1α	MMP-3
HS	46	13.7 ± 26.4	3.1 ± 0.7	16.0 ± 12.7	20.1 ± 4.6	0.6 ± 1.2	42.2 ± 30.8	18.0 ± 10.0
SpA	62	*52.5 ± 87.1	**4.8 ± 2.9	**24.2 ± 36.3	**48.2 ± 73.7	0.9 ± 2.9	**46.0 ± 23.2	21.4 ± 21.8
AS	19	*45.1 ± 58.05	*5.2 ± 3.9	*18.36 ± 8.0	**44.9 ± 42.6	1.0 ± 3.2	43.0 ± 7.7	16.2 ± 10
uSpA	30	**54.8 ± 109.4	**4.8 ± 2.7	*28.2 ± 49.2	**35.6 ± 50.7	0.93 ± 3.23	*44.7 ± 26.7	19.6 ± 17.2
ReA	13	**59.2 ± 44.5	3.73 ± 0.8	**22.0 ± 9.3	**96.3 ± 144.6	0.5 ± 1.1	*56.0 ± 29.5	37.5 ± 39

Values expressed as mean ±SD. *p-value <0,05 . **p-value ≤0,001

SpA: Spondyloarthritis; AS: ankylosing spondylitis; uSpA: undifferentiated spondyloarthritis; ReA: reactive arthritis Healthy subject: HS. Cytokine concentrations are expressed in pg/mL and for MMP-3 in ng/mL.

Table III. Categories by quartiles cytokines, Th-17 profile and activity index (Q1, Q2, Q3, Q4).

	IL-1α*	IL-17*	IL-23*	TNF-α*	IL-6*	IFN-γ*	MMP-3**	FSC-M**	LBP***	BASDAI
Lower quartile	38	9	3.3	14	22	4.1	8.6	54.9	4.94	4.8
Median	42	26.5	3.9	17	23.5	8.2	14.1	81.9	6.2	6.6
Upper quartile	45	63	4.8	22	35	12.3	27.4	132	8.9	7.6

*pg/ml, **ng/m, ***ug/mL.

2. Serum IL-17 levels and clinical manifestations of the disease although IL-17 levels were similar in the groups of patients with peripheral and axial involvement; values were increased in patients who reported enthesitis (p<0.05), regardless of the number of entheses involved. Similarly, patients who reported a history of infection at the initial stage of the disease had significantly higher IL-17 levels (p<0.05 U-test). Increased IL-17 levels in the serum of SpA patients positively correlated with IL-1 alpha (r=0.614, p<0.05) and TNF-alpha concentration. (r=0.518, p<0.05).

Canonical correlation analysis was applied in multivariate analysis (17, 18). To perform this correlation analysis, the first group of all laboratory variables was described according to the second group, and clinical parameters of activity were determined by selecting the presence or absence of arthritis, enthesitis, peripheral commitment, engagement and BASDAI. The canonical correlation obtained was 0.8, Wilks' lambda was 0.155 (p<0.05 by Chi-squared). The strongest correlation was between IFN-gamma variable with the compromise peripheral, followed by the correlation between the presences of serum amyloid (SAA) with axial involvement and, finally, CRP levels with the presence of arthritis.

A Fisher linear discriminant analysis was used to identify the most relevant variables (laboratory tests) in each of the groups examined (17, 18). A comparison of Th-17-related biomarkers in HS and patients with SpA revealed differences between the two groups, with a relative percentage of 100%, (p<0.05) and Wilks' lambda 0.794. Comparisons by disease subtype revealed no differences in biomarkers, with a relative percentage of 95.96%, (p>0.05) and Wilks' lambda 0.59.

