**TRANSLATIONAL SCIENCE**

**IL-23 skin and joint profiling in psoriatic arthritis: novel perspectives in understanding clinical responses to IL-23 inhibitors**

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**ABSTRACT**

**Objectives** To determine the relationship between synovial versus skin transcriptional/histological profiles in patients with active psoriatic arthritis (PsA) and explore mechanistic links between diseased tissue pathology and clinical outcomes.

**Methods** Twenty-seven active PsA patients were enrolled in an observational/open-label study and underwent biopsies of synovium and paired lesional/non-lesional skin before starting anti-tumour necrosis factor (TNF) (if biologic-naïve) or ustekinumab (if anti-TNF inadequate responders). Molecular analysis of 80-inflammation-related genes and protein levels for interleukin (IL)-23p40/IL-23p19/IL-23R were assessed by real-time-PCR and immunohistochemistry, respectively.

**Results** At baseline, all patients had persistent active disease as per inclusion criteria. At primary end-point (16-weeks post-treatment), skin responses favoured ustekinumab, while joint responses favoured anti-TNF therapies. Principal component analysis revealed distinct clustering of synovial tissue gene expression away from the matched skin. While IL12B, IL23A and IL23R were homogeneously expressed in lesional skin, their expression was extremely heterogeneous in paired synovial tissues. Here, IL-23 transcriptomic/protein expression was strongly linked to patients with high-grade synovitis who, however, were not distinguishable by conventional clinimetric measures.

**Conclusions** PsA synovial tissue shows a heterogeneous IL-23 axis profile when compared with matched skin. Synovial molecular pathology may help to identify among clinically indistinguishable patients those with a greater probability of responding to IL-23 inhibitors.

**INTRODUCTION**

Psoriatic arthritis (PsA) is a chronic heterogeneous inflammatory condition occurring in up to 30% of patients with skin and/or nail psoriasis (PsO), which variably affects the spine, peripheral synovial joints and enthesis.1 Although the mechanisms for such disease heterogeneity are not entirely clear, the interleukin (IL)-23/IL-17 axis is believed to be key in PsO and PsA pathogenesis.1 2 3 23

IL-23 is a proinflammatory cytokine composed of two subunits (p40, in common with IL-12, and p19, IL-23-specific) and mostly produced by keratinocytes, dendritic and myeloid cells. By binding its cognate receptors (IL-23R/IL-12Rβ1), it stabilises RAR-related-orphan-receptor-gamma-t (RORγt) in T-helper-17 cells, which, in turn, release their effector cytokines IL-17, IL-21 and IL-22 to initiate and amplify local autoimmune reactions and chronic inflammation.1 2

Several drugs targeting the IL-23/IL-17 axis have been successfully tested in the context of psoriasis and PsA but, while 50%–60% of patients achieve almost complete psoriasis clearance on treatment, the joint disease improvement is modest. To date, the mechanism for the divergent skin-joint response remains largely unexplained.

What is already known about this subject?

► Psoriatic arthritis (PsA) is a chronic heterogeneous inflammatory condition affecting patients with psoriasis, and the interleukin (IL) 23/IL-17 axis is believed to be key in psoriasis and PsA pathogenesis.

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What does this study add?

► It provides first-time detailed evidence of the expression of the IL-23 axis in matched skin and synovial tissue from active PsA patients demonstrating distinct gene expression clustering of the synovium away from paired skin. It reveals that, while IL23A, IL12B and IL23R are expressed at a high level in lesional skin, their expression in the synovium is hugely heterogeneous.

► It demonstrates that, while patients with diverse degrees of synovial inflammation could not be distinguished clinically by conventional clinimetric measures, the IL-23 axis signature is differentially expressed within the synovial tissue and strongly linked to high-grade synovitis.

**Key messages**

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► Psoriatic arthritis (PsA) is a chronic heterogeneous inflammatory condition affecting patients with psoriasis, and the interleukin (IL)-23/IL-17 axis is believed to be key in psoriasis and PsA pathogenesis.

