Decreased Th17 and Th1 cells in the peripheral blood of patients with early non-radiographic axial spondyloarthritis: a marker of disease activity in HLA-B27+ patients

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Abstract

Objective. To examine the frequency and phenotype of Th17 cells in the peripheral blood of patients with early non-radiographic axial SpA (early nrSpA).

Methods. CD4+ T cells were isolated from the peripheral blood of 30 early nrSpA patients, 11 AS patients and 41 age- and sex-matched healthy controls by Ficoll-Hypaque gradient and magnetic negative selection. After polyclonal stimulation, the frequency of Th17 and Th1 cells and of cells producing TNF-α or IL-10 was determined by cytometry and concentrations of IL-17, IL-22, IFN-γ, TNF-α, IL-10 and IL-4 were measured by ELISA.

Results. Early nrSpA but not AS patients demonstrated a significantly lower percentage of circulating Th17, Th1 and Th17/Th1 cells, together with lower CD4-derived IL-17 and IFN-γ secretion, as compared with controls. In contrast, the percentage of circulating cells producing IL-10 or TNF-α, and the secretion of CD4-derived IL-10, TNF-α, IL-22 and IL-4 in early nrSpA were not different from controls. All Th17 cells were CD45RO+CD45RA− and CCR6+. The frequency of circulating Th17, Th1 and Th17/Th1 was negatively correlated with BASDAI, BASFI, ASDAS-CRP, ASDAS-ESR, AS quality of life (ASQOL) and patient’s global assessment in HLA-B27+ but not in HLA-B27− early nrSpA patients. A positive correlation between circulating Th17 cells and BASDAI was observed in AS.

Conclusion. A decreased percentage of Th17, Th1 and Th17/Th1 cells is apparent in peripheral blood CD4+ T cells from early nrSpA. Th17, Th1 and Th17/Th1 cell numbers are related to disease activity indices in HLA-B27+, but not in HLA-B27−, early nrSpA patients.

Key words: spondyloarthritis, cytokines and inflammatory mediators, T cells, inflammation, lymphocytes.

Introduction

The term SpA designates a group of diseases characterized by inflammatory back pain, sacroilitis, enthesitis, asymmetric peripheral arthritis, dactylitis and uveitis. According to the newly developed Assessment of SpondyloArthritis International Society (ASAS) criteria, SpA can be classified based on clinical grounds as predominantly axial SpA or predominantly peripheral SpA [1, 2], with or without the presence of associated diseases such as psoriasis, IBD or a triggering infection. AS is the prototype of axial SpA, and its diagnosis is usually delayed 6–8 years after the onset of symptoms due to the late appearance of sacroilitis on plain radiographs in a majority of patients [1]. Therefore the ASAS classified those patients with axial SpA who do not demonstrate definite radiographic changes in the sacroiliac joints as non-radiographic axial SpA (nrSpA), in an attempt to improve previous criteria, especially for application in recent
onset SpA [1]. This facilitates the conduct of research in early disease and early initiation of treatment, which is pivotal to achieving good response and preventing chronic disability.

Numerous experimental data indicate that IL-17A may be implicated not only in human RA but also in SpA [3]. It was initially established that IL-17A plays an important role in the pathogenesis of animal models of inflammatory arthritis: IL-17-deficient mice demonstrate a markedly attenuated form of CIA [4], neutralization of IL-17 during the induction of rat adjuvant arthritis suppresses the onset of disease [5] and anti-IL-17 therapy in established CIA significantly reduces severity [6]. More recent observations indicate that joint ankylosis in a murine model is significantly associated with the number of popliteal lymph node T cells producing IL-17 [7], and Th17 cells are expanded in SpA-prone HLA-B27-transgenic rats [8]; in addition, blockade of IL-17 improves the onset of tarsal ankylosis in a murine model [9] and ameliorates the exacerbated uveitis in mice with proteoglycan-induced arthritis and spondyilitis [10].

In human studies, increased levels of IL-17 and Th17 cells have been detected in the peripheral blood and SF of SpA [11-17]. In addition, AS seems to be associated with genetic polymorphisms of the IL-23 receptor, and the protective R381Q gene variant is characterized by impaired Th17 responses [18, 19]. Furthermore, an anti-IL17A mAb has shown promising results in the treatment of RA, PsA and AS [20-23].