Finally, the continuous variables were categorised into quartiles (Q: 1, 2, 3 and 4) to find relationships between the individual responses to continuous clinical and laboratory variables and to use multiple correspondence analysis as a statistical method to generate a graphic representation of the associations between qualitative variables (17, 18) (Table III). There was a relationship between the presence of enthesitis and peripheral involvement in patients in the third quartile for IL-17 level (Q3, from 9 to 63 pg/mL). Levels greater than 63 pg/mL (Q4) were related to the presence of arthritis (Fig. 1). There was also a relationship between the presence of infection and IL-17 levels higher than 63 pg/mL (Fig. 2), confirming the result previously revealed by univariate analysis.

Discussion

SpA refers to a heterogeneous group of chronic inflammatory diseases characterised by bone formation that progressively leads to ankylosis and functional disability. It has been associated with HLA-B27 and environmental factors. Recent studies genome research regarding patients with SpA proposed the IL-23/IL-17 cytokine axis as a new factor in the pathogenesis (1). In this study, we found different behaviour in the biomarker profile associated with Th-17 (IL-1-alpha, TNF-alpha, IFN-gamma, IL-17, IL-23 and IL-6) for HS and patients with SpA. However, it was not possible to establish biomarker profile differences between subtypes of the disease, suggesting that the SpAs are similar diseases with different clinical manifestations and a common genetic predisposition (1). The sample size could be considered as an important factor.

As reported previously (11, 19, 20), cytokine concentrations are higher in AS patients than healthy controls. In their study in only 28 AS patients, Wendling *et al.* found a significant increase in the serum levels of IL-17 and not of IL-23p40 in AS patients compared with HS (21). In contrast, we found difference between the patient group and control group when we measured the p40/p19 subunits of IL-23.

In our patients, we found a strong correlation between IL-17 levels and IL-1 alpha and TNF-alpha, which have a clear role in recruiting different cell types to inflammation sites and inducing bone resorption, among other

