Ex vivo cytokine production in psoriatic disease: Towards specific signatures in cutaneous psoriasis and peripheral psoriatic arthritis

Guillaume Larid1,2, Adriana Delwail3, Thomas Dalle2, Philippe Vasseur2,4, Christine Silvain2,4, Jean-François Jégou2, Franck Morel2, Jean-Claude Lecron2,5 and Elisabeth Gervais1,2*

1Rheumatology Department, CHU de Poitiers, Poitiers, France, 2University of Poitiers, LITEC, Poitiers, France, 3UMR 6041, 4CS, Université de Poitiers, Poitiers, France, 5Gastroenterology Department, CHU de Poitiers, Poitiers, France

Objectives: Psoriatic arthritis (PsA) and cutaneous psoriasis (PsO) are different phenotypes of psoriatic disease (PsD), whose underlying specific mechanisms remain incompletely understood. As cytokines are key elements to induce and tune up immune responses to drive inflammatory diseases, our objective was to assess whether clinical features, disease phenotype and PsA and PsO activity were associated with a particular ex vivo cytokine production profile.

Methods: Forty-eight patients (37 PsA and 11 PsO) and 11 healthy subjects (HS) were studied. Cytokine production by peripheral blood mononuclear cells (PBMC) that were either unstimulated, or stimulated with LPS or anti-CD3/CD28 antibodies, were analysed by multiplex assay in the culture supernatants.

Results: Cytokine signature of PsD includes a high level of TNFα in supernatants of LPS-stimulated PBMC, higher levels of IL-6 and lower levels of IFN-γ and IL-17A after CD3/CD28 stimulation, as well as higher spontaneous IL-1RA and TNFα production compared to HS. High body mass index (BMI) was associated with lower levels of IL-1β, and metabolic syndrome with lower levels of IFN-γ after LPS stimulation. In PsD, dermatological activity was related with higher IL-17A level, while rheumatic activity was linked with lower levels of IFN-γ and TNFα. Comparing each PsD subtype to HS, IL-1β and IL-6 productions are higher when using LPS stimulation in PsO patients with higher levels of IL-1β and IL-1α in peripheral PsA patients after CD3/CD28 stimulation. LPS stimulation induced high levels of IL-17A in peripheral PsA compared to axial PsA. PsA patients with axial PsA share some features with PsO but shows a distinct cytokine pattern compared to peripheral PsA.

Conclusion: PsO and the different PsA subtypes exhibit distinct ex vivo cytokine production profiles and common features of the so-called PsD. Analysis of IL-1
cytokine family and IL-6 seems to be of particular interest to distinguish PsO and peripheral PsA since it depends on monocytes in PsO and T-lymphocytes in peripheral PsA. Peripheral cytokine profiles are influenced by rheumatic and dermatological activity of the disease, and also by metabolic syndrome features. Our results highlight the crucial role of immune cell interactions with different patterns of interaction depending on clinical phenotype.

KEYWORDS
psoriasis, psoriatic arthritis (PsA), psoriatic disease, cytokine signature, ex vivo stimulation

1 Introduction

Psoriasis is an inflammatory cutaneous disorder with prevalence up to 3% of people in industrialized countries (1). Originally described as a skin and/or articular pathology, psoriasis appears as a disease involving other tissue damage. In the last ten years, the concept of “psoriatic disease” (PsD) has emerged, which encompasses all the clinical aspects of so-called cutaneous psoriasis (PsO), psoriatic arthritis (PsA), and associated comorbidities such as cardio-metabolic disorders (2–5). As PsD is a systemic disease, patients can present features of PsO, PsA, or both. Numerous studies focusing on PsO patients do not indicate if patients have articular involvement or not. Likewise, among PsO patients, many patients suffering from PsA are undiagnosed (6). This common vagueness in population’s description creates a potential bias both for the clinical diagnosis which determines the follow-up and treatment of patients, and for studies describing the physiopathological mechanisms of PsD, in which the two clinical entities may not be clearly differentiated from one to another.

PsD is a polygenic inflammatory disorder of which the pathophysiology raises numerous questions (7, 8). Interestingly, among the multiple polymorphisms associated with PsO or PsA, a significant number involves genes encoding for cytokines such as IL-12B, IL-23A, IL-13, IL-36G, IL-20, TNFAIP3 or TNFIP1 (7–9), leading to investigations of cytokine profiles in PsO or PsA in order to determine specific cytokine signatures. To date, different biological approaches have been applied to PsD, including serum analysis, transcriptomic analysis, and ex vivo studies, but the majority of these studies do not discriminate between the two clinical entities. While transcriptomic approaches from inflamed skin or synovial tissue samples provide relevant information on the pathophysiology of both diseases, their use in routine practice is not possible for diagnosis, insofar it requires invasive techniques to obtain samples. Therefore, most studies have focused on cytokine assays from patient blood samples, even though it is known that circulating cytokines are the tip of the iceberg for tissue inflammatory diseases. The conflicting data between studies and/or between subjects have confirmed the fact that measurement of serum cytokines such as TNF-α, IL-1α, IL-1β, IL-17, IL-23, IL-18 or IL-33 is not a reliable and reproducible method to determine cytokine status in PsD (10–15).

An alternative approach would consist in analysing the cytokine secretion capacities of circulating immune cells so as to segregate between PsA and PsO. Studies investigating cytokine secretion profiles from ex vivo-stimulated PBMC have been reported in PsO and PsA patients. Nishibu et al. studied spontaneous secretions of a limited number of cytokines (IL-1β, IL-2, IL-6, IL-8 and IFN-γ) and reported higher secretions of IL-1β, IL-6, and IL-8 by peripheral blood mononuclear cells (PBMC) isolated from PsA patients compared to PsO patients and from PsO patients compared to healthy subjects (16). However, this cytokine panel did not include relevant cytokines such as TNF-α or IL-17A. In a second study by Bosé et al., the authors also used a limited panel of T cell cytokines composed of IL-2, IFN-γ, TNF-α, IL-4, IL-5, IL-10, and IL-17 and showed that higher IFN-γ and IL-10 levels were produced by activated PBMC from PsO compared to healthy subjects (17). Finally, cytokine secretion was induced after stimulation using an anti-CD3 monoclonal antibody (mAb). This approach does not take into account non-T cell populations as potential sources of cytokines, and hides possible interactions between activated non-T cells and T cells.