To our knowledge there are no published studies on Th17 cell frequencies in early nrSpA. This is attributable to the recent definition of this concept and the absence of previous appropriate and accepted classification criteria [1]. Existing reports on Th17 biology in undifferentiated SpA are limited to detection of this cytokine in serum and SF by ELISA [12, 13], with no data on the frequency of circulating Th17 cells.

Our objective was to examine the frequency and phenotype of Th17 cells in the peripheral blood of early nrSpA patients. Our early SpA clinic allowed the study of T cells from patients with early disease of <2 years, and who had not received DMARDs, steroids or biologics, thereby minimizing the interference of perpetuating inflammatory feedback loops and of drugs with ex vivo T cell responses. We observed that patients with early nrSpA demonstrate a decreased percentage of circulating Th17, Th1 and Th17/Th1 cells and, interestingly, Th17, Th1 and Th17/Th1 cell numbers are clearly correlated with disease activity indices in HLA-B27+ but not HLA-B27− early nrSpA.

Patients and methods

Patients

Peripheral blood was obtained from 30 early nrSpA, 11 patients with AS fulfilling modified New York criteria [24] and 41 age- and sex-matched healthy controls. Early nrSpA patients fulfilled ASAS criteria [1] and the inclusion criteria were inflammatory back pain with or without asymmetric peripheral arthritis, disease duration of 3-24 months, age of 18-45 years and being treatment naïve for disease-modifying drugs, corticosteroids and biologics. La Paz University Hospital in Madrid, Spain, has an early SpA unit that receives patients from eight primary care centres, corresponding to an area of 242 784 inhabitants, as part of the ESPERANZA programme, a nationwide health management programme designed to provide excellence in care for early SpA [25]; this facilitated recruitment of early nrSpA patients for the present study. At the baseline visit, medical history, physical examination, ESR, CRP, HLA-B27, BASDAI, BASFI, ASDAS-CRP, ASDAS-ESR, the AS Quality of Life (ASQOL) scale, patient’s global assessment and pelvis conventional X-ray were recorded for each patient. Among early nrSpA patients 9 were female and 21 were male; their mean (s.d.) age was 34.9 (7.2) years, median 35.6 years, maximum 45 years and minimum 19 years; 15 (50%) tested positive for HLA-B27. The mean (s.d.) disease duration was 12.7 (5.6) months, median 13 months, maximum 22 months and minimum 3 months. ASAS criteria fulfilled by HLA-B27+ and HLA-B27− early nrSpA patients are summarized in Table 1. The study was approved by the Hospital La Paz-IdiPAZ Ethics Committee, and all subjects provided written informed consent according to the Declaration of Helsinki.

Among AS patients, two were taking only NSAIDs and nine were receiving anti-TNF. There were 1 female and 10 males, their mean (s.d.) age was 49.5 (11.6) years, median 46.0 years, maximum 71 years and minimum 33 years; 10 (90.1%) tested positive for HLA-B27. The mean (s.d.) disease duration was 21.3 (13.4) years, median 23 years, maximum 51 years and minimum 3 years.

None of the patients had associated psoriasis, IBD or history of a preceding infection.

Isolation of CD4+ T cells

Mononuclear cells were isolated from human blood by density centrifugation using Ficoll-Paque Plus (GE Healthcare, Chalfont St Giles, UK). CD4+ T cells were subsequently purified by negative selection in an Automacs (Miltenyi Biotec, Bergisch Gladbach, Germany) using the CD4+ T Cell Isolation Kit II from Miltenyi Biotec, containing a cocktail of biotin-conjugated antibodies against CD8, CD14, CD16, CD19, CD36, CD56, CD123, γδ TCR and glycophorin A, followed by anti-biotin microbeads. Isolated CD4+ T cells were 98% pure and free of detectable CD14+ monocytes, CD8+ T cells, CD56+ NK cells, CD19+ B cells and γδ T cells.

