

Peripheral blood CD8+ T-cell profiles in patients with psoriatic arthritis: a cross-sectional case-control study

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Abstract. – **OBJECTIVE:** While CD4+ T-cells are traditionally regarded as the main pathogenic T-cell subpopulation in psoriatic arthritis (PsA), the role of circulating CD8+ T-cells remains poorly characterized. We evaluated the differential representation of CD8+ T-cell subpopulations in peripheral blood (PB) of PsA patients.

PATIENTS AND METHODS: CD8+IL-17+, CD8+IFN γ + and CD8+IL-17-IL-22+ T-cells were evaluated by flow-cytometry in 25 consecutive PsA patients, 7 rheumatoid arthritis (RA) patients, 16 patients with psoriasis, and 26 healthy controls (HC).

RESULTS: We observed a significant expansion of circulating IFN- γ producing CD8+ T-cells in PsA when compared to psoriasis [21.2 (6.9-55.8)% vs. 3.8 (0.7-11.8)%, $p < 0.0001$] and HC samples [21.2 (6.9-55.8)% vs. 4.05 (0.44-19.8)%, $p < 0.0001$]. A frequency of circulating IFN- γ producing CD8+T-cells $\geq 9\%$ distinguished PsA from psoriasis patients with a specificity of 84% and a sensitivity of 87.5% [AUC = 0.9 (0.80-0.99), $p < 0.0001$]. In addition, we found a significant expansion of circulating IL-17 producing CD8+ T cells in RA patients when compared to PsA, psoriasis and HC samples. By contrast, there were no significant between-group differences in the prevalence of circulating IL-22 producing CD8+ T-cells. In PsA patients there was a significant correlation between number of swollen joints and frequency of circulating IFN- γ producing CD8+ T-cells, and between extent and severity of psoriasis and frequency of circulating IL-17 producing CD8+ T-cells.

CONCLUSIONS: Circulating IFN γ -producing CD8+ T-cells are raised in PsA when compared to psoriasis, suggesting a potential pathogenetic

involvement of CD8+ T-cells and IFN γ production in chronic joint inflammation and damage. The significant enrichment of circulating IL-17 producing CD8+ T-cells in RA when compared to PsA warrants functional characterization and confirmation in larger studies. We found no significant enrichment of circulating IL-22 producing CD8+ T-cells in PsA, RA and psoriasis.

Key Words:

CD8+ T-cells, Psoriatic arthritis, Rheumatoid arthritis, Psoriasis.

Introduction

Psoriatic arthritis (PsA) is a chronic inflammatory disease belonging to the family of spondyloarthritis, a large group of disorders with specific genetic, clinical and radiographic features. Peripheral arthritis is the most common symptom of PsA followed by involvement of skin, nails, entheses and spine.

PsA is strongly linked to genes belonging to the MHC class I region (HLA-B*27, HLA-B*38, HLA-B*39, and HLA-C*06)¹ and weakly linked to several non-HLA genes, both within and outside the MHC region. Of interest, several genes associated to an increased susceptibility for PsA encode proteins functionally involved in the regulation of the immune response.

The key pathogenetic mechanism in PsA involves CD4+ T-cells secreting IL-17, also called T

helper 17 (Th17), under stimulation of IL-23 produced by dendritic cells.

Despite most research on the pathogenic involvement of T-cells in PsA has focused on CD4+ T-cells (IL-17 and IL-12/23 axis), recent evidence suggests a previously unsuspected role for CD8+ T-cells².

From a genetic point of view, the strong association of disease risk and disease severity with HLA class I molecules suggests that PsA is driven by the presentation of an “unknown arthritogenic peptide” by MHC class I molecules to self-reactive antigen-restricted CD8+ T-cells³.

