

Dynamics of interleukin-23/interleukin-17 axis and type 1 regulatory T cells across disease stages in axial spondyloarthritis

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Abstract Objective

The interleukin (IL)-23/IL-17 pathway is central to the pathogenesis of axial spondyloarthritis (axSpA); however, treatments targeting IL-23 have shown inconsistent effectiveness across spondyloarthritis subtypes. We hypothesised that the IL-23/IL-17 axis varies with disease stage and is modulated by its regulatory counterparts, such as type 1 regulatory T (Tr1) cells.

Methods

Serum levels of IL-17A and IL-23, along with peripheral CD4⁺IL-17A⁺ cells, monocytes (CD14⁺HLA-DR⁺), dendritic cells (DCs, CD11c⁺HLA-DR⁺), and Tr1 cells (CD4⁺CD49b⁺LAG3⁺FoxP3⁺IL-10⁺), were analysed in 20 patients with non-radiographic axSpA (nr-axSpA) and 24 with radiographic axSpA (r-axSpA) using enzyme-linked immunosorbent assay and flow cytometry. Additionally, we assessed IL-17A production by healthy CD4⁺ T cells stimulated by monocyte-derived DCs (moDCs) from patients with axSpA through co-culture experiments.

Results

Patients with nr-axSpA had higher serum IL-23 levels ($p=0.014$), whereas IL-17A levels were comparable between both groups ($p=0.912$). Peripheral DCs from the nr-axSpA group produced more IL-23 ($p=0.010$), while no significant differences were observed in Th17 cell proportions ($p>0.05$). MoDCs from patients with nr-axSpA induced higher pro-inflammatory cytokine production in healthy CD4⁺ T cells than those from patients with r-axSpA ($p<0.05$). Notably, Tr1 cells were reduced in the nr-axSpA group ($p=0.025$), and IL-10 selectively suppressed IL-23 production by nr-axSpA moDCs ($p=0.236$).

Conclusion

These findings indicate that IL-23-associated immune activity may be relatively more prominent in early axSpA and that differences in Tr1 cell-related regulation across disease stages could contribute to this pattern.

Key words

co-culture, dendritic cells, interleukin-23, spondyloarthritis, type 1 regulatory T cells

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Introduction

Axial spondyloarthritis (axSpA) is a chronic inflammatory disease of unknown aetiology that primarily affects the spinal and sacroiliac joints. It is further categorised based on the degree of radiographic progression into radiographic axSpA (r-axSpA), also known as ankylosing spondylitis, which is characterised by visible structural damage to the sacroiliac joints on radiographic imaging, and non-radiographic axSpA (nr-axSpA), which represents an early stage of the disease without radiographic evidence of sacroiliitis (1). The central role of the interleukin (IL)-23/IL-17 pathway in the pathogenesis of axSpA has been well-established through genetic, animal, and translational studies (2). However, treatments targeting IL-23 and IL-17 have shown varying degrees of efficacy across the subtypes. Notably, IL-23 inhibition did not yield significant benefits in patients with axSpA, whereas IL-17 inhibition effectively reduced inflammation and mitigated disease severity (3-6). The poor efficacy of IL-23 inhibitors in axSpA may be attributed to various factors, including challenges in trial design and outcome measures, emphasising the need to reassess the immunopathogenesis of axSpA.

Type 1 regulatory T (Tr1) cells are crucial for maintaining immune tolerance and preventing abnormal immune responses. Unlike other regulatory T cells, Tr1 cells secrete high levels of IL-10 but do not express FoxP3 (7). Recent studies have indicated that Tr1 cells play a crucial role in autoimmune diseases by modulating dendritic cell (DC) function, reducing their capacity to activate effector T cells, thereby diminishing the overall inflammatory response (8). IL-10 is essential for maintaining innate immune balance, and inborn errors in its pathway can cause severe early-onset intestinal inflammation, but its contribution to the skeletal inflammation of SpA remains uncertain and has not been demonstrated (9, 10). Given this gap in knowledge, we hypothesised that the inconsistent clinical efficacy of IL-23 inhibition may reflect heterogeneity within the IL-23/IL-17 axis according to the degree of

radiographic progression. Furthermore, the IL-23/IL-17 pathway may be differentially shaped by IL-10-producing Tr1 cells at distinct disease stages, potentially contributing to immunological differences between nr-axSpA and r-axSpA. To explore these hypotheses, we investigated the IL-23/IL-17 axis and Tr1 cell dynamics in nr-axSpA and r-axSpA using a co-culture system of DCs and CD4⁺ T cells.