T cell stimulation

Immediately after isolation, CD4+ T cells were cultured and stimulated in 24-well plates (105 cells/well) containing RPMI 1640 medium (Lonza, Allendale, NJ, USA) with 10% fetal calf serum (FCS), 2 mM L-glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin and 50 μM 2-mercaptopothenol (complete RPMI medium). Two different activation strategies were undertaken. T cells were stimulated for 16 h with phorbol myristate acetate (PMA) (10 nM) and ionomycin (2 μM) in the presence or absence of 4 μM monensin (all three from Sigma-Aldrich, St Louis, MO, USA). In addition,
cells were cultured for 1–4 days in the presence of a soluble anti-CD3 IgE subclass mAb (T3/4.E, Sanquin, Amsterdam, The Netherlands, formerly Central Laboratory of the Netherlands Red Cross Blood Transfusion Service) (0.5 μg/ml) plus anti-CD28 (1 μg/ml) (BD Pharmingen, San Diego, CA, USA) and anti-CD49d mAbs (1 μg/ml) (BD Pharmingen), and restimulated for the last 6 h with PMA and ionomycin, with or without monensin.

Intracellular cytokine staining, surface staining and flow cytometry

Fluorochrome-conjugated mAbs from BD Pharmingen were used to examine the expression of the phenotypical markers CD3, CD4, CD8, CCR6, CCR4, CD45RO, CD45RA, CD25 and CD127. Surface IL-23R was detected with a biotinylated goat anti-human IL-23R antibody (R&D Systems, Abingdon, UK), followed by an FITC-labelled avidin (BD Pharmingen). For intracellular cytokine staining, T cells were washed with PBS/2% FCS/0.01% NaN3, permeabilized for 10 min with FACS permeabilizing solution 2 (BD Pharmingen), washed again and incubated on ice for 1 h with an aliphycocyanin-labelled anti-IFN-γ mAb (clone B27; BD Pharmingen), a phycocythrin (PE)-labelled anti-IL17A mAb (clone eBio4DEC17; eBioscience, San Diego, CA, USA), an FITC-labelled anti-TNF-α mAb (clone Mab11, BD Pharmingen), a PE-labelled anti-IL10 mAb (clone B-T10, Miltenyi Biotec) or fluorochrome-labelled isotype control mAbs. FoxP3 was detected after permeabilization with a FoxP3 staining set (Miltenyi Biotec) and incubation with an anti-FoxP3-AlexaFluor 488 mAb (clone 236A/E7) (eBioscience). After washing once with PBS/2%FCS/0.01%NaN3 and once with PBS, cells were resuspended in 1% paraformaldehyde and analysed in a FACSCalibur flow cytometer using CellQuest software (BD Biosciences, San José, CA, USA).

ELISAs

Cell-free culture supernatants were collected and stored at −80°C. ELISAs for IL-17A and IL-22 were performed using kits from eBioscience. ELISAs for TNF-α, IL-10, IL-4 and IFN-γ were performed using kits from BD Biosciences following the manufacturer’s instructions.

Statistical analysis

Comparison between groups was by Mann–Whitney U-test. When appropriate, Bonferroni correction for multiple comparisons was applied. Correlations were analysed using Spearman’s rank correlation coefficients. All analyses were performed using Prism version 5.0 software (GraphPad Software, La Jolla, CA, USA).

Results

Expression of IL-17 and IL-22 by early nrSpA and by AS CD4+ T cells

The frequency of circulating CD4 T cells expressing IL-17 (Th17 cells) was significantly decreased among early nrSpA patients [median 0.63, interquartile range (IQR) 0.38–1.15%] in comparison with healthy controls (median 1.03, IQR 0.71–1.52%) (Fig. 1A). There were no differences between HLA-B27 + and HLA-B27– patients (Fig. 1A). In contrast, the percentage of Th17 cells in the peripheral blood of AS patients was not different from controls (Fig. 1A). All of the Th17 cells expressed the memory phenotypical marker CD45RO, were negative for CD45RA and positive for CCR6 expression (Fig. 1B). The expression of IL-23R and CCR4 could not be analysed together with IL-17, since these two molecules are down-regulated upon stimulation with PMA/ionomycin. Importantly, the proportion of circulating total CD4+ T cells was not different among early nrSpA, AS and control subjects.

