Differential expression of programmed death 1 (PD-1) on CD4+ and CD8+ T cells in rheumatoid arthritis and psoriatic arthritis

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KEY WORDS
programmed death 1, psoriatic arthritis, rheumatoid arthritis

ABSTRACT
INTRODUCTION Rheumatoid arthritis (RA) and psoriatic arthritis (PsA) are characterized by chronic inflammatory processes mediated by proinflammatory cytokines that affect the synovial lining. Programmed death 1 (PD-1) is a critical regulator of T-cell activation by downregulating immune responses.

OBJECTIVES The aim of the study was to investigate whether the expression of PD-1 on CD4+ and CD8+ T cells differs between patients with RA and those with PsA.

PATIENTS AND METHODS The study included 100 patients with RA, 31 patients with PsA, and 52 healthy controls. The percentages, absolute numbers, and mean fluorescence intensity (MFI) of CD4+PD-1+ and CD8+PD-1+ T cells from peripheral blood were analyzed using flow cytometry.

RESULTS The percentages and absolute numbers of CD4+ and CD8+ T cells with PD-1 expression were significantly higher in patients with RA than in controls. In patients with PsA, the percentages of CD4+PD-1+ and CD8+PD-1+ T cells were significantly lower than in controls. Because of the high frequency of PD-1-positive T cells in RA and their low frequency in PsA, we analyzed the expression level by analyzing the MFI. The median MFI of PD-1 on CD4+ and CD8+ T cells was significantly higher in patients with RA (median, 421 and 437, respectively) in comparison with patients with PsA (median, 222 and 198, respectively) and controls (median, 205 and 187, respectively).

CONCLUSIONS The differential expression of PD-1 in RA and PsA suggests that PD-1 might be involved in autoimmune mechanisms in RA and autoinflammatory mechanisms in PsA in a different manner.
are importantly involved in the immunopathogenesis of PsA, while the T cells and B cells play a decisive role in the development of RA. T cells, such as Th1 and Th17, their activation, and immune response are considered to be essential in the initiation and maintenance of RA and PsA. In both diseases, an imbalance between Th1, Th17, and regulatory T cells (Tregs) as well as the key role of various cytokines, including tumor necrosis factor, interleukin (IL)-1β, IL-6, IL-17A, IL-17F, IL-21, and IL-23, have been confirmed. Even though the pathogenesis of RA and PsA is fairly well known, the mechanism of chronic lymphocyte activation still awaits elucidation.

Since the programmed death 1 (PD-1), a protein which acts as an important negative regulator of T-cell activation and a crucial factor responsible for peripheral tolerance, is inducibly expressed on CD4+ T cells, CD8+ T cells, natural killer T cells, B cells, and activated monocytes, its role in the pathogenesis of RA and PsA might shed some light on this issue. PD-1 exerts its immune inhibitory action by binding to the ligands PD-L1 and PD-L2 on the antigen-presenting cells (APC). Upregulation of PD-1 and PD-L1 depends on the inflammatory cytokines, such as interferon-γ. Apart from PD-1 expressed on the cells, the soluble PD-1 (sPD-1) that lacks the transmembrane domain is encoded. Previous studies have demonstrated that sPD-1 blocks the PD-1/PD-L pathway and promotes the T-cell response.

The aim of this study was to characterize the expression of PD-1 on CD4+ and CD8+ T cells in patients with RA and those with PsA. Because a flare or exacerbation of both RA and psoriasis in patients treated with immune checkpoint inhibitors has been reported, the PD-1 expression should be comprehensively analyzed. Moreover, PD-1 involvement in the pathogeneses of the diseases might be differential because in RA the autoimmune reaction is more pronounced, while in PsA the autoimmune inflammatory mechanisms are responsible for the disease progression to a higher extent.

**PATIENTS AND METHODS**

**Characteristics of patients**

The study included 100 patients with RA and 31 patients with PsA treated either at the Department of Rheumatology and Connective Tissue Diseases or at the Department of Dermatology, Venereology and Pediatric Dermatology of the Medical University of Lublin, Poland. All patients with RA fulfilled the 1987 American College of Rheumatology criteria for the diagnosis of RA. PsA was diagnosed using the Classification of Psoriatic Arthritis (CASPAR) criteria. Peripheral blood was collected from all the studied patients and 52 healthy volunteers (controls). Both in patients with RA and those with PsA, the number of painful and swollen joints was counted and the Disease Activity Score 28 (DAS28) and visual analog scale score were calculated. In all patients, the following parameters were measured in laboratory tests: C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), anti–cyclic citrullinated peptide (anti–CCP) antibodies, and rheumatoid factor (RF).