Fig. 1. Perceptual graphic of the relationship between levels of IL-17 by quartiles and clinical manifestations at the time of evaluation.
There is a strong relationship between the presence of enthesitis and higher levels of quartile 2 and quartile 3 for the IL 17 (Q2: 9.0 to 63 pg/mL) and higher levels of 63 pg/mL and the presence of arthritis. Interpretation: Multiple correspondence analyses is represented by perceptual graphics and can be interpreted as the relationship between each category of variables. An acute angle between two points very close between two categories of different variables is synonymous of a very good association between them. The distance from one category of any variable to the origin is inversely proportional to their frequencies. Categories with low frequencies are far from the origin. For clinical variables: Number 1: present; Number 2 absent; for IL-17 Number 1, 2, 3 and 4 represent each quartile.
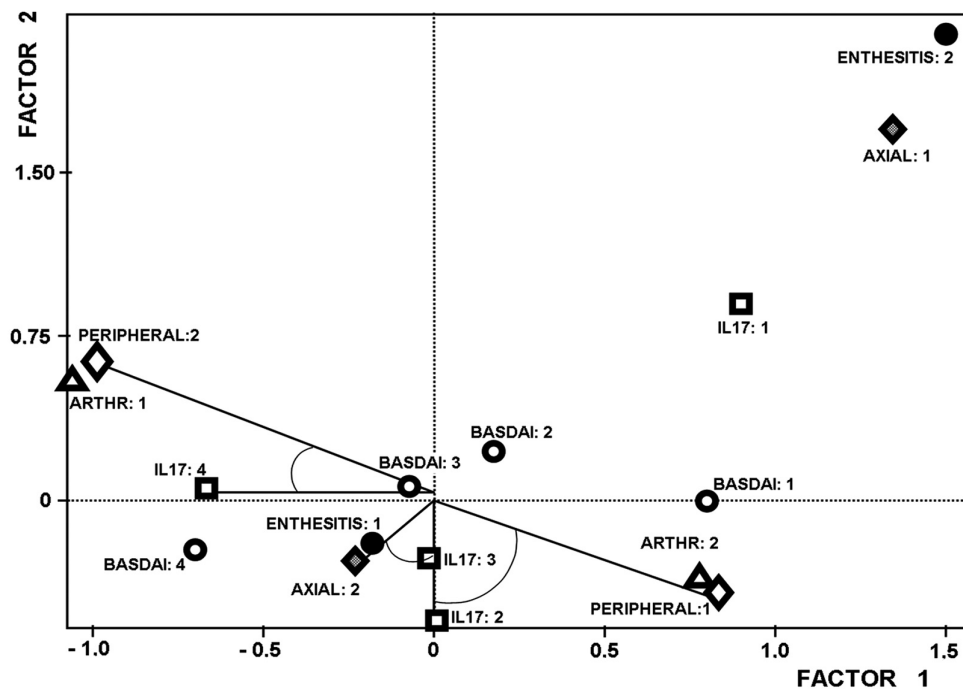
Fig. 2. Perceptual graphic of relationship between levels of IL-17 by quartiles and medical history in patients with spondyloarthritis. There is a strong relationship between a history of infection and higher levels of IL-17 in quartile 4 (Q4: greater than 63 pg/mL).
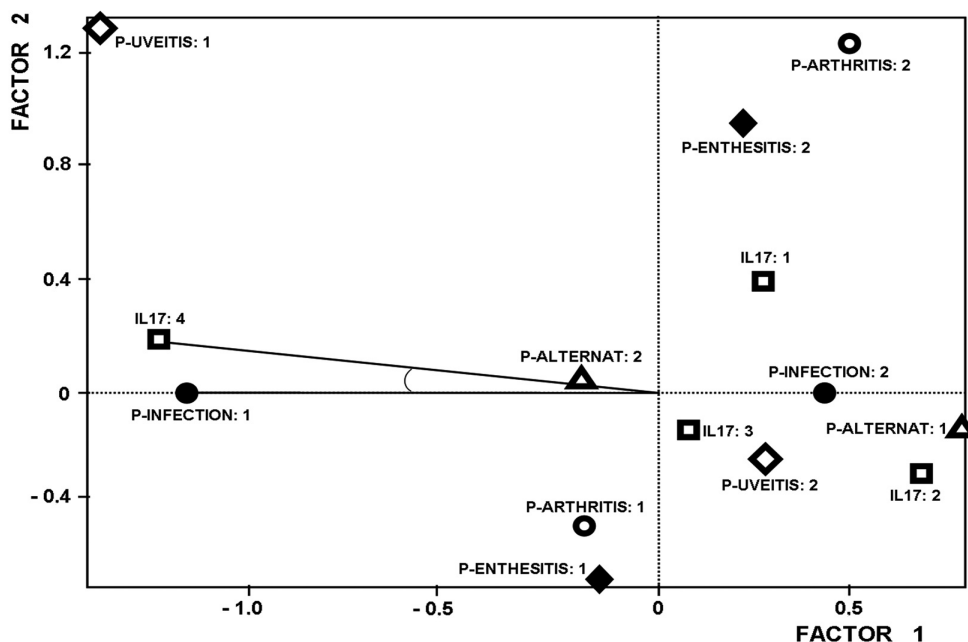
actions. It was reported a strong correlation between IL-17 with IL-1 and TNF-alpha, the last two are involved in the induction of IL-17, suggesting a synergistic action between theses cytokines and IL-17. There is evidence that IL-1alpha and TNF-alpha are involved in the processes of joint inflammation and cartilage and bone destruction. (22, 23).
In RA, the production of TNF-alpha, IL-1 and IL-17 by synovial cells is be-

lieved to predict joint destruction (10, 20, 24, 25). Koenders *et al.* found that IL-1 cells direct the development of pathogenic Th-17 during the onset of arthritis in IL-1Ra-/- mice (26). It has described that the stimulation whit IL-1 is crucial for the development and production of IL-17 and TNF-alpha by Th-17 cells (27).
Although the initial events in the development of SpA are unknown, it has recently been hypothesized that stress on