In parallel, the concentration of IL-17 detected by ELISA in culture supernatants of stimulated early nrSpA CD4+ T cells (median 1.34, IQR 0.45–2.23 ng/ml) was significantly decreased when compared with healthy controls (median 2.10, IQR 1.57–4.00 ng/ml) (Fig. 1C), and this was apparent not only when cells were stimulated overnight with PMA/ionomycin (see above data) but also after stimulation for 4 days with anti-CD3/CD28/CD49d (median 1.61, IQR 0.61–3.62 ng/ml for early nrSpA vs

| Table 1 ASAS classification criteria for axial SpA [1] in 30 patients with early nrSpA |
|---------------------------------|-----------------|-----------------|
| ASA classification criteria     | HLA-B27 (n = 15)| HLA-B27* (n = 15)|
| Definite sacroiliitis—modified New York criteria [24] | 0               | 0               |
| Sacroiliitis on MRI             | 15              | 7               |
| Inflammatory back pain          | 15              | 15              |
| Arthritis                       | 5               | 5               |
| Enthesitis (heel)               | 15              | 2               |
| Uveitis                         | 2               | 1               |
| Spondylitis                     | 1               | 0               |
| Psoriasis                       | 0               | 0               |
| Good response to NSAIDs         | 10              | 13              |
| Family history for SpA          | 4               | 5               |
| HLA-B27                         | 0               | 15              |
| Elevated CRP                    | 0               | 3               |

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CD4⁺ T cells were isolated from the peripheral blood of 41 healthy controls (HCs), 30 early nrSpA (e-nrSpA) patients (15 HLA-B27⁻ and 15 HLA-B27⁺) and 11 AS patients (1 HLA-B27⁻ and 10 HLA-B27⁺) and stimulated with PMA/ionomycin for 16 h or with anti-CD3/CD28/CD49d for 4 days. (A) Percentage of CD4⁺ T cells expressing IL-17A (Th17 cells) after a 16-h stimulation, as determined by flow cytometry. As the CD4 molecule is down-regulated upon stimulation with PMA, shown is CD3 expression on isolated CD4⁺ T cells. (B) Representative flow cytometry dot plots showing expression of CCR6, CD45RO and CD45RA vs IL-17A in isolated CD4⁺ T cells. (C) Secretion of IL-17A to the culture medium of CD4⁺ T cells stimulated for 16 h with PMA/ionomycin (left panel) or for 4 days with anti-CD3/CD28/CD49d (right panel). (D) Secretion of IL-22 to the culture medium of CD4⁺ T cells stimulated for 16 h with PMA/ionomycin (left panel) or for 4 days with anti-CD3/CD28/CD49d (right panel). Box plots represent the median and interquartile range of all studied subjects, whiskers represent the maximum and minimum values. *P < 0.05 vs HC; †P < 0.05 vs early nrSpA.
2.86, 2.05-6.52 ng/ml for controls) (Fig. 1C). Secretion of CD4-derived IL-17 was not different in HLA-B27+ vs HLA-B27− early nrSpA patients. In addition, the amount of IL-17 secreted by CD4 T cells of AS patients was not different from controls (Fig. 1C).

As several reports describe the production of IL-17 by other cell populations [26-29], we additionally isolated γ/δ T cells, CD8+ T cells and NK cells from patients and controls. We could not detect any IL-17 by cytomtery or ELISA in these cell subsets from the peripheral blood of early nrSpA, AS or healthy subjects. Moreover, after thorough depletion of CD4+ T cells from peripheral blood mononuclear cells, no IL-17 could be detected in culture supernatants after short or long-term stimulation periods, in agreement with work published by Shen et al. [15].

We additionally measured the secretion of IL-22, a cytokine secreted by Th17 cells [3], in culture supernatants of stimulated CD4+ T cells. No differences were observed among early nrSpA, AS and control subjects, after stimulation for 16 h or 4 days (Fig. 1D).

Expression of IFN-γ by early nrSpA and by AS CD4+ T cells

The frequency of circulating cells producing IFN-γ (Th1) was significantly decreased among early nrSpA patients (median 9.07, IQR 6.74-13.52%) in comparison with healthy controls (median 14.12, IQR 10.79-18.12%) (Fig. 2A). Among early nrSpA patients there were no differences between HLA-B27+ and HLA-B27− subjects (Fig. 2A). In contrast, the percentage of Th1 cells in the peripheral blood of AS patients was not different from controls (Fig. 2A).