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Severity Index (PASI75), success in treating joints is more modest, and a mere 20% improvement (American College of Rheumatology (ACR20)) is observed in 35±30% of patients.6 7 The new IL-23p19 selective inhibitors have been shown to be more effective, and ACR20 is reached in approximately 60%.8 9 However, while a similar proportion of patients achieve almost complete BO clearance (PASI90), high hurdles joint disease ACR50/ACR70 is achieved in only 33%–36% and 13%–20% of patients, respectively.8 9 10

To date, the mechanism for such divergent skin-joint response, consistent across multiple trials, remains largely unexplained. Boutet et al7 and Belasco et al10 have postulated that different target expression levels in skin and joints contribute to the diverse clinical response. For example, Belasco et al reported that gene expression patterns in skin and synovium are distinct, showing a stronger IL-17 signature in skin than in synovium, and more equivalent TNF signal across both tissues.10 Here, we present new evidence exploring the expression of the IL-12/IL-23 axis in psoriatic skin versus matched synovial tissue at both molecular and protein level.

METHODS
Full methods are included in online supplemental material. Briefly, 27 patients fulfilling the Classification Criteria for Psoriatic Arthritis (CASPAR)11 with active peripheral joint disease despite csDMARDs and either biologic-naïve/ failing TNFi were recruited in this observational/open-label study (REC15/LO/0584). Patients underwent a baseline ultrasound (US)-guided synovial biopsy12 and lesional/non-lesional skin punch-biopsies, and were then treated with TNFi/ustekinumab as per local guidelines. The chosen primary endpoint was 16 weeks. Gene expression was analysed by real-time PCR (Fluidigm). Paraffin-embedded skin/synovium samples were stained with H&E. Immune cells/IL-23-axis were quantified by immunohistochemistry. Synovial tissue were categorised in ‘low-grade’ (score 0–1) or ‘high-grade’ (score 2–7) synovitis13 and in pathotypes (lympho-myeloid/ diffusely myeloid/pauci-immune).14

RESULTS

Patients’ characteristics and treatment response
Baseline and 16 weeks demographic and clinical features are summarised in figure 1. The overall male to female ratio was 1:1 (59% female), the average age was 45±2.5 and disease duration >10 years. Seventy-eight per cent of patients had concomitant skin involvement, with a mean PASI of 7.8. As per inclusion criteria, all patients had active joint disease (68-tender joints count 30.9±19.2, 66-swollen joints count 13±10.4, Disease Activity Score (DAS) 4.3±1.1) despite treatment with csDMARDs±anti TNF. Following the baseline biopsy, patients were treated with anti-TNF (n=18) if they were biologic-naïve or ustekinumab (n=9) if they had not responded to at least one TNFi. The higher number of females in the ustekinumab-arm (8/9) reflects the gender differences in TNFi-treatment outcomes observed in registries15 (figure 1A). At 16 weeks, ESR, tender-joint scores, Ritchie Articular Index (RAI), Visual Analogue Scale (VAS)-pain, Likert-physician assessment and DAS were significantly higher in the ustekinumab-treated group; PASI-scores improved from baseline in both groups (−4.7±7.5 in TNFi treated vs −8.9±14.3 in ustekinumab treated) and were comparable between the two treatment arms (2.3±2.6 vs TNFi treated vs 2.3±2.3 in ustekinumab treated) (figure 1B). However, while significantly more patients in the anti-TNF group achieved EULAR(DAS)-response compared with ustekinumab-treated patients (70.6% vs 22.2%), there was a trend in favour of ustekinumab in terms of skin responses (figure 1C). Besides, as joint response to ustekinumab can be delayed up to 24–28 weeks, clinical responses were also assessed at 24 weeks. As shown in online supplemental figure S1, ustekinumab-treated patients maintained significantly higher tender joint scores, RAI, VAS-pain, Likert physician assessment and DAS; 50% and 68.8% of patients in the ustekinumab and TNFi arms achieved EULAR(DAS) response, respectively. Individual patient joint/ skin responses are summarised in online supplemental table S1.