The demographic and clinical characteristics of the participants are presented in Table 1. Most patients with RA had increased levels of CRP, ESR, RF, and anti–CCP antibodies, whereas patients with PsA had normal CRP and RF levels but increased ESR. No significant differences in the number of swollen and tender joints as well as the visual analog scale score were observed between the groups. However, DAS28 was significantly higher in patients with RA.

The systemic treatment differed significantly between patients with RA and PsA. On admission, most patients with PsA did not receive any systemic medications, whereas 94 patients with RA were on systemic treatment. Methotrexate was administered in 5 patients with PsA; sulfasalazine, in 2 patients; and cyclosporine A, in 1 patient. In the RA group, 18 patients were treated only with one DMARD (12 patients received methotrexate; 4 patients, chloroquine; and 2 patients, leflunomide); 4 patients were treated with glucocorticoid (GC); and 72 patients received a combination therapy (29 patients were treated with methotrexate + GC; 21 patients, methotrexate + chloroquine + GC; 7 patients, chloroquine + GC; 6 patients, chloroquine + GC; 6 patients, leflunomide + GC; 1 patient, sulfasalazine + GC; 1 patient sulfasalazine + methotrexate + chloroquine; and 1 patient, sulfasalazine + chloroquine). A median duration of RA treatment was 11 years (range, 0–40 years). No biological drugs were administered in any of the groups.

In patients with PsA, 3 clinical subsets of the disease were identified: oligoarticular, polyarticular, and distal interphalangeal predominant (Table 1).

The study protocol was approved by the Local Ethics Committee at the Medical University of Lublin (KE-0254/41/2012, KE-0254/81/2015). Informed consent was obtained from all participants.

**Cell isolation**

Using density gradient centrifugation on Ficoll-Hypaque, mononuclear cells were isolated from the peripheral blood obtained from patients with RA, with PsA, and controls. The interphase cells were removed, washed twice in phosphate-buffered saline without Ca2+ and Mg2+ and resuspended in RPMI (Roswell Park Memorial Institute) 1640 medium containing human albumin (2%). The viability of the obtained peripheral blood mononuclear cells (PBMCs) was always above 95%, as determined by trypan blue staining. The viable cells were quantified in the Neubauer chamber.

**Flow cytometry analysis**

A total of 500,000 cells were incubated with fluorochrome-labeled monoclonal antibodies (Mabs), that is, anti–CD3-PerCP, anti–CD4-FITC, anti–CD8-PE,
Statistical analysis A statistical analysis was performed using Statistica 10.0 PL (StatSoft Inc., Tulsa, Oklahoma, United States). The figures were created using GraphPad Prism 5 (GraphPad Software Inc., San Diego, California, United States). The median values with range (min–max) were calculated for continuous variables, whereas categorical variables were presented as absolute (n) and relative numbers (%).

To compare continuous variables between 2 study groups, the Mann–Whitney test was used, while the Kruskal–Wallis test was applied to compare continuous variables between more than 2 groups of patients. The comparison of categorical variables between the studied subgroups of

TABLE 1 Clinical data of patients with psoriatic arthritis and rheumatoid arthritis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PsA (n = 31)</th>
<th>RA (n = 100)</th>
<th>P value</th>
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<tbody>
<tr>
<td>Age, y, median (range)</td>
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<tr>
<td>Sex, n (%)</td>
<td>Female</td>
<td>Male</td>
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<td>Duration of the disease, y, median (range)</td>
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<td>Age of disease onset, y, median (range)</td>
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<td>Positive family history, n (%)</td>
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<td>Erythrocyte sedimentation rate, mm/h</td>
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<td>C-reactive protein, mg/dl</td>
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<td>RF positivity, n (%)</td>
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<td>Anti-CCP antibody positivity, n (%)</td>
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<td>Number of tender joints, median (range)</td>
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<tr>
<td>Number of swollen joints, median (range)</td>
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<tr>
<td>Patient visual analog scale, median (range)</td>
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<tr>
<td>Disease Activity Score 28</td>
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<tr>
<td>Clinical subsets of PsA, n (%)</td>
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<td></td>
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<td>Medications, n (%)</td>
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Abbreviations: anti-CCP, anti-citrullinated protein antibody; DMARD, disease-modifying antirheumatic drug; GC, glucocorticoid; PsA, psoriatic arthritis; RA, rheumatoid arthritis

and anti–PD-1-APC (clone MIH4) (all Becton Dickinson, Franklin Lakes, New Jersey, United States) at room temperature for 20 minutes. In each sample, approximately 300,000 stained cells were analyzed by flow cytometry using a FACS Canto II flow cytometer (BD Biosciences, San Jose, California, United States). Data analysis was performed by FACS Diva 8.0. Lymphocytes were gated from PBMCs by setting appropriate forward and side scatter parameters.