The objective of the present study was to assess the cytokine profiles of PsA and PsO patients by different ex vivo PBMC stimulations in order to highlight potential differences between these two distinct pathological entities. Correlation with the clinical variables of metabolic syndrome (MetS), dermatological activity, and rheumatic activity will likewise be explored, as will the differences before and after DMARD (disease modifying anti-rheumatic drugs) treatment initiation in PsA patients.

2 Materials and methods

2.1 Patients

Patients with PsA were included if they were 18-years-old or older and fulfilled the Classification Criteria for Psoriatic
Arthritis (CASPAR) of PsA (18). Patients of 18-years-old or older with PsO diagnosed by dermatologist without any joint complaint were included. Healthy controls were included if they did not suffer from any rheumatic, dermatological or inflammatory condition.

Patients were followed in outpatient clinics and included at any time point of the disease.

Characteristics of patients including dermatological activity, rheumatic activity, CRP sera levels, BMI, HOMA score, PASI, psoriasis duration, age, and abdominal circumference were obtained in the patients’ medical records. Detailed clinical characteristics were available for 31 out of the 37 PsA patients, who were classified as having axial disease, peripheral disease, or both patterns (mixed).

Dermatological activity (i.e. moderate to severe disease) was defined as PASI > 10 (19) and rheumatic activity as DAS28 ≥ 2.6 for peripheral PsA (20), BASDAI ≥ 40/100 for axial PsA (21), and either one of those two criteria for mixed PsA. Overall clinical activity was defined as an active disease that was either dermatological, or rheumatic, or both.

Focusing on metabolic syndrome (MetS) features, high abdominal circumference was defined as above 88 cm for women and 102 cm for men. HOMA score was considered elevated when superior to 3.

High CRP was defined as CRP ≥ 3 mg/L, and MetS was defined according to the ATP III definition (22).

We separately analysed 6 patients for whom we had samples taken before and after DMARD initiation, either csDMARD (methotrexate for 1) or bDMARD (TNF-α inhibitors for 5 of them).

2.2 Samples and cell culture

Ten millilitres of blood were collected from patients and healthy controls in Vacutainer tubes containing heparin for the isolation of PBMCs. Human PBMCs were isolated by density gradient centrifugation using Ficoll-Paque (GE Healthcare) and counted. Analysis of cell viability has been performed using Trypan Blue 0.4% (Merck-Sigma). All samples displayed at least 95% of cell viability. Isolated PBMCs were cultured in RPMI 1640 containing 2 mM glutamine, 100 U/ml penicillin-streptomycin (Invitrogen Life Technologies), and 10% heat-inactivated foetal calf serum (Gibco Life Technologies). Cells were seeded at 1 × 10⁶ cells/ml in 24-well plates and incubated 24 h at 37°C in a humidified atmosphere containing 5% CO₂. Cultures were either left unstimulated to reflect the intrinsic state of cell activation or stimulated throughout the 24 h culture period with E. coli LPS (1 µg/ml; Sigma L2654 – Escherichia Coli serotype 026:B6) for monocyte activation or anti-CD3/CD28 mAb coated-beads for T cell activation (2.5 × 10⁵ beads/10⁶ cells/ml; Invitrogen Life Technologies) (23, 24). After incubation, the plates were centrifuged and supernatants were stored at -80°C until analysis. To estimate lymphocyte activity, the secretion of pro- and anti-inflammatory cytokines has been measured with a set of cytokines representative of Th subsets. Even if they were not exclusively secreted by these Th subsets, IFN-γ, IL-4, IL-17, and IL-10 were representative of Th1 cell, Th2 cell, Th17 cell, and Treg cell, respectively (25).

2.3 Cytokine measurements

The concentrations of IL-1α, IL-1β, IL-1RA, IL-4, IL-17, IL-6, TNF-α, IL-10, and γ interferon (IFN-γ) were quantified in cell culture supernatants using the MILLIPLEX MAP Human Cytokine/Chemokine magnetic bead panel kit (Millipore Corporation, Billerica, MA) according to the manufacturer’s instructions. Data were obtained with the Luminex 200™ platform (Luminex Xmap Technology) and analysed with the xPONENT™ software. All samples were assayed in duplicates.

2.4 Ethics

All patients gave informed consent. The study was conducted in accordance with the declaration of HELSINKI and was approved by the Poitiers University Hospital research board and local ethics committee.

2.5 Statistical analysis

All statistical analyses were performed using GraphPad Prism 9 (GraphPad Software, Inc.). Quantitative data were described by means and standard deviation or median. Qualitative data were presented as absolute frequencies and percentages.

The nonparametric Mann-Whitney U test was used to evaluate the difference of quantitative variables between two groups. For comparison of qualitative variables between three groups, Kruskal-Wallis test with post-hoc Dunn’s multiple comparison tests were performed. Paired observations were compared with the Wilcoxon matched-pairs test. Chi-square tests or Fisher’s exact test were conducted to compare qualitative variables between groups. All box plots showed medians with the minimal and maximal values. P values less than 0.05 were considered statistically significant.

3 Results

3.1 Description of the patient population

Thirty-seven PsA patients and 11 patients with PsO were included in the study, as were 11 healthy subjects. The population had a mean age of 53 years and 62.5% were women. MetS was present in 33.3% of PsD patients, with a
mean BMI of 28 kg/m², a mean HOMA score of 3.74, and a mean abdominal circumference of 96.4 cm for women and 101.5 cm for men. In PsA patients, 37.8% had a purely peripheral form of the disease, 16.2% a purely axial disease, and 46% a mixed pattern. In PsO and PsA respectively, 63.6% and 67.6% of patients received csDMARDs, while 54.5% and 40.5% received bDMARDs. The mean PASI score was higher in PsO (7.42) than in PsA patients (3.69) (p = 0.030), with 27.2% and 12.9% respectively having a dermatologically active disease. There were no other significant differences between PsO, PsA, and PsD groups (Table 1).

### 3.2 Cytokine profiles of psoriatic disease

Since PsD is a systemic disorder, analysis of all patients taken together was first performed before deeper analysis depending on clinical phenotype.