Gene expression profiles in paired skin and synovium reveal tissue-specific signatures and divergent expression patterns
Gene expression analysis was performed on 14 matched synovial tissue, lesional and adjacent non-lesional skin. As shown in figure 2A, principal component analysis (PCA), built on the expression of 80 inflammation-related genes (online supplemental table S2), showed that the synovium clusters away from the skin, with a partial overlapping of lesional and non-lesional skin. To further investigate the gene variance contributing to the diversity of expression within each anatomic site (skin/synovium), related PCA plots were covisualised with loading plots (biplots) (figure 2B and C). IL17A/F, IL23R and IL21 were the major contributors of PC1/2 variation in lesional skin. In synovium, genes related to ectopic lymphoid structure (ELS) formation (CXCL13, CXCR5) and the IL-23 axis (IL23A, IL12B, IL23R) together strongly contributed to the PC variation. For instance, CXCR5 and IL23A robustly aligned with PC1 in accounting for 35.4% of the variance within the synovium data set and CXCL13 strongly and equally contributed to PC1 and PC2 variation. We next assessed the relative gene expression of the drug-targets of TNF- and IL-23/IL-12-inhibitors, that is, TNF, IL23A (encoding IL-23p19), IL12B (encoding IL-23p40) and IL23R (figure 2D). TNF was generally homogeneously expressed in both skin and synovial tissue. Conversely, IL23A, IL12B and IL23R showed higher expression in lesional skin compared with both non-lesional skin and synovium. Interestingly, we observed that while some patients did express IL-23 cytokines/receptor in both skin and joint, others had discordant expression, that is, active IL-23 pathway in the lesional skin but not in the synovium. To investigate potential mechanisms for the diverse expression of the IL-23-axis within the synovium, we stratified patients based on the degree of synovial inflammation.14 Both IL12B and IL23R genes, but not IL23A, were significantly more expressed in patients with higher synovitis scores (figure 2E). Notably, despite the major variance in the degree of synovial inflammation and histopathological phenotypes, there were no significant clinical differences in the two patient groups (online supplemental table S3,S4).

Key messages

How might this impact on clinical practice or future developments?

- This study demonstrates that psoriatic arthritis synovial tissue shows a heterogeneous interleukin 23 (IL-23) axis profile independently of its expression in paired-skin samples, thus providing a plausible mechanistic explanation for the divergent skin and joint clinical response to IL-23 inhibitors. It supports the need to test in larger appropriately designed and powered studies whether drug-target bioavailability correlates with the likelihood of response. Identifying biomarkers of joint response to therapy in patients clinically indistinguishable is going to be vital to improve disease outcomes, prevent disability and reduce healthcare and societal costs.

Psoriatic arthritis


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### Psoriatic arthritis

#### Table A

<table>
<thead>
<tr>
<th></th>
<th>TOTAL POPULATION (27 PATIENTS)</th>
<th>ANTI-TNF (18 PATIENTS)</th>
<th>USTEKINUMAB (9 PATIENTS)</th>
<th>p-values</th>
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<tr>
<td><strong>Female % (n)</strong></td>
<td>58% (16)</td>
<td>44.4% (8)</td>
<td>88.9% (8)</td>
<td>p = 0.63</td>
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<td><strong>Age years (mean ± SD)</strong></td>
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<td>42.2 ± 11.2</td>
<td>51.8 ± 12.9</td>
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<td><strong>Disease duration years (mean ± SD)</strong></td>
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<td>7.3 ± 7.2</td>
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<tr>
<td><strong>ESR mm/h (mean ± SD)</strong></td>
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<td>22.8 ± 15.4</td>
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<td><strong>CRP mg/l (mean ± SD)</strong></td>
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<td>11.4 ± 22.7</td>
<td>11.7 ± 16.6</td>
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<tr>
<td><strong>TJC70 (mean ± SD)</strong></td>
<td>34.2 ± 22.6</td>
<td>31 ± 25.1</td>
<td>40.7 ± 15.9</td>
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<tr>
<td><strong>TJC68 (mean ± SD)</strong></td>
<td>30.9 ± 19.2</td>
<td>27.7 ± 21.2</td>
<td>37 ± 13.1</td>
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<td><strong>RAI (mean ± SD)</strong></td>
<td>18.7 ± 9.8</td>
<td>16.6 ± 10.7</td>
<td>23.1 ± 5.6</td>
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<tr>
<td><strong>SJC76 (mean ± SD)</strong></td>
<td>13.6 ± 10.6</td>
<td>11.6 ± 8.5</td>
<td>17.4 ± 13.9</td>
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<tr>
<td><strong>SJC66 (mean ± SD)</strong></td>
<td>13 ± 10.4</td>
<td>11.3 ± 8.6</td>
<td>16.5 ± 12.1</td>
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<tr>
<td><strong>SJC44 (mean ± SD)</strong></td>
<td>10.3 ± 8.3</td>
<td>9.2 ± 7.8</td>
<td>12.6 ± 5.3</td>
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### Table B