the enthesis activates cells in this organ, which could allow a dual phenomenon. First, the response leads to the formation of new tissue repair and the production of pro-inflammatory molecules, which allows the repair of tissue integrity (28, 29). Second, the response favours the development of chronic inflammatory conditions in which cytokines such as TNF-alpha, IL-1 and IL-17 may have a role. Actually, first insights into the molecular mechanisms between inflamma-

tion and bone destruction or new bone formation, contrary to expectations, bone loss associated with inflammation and immune activation occurs, but with a deleterious effect on bone apposition. This result is related to the role of TNF-alpha in regulating the Wnt pathway and negatively regulating the expression of Dickkopf-related protein 1 (DKK-1), the main inhibitor of the osteoblast differentiation pathway and suppressor of bone formation (28, 29).

Data on cytokine levels in Th-17 SF is still limited and contradictory. A previous report (20) measured IL-17, CCL-20 and IL-23 levels with ELISA in patients with RA and SpA and correlated them with clinical markers of disease activity in RA. In this study, they found higher IL-17 levels in the SF of SpA patients compared with PsA and RA patients; IL-23 concentrations were similar in the three groups analysed (20). in contrast our results the predominant was IL-23 levels in SF. Similar to our results, they have not found correlation with the level of disease activity.

The production of IL-23 is dependent on the signalling pathway of NF-kappa B (30). This cytokine induces the release of IL-1 beta and IL-6, supporting the continued activation of Th-17 cells (32). These observations suggest a local involvement for IL-23, which could favour the perpetuation of joint inflammation and remodelling for the maintenance of Th-17 cells. Given the recently reported association, this finding highlights the need for studies of IL-23 receptor gene polymorphisms in these patients (5). A recent report (9) showed that increased secretion of IL-23 in a murine model of HLA-B27 is a consequence of altered folding in the endoplasmic reticulum, which leads to increased production and maintenance of the Th-17 population (9).

On the other hand, in patients with SpA, IFN-gamma levels were not increased, their concentrations were low than HS. For several years, various studies have proposed a possible pathogenic role of IFN-gamma in patients with AS, who showed a lower capacity than healthy individuals to produce IFN-gamma after stimulation (34, 35). Moreover, it has been proposed that

IFN-gamma antagonises the development of Th-17 cells. Individuals with low IFN-gamma levels may be more susceptible to developing a Th-17-type response (3). Our data suggest that low IFN-gamma levels could be related to the occurrence of peripheral engagement, a clinical finding that is indeed present in ReA patients with prior exposure to infectious agents. This finding supports the previously described pattern of cytokine production, with increased IL-4 and IL-10 and a relative decrease in TNF-alpha and IFN-gamma (34), predisposing patients to a chronic course and shifting the focus of management toward eliminating associated pathogens (36, 37).

We found a strong relationship between increased IL-17 levels in patients who reported previous infections. IL-17 is a proinflammatory cytokine that plays an important role against the host response to extracellular bacteria, protozoa and fungi (38). However, in contrast to this protective role during the initial phase of host defense, Th-17 cells promote joint destruction during inflammation, and the IL-17/IL-23 axis often appears more engaged in the development and maintenance of chronic diseases (39, 40).

We should note the strong relationship between high serum IL-17 levels and the presence of enthesitis and the commitment and presence of peripheral arthritis has not been reported previously. In a study of paediatric patients with enthesitis-related arthritis (ERA), Mahendra et al. (41) found higher IL-17 levels in synovial fluid, which could be due to local production of MMP-induced IL-17 (42).

In 1971 an histopathological study of enthesopathy in RA and AS, Ball et al. (43), described the prototype inserts and proved the existence of perivascular lymphocytic infiltration in adipose tissue around the enthesis and bone oedema. More recently, they had access to tissue of the zygapophyseal hip and sacroiliac joints that allowed us to describe the presence of abundant CD3 T cells in the iliac bone marrow and connective tissue of patients with recently developed EA, with the presence of CD163 macrophages, B cells

and osteoclast formation (6, 44, 45). Recent-onset patients had a greater number of T cells, TNF-alpha and IL-6-positive and active lesions of longer duration and showed cells that were positive for TGF-beta (6).

