

Decreased blood CD4+PD-1+ and CD8+PD-1+ T cells in psoriatic patients with and without arthritis

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Abstract

Introduction: Psoriasis with and without arthritis have common immunological mechanisms which among others involve the interactions between cytokines produced by T cells, including Th1, Th17 and Th22. Although quite a lot is known about psoriasis pathogenesis, the cause of chronic immune activation and response in the disease remains unclear. One of the negative regulators of the immune system is programmed death 1 (PD-1).

Aim: To assess the expression level of PD-1 in the peripheral T cells of psoriatic patients with and without arthritis.

Material and methods: The study included 23 psoriatic patients with arthritis, 52 psoriatic patients without arthritis and 52 healthy controls. The percentages of CD3+, CD4+, CD8+, CD4+PD-1+ and CD8+PD-1+ T cells were analyzed using flow cytometry.

Results: The percentages of CD4+PD-1+ as well as CD8+PD-1+ T cells in the psoriatic patients both with and without arthritis were significantly lower than in the control group. The percentages of CD4+PD-1+ as well as CD8+PD-1+ T cells were not significantly different between the psoriatic patients with and without arthritis. A significant positive correlation between PD-1 expression on the CD4+ and CD8+ T cells was found in the psoriatic patients without arthritis.

Conclusions: Impairment of the negative co-stimulation from PD-1 may be another common characteristic of psoriasis both with and without arthritis.

Key words: psoriasis, psoriatic arthritis, programmed death 1.

Introduction

An erythematous plaque covered with silvery scales is a typical skin lesion of psoriasis, a chronic systemic inflammatory disease which presents itself in various clinical forms. The disease is heterogeneous, and whether it will assume a mild or more severe course may depend on genetic variants of psoriasis [1]. Even though in about 30% of psoriatic patients psoriatic arthritis (PsA) is also diagnosed, it is debatable whether PsA may be regarded as one of the psoriasis manifestations or whether it is a separate and independent form of arthritis. These two types of psoriasis, i.e. psoriasis with and without arthritis, share plenty of immunological mechanisms, yet their pathogenesis has not been fully explained so far. It is thought that innate immunity plays a certain role in psoriasis initiation with a subsequent response of various T cell subsets, not only Th1 but also Th17, Th22 and

their cytokines, which will ultimately lead to formation of psoriasis auto-inflammatory pathways [2]. Nevertheless, the cause of immune imbalance and chronic activation of the T cell subsets in psoriasis should be further investigated. The recently published reports indicate that during anti-programmed death 1 (PD-1) and anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) therapies, skin and articular complications, including psoriasis, may occur; therefore, it seems plausible that negative T cell co-stimulation may be impaired in psoriasis [3–5]. In experimental studies PD-1^{-/-} T cells were able to produce increased amounts of interferon γ (IFN- γ) and interleukin (IL)-17, thereby confirming the key role of PD-1 in the regulation of Th1 and Th17 responses [6]. In order to detect immunomodulatory agents capable of reducing an inflammatory process in the course of psoriasis, an in-depth investigation of immune tolerance mecha-

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nisms in psoriasis, both with and without arthritis, is worth launching.

Aim

The aim of the study was to assess the expression level of PD-1 in the peripheral T cells of psoriatic patients with and without arthritis.

Material and methods

Study groups

The study included 75 patients: 23 (30.67%) psoriatic patients with arthritis and 52 (69.33%) psoriatic patients without arthritis hospitalized in the Department of Dermatology, Venereology and Pediatric Dermatology at the Medical University of Lublin, Poland, as well as 52 age- and gender-matched healthy volunteers. Psoriatic arthritis (PsA) was diagnosed using the Classification of Psoriasis Arthritis (CASPAR) criteria.

The inclusion criteria were: the duration of psoriasis for at least one year, active psoriatic skin lesions, age at least 18 years. The exclusion criteria were: cardiovascular, cerebrovascular, hematologic, hepatic or renal disease, neoplasm, chronic viral infections, erythrodermic, pustular or guttate psoriasis, addiction to drugs, systemic anti-psoriatic treatment.

The study was approved by the Local Ethic Committee at the Medical University of Lublin (KE-0254/81/2015). Informed consent was obtained from all the participants.

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Assessment of psoriasis severity

The severity of psoriatic skin lesions was assessed with Psoriasis Area and Severity Index (PASI), body surface area (BSA), Patient Global Assessment (PGA), and Dermatology Life Quality Index (DLQI), and nail plate changes with Nail Psoriasis Severity Index 80 (NAPSI 80).