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<th>TOTAL POPULATION (26 PATIENTS)</th>
<th>ANTI-TNF (17 PATIENTS)</th>
<th>USTEKINUMAB (9 PATIENTS)</th>
<th>p-values</th>
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<tr>
<td><strong>ESR mm/h (mean ± SD)</strong></td>
<td>17.6 ± 14.6</td>
<td>12.2 ± 8.8</td>
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<td>p = 0.06</td>
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<td><strong>CRP mg/l (mean ± SD)</strong></td>
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<td><strong>TJC70 (mean ± SD)</strong></td>
<td>20.7 ± 22.1</td>
<td>20.2 ± 20.9</td>
<td>30.7 ± 17.9</td>
<td>p = 0.01</td>
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<tr>
<td><strong>TJC68 (mean ± SD)</strong></td>
<td>23.3 ± 19.5</td>
<td>16.5 ± 14.0</td>
<td>36.6 ± 15.9</td>
<td>p = 0.07</td>
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<td><strong>RAI (mean ± SD)</strong></td>
<td>13.9 ± 10.6</td>
<td>10.9 ± 11.1</td>
<td>19.4 ± 7.2</td>
<td>p = 0.02</td>
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<td><strong>SJC76 (mean ± SD)</strong></td>
<td>8.1 ± 9.3</td>
<td>7.4 ± 8.0</td>
<td>10.1 ± 11.8</td>
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<tr>
<td><strong>SJC66 (mean ± SD)</strong></td>
<td>7.8 ± 9.0</td>
<td>7.0 ± 8.0</td>
<td>9.4 ± 11.0</td>
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<tr>
<td><strong>SJC44 (mean ± SD)</strong></td>
<td>5.8 ± 6.2</td>
<td>5.6 ± 6.0</td>
<td>6.3 ± 6.9</td>
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<td><strong>PASI (mean ± SD)</strong></td>
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<td>2.3 ± 2.6</td>
<td>2.3 ± 2.3</td>
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<td><strong>VAS tenderness (mean ± SD)</strong></td>
<td>54.1 ± 31.6</td>
<td>50.1 ± 31.9</td>
<td>61.3 ± 31.6</td>
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<td><strong>VAS pain (mean ± SD)</strong></td>
<td>46.7 ± 32.2</td>
<td>37.2 ± 30.2</td>
<td>64.6 ± 29.4</td>
<td>p = 0.06</td>
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<td><strong>VAS GH Patient (mean ± SD)</strong></td>
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<td>46.5 ± 33.9</td>
<td>51.7 ± 33.3</td>
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<td><strong>VAS GH Physician (mean ± SD)</strong></td>
<td>45.0 ± 26.0</td>
<td>38.8 ± 24.3</td>
<td>56.7 ± 26.1</td>
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<td><strong>LIKERT Patient (mean ± SD)</strong></td>
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<td>2.8 ± 1.1</td>
<td>3.1 ± 1.2</td>
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<tr>
<td><strong>LIKERT Physician (mean ± SD)</strong></td>
<td>2.0 ± 1.3</td>
<td>2.5 ± 1.2</td>
<td>3.6 ± 1.1</td>
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<td><strong>HAQ (mean ± SD)</strong></td>
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### Table C

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<th>PASI50-response</th>
<th>EULAR-response</th>
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<td><strong>Number of Patients</strong></td>
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<td>PASI50 Yes</td>
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<tr>
<td><strong>50%</strong></td>
<td>16.7%</td>
<td>83.3%</td>
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<tr>
<td><strong>50%</strong></td>
<td>50%</td>
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#### Figure 1