#### 3.2.1 Comparison of PsD patients with healthy subjects

In comparison with healthy subjects, unstimulated PBMC of PsD patients exhibited a monocyte-predominant pro-inflammatory pattern with higher spontaneous release of IL-6 and TNF-α and higher release of IL-1RA. Stimulation of the monocytes by LPS induced higher levels of IL-1β and higher secondary activation of Th1 (IFN-γ), Th2 (IL-4), and Th17 (IL-17A) cytokines. In addition, the activation of T lymphocytes by anti CD3/CD28 mAb indirectly stimulated monocyte production of IL-1β at a higher level than in healthy controls. IL-6 higher production could result from either directly stimulated T-cells or indirectly activated monocytes. Surprisingly, these stimulated lymphocytes displayed lower levels of IFN-γ and IL-17A than healthy controls (Figure 1 and Supplementary Table 1).

**TABLE 1** Description of the population.

| PsO (n = 11) | PsA (n = 37) | PsD (n = 48) | p | PsA vs PsO | p | PsA vs PsD | p | PsO vs PsD |
|--------------|-------------|-------------|---|------------|---|-----------|---|-----------|
| Age [mean (SD); years] | 57.82 (12.41) | 51 (13.17) | 53 (13.19) | 0.215 | 0.239 | > 0.999 | 0.495 |
| Female patients (n; %) | 9 (81.8%) | 21 (56.8%) | 30 (62.5%) | 0.321 | 0.171 | 0.592 | 0.302 |
| PsA Phenotype (n; %) | Axial PsA | 6 (16.2%) | -- | -- | -- | -- | -- |
| Peripheral PsA | 14 (37.8%) | -- | -- | -- | -- |
| Mixed PsA | 17 (46%) | -- | -- | -- | -- |
| Treatment | csDMARD (n; %) | 7 (63.6%) | 25 (67.6%) | 32 (66.7%) | 0.971 | 0.843 | 0.930 | 0.848 |
| bDMARD (n; %) | 6 (54.5%) | 15 (40.5%) | 21 (43.8%) | 0.713 | 0.498 | 0.767 | 0.517 |
| TNF inhibitors (n; %) | 4 (66.7%) | 13 (86.7%) | 17 (71.0%) | 0.997 | > 0.999 | 0.997 | > 0.999 |
| Ustekinumab (n; %) | 2 (33.3%) | 1 (6.7%) | 3 (14.3%) | -- | NR | NR | NR |
| Rituximab (n; %) | 0 (0%) | 1 (6.7%) | 1 (4.8%) | -- | NR | NR | NR |
| Disease activity | PASI [mean (SD)] | 7.42 (7.45) | 3.69 (6.79) | 4.62 (7.05) | 0.037 | 0.030 | 0.993 | 0.139 |
| Dermatological activity (n; %) | 3 (27.2%) | 4 (12.9%) | 7 (16.7%) | 0.547 | 0.353 | 0.750 | 0.416 |
| Rheumatic activity (n; %) | -- | 10 (32.3%) | 10 (23.8%) | -- | -- | -- | -- |
| Metabolic parameters | BMI [mean; kg/m²; (SD)] | 28.72 (9.52) | 27.73 (4.78) | 27.99 (6.27) | 0.785 | > 0.999 | > 0.999 | > 0.999 |
| BMI > 25 kg/m² (n; %) | 8 (72.7%) | 23 (70.1%) | 31 (77.0%) | 0.967 | > 0.999 | 0.918 | > 0.999 |
| HOMA (mean [SD]) | 3.93 (3.73) | 3.74 (3.41) | 0.953 | > 0.999 | > 0.999 | > 0.999 |
| HOMA > 3 (n; %) | 4 (76.4%) | 12 (40%) | 16 (41.3%) | 0.978 | > 0.999 | 0.934 | > 0.999 |
| Abdominal circumference [mean, cm (SD)] | Female | 116 (35.36) | 92.8 (12.47) | 96.4 (17.60) | 0.539 | 0.800 | > 0.999 | > 0.999 |
| Male | 99.3 (20.93) | 103.2 (12.10) | 101.5 (15.99) | 0.799 | > 0.999 | > 0.999 | > 0.999 |
| NR, not relevant. Significant values are bold.
in PsD patients when compared to healthy controls, whereas direct T cell activation in PsD patients was reduced.

3.2.2 Cytokine levels according to biological and clinical parameters of MetS

Differences in cytokine secretion between PsD patients with high circulating CRP (> 3 mg/L), BMI above 25 kg/m², HOMA-score above 3, presence of MetS, or elevated abdominal circumference and PsD patients without these features were analysed (Table 2).

In patients with high CRP, LPS-induced monocyte production of IL-17A and IL-4 was higher than in patients with low CRP, while direct stimulation of T cells had an indirectly stronger monocyte-stimulating effect with higher secretion of IL-1α and IL-6. PBMC from patients with high BMI secreted lower levels of IL-1β after LPS stimulation than those from normal BMI patients. When considering patients with MetS, stimulated monocytes were less prone to stimulate Th1 cells (less IFN-γ release) than those without MetS. Regarding these parameters, no difference was observed regarding HOMA-score or abdominal circumference.

3.2.3 Cytokine levels according to cutaneous or rheumatic activity in PsD patients

PBMC from patients with rheumatic activity (10 of 42) showed lower IFN-γ and TNF-α production after LPS stimulation (Figure 2A and Table 3). LPS-stimulated PBMC from patients with dermatological activity (7 of 42 PsD patients) displayed higher IL-17A production (Figure 2B).

3.2.4 Comparison between PsO and PsA patients

PsO and PsA patients (with all rheumatologic phenotypes encompassed) were compared (Figure 3 and Supplementary Table 2). Unstimulated PBMC from both PsO and PsA displayed higher levels of spontaneous release of IL-6, TNF-α,
and IL-1RA without difference between the two. Stimulation of monocytes by LPS induced higher activation of Th1 and Th2 cells, while T cell activation induced higher production of IL-6 in the two groups. A spontaneous inflammatory state (IL-6, TNF-α and IL-1RA) with monocyte-mediated activation of Th1 (IFN-γ, IL-6) cells is a common feature of PsO and PsA patients. However, only PsO patients displayed an increased production of IL-1α, IL-1β, and IL-6 by stimulated monocytes, indicating a monocyte-predominant cytokine signature of PsO.

In PsA patients, stimulated monocytes activated Th17 cells, while T lymphocyte-stimulation reduced IL-17A but enhanced IL-1β production when compared to healthy controls. This finding suggests that Th17 cells need interaction with stimulated monocytes to produce IL-17A, while their own stimulation activated only monocytes in PsA.