These exploratory results suggest a possible relationship between T lymphocyte-produced IL-17 and entheses in patients with EAS. Enthesitis is a distinctive pathological feature of spondyloarthritis and may involve synovial joints, fibrocartilaginous joints, syndesmoses and extra-articular entheses (46). Pathological studies revealed that the various components of the enthesis organ, aside from the underlying bone, are involved in inflammatory joint (47).

Conclusions

This report describes the behaviour of Th-17 cytokines and inflammatory markers in a group of patients with various subtypes of SpA. Increased IL-23 levels in SF could suggest a local amplification of the Th-17 profile in these patients. The reduction of IFN-gamma during immune responses may predispose patients with SpA to develop an increased Th-17 response rate. Furthermore, these results suggest the need to investigate the relationship between IL-17 and enthesis.

References

1. ZOCHLING J, BRANDT J, BRAUN J: The current concept of spondyloarthritis with special emphasis on undifferentiated spondyloarthritis. *Rheumatol* 2005; 44: 1483-91.
2. ROMERO-SANCHEZ C, TSOU HK, JAN MS *et al.*: Serum monocyte chemotactic protein-1 concentrations distinguish patients with ankylosing spondylitis from patients with mechanical low back pain. *J Spinal Disord Tech* 2010; Aug 27. [Epub ahead of print].
3. LAYH-SCHMITT G, COLBERT RA: The interleukin-23/interleukin-17 axis in spondyloarthritis. *Curr Opin Rheumatol* 2008; 20: 392-7.
4. BROWN MA: Genetics of ankylosing spondylitis. *Curr Opin Rheumatol* 2010; 22: 126-32.
5. AUSTRALO-ANGLO-AMERICAN SPONDYLOARTHRITIS CONSORTIUM (TASC); REVEILLE JD *et al.*: Genome – wide association study of ankylosing spondylitis identifies non-MHC susceptibility loci. *Nat Genet* 2010; 42: 123-7.
6. FRANÇOIS RJ, NEURE L, SIEPER J, BRAUN J: Immunohistological examination of open sacroiliac biopsies of patients with ankylosing spondylitis: detection of tumor necrosis factor alpha in two patients with early disease and transforming growth factor beta in three more advanced cases. *Ann Rheum Dis* 2006; 65: 713-20.

7. BOLLOW M, FISHER T, REISSHAUER H *et al*.: Quantitative analyses of sacroiliac biopsies in spondyloarthropathies T-cells and macrophages predominate in early and active sacroilitis-cellularity correlates with the degree of enhancement detected by magnetic resonance imaging. *Ann Rheum Dis* 2000; 59; 135-40.

8. VEALE D, YANINI G, ROGERS S *et al*.: Reduce Synovial membrane macrophage numbers, ELAM-1 expression, and lining layer hiperplasia in psoriatic arthritis as compared with rheumatoid arthritis *Arthritis Rheum* 1993; 36: 893-900.

9. DeLAY ML, TURNER MJ, KLENK EI, SMITH JA, SOWDERS DP, COLBERT RA: HLA-B27 misfolding and the unfolded protein response augment interleukin-23 production and are associated with Th17 activation in transgenic rats. *Arthritis Rheum* 2009; 60: 2633-43.

10. SHEN H, GOODALL JC, HILL GASTON JS: Frequency and phenotype of peripheral blood Th17 cells in ankylosing spondylitis and rheumatoid arthritis. *Arthritis Rheum* 2009; 60: 1647-56.

11. JANDUS C, BIOLEY G, RIVALS JP, DUDLER J, SPEISER D, ROMERO P: Increased numbers of circulating polyfunctional Th17 memory cells in patients with seronegative spondylarthritides. *Arthritis Rheum* 2008; 58: 2307-17.