Baseline and 16 weeks characteristics of the patients included in the psoriatic arthropathy and its relationship with clinical disease activity (PsABRE) study. (A) Baseline features of the whole cohort (n=27) and comparison of variables between patients receiving anti-TNF (n=18) or ustekinumab (n=9). (B) Patients’ characteristics at the chosen primary endpoint, that is, 16-weeks post-treatment (n=26, one patient lost to follow-up) and comparison between TNFi- (n=17) and ustekinumab-treated patients (n=9). (A, B) P values calculated using Mann-Whitney U test or Fisher’s exact test as required (TNFi-arm vs ustekinumab-arm). (C) Skin (PASI50) and joints (EULAR(DAS) good/moderate vs none) response at 16 weeks. P values calculated using Fisher’s exact test. CRP, C reactive protein; DAS, Disease Activity Score; DMARDs, disease-modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; N, number; NS, non-significant; PASI, Psoriasis Area and Severity Index; RAI, Ritchie Articular Index; SJC, swollen joints count; TJC, tender joints count; TNF, tumour necrosis factor; VAS, Visual Analogue Scale (0–100).

### Notes

Synovial IL-23p40/p19 and IL-23R protein expression correlates with the histological inflammatory status

To confirm the molecular findings, we next evaluated protein expression levels of IL-23p40, IL-23p19 and IL-23R in skin and synovium by immunohistochemistry. As expected, the percentage of IL-23p40-, IL-23p19- and IL-23R-positive cells was significantly higher in lesional skin compared with paired non-lesional skin (figure 3A, B); within the synovium, it was greater in patients with higher degree of inflammation (figure 3C, D) and in lympho-myeloid and diffuse-myeloid pathotypes (online supplemental figure S2). This result was in line with the positive correlation observed between the synovial inflammatory score and the proportion of IL-23p40/IL-23p19/IL-23R-positive cells (figure 3E), as well as their correlation with each other’s (data not shown). Of note, the percentage of IL-23p40/IL-23p19/IL-23R-positive cells at baseline was, on average, comparable between the treatment groups despite different drug exposure (online supplemental table S5). Except for the LIKERT patient score, we did not detect other significant correlations between IL-23-axis expression and clinical parameters at baseline, suggesting that patients with comparable disease severity may have, in fact, heterogeneous histopathological features and expression of drug...
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To further assess whether the IL-23-axis heterogeneity tracks across different stages of the disease, we analysed IL-23 expression pattern in the synovium of 21 treatment-naïve PsA patients with <12 months symptoms. As shown in online supplemental figure S3, overall, there was a positive correlation between IL-23p40/IL-23p19/IL-23R-positive cells and synovitis scores, and lower

Figure 2  Gene expression analysis in matched skin and synovium from PsA patients. (A) Principal component (PC) analysis (PCA) performed on the expression data of a set of 80 selected genes in 14 matched non-lesional (non les.) and lesional skin and synovium. The first two eigenvalues were plotted with data ellipses for each tissue type using a CI of 0.95. The PCA clearly separates synovium (blue dots) from non-lesional skin (non les., green dots) and lesional skin (red dots). (B, C) Biplots showing individuals repartition in PC1 and 2 (black dots) and loading plots assessing the contribution of each of the 80 genes analysed in the PC, displayed for the lesional skin (B) and synovial tissues (C). Genes names are indicated if their contribution to the PC variance is >1. The PCA and biplots were created using function prcomp from the stats package within R statistics (version 3.5.3) and factoextra R package20 (D) Heatmap representing TNF, IL12B (IL-23p40 protein), IL23A (IL-23p19 protein) and IL23R expression in 14 matched non-lesional (non les.) and lesional skin and synovium samples. dd-threshold cycles (ddCTs) are shown in colorimetric scale (low expression in blue, high expression in red). Lines 1–11 represent anti-TNF-treated patients, lines 12–14 ustekinumab-treated patients. E, IL12B, IL23A and IL23R gene expression in synovial biopsies classified as ‘low’ (0–1) and ‘high’ (2–7) synovial inflammatory score (Krenn’s score). P values were calculated using Mann-Whitney U test, *P<0.05, mean and SD are shown. IL-23, interleukin 23; TNF, tumour necrosis factor.
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IL-23 cytokines/receptor tissue-availability in the pauci-immune compared with macrophage-rich pathotypes. Similarly to established PsA, we did not find significant correlations between clinical parameters and IL-23 axis expression. Finally, to investigate whether the differential IL-23-expression observed in PaA synovium was disease-specific or related to synovial histopathology, we quantified IL-23p40/IL-23p19/IL-23R in a cohort of 17 treatment-naive rheumatoid arthritis (RA) patients spanning diverse degrees of synovial inflammation and histopathotypes, and confirmed that, at least in the early phases of RA, IL-23 expression pattern is pathology related and significantly associates with the presence of ELS (online supplemental figure S4).