Patients and methods

Patient recruitment and blood/synovial fluid sample collection

Blood samples were collected from 20 patients with nr-axSpA and 24 with r-axSpA at Gachon University Gil Medical Center. All the participants fulfilled the Assessment of SpondyloArthritis International Society classification criteria for axSpA (11). The classification of patients with axSpA into nr-axSpA and r-axSpA was based on the radiographic criteria of the modified New York Criteria for ankylosing spondylitis (12). In addition, synovial fluid samples were obtained during therapeutic arthrocentesis (nr-axSpA, n=3; and r-axSpA, n=3). This study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the Institutional Review Board of Gachon University Gil Medical Center (IRB no. GBIRB2021-209). Additional details on the materials and methods, as well as statistical analyses, are given in Supplementary Materials and Methods S1.

Cytokine level analysis in serum

Serum samples were collected and stored at -80°C until analysis. The levels of inflammatory cytokines, including interleukin (IL)-23, tumour necrosis factor- α (TNF- α), granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-17A, and IL-22 in serum were measured using enzyme-linked immunosorbent assay (ELISA) kit (DuoSet, R&D systems) according to the manufacturer's instructions. In addition, IL-6, IL-1 β , and IL-18 were quantified using a cytometric bead array (CBA) assay (Biolegend, CA, USA). Data were analysed using the accom-

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panying bead array analysis software in accordance with the manufacturer's recommendations.

Allogenic co-culture of dendritic

cells with CD4⁺ T cells or Tr1 cells

Supplementary Figure S1 shows the co-culture process. CD14⁺ monocytes were isolated from peripheral blood mononuclear cells (PBMCs) of patients with axSpA via Magnetic-Activated Cell Sorting (MACS) using CD14 MicroBeads. The isolated CD14⁺ cells were seeded in a 12-well plate and cultured in Roswell Park Memorial Institute medium, supplemented with 50 ng/mL recombinant human GM-CSF and 533 U/mL recombinant human IL-4, at 37°C and 5% CO₂ condition for 7 days. The medium was refreshed every 2–3 days. The monocyte-derived DCs (moDCs) were stimulated with 1 µg/mL lipopolysaccharide (LPS) on day 6. On day 7, CD4⁺ T cells were negatively isolated from the PBMCs of healthy donors via MACS using CD4 Microbeads (Miltenyi Biotec) and the CD4 enriched cells were labelled with CellTrace™ Violet (BD Biosciences) to track cell proliferation. A total of 1×10^4 moDCs per well were seeded in 96-well plates with CD4⁺ T cells at a ratio of 1:20 (moDC: CD4⁺ T cell) (Suppl. Fig. S2). The cells were treated with 5 µg/mL CD3 (clone: OKT3) and 10 µg/mL CD28 (clone: CD28.6) monoclonal antibodies and cultured for 4 days.

To evaluate the functional impact of Tr1 cells, moDCs were generated from a healthy control donor using the same differentiation protocol. Tr1 cells were induced from PBMCs using anti-CD3, anti-CD28, and IL-10 stimulation, and Tr1 cells (CD4⁺CD49b⁺PD-1⁺LAG-3⁺) were purified by FACS (Suppl. Fig. S3). FACS-sorted Tr1 cells from nr-axSpA and r-axSpA were then co-cultured with healthy control-derived moDCs for 48 hours to examine their effects on moDC cytokine production.

Flow cytometry analysis of surface molecules and intracellular cytokines

Flow cytometry was used to examine cytokine production in PBMCs/synovial fluid mononuclear cells (SFMCs) and co-cultured cells. For intracellular

Table I. Clinical characteristics of enrolled patients with axial spondyloarthritis.