The severity of psoriatic arthritis was assessed with the Disease Activity Score 28 (DAS 28), Patient Visual Analog Scale (VAS), number of tender joints, number of swollen joints, and physician and patient Likert scales.

In studied patients laboratory tests, i.e. blood morphology, C-reactive protein (CRP), and (erythrocyte sedimentation rate (ESR), were performed in the hospital central laboratory.

Flow cytometry analysis of peripheral blood mononuclear cell populations and expression of PD-1

Flow cytometry analysis was performed in the Experimental Hematooncology Department at the Medical University of Lublin, Poland, with staff professionally trained

and experienced in such analyses in patients with lymphoproliferative diseases.

Eight ml of peripheral venous blood from the psoriatic patients with and without arthritis as well as healthy volunteers were collected into anti-coagulated tubes. We isolated mononuclear cells using density gradient centrifugation on Ficoll-Hypaque (Biochrom AG, Berlin, Germany). Interphase cells were removed, washed twice in phosphate-buffered saline (PBS) without Ca^{2+} and Mg^{2+} and resuspended in RPMI 1640 containing 2% human albumin. The viability of obtained peripheral blood mononuclear cells (PBMCs) was always > 95%, as determined by trypan blue staining. Viable cells were quantified in a Neubauer chamber. 5×10^5 cells were incubated for 20 min at room temperature with fluorochrome-labeled monoclonal antibodies (Mabs): anti-CD3-PerCP (Becton Dickinson), anti-CD4-FITC (Becton Dickinson), anti-CD8-PE (Becton Dickinson) and anti-PD-1-APC (clone MIH4) (Becton Dickinson). Approximately 100,000 stained cells in each sample were analyzed by flow cytometry using a FACS Canto II flow cytometer (BD Biosciences, San Jose, CA, USA). Unstained cells were used as a negative control (for each patient). Data analysis was accomplished by FACS Diva 8.0.

For each person, lymphocytes were identified and gated from PBMCs by setting appropriate forward and side scatter parameters. Next, 3 steps of gating were applied in flow cytometry analysis. The first dot plot showed CD3+ cells selected from all lymphocytes and as a result we obtained percentages of CD3+ cells of all lymphocytes. The two gates at the second step of the cytometry selected: one gate CD4+ and the other gate CD8+ T cells separately, resulting in percentages of CD4+ and CD8+ T cells, respectively, of all CD3+ cells. The two gates at the third step of the cytometry selected: one gate CD4+PD-1+ T cells and the other gate CD8+PD-1+ T cells separately, resulting in percentages of CD4+PD-1+ T cells and CD8+PD-1+ T cells of CD4+ and CD8+ T cells, respectively (Figure 1 shows it for 3 exemplary persons: 1 patient with psoriatic arthritis, 1 patient with psoriasis without arthritis and 1 healthy volunteer).

Statistical analysis

Mean values (M) and standard deviations (SD) were calculated for continuous variables, or absolute numbers (n) and relative numbers (%) of occurrence of items of categorical variables. We used the stochastic independence χ^2 test to compare sex between the control group and psoriatic patients with and without arthritis, as well as positive family history of psoriasis of psoriatic patients with and without arthritis. We used the Mann-Whitney U test to compare: age, percentages of PBMCs and PD-1 between the control group and psoriatic patients with and without arthritis, and other clinical data (measured as continuous variables) between psoriatic patients with and without arthritis.