Due to the exceptionally high percentage of CD4+PD-1+ and CD8+PD-1+ T cells in patients with RA, the mean fluorescence intensity (MFI) of PD-1 on CD4+ and CD8+ T cells was analyzed. The cut-off values were based on a negative control in the analyzed samples.
patients was conducted by the Pearson χ² test. A P value of less than 0.05 was considered significant.

RESULTS  Comparison of PD-1 expression on peripheral CD4⁺ and CD8⁺ T cells  The percentage of CD4⁺ T cells with PD-1 expression was higher in patients with RA as compared with controls (median, 82.72% [range, 9.49–98.53] vs 2.57% [range, 0.32–8.74], P < 0.001). Similarly, an increased percentage of PD-1⁺ cells was detected on CD8⁺ T cells in RA patients compared with controls (median, 88.08% [range, 16.16–99.70] vs 4.99% [range, 1.30–20.13], P < 0.001). Contrary to the findings in RA patients, the percentage of CD4⁺PD-1⁺ T cells was lower in patients with PsA, as compared with controls (median, 1.56% [range, 0.55–4.24] vs 2.57% [range, 0.32–8.74], P < 0.001). The expression of PD-1 on CD8⁺ T cells was downregulated in patients with PsA, as compared with controls (median, 2.73% [range, 1.04–8.60] vs 4.99% [range, 1.30–20.13], P < 0.001).

The absolute number of CD4⁺ T cells with PD-1 expression was higher in patients with RA, as
compared with controls (median, 158 cells/μl [range, 3–926 cells/μl] vs 13 cells/μl [range, 1–44 cells/μl], P < 0.001). Similarly, a higher absolute number of CD8+PD-1+ T cells was detected in patients with RA, as compared with controls (median, 155 cells/μl [range, 3–744 cells/μl] vs 10 cells/μl [range, 1–68 cells/μl], P < 0.001). The absolute number of CD4+PD-1+ T cells was not different in patients with PsA when compared with controls (median, 10 cells/μl [range, 2–43 cells/μl] vs 13 cells/μl [range, 1–44 cells/μl], P = 0.437). The expression of PD-1 on CD8+ T cells also did not differ between patients with PsA and controls (median, 9 cells/μl [range, 1–24 cells/μl] vs 10 cells/μl [range, 1–68 cells/μl], P = 0.26).

The MFI of PD-1 on CD4+ T cells was higher in patients with RA as compared with controls (median, 421 [range, 132–969] vs 205 [range, 138–300], P < 0.001). Similarly, the MFI of PD-1 on CD8+ T cells was higher in patients with RA as compared with controls (median, 437 [range, 127–977] vs 187 [range, 98–326], P < 0.001).

The median MFI of PD-1 on CD4+ and CD8+ T cells in patients with PsA was not different from that in controls (222 [range, 163–309], P = 0.226 and 198 [range, 93–262], P = 0.754, respectively).

A comparative analysis of PD-1 expression on CD4+ and CD8+ T cells between patients with RA and those with PsA and controls is shown in FIGURE 1. A representative flow cytometry analysis of PD-1 expression on CD4+ and CD8+ T cells from 1 healthy volunteer (control), 1 patient with psoriatic arthritis (PsA), and 1 patient with rheumatoid arthritis (RA) compared with controls (median, 3.2% [range, 0–26%] vs 0% [range, 0–26%], P < 0.001).

Similarly, the MFI of PD-1 on CD8+ T cells was higher in patients with RA as compared with controls (median, 6.0% [range, 0–18%] vs 0% [range, 0–18%], P < 0.001).

The median MFI of PD-1 on CD8+ T cells in patients with PsA was not different from that in controls (265 [range, 102–103] vs 252 [range, 102–103], P = 0.26).