In parallel, the concentration of IFN-γ detected by ELISA in culture supernatants of stimulated early nrSpA CD4+ T cells (median 8.41, IQR 4.95-13.91 ng/ml) was significantly decreased as compared with healthy controls (median 17.67, IQR 7.94-26.11 ng/ml) (Fig. 2B), and this was apparent not only when cells were stimulated overnight with PMA/ionomycin (see above data), but also after stimulation for 4 days with anti-CD3/CD28/CD49d (median 208.7, IQR 30.0-328.0 ng/ml for early nrSpA vs 296.3, 174.5-934.1 ng/ml for controls) (Fig. 2B). Secretion of CD4-derived IFN-γ was not different in HLA-B27+ vs HLA-B27− early nrSpA patients. In addition, the amount of IFN-γ secreted by CD4 T cells of AS patients was not different from controls (Fig. 2B).

Frequency of Th17/Th1 cells in early nrSpA and in AS

A population of cells simultaneously producing IL-17 and IFN-γ (Th17/Th1 cells) [30] was detected in the peripheral blood of controls, early nrSpA and AS patients, and their frequency was significantly lower in early nrSpA (median 0.11, IQR 0.05-0.22%) as compared with controls (median 0.20, IQR 0.13-0.28%) (Fig. 2C). The percentage of Th17/Th1 cells was not different in HLA-B27+ vs HLA-B27− early nrSpA patients. In addition, the percentage of Th17/Th1 cells in the peripheral blood of AS patients was not different from controls (Fig. 2C).

Expression of TNF-α, IL-10 and IL-4 by early nrSpA CD4+ T cells

We were next interested in determining whether early nrSpA is characterized by a generalized defect in cytokine production. To this end, the expression of additional cytokines was investigated in these patients. Interestingly, the frequency of circulating cells producing TNF-α or IL-10 (Fig. 3A and B), together with the concentration of TNF-α, IL-10 and IL-4 in supernatants of stimulated CD4+ T cells (Fig. 3C) were comparable in early nrSpA and control subjects, and there were no differences between HLA-B27+ vs HLA-B27− patients. In addition, the frequency of circulating CD4+ T cells expressing both IL-17 and TNF-α was not different in early nrSpA as compared with controls (Fig. 3D).

Frequency of circulating CD25+/CD127− CD4+ T cells and of FoxP3+ CD4+ T cells in early nrSpA and in AS

Numerous reports indicate that there is a close relationship between Th17 cells and CD4+CD25+ Tregs [3]. Therefore we decided to examine the numbers of this Th subset in our patients. The frequencies of circulating CD25+/CD127− CD4+ T cells and of FoxP3+ CD4+ T cells were not different among early nrSpA, AS and control subjects (Fig. 4A and B).

Relation of circulating Th17, Th1 and Th17/Th1 cells with clinical and analytical parameters in early nrSpA and AS

We then decided to analyse the relationship of the circulating Th17, Th1 and Th17/Th1 frequencies with clinical and analytical data. Interestingly, in HLA-B27+ early nrSpA patients, the percentage of peripheral blood Th17 cells, Th1 cells and Th17/Th1 cells was negatively correlated with the following parameters: BASDAI, BASFI, ASDAS-CRP, ASDAS-ESR, ASQOL and patient’s global assessment (Fig. 5). In contrast, these parameters were not correlated with circulating Th17, Th1 or Th17/Th1 numbers in HLA-B27− early nrSpA patients (supplementary Fig. S1, available as supplementary data at Rheumatology Online), even though the percentage of circulating Th17 cells was not different between HLA-B27+ and HLA-B27− patients (Fig. 1A). Importantly, no differences in BASDAI, BASFI, ASDAS-CRP, ASDAS-ESR, ASQOL or patient’s global assessment were observed between HLA-B27+ and HLA-B27− patients. Strikingly, in AS patients, a significant correlation was observed between the percentage of circulating Th17 cells and the BASDAI index, but this was a positive correlation as opposed to the negative correlation seen in early nrSpA (Fig. 6A). In addition, a good positive correlation between the percentage of circulating Th1 cells and the BASDAI index was apparent in AS, although it did not reach statistical significance (P=0.08) (Fig. 6B). No such correlations were found in AS with BASFI, ASDAS-CRP, ASDAS-ESR, ASQOL or patient’s global assessment.
Fig. 2 Expression of IFN-γ by early nrSpA and AS CD4⁺ T cells.