### 3.3 Cytokine profiles in psoriatic arthritis

#### 3.3.1 Cytokine profile of each clinical phenotype of PsA

Considering patients with PsA, we further identified the specific signature of each clinical phenotype that would reveal distinctive underlying pathways (Figure 4 and Supplementary Table 3). Peripheral PsA exhibited a cytokine profile distinct from axial PsA, characterized by Th17 and Th2 activation by activated monocytes and higher activation of monocytes by stimulated lymphocytes. Therefore, a specific peripheral PsA cytokine signature is characterised by a stronger monocyte activation with higher IL-6, IL-1α, and IL-1β levels after T lymphocyte stimulation, as well as greater activation of Th17 and Th2 cells after monocyte stimulation.

### Table 2: Comparison of ex vivo cytokine production depending on MetS and related parameters in PsD patients (median; pg/mL).

| Cytokine | Stimulation | High CRP (> 3 mg/L) | BMI > 25 kg/m² | HOMA score > 3 | MetS | High abdominal circumference |
|----------|-------------|---------------------|----------------|----------------|------|-----------------------------|
|          |             | + | - | + | - | + | - | + | - | + | - | + | - |
| IFN-γ    | None        | 1 | 1 | ns | 1 | 3 | ns | 1 | 1 | ns | 1 | 1 | ns | 1 | 1 | ns |
|          | LPS         | 55.50 | 51.70 | ns | 55.5 | 45.55 | ns | 53.90 | 58.00 | ns | 36 | 132.5 | 0.014 | 55 | 58 | ns |
|          | CD3-CD28    | 178.8 | 2209 | ns | 390.5 | 1249 | ns | 1847 | 190.6 | ns | 947.8 | 406.3 | ns | 789 | 565 | ns |
| IL-10    | None        | 8.30 | 5.75 | ns | 7.60 | 6.65 | ns | 7.3 | 7.6 | ns | 5.6 | 7.6 | ns | 7 | 8 | ns |
|          | LPS         | 2212 | 2500 | ns | 2364 | 1758 | ns | 2495 | 2076 | ns | 1569 | 2791 | ns | 2860 | 2765 | ns |
|          | CD3-CD28    | 809.2 | 589 | ns | 848.5 | 483 | ns | 692.2 | 809.2 | ns | 692.2 | 706.2 | ns | 809 | 501 | ns |
| IL-17A   | None        | 1.0 | 1.20 | ns | 1.0 | 1.0 | ns | 1.0 | 1.0 | ns | 1 | 1 | ns | 1 | 1 | ns |
|          | LPS         | 19.80 | 9.30 | 0.020 | 10.30 | 19.75 | ns | 9.55 | 20.40 | ns | 10 | 19 | ns | 10 | 20 | ns |
|          | CD3-CD28    | 21.30 | 34.80 | ns | 23.70 | 28.70 | ns | 36.25 | 17.00 | ns | 36 | 20 | ns | 35 | 70 | ns |
| IL-1RA   | None        | 9.0 | 5.45 | ns | 8.25 | 7.85 | ns | 4.65 | 7.85 | ns | 7 | 8 | ns | 7 | 8 | ns |
|          | LPS         | 112.2 | 107.5 | ns | 104.4 | 102.3 | ns | 124.6 | 97.20 | ns | 125 | 101 | ns | 137 | 111 | ns |
|          | CD3-CD28    | 113.0 | 62.70 | < 0.001 | 98.70 | 72.90 | ns | 79.65 | 81.70 | ns | 77 | 88 | ns | 99 | 77 | ns |
| IL-1α    | None        | 3.3 | 3.2 | ns | 3.4 | 1.30 | ns | 3.50 | 2.60 | ns | 3 | 3 | ns | 3 | 3 | ns |
|          | LPS         | 521.5 | 665 | ns | 521.5 | 770.2 | ns | 721.4 | 513.7 | ns | 510 | 710 | ns | 598 | 886 | ns |
|          | CD3-CD28    | 128 | 32.50 | na | 58.90 | 49.75 | ns | 47.10 | 59.60 | ns | 33 | 59 | ns | 42 | 40 | ns |
| IL-1β    | None        | 4.10 | 2.45 | ns | 3.6 | 3.4 | ns | 2.75 | 4.2 | ns | 3 | 4 | ns | 3 | 5 | ns |
|          | LPS         | 3746 | 3363 | ns | 2723 | 5592 | 0.017 | 3363 | 2971 | ns | 3059 | 3641 | ns | 3190 | 5369 | ns |
|          | CD3-CD28    | 156.9 | 77.85 | ns | 90.90 | 119.3 | ns | 83.45 | 149.6 | ns | 125 | 110 | ns | 80 | 139 | ns |
| IL-4     | None        | 3.40 | 2.850 | ns | 3.2 | 3.0 | ns | 3.15 | 3.40 | ns | 3 | 3 | ns | 3 | 4 | ns |
|          | LPS         | 38.40 | 19.40 | < 0.001 | 25.10 | 24.0 | ns | 21.10 | 26.50 | ns | 28 | 25 | ns | 23 | 27 | ns |
|          | CD3-CD28    | 66.0 | 143.1 | ns | 53.70 | 144.0 | ns | 86.95 | 66.0 | ns | 160 | 52 | ns | 90 | 283 | ns |
| IL-6     | None        | 22.80 | 11.70 | ns | 18.60 | 7.85 | ns | 15.75 | 17.70 | ns | 12 | 16 | ns | 13 | 11 | ns |
|          | LPS         | 6144 | 16420 | ns | 12084 | 10428 | ns | 17133 | 10000 | ns | 9939 | 12248 | ns | 13693 | 16098 | ns |
|          | CD3-CD28    | 1911 | 482.7 | 0.030 | 1409 | 569 | ns | 963.5 | 1059 | ns | 1473 | 902 | ns | 870 | 567 | ns |
| TNF-α    | None        | 12.40 | 11.45 | ns | 12.50 | 10.10 | ns | 12.40 | 11.20 | ns | 11 | 12 | ns | 12 | 11 | ns |
|          | LPS         | 6875 | 3389 | ns | 5878 | 3185 | ns | 5984 | 3945 | ns | 3135 | 4597 | ns | 3576 | 3945 | ns |
|          | CD3-CD28    | 2196 | 3150 | ns | 2374 | 2336 | ns | 3026 | 2125 | ns | 2285 | 2834 | ns | 2546 | 3926 | ns |

ns, not significant. Significant values are in bold.
The features distinguishing axial PsA from peripheral PsA were higher production of IL-1α by stimulated monocytes, which is part of the PsO cytokine signature, and a lower production of IL-4 after monocyte stimulation. The singularity of an axial PsA cytokine profile is the absence of Th2 cell activation by stimulated monocytes.