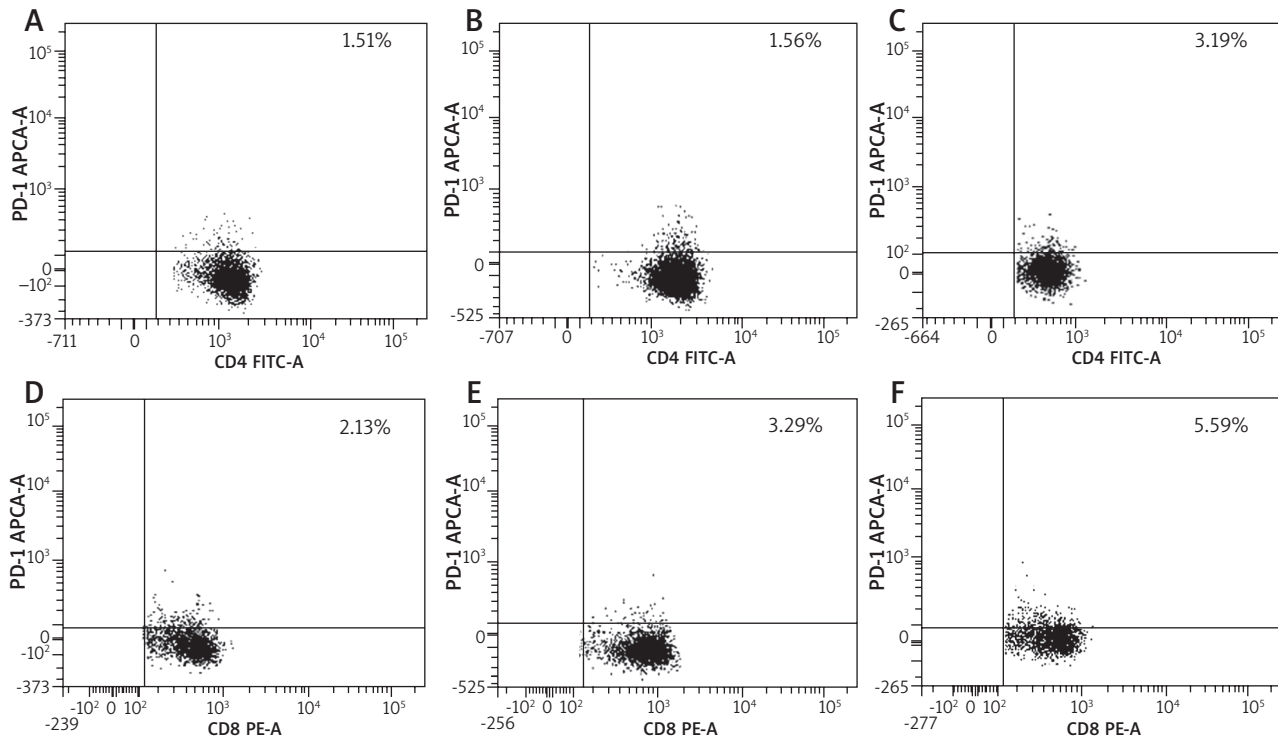


Figure 1. Representative flow cytometry analysis of PD-1 expression on CD4+ (A–C) and CD8+ (D–F) T cells from psoriatic patients without arthritis (A, D), with arthritis (B, E) and healthy controls (C, F)

We used Spearman’s correlation coefficient to investigate mutual correlations between the expression of PD-1 on CD4+ and CD8+ T cells and between this expression and clinical data in psoriatic patients, separately with and without arthritis.

Statistical analysis was performed using Statistica software. The significance level was set at 0.05.

Results

Characteristics of studied psoriatic patients with and without arthritis and healthy controls

Demographic characteristics and clinical data, duration, severity and family history and laboratory blood tests (ESR and CRP) of the patients are described in Table 1. Psoriatic patients with and without arthritis and the control group did not significantly differ in sex ($p < 0.05$). In the control group 43 out of 52 were males, that is 82.69%. Mean age of the control group was 48.7 ± 15.2 years and did not significantly differ from patients with and without arthritis ($p < 0.05$). However, patients with arthritis were significantly older than patients without arthritis. Psoriasis duration was significantly longer in psoriatic patients with arthritis compared to patients without arthritis. Psoriasis severity did not significantly differ between psoriatic patients with and without arthritis.

Clinical data and psoriasis arthritis severity are also presented in Table 1: mean duration of PsA 9.3 years, mean age of disease onset 42 years, mean number of tender joints 4.6, mean number of swollen joints 1.3, mean value of VAS 40, mean value of DAS 3.2. The majority of patients (65.2%) had the oligoarticular subset of PsA.

Comparison of PBMC populations’ distribution between psoriatic patients with and without arthritis and healthy controls

The percentages of CD3+, but not CD4+ and CD8+, T cells were significantly higher in the psoriatic patients with arthritis in comparison with the control group. The percentages of CD3+ and CD8+, but not CD4+, T cells were significantly higher in the psoriatic patients without arthritis in comparison with the control group. The expression of PD-1 on CD4+ and CD8+ T cells was significantly lower in psoriatic patients with arthritis in comparison with the control group as well as in psoriatic patients without arthritis in comparison with the control group (Table 2).

Percentages of CD3+, CD4+, CD8+, CD4+PD-1+ and CD8+PD-1+ T cells were not significantly different between psoriatic patients with and without arthritis ($p > 0.05$).