Genes involved in CD8+ T-cells differentiation, such as RUNX³, have been reported to be strongly associated with the risk of PsA⁴. Recently, a putative new PsA-specific locus has been identified in chromosome 5q31⁵; of note, the index SNP, rs10065787, maps close to CD8+ T-cell differentiation transcription factors, including RUNX3⁶ and BATF^{7,8}.

Additional insights about the potential relevance of CD8+ T cells in PsA pathogenesis are provided by studies showing significant correlations between disease severity and CD8+ T-cell number and phenotype, either in the peripheral blood (PB) or in the inflamed tissue. These studies reported that IL-17 producing CD8+ T-cells are prevalent in the synovial fluid of patients with PsA with respect to PB, and are linked to a more active and erosive disease⁹. Moreover, a strong T-cell receptor repertoire oligoclonality of infiltrating CD8+ T-cells has been identified in PsA synovium¹⁰ and synovial fluid¹¹.

From a clinical perspective, the seronegativity for specific autoantibodies and the emergence of severe and frequently intractable PsA in HIV-patients, typically characterized by a low CD4+ count, further support the role of CD8+ T-cells in the pathogenesis of PsA.

Notably, apart from IL-17 producing CD8+ T-cells, there is a paucity of data about CD8+ T-cell profiles in PB of PsA. To address this issue, we performed a cross-sectional study to compare the CD8+ T-cell subset (CD8+IL-17+, CD8+IFN γ + and CD8+IL-17-IL-22+ T-cells) representation and relative frequency in PB from PsA to that of patients with psoriasis, rheumatoid arthritis (RA), and healthy controls (HC).

Patient and Methods

Patients

A consecutive series of patients fulfilling the Classification Criteria for Psoriatic Arthritis (CA-

SPAR) were enrolled in the study. A consecutive series of patients with RA, according to the criteria of the American College of Rheumatology, patients with psoriasis seen at our Dermatology outpatient clinic and diagnosed by a consultant dermatologist, and a group of HC were also enrolled for comparison purposes. Exclusions criteria were recent infections and pregnancy.

To assess potential correlations between PsA severity and CD8+T-cell number and phenotype, the following disease specific scores, disease descriptors and treatment data were recorded and collected: steroid treatment; treatment with synthetic or biological disease-modifying anti-rheumatic drugs (DMARDs); number of swollen joints; number of tender joints; C-reactive protein (CRP) concentrations, mg/dL; erythrocyte sedimentation rate (ESR), mm/h; enthesitis score and dactylitis score; the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI).

In patients with psoriasis (with or without PsA) we also recorded topical treatment and/or PUVA, and the Psoriasis Activity And Severity Index (PASI).

In RA the following measures of disease activity and severity were recorded and collected: treatment with steroids, synthetic and biological DMARDs; C-reactive protein (CRP) concentrations, mg/dL; erythrocyte sedimentation rate (ESR), mm/h; Disease Activity Score-28 (DAS-28); Health Assessment Questionnaire (HAQ); number of swollen joints; and number of tender joints.

Ethical Approval

The protocol of the study was approved by the local Ethics Committee (ASLI Sassari) and all patients gave written informed consent. The study was conducted according to Helsinki's declaration and good clinical practice.

Flow-Cytometry

Peripheral blood mononuclear cells (PBMCs) were stimulated with phorbol-12-myristate 13-acetate (25 ng/ml) (Sigma Aldrich, St. Louis, MO, USA) and calcium ionomycin (1 μ g/ml) (Sigma Aldrich, St. Louis, MO, USA) and then cytokine secretion was blocked with A Brefeldin (10 μ g/ml) (Sigma Aldrich, St. Louis, MO, USA). The samples were incubated for 4 h in atmosphere with 5% CO₂ at 37°C. Cells were stained with antibodies anti-CD3, anti-CD8, anti-IL-22, anti-IL-17 and anti-IFN- γ (all antibodies from BD Biosciences, Franklin Lakes, NJ, USA). Flow-cytometry was performed with FACS-calibur (BD Biosciences, Franklin Lakes, NJ, USA)

and DIVA software (BD Biosciences, Franklin Lakes, NJ, USA).