12. DOUGADOS M, VAN DER LINDEN S, JUHLIN R *et al*.: The European Spondylarthropathy Study Group preliminary criteria for the classification of spondylarthropathy. *Arthritis Rheum* 1991; 34: 1218-27.

13. VAN DER LINDEN S, VALKENBURG HA, CATS A: Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. *Arthritis Rheum* 1984; 27: 361-8.

14. GARRETT S, JENKINSON T, KENNEDY LG, WHITELOCK H, GAISFORD P, CALIN A: A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. *J Rheumatol* 1994; 21: 2286-91.

15. CALIN A, GARRETT S, WHITELOCK H *et al*.: A new approach to defining functional ability in ankylosing spondylitis: the development of the Bath Ankylosing Spondylitis Functional Index. *J Rheumatol* 1994; 21: 2281-5.

16. ARNETT FC, EDWORTHY SM, BLOCH DA *et al*.: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.

17. PEÑA D: Análisis de datos multivariantes. 1 Edición 2002; S.A McGraw-Hill/Interamericana, Madrid: Cap 13.

18. JOHNSON RA, WICHERN DW: Multivariate Statistical Data Analysis (6th ed.) 2007, Prentice Hall. New Jersey.

19. WENDLING D, CEDOZ JP, RACADOT E, DUMOULIN G: Serum IL-17, BMP-7, and bone turnover markers in patients with ankylosing spondylitis. *Joint Bone Spine* 2007; 74: 304-5.

20. MELIS L, VANDOOREN B, KRUITHOF E *et al*.: Systemic levels of IL-23 are strongly associated with disease activity in rheumatoid arthritis but not spondyloarthritis. *Ann Rheum Dis* 2010; 69: 618-23.

21. WENDLING D, CEDOZ JP, RACADOT E: Serum and synovial fluid levels of p40 IL12/23 in spondyloarthropathy patients. *Clin Rheumatol* 2009; 28: 187-90.

22. ZWERINA J, HAYER S, TOHIDAST-AKRAD M *et al*.: Single and combined inhibition of tumor necrosis factor, interleukin-1, and RANKL pathways in tumor necrosis factor–induced arthritis: effects on synovial inflammation, bone erosion, and cartilage destruction. *Arthritis Rheum* 2004; 50: 277-90.

23. HORAI R, SAIJO S, TANIOKA H *et al*.: Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice. *J Exp Med* 2000; 191: 313-20.

24. SUTTON C, BRERETON C, KEOGH B, MILLS KH, LAVELLE EC: A crucial role for interleukin (IL)-1 in the induction of IL-17–producing T cells that mediate autoimmune encephalomyelitis. *J Exp Med* 2006; 203: 1685-91.

25. KORN T, BETTELLI E, OUKKA M, KUCHROO VK: IL-17 and Th17 Cells. *Annu Rev Immunol* 2009; 27: 485-517.

26. KOENDERS MI, DEVESA I, MARIJNISSEN RJ *et al*.: Interleukin-1 drives pathogenic Th17 cells during spontaneous arthritis in interleukin-1 receptor antagonist–deficient mice. *Arthritis Rheum* 2008; 58: 3461-70.

27. JOOSTEN LA: Excessive interleukin-1 signaling determines the development of Th1 and Th17 responses in chronic inflammation. *Arthritis Rheum* 2010; 62: 320-2.

28. DIARRA D, STOLINA M, POLZER K, ZWERINA J, OMINSKY MS, DWYER D: Dickkopf-1 is a master regulator of joint remodeling. *Nat Med* 2007; 13: 156-63.