### 3.3.2 Ex vivo cytokine profiles according to disease activity

As regards rheumatic activity in the 31 PsA patients, a Th1 and monocytic-predominant signature appeared. Higher production of IL-1RA after LPS stimulation was found, as was the case for IFN-γ and TNF-α as previously described in the PsD patients. No difference was observed according to the dermatological activity of PsA (Table 3).

### 3.3.3 Impact of DMARD initiation on cytokine profiles in psoriatic arthritis

Results of ex vivo cytokine production by PBMC before and after DMARD initiation were available for 6 patients (Figure 5). After treatment, lower production of IL-17A after anti-CD3/CD28 mAb stimulation and higher production of IL-1RA after LPS stimulation were observed, suggesting that the PsA treatment reduced Th17 pro-inflammatory state and reinforced the control of inflammation by monocytes through IL-1RA release.

### 4 Discussion

This exploratory study analysed the ex vivo cytokine production by PBMC stimulated with either LPS or anti-CD3/CD28 mAb in PsD patients and PsA patients. The results showed a Th1 and monocytic-predominant signature for rheumatic activity in PsA patients, with higher production of IL-1RA after LPS stimulation compared to Th2 activation by stimulated monocytes. After DMARD initiation, the PsA treatment reduced Th17 pro-inflammatory state and reinforced the control of inflammation by monocytes through IL-1RA release.
CD28 mAb in clinically well-described populations of PsA and PsO patients (26, 27). PBMCs have the advantage of being easily accessible to perform cytokine measurements after stimulation, although in inflammatory arthritis many other cell types produce cytokines, including fibroblasts, synoviocytes, immune infiltrated cells and osteoblasts. These cells can contribute to the cytokine network of the disease, particularly inside the synovium, and may have different cytokine production patterns (28–31). However, isolating these infiltrated cells is not easy since it requires invasive techniques, making it not suitable for a daily analysis, by contrast to PBMC ex vivo cytokine production (32).

In our study, LPS and anti-CD28/CD28 mAb stimulation of PBMCs were used to stimulate monocytes and T-cells, respectively (23, 24). LPS is a TLR4 agonist traditionally considered as a monocyte activator since TLR4 expression is mainly expressed in myeloid cells in humans although minor expression level of TLR4 has been also described in some lymphoid cell subsets (33). Concerning the activation of T cells following LPS stimulation, the literature is contradictory. Indeed some authors found a direct activation of T-cell by LPS through TLR4 (34) meanwhile some authors hypothesize that it is the result of cell-cell interactions with monocytes. Recent single cell analyses of LPS or anti-CD3/CD28 stimulated-PBMC confirm that LPS only activate monocytes (35). Considering this recent literature, we analysed our results considering that LPS activates monocytes and not T-cells, even if a marginal activation of T-cells could not be ruled out.

Several studies have attempted to determine specific cytokine profiles of PsD. Increased IL-17A production by PBMC after 4 days of anti-CD3/CD28 mAb stimulation has been described in PsO and PsA patients (36). Another study reported high IL-17A in supernatants of CD4+ T cells after 6 days of culture with anti-CD3/CD28 mAb stimulation in very early PsA (37), while we report lower IL-17A levels with similar conditions of stimulation. In our study, 24h of stimulation were used to limit secondary or tertiary production of cytokines that can modify the profile following longer stimulation. Another explanation for this result may be the need for interactions with other cell types and not only T-cell stimulation to induce higher immune system to alleviate systemic inflammation. Interestingly, while LPS stimulation induced increased production of all the cytokines studied in PsD patients, no

TABLE 3  Comparison of ex vivo cytokine production depending on disease activity (dermatological or rheumatic) in PsO patients, PsA patients, and PsD (median; pg/mL).

| Cytokine | Stimulation | PsO | PsD | PsA | Dermatological Activity PsO | Dermatological Activity PsA | Rheumatic Activity PsO | Rheumatic Activity PsA | Overall Activity PsO |
|----------|-------------|-----|-----|-----|--------------------------|--------------------------|------------------------|------------------------|----------------------|
| IFN-γ    | None        | 4736 | 2700 | 3000 | 3100                     | 3200                     | 3400                   | 3400                   | 3500                 |
|          | LPS         | 14366| 2700 | 3000 | 3100                     | 3200                     | 3400                   | 3400                   | 3500                 |
|          | CD3-CD28    | 3005 | 2421 | 2421 | 2500                     | 2600                     | 2800                   | 2800                   | 2900                 |
| IL-10    | None        | 3724 | 5130 | 6360 | 6500                     | 6600                     | 6800                   | 6800                   | 6900                 |
|          | LPS         | 10120| 1550 | 1750 | 1850                     | 1950                     | 2150                   | 2150                   | 2250                 |
|          | CD3-CD28    | 3204 | 3822 | 4321 | 4500                     | 4700                     | 4900                   | 4900                   | 5100                 |
| IL-17A   | None        | 1380 | 2290 | 2690 | 2900                     | 3100                     | 3300                   | 3300                   | 3500                 |
|          | LPS         | 3181 | 5293 | 7893 | 8100                     | 8300                     | 8500                   | 8500                   | 8700                 |
|          | CD3-CD28    | 1384 | 2590 | 2790 | 2890                     | 2990                     | 3000                   | 3000                   | 3100                 |
| IL-1RA   | None        | 3029 | 4390 | 4890 | 5100                     | 5300                     | 5500                   | 5500                   | 5600                 |
|          | LPS         | 1464 | 2481 | 2781 | 2890                     | 2990                     | 3000                   | 3000                   | 3100                 |
|          | CD3-CD28    | 1094 | 1490 | 1790 | 1900                     | 2000                     | 2100                   | 2100                   | 2200                 |
| IL-4     | None        | 3590 | 5290 | 5890 | 6100                     | 6200                     | 6300                   | 6300                   | 6400                 |
|          | LPS         | 1064 | 1750 | 2050 | 2150                     | 2200                     | 2300                   | 2300                   | 2400                 |
|          | CD3-CD28    | 1014 | 1550 | 1750 | 1850                     | 1950                     | 2000                   | 2000                   | 2100                 |
| IL-10n   | None        | 3800 | 5500 | 6200 | 6500                     | 6600                     | 6800                   | 6800                   | 6900                 |
|          | LPS         | 1064 | 1650 | 1950 | 2000                     | 2100                     | 2200                   | 2200                   | 2300                 |
|          | CD3-CD28    | 1124 | 1750 | 1950 | 2000                     | 2100                     | 2200                   | 2200                   | 2300                 |
| IL-1β    | None        | 4100 | 5700 | 6400 | 6700                     | 7000                     | 7300                   | 7300                   | 7500                 |
|          | LPS         | 1464 | 2481 | 2781 | 2890                     | 2990                     | 3000                   | 3000                   | 3100                 |
|          | CD3-CD28    | 1014 | 1490 | 1790 | 1900                     | 2000                     | 2100                   | 2100                   | 2200                 |
| IL-4     | None        | 4200 | 6600 | 7200 | 7500                     | 7700                     | 7900                   | 7900                   | 8000                 |
|          | LPS         | 1064 | 1650 | 1950 | 2000                     | 2100                     | 2200                   | 2200                   | 2300                 |
|          | CD3-CD28    | 1124 | 1750 | 1950 | 2000                     | 2100                     | 2200                   | 2200                   | 2300                 |
| TNFα     | None        | 4288 | 5600 | 6500 | 6800                     | 7200                     | 7700                   | 7700                   | 7900                 |
|          | LPS         | 1612 | 2100 | 2500 | 2600                     | 2700                     | 3000                   | 3000                   | 3200                 |
|          | CD3-CD28    | 1282 | 1800 | 2100 | 2300                     | 2500                     | 2800                   | 2800                   | 3000                 |