(A) Percentage of CD4⁺ T cells expressing IFN-γ (Th1 cells) after 16 h of stimulation. (B) Secretion of IFN-γ to the culture medium of CD4⁺ T cells stimulated for 16 h (left panel) or for 4 days (right panel). (C) Percentage of CD4⁺ T cells expressing both IL-17A and IFN-γ (Th17/Th1 cells) after 16 h of stimulation. *P < 0.05 vs HC. See Fig. 1 for details and definitions.
Discussion

Several previous studies indicated that Th17 cells may play an important role in the pathogenesis of SpA [7–23], but limited data exist on Th17 biology in patients with early nrSpA. We detected a significantly decreased frequency of circulating Th17 cells in early nrSpA, and there were no differences between HLA-B27+ and HLA-B27– patients. Furthermore, the frequency of circulating Th1 cells, of circulating Th17/Th1 cells and the secretion of CD4-derived IL-17 and IFN-γ were also decreased in early nrSpA. This was not attributable to a generalized defect of cytokine production, since the frequency of circulating cells expressing TNF-α or IL-10, together with the secretion of CD4-derived TNF-α, IL-10, IL-4 and IL-22, were not different in early nrSpA patients as compared with healthy controls. That is, CD4+ T cells of early nrSpA patients demonstrated a selectively decreased secretion of both IL-17 and IFN-γ. In contrast, our patients with AS of long duration demonstrated expression of IL-17 and IFN-γ that was comparable to that observed in healthy controls, suggesting that the findings in early nrSpA may be related to the early disease stage and/or different medication. Previous studies on the frequency of Th17 cells in the peripheral blood of AS patients have yielded conflicting results: while some have described increased Th17 frequencies [11, 15, 16], others have reported frequencies comparable to those observed in control subjects [29], and the latter are consistent with our findings.

To date, limited information exists regarding Th17 biology in patients with early nrSpA, due in part to the previous absence of appropriate classification criteria [1]. Our finding that circulating Th17 cells are decreased in early nrSpA naïve for treatment with DMARDs, steroids or...
Fig. 5 Relationship of Th17, Th1 and Th17/Th1 periperal blood cell frequencies with clinical parameters in HLA-B27+ early nrSpA patients.

The left panels show correlations of Th17, the central panels show correlations of Th1 and the right panels show correlations of Th17/Th1 cells with the BASDAI index (A), BASFI index (B), ASDAS-CRP (C), ASDAS-ESR (D), ASQOL (E) and patient’s global assessment (F) in HLA-B27+ early nrSpA patients (n = 15). Each filled square represents one patient.
biologics is striking and not easy to explain from a pathomechanistic perspective. Th17 cells are found in the facet joints of AS patients [29], and it is possible that in early disease, recirculation of Th17 cells through the peripheral blood is limited due to sequestration at the inflammatory site. As an abundance of IL-17-producing neutrophils has been observed in AS facet joints [29], and mast cells seem to be the major source of IL-17 in SpA synovial membranes [28], it has been suggested that these cell types could play a leading role in the pathogenesis of SpA [31]. It has been described that additional sources of IL-17, such as γ/δ T cells and NK cells, may also be pathogenic [26, 27]. In this context we could not detect any IL-17 expression by γ/δ T cells, CD8+ T cells or NK cells from the peripheral blood of healthy controls, AS or early nrSpA patients, consistent with work published by Shen et al. [15].

Interestingly, we also observed decreased frequency of circulating Th1 cells in early nrSpA, with no difference between HLA-B27+ and HLA-B27− patients, whereas the percentage of Th1 cells in the peripheral blood of our patients with AS was comparable to that observed in controls. Previous work has described either decreased [32–34] or normal [15] circulating Th1 frequencies in AS. Again, our data indicate that patients with early disease are characterized by a distinct immunological status.