29. SCHETT G, SIEPER J: Inflammation and repair mechanisms. *Clin Exp Rheumatol* 2009; 27 (Suppl. 55): S33-5.

30. MISE-OMATA S, KURODA E, NIIKURA J, YAMASHITA U, OBATA Y, DOI TS: A proximal kappaB site in the IL-23 p19 promoter is responsible for RelA- and c-Rel-dependent transcription. *J Immunol* 2007; 179: 6596-603.

31. OPPMANN B, LESLEY R, BLOM B *et al*.: Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity* 2000; 13: 715-25.

32. LEIBUNDGUT-LANDMANN S, GROSS O, ROBINSON MJ *et al*.: Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. *Nat Immunol* 2007; 8: 630-8.

33. DARDALHON V, KORN T, KUCHROO VK, ANDERSON AC: Role of Th1 and Th17 cells in organ-specific autoimmunity. *J Autoimmun* 2008; 3: 252-6.

34. RUDWALEIT M, SIEGERT S, YIN Z *et al*.: Low T cell production of TNF-α and IFNγ in ankylosing spondylitis: its relation to HLA-B27 and influence of the TNF-308 gene polymorphism. *Ann Rheum Dis* 2001; 60: 36-42.

35. SMITH JA, BARNES MD, HONG D, DeLAY ML, INMAN RD, COLBERT RA: Gene expression analysis of macrophages derived from ankylosing spondylitis patients reveals interferon-gamma dysregulation. *Arthritis Rheum* 2008; 58: 1640-9.

36. YIN Z, BRAUN J, NEURE L *et al*.: Crucial role of interleukin -10/interleukin-12 balance in the regulation of the type 2 helper cytokine response in reactive arthritis. *Arthritis Rheum* 1997; 40: 1788-97.

37. BRAUN J, YIN Z, SPILLER I *et al*.: A low TNF a secretion of peripheral blood mononuclear cells but no other T helper 1 o 2 cytokines correlates with chronicity in reactive arthritis. *Arthritis Rheum* 199; 42: 2039-44.

38. BOEHM U, KLAMP T, GROOT M, HOWARD JC: Cellular responses to interferon-gamma *Annu Rev Immunol* 1997; 15: 749-95.

39. KATZ Y, NADIV O, RAPOPORT MJ, LOOS M: IL-17 regulates gene expression and protein synthesis of the complement system, C3 and factor B, in skin fibroblasts. *Clin Exp Immunol* 2000; 120: 22-9.

40. LOUTEN J, BONIFACE K, DE WAAL MALEFYT R: Development and function of TH17 cells in health and disease. *J Allergy Clin Immunol* 2009; 123: 1004-11.

41. ROMAGNANI S: Human Th17 cells. *Arthritis Res Ther* 2008; 10: 206.

42. MAHENDRA A, MISRA R, AGGARWAL A: Th1 and Th17 predominance in the enthesitis-related arthritis form of juvenile idiopathic arthritis. *J Rheumatol* 2009; 36: 1730-6.

43. BALL J: Enthesopathy of rheumatoid and ankylosing spondylitis. *Ann Rheum Dis* 1971; 30: 213-23.

44. APPEL H, KUHNE M, SPIEKERMANN S *et al*.: Immunohistologic analysis of zygapophyseal joints in patients with ankylosing spondylitis. *Arthritis Rheum* 2006; 54: 2845-51.

45. APPEL H, KUHNE M, SPIEKERMANN S *et al*.: Immunohistochemical analysis of hip arthritis in ankylosing spondylitis: evaluation of the bone-cartilage interface and subchondral bone marrow. *Arthritis Rheum* 2006; 54: 1805-13.

46. D'AGOSTINO MA, PALAZZI C, OLIVIERI I: Entheseal involvement. *Clin Exp Rheumatol* 2009; 27 (Suppl. 55): S50-5.

47. OLIVIERI I, BAROZZI L, PADULA A *et al*.: Retrocalcaneal bursitis in spondyloarthropathy: assessment by ultrasonography and magnetic resonance imaging. *J Rheumatol* 1998; 25: 1352-7.