	nr-axSpA (n=20)	r-axSpA (n=24)	p-value
Age, years, mean (SD)	41.1 (12.1)	45.5 (12.4)	0.243
Male, n (%)	17 (85.0)	19 (79.2)	0.617
Disease duration, years, mean (SD)	5.2 (4.4)	13.9 (10.1)	0.001
HLA-B27 positivity, n (%)	18 (90.0)	23 (95.8)	0.445
Current smoker, n (%)	7 (35.0)	3 (12.5)	0.204
Clinical manifestations, n (%)			
Only axial symptom	1 (5.0)	8 (33.3)	0.020
+ Enthesitis	8 (40.0)	4 (16.7)	0.084
+ Peripheral arthritis	13 (65.0)	8 (33.3)	0.036
+ Dactylitis	2 (10.0)	0 (0.0)	0.113
+ Uveitis	2 (10.0)	8 (33.3)	0.066
+ Inflammatory bowel disease	3 (15.0)	0 (0.0)	0.049
+ Psoriasis	1 (5.0)	1 (4.2)	0.895
Syndesmophyte, n (%)	0 (0.0)	11 (50.0)	0.001
Laboratory findings			
ESR, mm/h, mean (SD)	18.0 (22.8)	10.4 (16.3)	0.216
CRP, mg/dL, mean (SD)	1.8 (3.0)	0.6 (0.7)	0.091
Disease activity			
BASDAI, mean (SD)	3.9 (2.5)	3.0 (2.1)	0.175
BASFI, mean (SD)	1.5 (1.9)	1.4 (1.4)	0.861
ASDAS, mean (SD)	2.6 (1.3)	2.0 (0.8)	0.085
Treatment, n (%)			
NSAID use	15 (88.2)	21 (87.5)	0.943
TNF inhibitor naive	17 (85.0)	20 (83.3)	0.880

nr-axSpA: non-radiographic axial spondyloarthritis; r-axSpA: radiographic axial spondyloarthritis; SD: standard deviation; HLA: human leukocyte antigen; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index; ASDAS: Ankylosing Spondylitis Disease Activity Score; NSAIDs: non-steroidal anti-inflammatory drugs; TNF: tumour necrosis factor.

Data are expressed as the mean (SD) or n (%). Comparisons between the two groups were performed using Student's t-test or chi-square test.

lar cytokine analyses, the cells were stimulated with 50 ng/mL PMA, 1 µg/mL Ionomycin, and Golgistop for 4 h at 37°C in a 5% CO₂ environment. Additionally, moDCs were cultured with or without 50 ng/mL recombinant human IL-10. The cells were immunostained to evaluate IL-23p19 and IL-27 protein production using flow cytometry. The specific antibodies and their sources are listed in Supplementary Table S1. Fluorescence data were collected using a BD LSRII Cell Analyzer (BD Biosciences) and analysed using FlowJo software (v. 10.8.1; FlowJo, Ashland, OR, USA). To evaluate the CD4⁺ T cells expressing IFN-γ, IL-17A, GM-CSF, and TNF-α, we used OMIQ and visualised flow cytometry data in high dimensional space using uniform manifold approximation and projection (UMAP).

Statistical analysis

Statistical analyses were performed using the unpaired t-test, Mann-Whitney U-test, chi-square test, Fisher's exact test to compare the nr-axSpA and r-ax-

SpA groups. *p*-values were adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR) method, and the adjusted values are reported as *q*-values. Spearman's correlation analyses were conducted to evaluate the relationships between immunological parameters and clinical indices of disease activity. Additional multivariable linear regression analyses were performed adjusting for disease duration and extra-spinal manifestations. All statistical analyses were performed using SPSS Statistics, Version 26.0 (IBM Corp., Armonk, NY, USA), and data were visualised by GraphPad Prism, v. 8.0.1 (GraphPad Software, Boston, MA, USA). Statistical significance was defined as *p*<0.05.

Results

Patients' characteristics

Blood samples were collected from 20 and 24 patients with nr-axSpA and r-axSpA, respectively. The main clinical characteristics are summarised in Table I. The mean (standard deviation)