DISCUSSION

To our knowledge, this study provides first-time detailed evidence of the expression of the IL-23 axis (IL-23p40/IL-23p19/IL-23R), both at transcript and protein level, in matched skin-synovium obtained from clinically active PsA patients before undergoing anti-TNF or ustekinumab.

Figure 3 Expression of IL-23p40, IL-23p19 and IL-23R in skin and synovium from PsA patients. (A, C) representative images of sections of PaA non-lesional (non les.) and lesional skin (A) and synovial tissue of different degree of inflammatory scores (C) immunostained for IL-23p40, IL-23p19 and IL-23R. Scale bar=200 µm. Enlarged images correspond to the respective boxed areas. (B, D) Digital image analysis was performed on non-lesional and lesional skin (B) (n=11–12) and synovium (D) (low inflammatory score, n=4–8; high inflammatory score, n=13–14) sections. IL-23p40, IL-23p19 and IL-23R positive cells were determined using QuPath software21 and are presented as % of the total number of cells. Results are shown as mean±SD. *P<0.05, **P<0.01 as assessed by Mann-Whitney U test. (E) Correlations between inflammatory scores and IL-23p40, IL-23p19 or IL-23R percentages of positive cells within the synovial tissue. P values, calculated by Spearman’s bivariate correlation analysis, are indicated on each graph.

IL-23, interleukin 23; PsA, psoriatic arthritis.
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Using a PCR-Fluidigm-assay of 80 inflammation-related genes, first, we demonstrated distinct synovial gene expression clustering away from paired skin but a partial overlapping between lesional and non-lesional skin profiles. We also showed that IL-17 and IL-23 cytokines together with CXCL13/CXCR5, key chemokines involved in ELS formation, significantly contribute to the gene expression variance within skin and joint sites, respectively. These results are in line with those reported by Belasco et al demonstrating that IL-17 is a major contributor of the gene expression variability within the lesional skin, and Celis et al who showed that in synovial biopsies (unmatched for skin samples) the expression of IL-23 correlates with ELS-positive samples.

The analysis of the expression profiles of biological DMARDS targets demonstrated that TNF was more homogeneously expressed in skin and synovial tissue, while IL23A/IL12B/IL23R were generally higher-expressed in lesional skin compared with both non-lesional skin and synovium. The synovial expression of IL23A/IL12B/IL23R was, in fact, greatly heterogeneous and could be either similar to or more lower than the paired lesional skin. Notably, IL12B and IL23R transcripts levels were dependent on the degree of tissue inflammation, being more expressed in the presence of higher synovitis scores. Similarly, we confirmed a preferential expression of IL-23p40/IL-23p19/IL-23R proteins in patients with high-grade synovitis and immune-cells-rich histopathotypes. Importantly, patients with variable degrees of synovial inflammation and diverse pathotypes, as well as different levels of IL-23-cytokines/receptor could not be phenotypically distinguished by conventional clinical scores. Furthermore, despite variable drug exposure, the pathology of the IL-23 axis in active patients was comparable at baseline. We confirmed that IL-23-axis expression relates to the synovial histopathology not only in PsA at different stages of the disease, including early treatment-naïve patients, but also in the early phase of RA, investigated as disease control. Therefore, the pattern of expression of the IL-23 axis does not seem to be disease-specific but rather dependent on the inflammatory status and histological features of the synovial tissue in both PsA and RA.

While it is generally accepted that patients with high disease activity respond better to biologics, clinimetric measures cannot of the synovial tissue in both PsA and RA.

The heterogeneous synovial expression of the IL-23-axis provides a plausible mechanistic explanation for the divergent outcomes consistently observed in clinical trials whereby IL-23 inhibitors have better results in PsA skin than in joints. This hypothesis needs to be tested in larger, appropriately designed and powered studies. Identifying biomarkers of joint-response to therapy in patients clinically indistinguishable is going to be vital to refine PsA clinical classification and enrich for treatment response while reducing unnecessary exposure to costly and potentially toxic medications.

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