Statistical Analysis

All data are expressed as median [10°-90° percentile]. Mann-Whitney test was used to detect differences for non-normally distributed groups. Correlations were assessed using bivariate correlation analysis. A p-value less than 0.05 indicated statistical difference. All analyses were performed using GraphPad Prism 7 software (GraphPad Software, La Jolla, CA, USA).

Results

Patients and Controls

Twenty-five PsA patients (eight of them without skin involvement), 16 patients with psoriasis, 7 RA patients and 26 HC were enrolled in the study (Table I).

Circulating IFN- γ producing CD8+T-cells

We demonstrated a significant expansion of circulating IFN- γ producing CD8+T-cells in PsA when compared to psoriasis [21.2 (6.9-55.8)% vs. 3.8 (0.7-11.8)%, $p < 0.0001$] and HC [21.2 (6.9-55.8)% vs. 4.05 (0.44-19.8)%, $p < 0.0001$] (Figure 1). A frequency of circulating IFN- γ producing CD8+ T-cells $\geq 9\%$ distinguished PsA from psoriasis patients with a specificity of 84% and a sensitivity of 87.5% [AUC = 0.9 (0.80-0.99), $p < 0.0001$].

Similarly, RA patients showed a significant increase in IFN- γ producing CD8+ T-cells when compared to psoriasis [15.0 (10.4-58.1)% vs. 3.8 (0.7-11.8)%, $p < 0.001$] and HC [15.0 (10.4-58.1)% vs. 4.05 (0.44-19.8)%, $p < 0.01$] (Figure 1).

The frequency of circulating IFN- γ producing CD8+ T-cells was not significantly different in PsA and RA patients [21.2 (6.9-55.8)% vs. 15.0 (10.4-58.1)%; $p > 0.05$] (Figure 1).

Circulating IL-17 Producing CD8+ T-Cells

We observed no significant differences in the prevalence of circulating IL-17 producing CD8+ T-cells in PsA when compared to psoriasis [0.5 (0.1-0.7)% vs. 0.3 (0.2-0.7)%, $p > 0.05$] and HC [0.5 (0.1-0.7)% vs. 0.3 (0.1-1.1)%, $p > 0.05$] (Figure 1).

However, RA patients showed a significant expansion of circulating IL-17 producing CD8+ T-cells when compared to PsA [0.9 (0.4-1.6)% vs. 0.5 (0.1-0.7)%, $p < 0.01$], psoriasis [0.9 (0.4-1.6)% vs. 0.3 (0.2-0.7)%, $p < 0.01$] and HC [0.9(0.4-1.6)% vs. 0.3 (0.1-1.1)%, $p < 0.05$] (Figure 1).

Circulating CD8+IL-22+IL-17- T-Cells

We observed no significant difference in the frequency of circulating CD8+IL-22+IL-17-T-cells in PsA when compared to the other three groups [PsA 6.6 (1.1-14.2)% vs. RA 9 (3.8-14.8)% vs. psoriasis 5.5 (1.0-15.8)% vs. HC, $p > 0.05$ for all comparisons] (Figure 1).

Table I. Clinical and demographic characteristics of PsA, RA, psoriasis patients and HC.