We additionally observed that the frequency of circulating CD4+CD25+CD127− T cells and CD4+FoxP3+ T cells in early nrSpA patients was not different from control subjects. To our knowledge, this is the first report on the frequency of this T cell subset in patients with early nrSpA. Tregs may be implicated in the pathogenesis of autoimmune and inflammatory conditions and their numbers have been found altered in the peripheral blood of patients with SLE and RA [35]. Previous work by Appel et al. [36] indicated that the frequency of CD4+FoxP3+ in the peripheral blood of established SpA, including 11 subjects with undifferentiated SpA, is not different from controls, which is consistent with our results.

Our most striking finding was the good negative correlation between clinical parameters of disease activity or function and the numbers of Th1, Th1 and Th17/Th1 cells in the peripheral blood of HLA-B27+ early nrSpA patients. This is especially remarkable given the difficulty in assessing clinical activity in SpA due to the lack of a universally accepted gold standard [37], and given that some of the currently used indices measure only one aspect of the disease or are fully patient or physician oriented. Among HLA-B27+ early nrSpA patients, the circulating Th17, Th1 and Th17/Th1 frequencies were negatively correlated with the BASDAI, BASFI, ASDAS-CRP, ASDAS-ESR and ASQOL indices, and with patient’s global assessment. Of note, these correlations were not detected in HLA-B27− early nrSpA subjects, and reasons to explain this observation are not apparent to us at present. In contrast, and more remarkably, the BASDAI index was positively correlated with circulating Th17 cells in AS patients, even though Th17 cells were not overrepresented in their peripheral blood. That is, those AS patients who demonstrated the highest disease activities tended to show higher levels of circulating Th17 cells. This was in contrast to the observation in early nrSpA, where the highest disease activities were associated with the lowest rates of circulating Th17 cells. This observation reinforces the finding that Th17 cell biology is different in early nrSpA vs AS, and indicates that further investigation is needed on this matter. A negative correlation between patient’s global assessment of disease activity and circulating Th1 cells was previously reported by Baeten et al. [33] in patients with long-term established SpA, which confirms that values of certain Th cell subsets in peripheral blood can be used as alternative markers of disease activity.

Conclusions

In summary, we have observed a significantly decreased frequency of circulating Th17, Th1 and Th17/Th1 cells in early nrSpA patients together with a preserved frequency of circulating CD4+ T cells producing IL-10 or TNF-α; in addition, the frequency of circulating CD4+CD25+CD127− T cells and CD4+FoxP3+ T cells in early nrSpA was not different from that observed in control subjects. The circulating Th17, Th1 and Th17/Th1 frequencies were negatively correlated with BASDAI, BASFI, ASDAS and ASQOL indexes, and with patient’s global assessment, in HLA-B27− but not in HLA-B27+ early nrSpA patients. This indicates that further studies are needed to fully understand the complex biology of Th17 cells in early nrSpA. In addition, the circulating Th17 frequency can be considered as a surrogate measure of disease activity in HLA-B27+ patients with early nrSpA.

Rheumatology key messages

- The frequency of circulating Th17, Th1 and Th17/Th1 cells is decreased in patients with early nrSpA.
- Circulating Th17, Th1 and Th17/Th1 frequencies are negatively correlated with disease activity in HLA-B27+ early nrSpA.

Acknowledgements

M.-E.M.-C. conceived and designed the experiments; M.-B.B.-C. and I.A.-V. performed the experiments; M.-E.M.-C., M.-B.B.-C., I.A.-V., E.dM., C.C.-G., D.P.L. and E.M.-M. analysed the data; E.dM., C.C.G., D.P. and
E.M.-M. contributed reagents, materials and analysis tools; M.-E.M.-C., M.-B.B.-C., I.-A.-V. and E.dM. wrote the manuscript.

Funding: This work was supported by Ministerio de Ciencia e Innovación grant SAF 2009-07100; by the Fundación Española de Reumatología—ESPERANZA programme; and by the RETICS programme, RD08/0075 (RIER) from Instituto de Salud Carlos III (ISCIII). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Disclosure statement: The authors have declared no conflicts of interest.

Supplementary data

Supplementary data are available at Rheumatology Online.

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