Table 1. Clinical data of psoriatic patients with and without arthritis

Clinical data	Psoriasis with arthritis (n = 23)	Psoriasis without arthritis (n = 52)	P-value
Age, M ± SD [years]	53.1 ±8.9	44.4 ±15.9	0.011
Sex – male, n (%)	19 (82.6)	46 (88.5)	0.500
Duration of psoriasis, M ± SD [years]	28.8 ±11.6	16.1 ±12.7	< 0.001
Age of psoriasis onset, M ± SD [years]	24.3 ±9.4	28.3 ±16.3	0.573
Positive family history of psoriasis, n (%)	12 (52.2)	16 (30.8)	0.080
PASI, M ± SD	15.0 ±11.5	13.5 ±8.3	0.998
BSA (%), M ± SD	25.3 ±18.6	23.4 ±17.6	0.877
PGA, M ± SD	3.0 ±0.8	3.1 ±0.8	0.909
NAPSI, M ± SD	21.1 ±17.2	17.4 ±16.2	0.177
DLQI, M ± SD	15.7 ±7.4	13.3 ±8.0	0.323
Erythrocyte sedimentation rate, M ± SD [mm/h]	12.1 ±8.4	12.2 ±12.2	0.346
C-reactive protein, M ± SD [mg/dl]	4.2 ±5.8	3.7 ±8.3	0.283
Duration of PsA (psoriatic arthritis), M ± SD [years]	9.3 ±5.9		
Age of PsA onset, M ± SD [years]	42.0 ±12.1		
Clinical subsets of PsA n (%):			
Oligoarticular (≤ 5 joints)	15 (65.2)		
Polyarticular	6 (26.1)		
Distal interphalangeal predominant	2 (8.7)		
Spondylitis predominant	0 (0.0)		
Mutilans	0 (0.0)		
Tender Joint count, M ± SD	4.6 ±3.6		
Swollen Joint count, M ± SD	1.3 ±1.9		
5-point Likert scale (physician), M ± SD	2.1 ±0.8		
5-point Likert scale (patient), M ± SD	2.4 ±0.9		
Patient Visual Analog Scale (VAS), M ± SD	40.0 ±25.0		
Disease Activity Score (DAS28), M ± SD	3.2 ±1.1		

Table 2. Comparison of the cell populations of PBMCs and PD-1 expression in psoriatic patients with and without arthritis as well as the control group

PBMC and PD-1 expression	Psoriasis with arthritis (n = 23) M ± SD	Psoriasis without arthritis (n = 52) M ± SD	Control (n = 52) M ± SD	P-value		
				Psoriasis with arthritis versus control	Psoriasis without arthritis versus control	Psoriasis with arthritis versus psoriasis without arthritis
CD3+ (%)	60.1 ±12.6	58.8 ±13.3	48.2 ±17.0	0.002	0.001	0.694
CD4+ (%)	61.4 ±19.8	58.5 ±17.6	54.0 ±17.5	0.143	0.257	0.533
CD8+ (%)	29.6 ±18.7	31.7 ±16.0	23.3 ±10.1	0.405	0.011	0.623
CD4+PD-1 (%)	1.6 ±0.7	2.1 ±1.3	2.9 ±1.7	0.001	0.022	0.084
CD8+PD-1 (%)	3.4 ±2.3	3.2 ±2.1	6.6 ±4.4	0.001	< 0.001	0.704