A comparative analysis of PD-1 expression on CD4+ and CD8+ T cells between patients with RA and those with PsA and controls is shown in FIGURE 1. A representative flow cytometry analysis of PD-1 expression on CD4+ and CD8+ T cells in 1 patient with RA, 1 patient with PsA, and 1 control subject is presented in FIGURE 2.

Comparison of PD-1 on CD4+ and CD8+ T cells between treated and untreated patients. No significant differences were found in the absolute numbers, MFI, and percentages of CD4+ and CD8+ T cells with PD-1 expression between patients with PsA, either treated or untreated (TABLE 2).
Interestingly, in our study, we found opposite results for PD-1 expression on CD4+ and CD8+ T-cells in patients with RA and PsA, because in patients with RA the expression was significantly higher, whereas in those with PsA, it was significantly lower in comparison with the control group. In patients with RA, the absolute numbers of CD4+PD-1+ and CD8+PD-1+ T-cells were also significantly higher than in controls. These findings might confirm the presence of different mechanisms of PD-1 regulation, which will ultimately compromise its function and enhance T-cell response both in RA and PsA. Interestingly, in patients with RA, the increased MFI of PD-1 on CD4+ and CD8+ T-cells was also observed, which may suggest that an increased expression of PD-1 in these patients is due to increased absolute numbers and percentages of CD4+PD1+ and CD8+PD-1+ T-cells as well as the high intensity of PD-1 expression. It seems plausible that high expression of PD-1 on T-cells in RA might be a result of the induction of follicular helper T cells (Tfh), a recently discovered subset of CD4+ T-cells characterized by the expression of PD-1. This appears to contribute to B-cell activation, differentiation, and survival, and may explain the production of specific serum autoantibodies (RF and anti-CCP antibodies) by the B-memory cells in RA.

Data are presented as median (min–max).

Abbreviations: MFI, mean fluorescence intensity; others, see **Table 1**

**Table 3** The distribution of CD4+ and CD8+ T-cells with programmed death-1 (PD-1) expression in patients with RA who received or did not receive treatment with systemic medications

<table>
<thead>
<tr>
<th>PD-1 expression</th>
<th>IU</th>
<th>RA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No treatment or GC (n=10)</td>
<td>1 DMARD only (n=18)</td>
</tr>
<tr>
<td>CD4+PD-1+ Cells/μl</td>
<td>213 (20–926)</td>
<td>172 (21–509)</td>
</tr>
<tr>
<td>%</td>
<td>81.17 (19.15–94.49)</td>
<td>78.51 (49.49–98.40)</td>
</tr>
<tr>
<td>MFI</td>
<td>419 (169–969)</td>
<td>342 (259–664)</td>
</tr>
<tr>
<td>CD8+PD-1+ Cells/μl</td>
<td>165 (36–504)</td>
<td>168 (16–457)</td>
</tr>
<tr>
<td>%</td>
<td>85.51 (27.54–99.35)</td>
<td>83.69 (18.45–99.70)</td>
</tr>
<tr>
<td>MFI</td>
<td>467 (174–837)</td>
<td>353 (219–716)</td>
</tr>
</tbody>
</table>

Data are presented as median (min–max).

Abbreviations: see **Tables 1 and 2**
Bautista-Caro MB et al \(^{21}\) observed decreased frequencies of blood Tfh cells in patients with ankylosing spondylitis. Therefore, it can be speculated that the decreased PD-1-positive T cells, including Tfh cells, are found in seronegative arthritis and may reflect their possible inability to produce autoantibodies.\(^{22,23}\) There have been few studies assessing the frequency of Tfh cells in patients with psoriasis. Shin et al \(^{22}\) found a decreased number of PD-1+ Tfh cells in these patients. However, Niu et al \(^{24}\) revealed that Tfh cells and activated B cells were increased in the peripheral blood of psoriatic patients and positively correlated with the disease severity. Since the role of humoral immunity in psoriasis and other seronegative spondyloarthropathies is not well-established, its contribution to the pathogenesis of these diseases requires further investigation.