ns, not significant. Significant values are in bold. DA, dermatological activity; RA, rheumatic activity; OA, overall activity.
increase of IL-4, IL-17, and IFN-γ was observed in healthy controls. All in all, this suggests a particular ability of circulating T cells to be activated via monocytes in PsD.

Patients suffering from PsD are known to present MetS features, a clinical entity with complex and multiple impacts on the immune system, as reviewed by Andersen et al. (39). There is no literature on ex vivo PBMC-derived cytokine profiles comparing patients according to their BMI status. A recent study showed an exhaustion of T cells inside adipose tissue of obese patients, which is concordant with our findings (40). As reported by Diehl et al., IL-6 exerts an inhibitory effect on Th1 differentiation, thereby lowering IFN-γ production (41). Therefore, since LPS stimulates production of IL-6 by PBMC, we can hypothesize that stimulated monocytes polarize toward a pro-inflammatory phenotype with increased production of IL-6, which hampers IFN-γ production by Th1 cells, especially as IL-6 levels did not differ between patients with and without MetS.

Comparison of the cytokine profiles of the different clinical phenotypes of PsD patients demonstrated features distinctive to each subtype. In PsO patients, our results confirm previous studies highlighting the pivotal role of monocytes and their cytokines (IL-1α, IL-1β, and IL-6) (42–44). Likewise, clinical improvement of PsO with biological therapies has been associated with decreased monocyte activity (45). Since IL-1β induces IL-6 secretion by monocytes (46), we hypothesize that a cascade of cell activation might contribute to IL-6 production. Nevertheless, IL-6 implication in PsO pathogenesis remains unclear, and its inhibition in clinical practice is not efficient (47). Intriguingly, IL-17A is not present in PsO cytokine signature, whereas IL-17A is one of the main cytokines that drives cutaneous inflammation in psoriasis (48). However, activated monocytes may be one of the missing links. In fact, IL-1β enhances the differentiation and expansion of Th17 cells (49). Moreover, the IL-1β/IL-1R pathway regulates dermal IL-17A-producing cells and the stimulation of keratinocytes in PsO.

FIGURE 3 Overall comparison of ex vivo cytokine production between healthy subjects (n = 11), PsO (n = 11), and PsA (n = 37) patients. PBMCs from healthy subjects (n = 11), PsO (n = 11), and PsA (n = 37) patients were cultured at 1x10^6 cells/ml for 24 h at 37°C, 5% CO2, with and without stimulation of monocytes by E. coli LPS (1 µg/mL) or T lymphocytes by anti-CD3/CD28 mAb (2.5 x 10^5 beads/10^6 cells/ml). Supernatants were collected and cytokine levels were assayed by Luminex Multiplex ELISA. Results are presented using median values with minimum to maximum box-plot. Difference between the groups using non-parametric Kruskal-Wallis test with Dunn’s multiple comparison test is presented at the top of the figure for each condition. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.
Considering the absence of IL-17A expression by PBMCs from PsO patients, we hypothesize that IL-17A might be produced essentially by tissue-resident cells such as type 3 innate lymphoid cells (ILC3) for instance. The specific cytokine profile of peripheral PsA raises the interesting concept of crosstalk between immune cells subtypes as a crucial element of disease pathophysiology. Chizzolini et al. demonstrated that activated cells could induce the production of cytokines by monocytes through cell-cell contact. Evans et al. demonstrated that LPS-activated monocytes induce Th17 responses when co-cultured with CD4+ T cells, thereby highlighting the importance of monocyte/T cell interaction in the shaping of inflammatory T cell responses. These interactions seem to be the key to the vicious circle of inflammation found in peripheral but not in axial PsA. It is also well-known that peripheral PsA patients present a more inflammatory phenotype, reflected by higher levels of erythrocyte sedimentation rate or CRP than axial PsA patients. We can parallel a study focusing on innate lymphoid cells (ILCs) in different rheumatic diseases with either a synovitis-predominant pattern or not. Differences in ILC populations were found between these two groups, but not between the different rheumatic diseases, implying that the clinical profile may be more important than the disease itself to distinguish particular immune patterns. The importance of T cell activation in PsA has also been highlighted in a study reporting a therapeutic strategy based on targeted treatments in which patients with an activated Th17 predominant pattern were treated by an IL-17A inhibitor, while patients harbouring an activated Th1 predominant pattern received an anti-p40 treatment. This study showed better treatment efficacy, highlighting the importance of T cells in the pathophysiology of peripheral PsA.