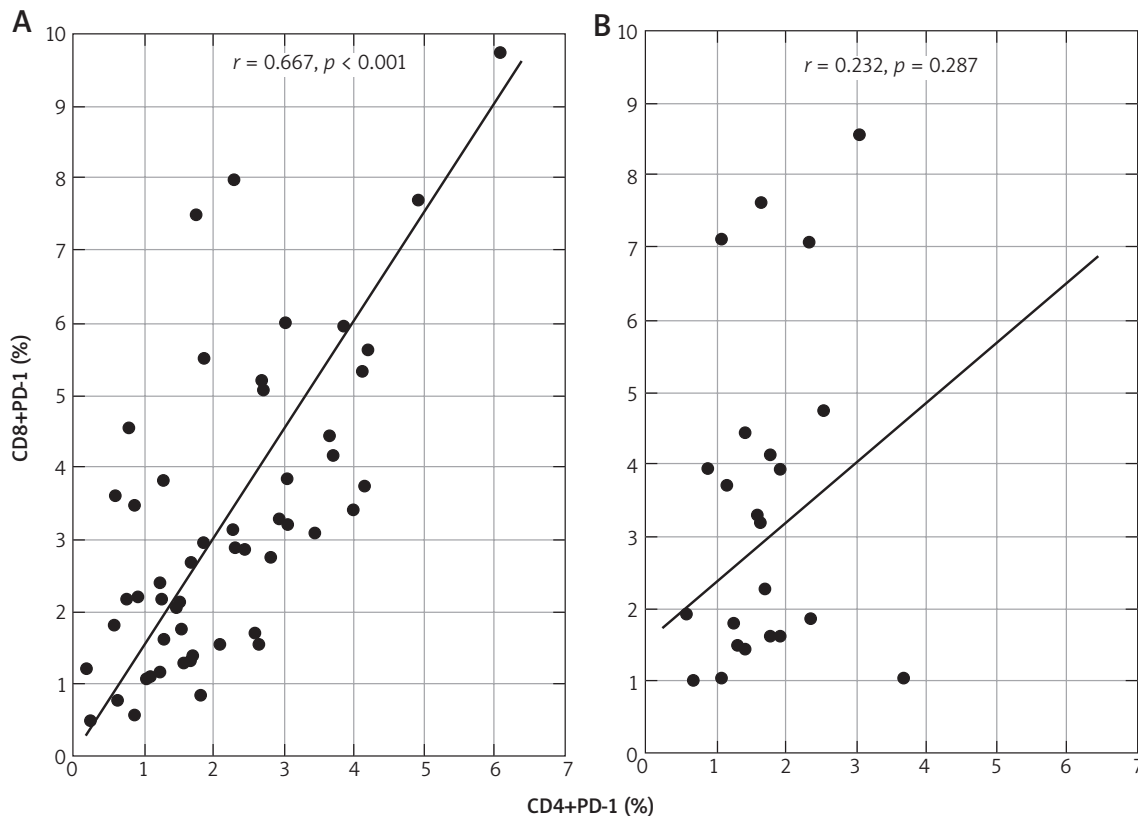


Figure 2. Correlation between CD4+PD-1 and CD8+PD-1 for psoriatic patients without (A) and with (B) arthritis

Analysis of mutual correlations between CD4+PD-1+ and CD8+PD-1+ in patients with and without arthritis

The percentages of CD4+PD-1+ correlated positively with the percentages of CD8+PD-1 in patients without arthritis, but not in patients with arthritis (Figure 2).

Analysis of correlations between PBMC populations' distribution and clinical data in psoriatic patients with and without arthritis

The expression of PD-1 on CD4+ and CD8+ T cells did not correlate with any of the studied clinical data in the psoriatic patients with and without arthritis ($p > 0.05$).

Discussion

In our study we assessed the whole spectrum of T cells with respect to PD-1 in the psoriatic patients with and without arthritis in comparison to the control group.

In this study, we provided evidence that the expression of PD-1 on the CD4+ and CD8+ T cells is altered in psoriatic patients; therefore, the PD-1/PDL-1 pathway may be involved in the regulation of T-cell activation and function in psoriatic patients both with and without arthritis. Therefore, the finding may also confirm the concept of “psoriatic disease” and allow psoriasis to be

considered as a single entity together with identified comorbidities [7].

In psoriatic arthritis (PsA) chronic inflammation results in enthesitis, cartilage and bone damage, leading to joint and bone destruction and remodeling. In the majority of patients a skin manifestation of psoriasis occurs prior to the joint involvement, which may be suggestive of some skin pathological inflammatory processes playing a role in the development of arthritis [8].

Both psoriasis and PsA were initially regarded as Th1-mediated diseases, due to differentiation of the naïve T cells after IL-12 and IFN- γ release by antigen-presenting cells (APCs). The psoriatic skin and synovium are infiltrated by the T cells, dendritic cells, neutrophils and macrophages. However, it is well known that Th17 cells, activated by IL-23, IL-21, IL-6 and transforming growth factor- β (TGF- β), play a vital role in the pathogenesis of psoriasis and PsA. Th17 cells are present not only in the skin but also in the synovial tissue and synovial fluid [9, 10]. Some animal studies have revealed that mice deficient in IL-17 showed decreased development of collagen-induced arthritis [11]. Nevertheless, the mechanism of chronic T cell activation by APCs in psoriasis still awaits elucidation. Impaired interaction of the PD-1 on T cells, B cells and macrophages with its ligand PD-L1 or PD-L2 on the APCs seems to be responsible for an intense, up-regulating T cell response [12, 13].