	HC n=26	Psoriasis n=16	PsA n=25	RA n=7
Age, yrs median (10°-90°)	35.5 (26-63)	49 (37-67)	46 (37-72)	55 (39-69)
Females, n	14	5	10	4
Treatment				
No treatment	-	0	4	2
Biological DMARDs	-	0	6	1
Synthetic DMARDs	-	0	9	2
Corticosteroids	-	0	6	2
Topical treatment	-	11	-	-
PUVA	-	5	-	-
PASI score, mean \pm 1SD	-	9.7 (5.8-23.5)	1 (0-12)	-
Tender joints, mean \pm 1SD	-	-	3 (0-12)	3 (0.6-7.8)
Swollen joints, mean \pm 1SD	-	-	0 (0-3)	0 (0-2.8)
Dactylitis, mean \pm 1SD	-	-	0 (0-2)	-
Enthesitis, mean \pm 1SD	-	-	0 (0-2)	-
BASDAI, mean \pm 1SD	-	-	5.25 (0.5-7.7)	-
DAS28, mean \pm 1SD	-	-	-	4 (1.7-4.4)
HAQ, mean \pm 1SD	-	-	0.9 (0-2.2)	1.12 (0.1-1.9)

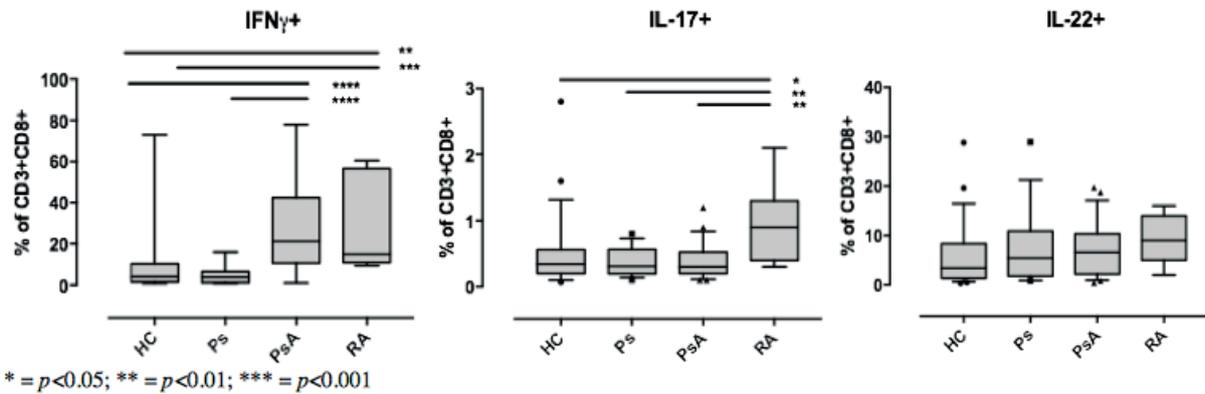


Figure 1. Frequencies of peripheral blood CD8+ T cell subsets across all groups.

Frequency of Circulating IFN- γ and IL-17 CD8+ T-cells and PsA Disease Descriptors

We observed no significant correlations between the descriptors of PsA activity and severity, and the levels and phenotype of circulating CD8+ T-cells, barring a significant correlation between number of swollen joints and frequency of circulating IFN- γ producing CD8+ T-cells ($R = 0.53$, $p < 0.01$) and between PASI and frequency of circulating IL-17 producing CD8+ T-cells ($R = 0.49$, $p < 0.05$).

Discussion

We conducted for the first time a comprehensive PB analysis of circulating CD8+IL-17+, CD8+IFN γ + and CD8+IL-17-IL-22+ T-cells in patients with PsA, RA, psoriasis and HC.

The main finding is a significant enrichment of IFN γ -producing CD8+ T-cells in PsA, as well as RA, when compared to patients with psoriasis. Moreover, the levels of circulating IFN γ -producing CD8+ T-cells were correlated with the number of inflamed joints, suggesting that increased circulating levels of IFN γ -producing CD8+ T-cells are linked to joint inflammation and damage in PsA. Pending further confirmation, it is possible that the increase in IFN γ -producing CD8+ T-cells in PB is secondary to the transfer of these proliferating cells from the synovium to the lymphatic system and, then, to the blood circulation.