Focusing on disease activity, the spontaneous production of IL-6 and TNF-α was increased in patients with dermatological activity, as previously reported for TNF-α, but not for IL-6.
In accordance with our results showing an increase of IL-17A after LPS stimulation in PsD patients with dermatological activity, it has been reported that IL-17+ T cells were increased in active psoriasis (58, 59) and that elevated concentrations of IL-17A in serum were correlated with the dermatological activity (60). In PsA, in agreement with our results, serum IL-1RA was associated with joint severity and correlated with tender and swollen joints (12). Lower levels of IFN-γ associated with rheumatic disease activity was to be expected, given the accumulating evidence of a “yin and yang” effect of IFN-γ on the inflammatory process (61), particularly with negative regulation of IL-17 expression by Th17 cells (62).

After DMARD initiation, we observed decreased IL-17A production and increased IL-1RA production, in accordance with the decreased inflammatory process. Previous studies reported not only decreased serum IL-17A levels (63), but also decreased TNF-α after TNF-α inhibitor treatment, which was not observed in our ex vivo analysis (64, 65). It is noteworthy that the approaches were different, and that, to our knowledge, no ex vivo PBMC stimulation study has focused on this issue.

One of the limits of this study is the inclusion of subjects who, for the most of part, already benefited from DMARDs therapies, which have an impact on cytokines production (66). However, this feature equally concerns both PsO and PsA patients, thereby limiting the bias. The number of patients included is also limited but comparable to other studies in the field (10, 11, 13, 14, 16, 17). In any event, our results should be viewed with all the necessary precautions. While the ex vivo approach is dynamic and easy to implement to study the contribution of circulating cells to the diseases, it excludes the
contribution of resident cells. Another limit of this global approach is that the underlying mechanisms of the complex interactions between immune cells and their subsequent cytokine productions were not fully elucidated.

Taken together, our results lead us to propose a core cytokine profile for all PsD patients and specific cytokine patterns distinguishing the different PsD sub-phenotypes, as summarized in Figure 6. The perspective of characterizing distinct particular immune profiles might be of great interest to tailor patient treatment with a potential clinical impact on the effectiveness of biologic DMARDs (56).

In conclusion, PsO and PsA share not only the common cytokine patterns common to PsD, but a number of specific cytokine patterns, as well. PsO exhibits a monocyte-predominant cytokine signature, whereas peripheral PsA signature is characterized by strong monocyte activation by stimulated T cells and Th17 cell activation by stimulated monocytes. Our study highlights the crucial role of immune cell interaction potentially involving soluble mediators and/or cell-cell contact with different patterns of interaction depending on clinical phenotype. Further studies should be performed to increase knowledge of the immunological mechanisms that underlie every sub-phenotype of PsD.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Poitiers University Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

AD, TD, and PV performed the experiments. CS, EG, and PV acquired data. GL, AD, TD, PV, CS, FM, J-CL, and EG analysed data. GL performed statistical analysis. GL wrote first draft of the manuscript. GL, EG, FM, J-CL, and J-FJ corrected manuscript. All authors contributed to the article and approved the submitted version.

Funding

This research was supported by Poitiers University Hospital and Poitiers University.

Acknowledgments

Pr. Stéphanie RAGOT for her statistical advices and validation. M. Jeffrey Arsham for language editing of the manuscript. The authors thank the European Union for an equipment grant program for research laboratories and platforms.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.993363/full#supplementary-material

References

1. Rech J, Sticherling M, Stoessel D, Biermann MHC, Häberle BM, Reinhardt M. Psoriatic arthritis epidemiology, comorbid disease profiles and risk factors: Results from a claims database analysis. Rheumatol Adv Pract (2020) 4(2):rkaa033. doi: 10.1093/rap/rkaa033

2. Scarpa R, Ayala F, Caporaso N, Olivieri I. Psoriasis, psoriatic arthritis, or psoriatic disease? J Rheumatol (2006) 33:210–2.

3. Ritchlin C. Psoriatic disease—from skin to bone. Nat Rev Rheumatol (2007) 3:698–706. doi: 10.1038/nrrheum670

4. Lubrano E, Sciriha S, Perrotta FM. Psoriatic arthritis, psoriatic disease, or psoriatic syndrome? J Rheumatol (2019) 46:1428–30. doi: 10.3899/jrheum.190054