In our study psoriatic patients with arthritis constituted 30.67% of all studied subjects. In the psoriatic patients with arthritis, similarly to the patients without arthritis, the percentages of both CD4+PD-1+ and CD8+PD-1+ T cells were significantly lower than in the control group. This finding is in agreement with the concept of the PD-1/PD-L1 axis in the down-regulating T cell response. The observation may confirm the hypothesis of the convergent role of PD-1 in psoriasis with and without arthritis. However, it is generally assumed that the development of psoriatic changes in the joints, cartilages and tendons may be insidious and some discrete inflammatory processes are slowly progressing, bringing about subclinical changes sometimes hard to detect even by high-tech diagnostic tools.

Peled *et al.* [14], in 20 PsA patients, found that the percentage of CD3+PD-1+ T cells was higher in patients than healthy controls. However, the authors observed an inverse correlation with disease activity: the higher the number of swollen and/or tender joints, the lower the percentage of PD-1 expressing T cells. In our study we did not find any correlation between the severity of PsA, including the number of swollen or tender joints, Likert score, VAS and DAS28 and the expression of PD-1 on CD4+ and CD8+ T cells. However, in our group of psoriatic patients with arthritis the mean values of both the VAS and DAS28 were lower than those reported by Peled *et al.* [14] (VAS 40.0 \pm 25.0 vs. 46 \pm 5.5 and DAS28 3.2 \pm 1.1 vs. 3.6 \pm 0.4), whereas PASI was higher in our patients (15.0 \pm 11.5) than in the studied group of Peled *et al.* [14] (8.1 \pm 5.7). Similarly, the authors did not find any correlation between the level of PD-1 expressing T cells and ESR, CRP or PASI in the PsA patients. Moreover, in our study, PASI, BSA, PGA, NAPSI, and DLQI did not significantly differ between the psoriatic patients with arthritis and without arthritis. Therefore, we suspect that some other factors, e.g. genetic, might influence expression of the negative co-stimulators of T cells in psoriasis, thereby resulting in various severity and different clinical presentation of the disease.

The studies which provide evidence that the Th17 cell cytokines are promoters of osteoclastogenesis via induction of the receptor activator of the NF- κ B ligand (RANKL) give grounds to postulate that the role of decreased PD-1 expression in Th17 cytokine/RANKL axis activation is a possible explanation of PsA development [15]. In our previous study we observed that the osteoprotegerin, which is an important osteoclastogenesis inhibitor, to sRANKL ratio was significantly lower in the PsA than in the psoriatic patients without arthritis [16].

Although activation and hyperactivity of Th1 and Th17 in joint inflammatory diseases is well known, the results of the studies on the role of PD-1 in progression of arthritis and joint destruction in the pathogenesis of various arthropathies are inconclusive.

Earlier research into defective PD-1 expression in various rheumatologic diseases, i.e. PsA, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and ankylosing spondylitis (AS), concerned the PD-1 gene polymorphisms [17–19]. Recently, studies have been focusing on the relationship of the expression levels of PD-1 and T cell activity and function.

Contrary to PsA, in RA increased PD-1 expression on T cells was found [20, 21].

However, in RA patients there were found high concentrations of serum and synovial sPD-1, a soluble form of PD-1, able to neutralize the increased PD-1 T cell expression. Therefore, even though PD-1 expression in RA is increased, it seems plausible that its capacity to regulate T cell activation is insufficient [20]. In SLE patients, some authors [22, 23] have also revealed increased expression of PD-1 on CD3+ and CD4+ T cells. Dolff *et al.* [23] in SLE patients did not observe any correlation between PD-1 expression as well as renal involvement and disease activity. However, in patients with AS, a disease which belongs to the same group as PsA, i.e. seronegative spondyloarthropathies (SpAs), the authors found decreased amounts of PD-1+ T cells, including follicular T cells, a subset of CD4+ T cells characterized by expression of PD-1 [24].

Conclusions

The findings reported in this paper are important for understanding the role of the negative co-stimulatory molecule PD-1 in T-cell activation in psoriasis with and without arthritis. Although the clinical presentations vary in many respects, psoriatic patients, both with and without arthritis, share some common inflammatory mediators and pathways. Thus, targeting the PD-1/PDL-1/Th17 axis/pathway may appear to be a potential therapeutic strategy in psoriatic patients.

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Conflict of interest

The authors declare no conflict of interest.

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