Intriguingly, IFN γ producing CD8+ T-cells were found to be enriched at the edge of the mantle zone of ectopic germinal centres located in RA synovial tissue. Experiments of CD8+ T-cells depletion demonstrated that IFN γ producing CD8+ T-cells are

fundamental for the architectural and functional integrity of tertiary lymphoid structures in RA synovial tissue. Furthermore, it has been proposed that IFN γ -producing CD8+ like CD4+ T-cells may interact with dendritic cells and B cells¹². Of interest, similar ectopic lymphoid structures with clear B/T cells segregation have been demonstrated in inflamed PsA synovial tissue¹³, suggesting a potential role for dendritic cells and B-cells in driving antibody-independent synovial inflammation through the development of CD8+ T-cell response followed by pro-inflammatory cytokine release.

Moreover, it has been reported that the beneficial effects of treatment with Abatacept (Orencia® Bristol Myers Squibb, Rome, Italy), a T-cell inhibitor, on joint manifestations of RA and PsA, are accompanied by a significant reduction of the levels of circulating IFN γ producing CD8+ T-cells¹⁴.

Notably, in our study the frequency of IFN γ -producing CD8+ T-cells was shown to discriminate PsA patients from psoriatic patients without joint involvement; as a consequence, the role of CD8+ T-cells phenotypes and IFN γ production in psoriatic patients as a biomarker predictive of concurrent subclinical or future joint involvement warrants further evaluation in large prospective studies. In this context, it is noteworthy the demonstration that serum levels of CXCL10, a chemokine secreted by multiple cell lineages in response to IFN γ , are raised in PsA patients¹⁵ and predict future articular involvement in patients with psoriasis¹⁶.

Collectively taken, these data support a differentiating role for IFN γ -producing CD8+ T-cell in joint inflammation in PsA.

A rapidly increasing number of experimental and clinical studies highlight the importance of IL-

17 in the pathogenesis of PsA, RA and psoriasis¹⁷. Apart from T helper type 17 cells, IL-17 is produced by several cell lines including CD8⁺ T cells.

Our data show a significant enrichment of PB CD8⁺IL-17⁺ T-cells in RA when compared to PsA, psoriasis and HC. Likewise, increased levels of circulating IL-17 producing CD8⁺ T-cells have been reported in RA patients^{14,18,19}. On the contrary, in PsA the levels of IL-17 producing CD8⁺ T-cells in synovial fluid were higher than PB⁹. It is possible that a differential synovial homing pattern between PsA and RA IL-17 producing CD8⁺ T-cells may explain the differences in PB.

Of note, we demonstrated a significant correlation between the extent and severity of psoriasis, as measured by PASI, and the frequency of circulating IL-17 producing CD8⁺ T-cells in PsA. Our findings are in agreement with earlier studies reporting a close correlation between the frequency of circulating IL-17 producing CD8⁺ T-cells and the severity of psoriasis^{20,21}.

Recently, a new CD4⁺ T-cells subset producing IL-22, but not IL-17 or IFN- γ (named Th22), has been found to be significantly enriched in PB of psoriasis, RA and PsA^{22,23}. Apart from Th22, IL-22 is secreted by CD8⁺ T-cells, termed Tc22 cells, that have not yet been investigated in PsA. Our study report for the first time the absence of significant enrichment of circulating IL-22 producing CD8⁺ T-cells in PsA, as well as RA and psoriasis, when compared to HC.

The limitations of our study include the relatively small sample size, the cross-sectional design, the inclusion of patients receiving immunosuppressive and/or anti-inflammatory treatment, and the lack of data on other tissue (skin, synovium, synovial fluids) samples.

Conclusions

Our data provide new evidence supporting the role of CD8⁺ T-cells in the pathogenesis of PsA. In particular, the role of IFN γ -producing CD8⁺ T-cells warrants functional studies to assess their involvement in boosting inflammation and joint damage in the articular micro-environment. A better understanding of the pathogenic role of CD8⁺ T-cells in PsA is essential for the identification and development of new cellular therapeutic targets.

Conflict of interest

The authors declare no conflicts of interest.

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