5. Sobchak C, Eder L. Cardiometabolic disorders in psoriatic disease. Curr Rheumatol Rep (2017) 19:63. doi: 10.1007/s11926-017-0692-2
increased production of monocytes TNF-α. 58:336
phenotypes of peripheral T helper cells in psoriatic arthritis. J Invest Dermatol (2019) 153:531–6. doi: 10.1111/jid.202003.06648.x
Eur Rev Med Pharmacol Sci (2017) 21:5166–71. doi: 10.26355/eurev_201711_13834
A critical role of the IL-1β-IL-1R signaling pathway in skin inflammation and psoriasis pathogenesis. J Invest Dermatol (2019) 153:531–6. doi: 10.1111/jid.202003.06648.x
55. Kato M, Ikeda K, Sugiyama T, Tanaka S, Iida K, Suga K, et al. Associations of inflammation patterns with peripheral innate lymphoid cell characterization of psoriatic patients in long term etanercept treatment. J Autoimmun (2019) 106:6232–6. doi: 10.1016/j.jauto.2020.03.008
54. Feld J, Ye JY, Chandran V, Inman RD, Haroon N, Cook R, et al. Axial psoriatic arthritis distinct from ankylosing spondylitis with and without concomitant psoriasis? Rheumatology (2010) 58:336–44. doi: 10.1093/rheumatology/key069
53. Mizutani H, Ohmoto Y, Mizutani T, Murata M, Shimizu M. Role of increased production of monocytes TNF-α, IL-1β and IL-6 in psoriasis: Relation to focal infection, disease activity and responses to treatments. J Dermatol Sci (1997) 14:145–53. doi: 10.1016/S0923-1816(96)00562-2
52. Chizzolini C, Chicheportiche R, Burger D, Dayer J-M. Human Th1 cells preferentially induce interleukin (IL)-1β while Th2 cells induce IL-1 receptor antagonist production upon cell/cell contact with monocytes. Eur J Immunol (1997) 27:171–7. doi: 10.1002/eji.1830270125
51. Bielecki P, Riesenfeld SJ, Hütter J-C, Tozlau Triglia E, Kowalczyk MS, Ricardò-Gonzalez RR, et al. Skin-resident innate lymphoid cells converge on a pathogenic effector state. Nature (2021) 592(7852):128–132. doi: 10.1038/s41586-021-03188-w
50. Cai Y, Xue F, Quan C, Qu M, Liu N, Zhang Y, et al. A critical role of the IL-17A in the psoriatic patients IL-17A in the psoriatic patients pathogenic Th17 cells with the disease severity and its potential implication for biological treatment selection in psoriasis patients. Mediators Inflammation (2020) 2020:1–6. doi: 10.1155/2020/8065147
49. Wilson NJ, Boniface K, Erre GL, Piera C, Bondi G, Taras L, et al. Peripheral blood CD8+ T-cell profiles in patients with psoriatic arthritis: A cross-sectional case-control study. Eur Rev Med Pharmacol Sci (2017) 21:5166–71. doi: 10.26355/eurev_201711_13834
48. Blauvelt A, Chiricuzzi A. The immunologic role of IL-17 in psoriasis and psoriatic arthritis pathogenesis. Clin Rev Allergy Immunol (2018) 55:379–90. doi: 10.1007/s12016-018-8072-3
47. Blauvelt A, Chiricuzzi A. The immunologic role of IL-17 in psoriasis and psoriatic arthritis pathogenesis. Clin Rev Allergy Immunol (2018) 55:379–90. doi: 10.1007/s12016-018-8072-3
46. Cordiali-Fei P, Ardigò M, Mastroianni A, Giuliani A, D’Agosto G, Bordignon V, et al. Serum cytokines and biohumoral immunological characterization of psoriatic patients in long term etanercept treatment. Int J Immunopathol Pharmacol (2008) 21:643–9. doi: 10.1177/093452080802100318
45. Kato M, Ikeda K, Sugiyama T, Tanaka S, Iida K, Suga K, et al. Associations of ultrasound-based inflammation patterns with peripheral innate lymphoid cell populations, serum cytokines/chemokines, and treatment response to methotrexate in rheumatoid arthritis and spondyloarthritis. PloS One (2021) 16:e0252116. doi: 10.1371/journal.pone.0252116
44. Cordiali-Fei P, Ardigò M, Mastroianni A, Giuliani A, D’Agosto G, Bordignon V, et al. Serum cytokines and biohumoral immunological characterization of psoriatic patients in long term etanercept treatment. Int J Immunopathol Pharmacol (2008) 21:643–9. doi: 10.1177/093452080802100318
43. Sakkas LI, Bogdanos DP. Are psoriasis and psoriatic arthritis the same disease? the IL-23/IL-17 axis data. J Autoimmun (2017) 66:10–5. doi: 10.1016/j.jaut.2016.09.015
42. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. Interleukin 17–producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol (2005) 6:1123–32. doi: 10.1038/nijm1254
41. Kelchtermans H, Billiau A, Matthys P. How interferon-γ keeps autoimmune diseases in check. Trends Immunol (2008) 29:479–86. doi: 10.1016/j.it.2008.07.002
40. Evans HG, Gullick NJ, Kelly S, Pitizali C, Lord GM, Kirkham BW, et al. In vivo activated monocytes from the site of inflammation in humans specifically promote Th17 responses. Proc Natl Acad Sci USA (2009) 106:6232–7. doi: 10.1073/pnas.0808144106
39. Agrawal N, Grewal HS, Sethi S, Kochhar P, Gupta R, et al. Psoriasis: Update on diagnosis and management. J Clin Diagn Res (2014) 8:146–50. doi: 10.7024/jcdr.2014.8.146
38. Blauvelt A, Chiricuzzi A. The immunologic role of IL-17 in psoriasis and psoriatic arthritis pathogenesis. Clin Rev Allergy Immunol (2018) 55:379–90. doi: 10.1007/s12016-018-8072-3
37. Herrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. Interleukin 17–producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol (2005) 6:1123–32. doi: 10.1038/nijm1254
36. Sakkas LI, Bogdanos DP. Are psoriasis and psoriatic arthritis the same disease? the IL-23/IL-17 axis data. J Autoimmun (2017) 66:10–5. doi: 10.1016/j.jaut.2016.09.015
35. Kato M, Ikeda K, Sugiyama T, Tanaka S, Iida K, Suga K, et al. Associations of ultrasound-based inflammation patterns with peripheral innate lymphoid cell populations, serum cytokines/chemokines, and treatment response to methotrexate in rheumatoid arthritis and spondyloarthritis. PloS One (2021) 16:e0252116. doi: 10.1371/journal.pone.0252116
34. Miyagawa I, Nakaayama S, Nakano K, Kubo S, Iwata S, Miyazaki Y, et al. Precision medicine using different biological DMARDs based on characteristic phenotypes of peripheral T helper cells in psoriatic arthritis. Rheumatology (2019) 58:336–44. doi: 10.1093/rheumatology/key069
33. Mizutani H, Ohmoto Y, Mizutani T, Murata M, Shimizu M. Role of increased production of monocytes TNF-α, IL-1β and IL-6 in psoriasis: Relation to focal infection, disease activity and responses to treatments. J Dermatol Sci (1997) 14:145–53. doi: 10.1016/S0923-1816(96)00562-2
32. Colombo E, Garry G, Erre GL, Piera C, Bondi G, Taras L, et al. Peripheral blood CD8+ T-cell profiles in patients with psoriatic arthritis: A cross-sectional case-control study. Eur Rev Med Pharmacol Sci (2017) 21:5166–71. doi: 10.26355/eurev_201711_13834
31. Aguilar-Nieves C, Castro-Escamilla O, Ortega-Rocha EM, Maldonado-García C, Jurado-Santa Cruz F, Pérez-Montesinos G, et al. Association of pathogenic Th17 cells with the disease severity and its potential implication for biological treatment selection in psoriasis patients. Mediators Inflammation (2020) 2020:1–6. doi: 10.1155/2020/8065147
30. Michalak Stoma A, Bartosinska J, Kowal M, Raczkiewicz D, Krasowska D, Chodorowska G. IL-17A in the psoriatic patients’ serum and plaque scales as potential marker of the diseases severity and obesity. Mediators Inflammation (2020) 2020:1–9. doi: 10.1155/2020/7420823
29. Kelchtermans H, Billiau A, Matthys P. How interferon-γ keeps autoimmune diseases in check. Trends Immunol (2008) 29:479–86. doi: 10.1016/j.it.2008.07.002