In most cases, it is not difficult to differentiate between RA and PsA on the basis of the patient’s medical history and physical examination complemented by laboratory testing of RF and/or anti-CCP antibodies. Nevertheless, in patients with polyarticular symmetric joint involvement or in rare cases of the simultaneous presence of RA and PsA, the differential diagnosis may be more challenging.\(^{25}\) Moreover, it is known that both RF and anti-CCP antibody titers may be negative in about 12% to 30% of patients with RA, whereas in patients with PsA, the positive anti-CCP antibody titer is present in 5.6% to 20% of patients while from 5% to 10% of patients are positive for RF.\(^{26,27}\) Also, the majority of patients positive for anti-CCP antibodies showed polyarticular joint involvement or erosive changes and other forms of bone destruction mimicking RA; thus, sensitive and specific diagnostic tools are still needed. In our small group of 9 patients (29.03%) with polyarticular symmetric PsA, PD-1 expression on CD4+ and CD8+ T cells was not significantly different from that observed in patients with other clinical subsets of PsA.

Contrary to our results, Li et al \(^{29}\) observed that patients with RA had a decreased expression of PD-1 on CD4+ and CD8+ T cells, which inversely correlated with positive CRP levels and DAS28 score. They also observed decreased serum levels of sPD-1. The discrepancies between our results might be related to using a different clone of anti-PD-1 for T-cell labeling. In our study, all participants were Caucasians, while in the study of Li et al, they were of Han Chinese ethnicity.

Since we were aware that our results showed an exceptionally high PD-1 expression on the peripheral CD4+ and CD8+ T cells in our patients with RA, we decided to analyze the MFI. The obtained results confirmed high fluorescence intensity of PD-1 with a median value of 421 on CD4+ and 437 on CD8+.

In the light of the published reports,\(^{23,30}\) it seems that in patients with RA the increased serum and synovial sPD-1 might contribute to T-cell hyperactivity and inflammatory response in the joints. Thus, an increased PD-1 T-cell expression may not be capable of counteracting the inhibitory effect of sPD-1. Therefore, even though the PD-1 expression in RA is high, it is likely that has insufficient ability to downregulate the T-cell activation.

Importantly, an increased expression of PD-1 was reported in other autoimmune connective tissue diseases: on T cells in SLE and on salivary lymphocytes in Sjögren syndrome.\(^{31,32}\) Dolf et al,\(^{21}\) who investigated a group of 32 patients with SLE, found higher percentages of PD-1 expression on CD4+ T cells in patients with SLE (mean [SD], 18.6% [11.2%]) in comparison with controls (mean [SD], 10.3% [4.3%]) (\(P = 0.008\)). The MFI of PD-1 on CD4+ T cells was also significantly higher in patients with SLE than in the control group.

As for PsA, the decreased PD-1 expression on CD4+ and CD8+ T cells seems to be justified since without the negative regulatory role of PD-1, the sustained activation of T cells will lead to chronic cytokine production.

Peled et al,\(^{33}\) in the group of 20 patients with PsA, found that the percentage of CD3–PD-1+ T cells was higher in these patients, as compared with healthy controls. However, the authors observed an inverse correlation with the disease activity: the higher the number of swollen and/or tender joints, the lower the percentage of PD-1-expressing T cells.
It is noteworthy that in our study the absolute numbers, percentages, and MFI of PD-1 on CD4+ and CD8+ in patients with PsA failed to show a significant difference regardless of whether they were treated or not with systemic medications. As for patients with RA, depending on the treatment regimen (ie, 1 DMARD, 2 or 3 DMARDs with or without GC, etc), we found some significant differences in the percentages and MFI of CD4+PD-1- and CD8+PD-1-, but the differences in the absolute numbers were not significant. However, all the median values of the percentages and MFI of CD4+PD-1- and CD8+PD-1- in patients with RA were still significantly higher in comparison with the control group. Chen et al,26 who investigated patients with ankylosing spondylitis and RA treated with various systemic medications, did not observe significant differences in PD-1 levels regardless of whether they received tumor necrosis factor inhibitors or not.

One possible limitation of our study is a smaller number of patients with PsA in comparison with the number of patients with RA.

In conclusion, a thorough elucidation of the molecular mechanisms involved in the PD-1/PD-L-1 pathway and the role of this pathway in RA and PsA would undoubtedly improve the diagnosis and possibly provide new and more effective therapeutic options.

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Contribution statement JB and KG conceived the idea for the study; JB, EZ, AK, GC, and MM contributed to the design of the research; JB, AK were involved in data collection; DR performed statistical analysis; JB, EZ, AK, JP, GC, MM, and KG analyzed the data; GC, AK, MM, and KG coordinated funding for the project; JB, KG, GC, and DR made the final revision of the manuscript. All authors edited and approved the final version of the manuscript.

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