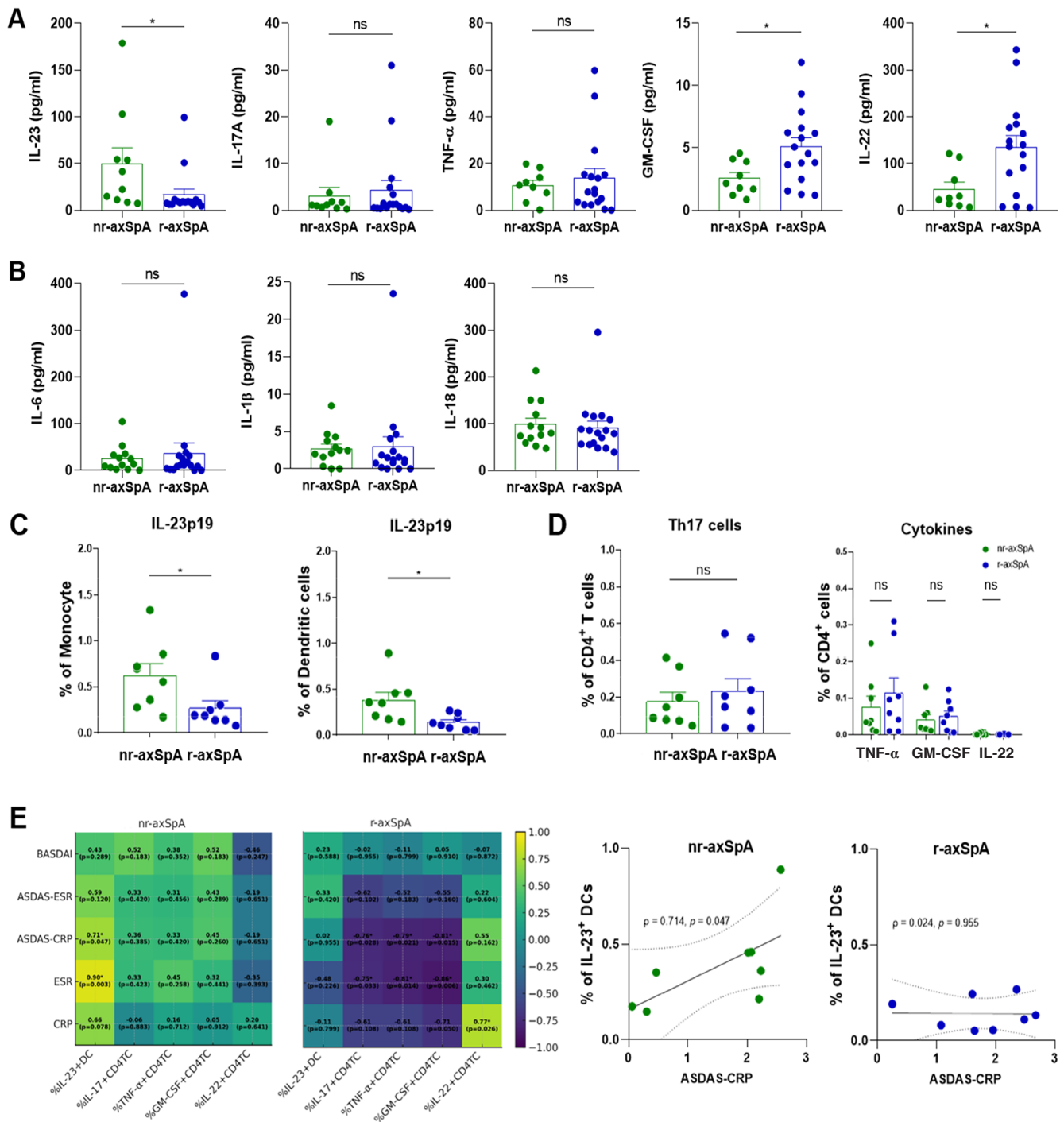


Fig. 1. Comparison of inflammatory cytokines between non-radiographic and radiographic axial spondyloarthritis.

A: Serum levels of IL-23, IL-17A, TNF-α, GM-CSF, and IL-22 are measured using ELISA in patients with nr-axSpA (n=10) and r-axSpA (n=17). IL-23 levels are higher in patients with nr-axSpA compared to those with r-axSpA. No significant differences are observed for IL-17A and TNF-α levels between the two groups. GM-CSF and IL-22 levels are significantly higher in patients with r-axSpA compared to those with nr-axSpA.

B: Serum levels of IL-6, IL-1β, and IL-18 were measured using a cytometric bead array in patients with nr-axSpA (n=13) and r-axSpA (n=17). IL-1β and IL-18 levels were comparable between the two groups, and IL-6 tended to be lower in patients with nr-axSpA, although no statistically significant differences were observed.

C: IL-23 protein expression is assessed in monocytes (CD14⁺HLA-DR⁺) and dendritic cells (DCs, CD11c⁺HLA-DR⁺) from the PBMCs of patients with nr-axSpA (n=8) and r-axSpA (n=8). The frequency of IL-23-producing monocytes and DCs is higher in patients with nr-axSpA compared to those with r-axSpA.

D: Th17 cells and TNF-α, GM-CSF, and IL-22-producing Th17 cells are assessed in the PBMCs from patients with nr-axSpA (n=8) and r-axSpA (n=8). No significant differences are observed between the two groups.

E: Heatmaps and scatter plot show the correlations between clinical indices and the proportions of immune cell subsets analysed from PBMCs by flow cytometry in patients with nr-axSpA (n=8) and r-axSpA (n=8). Significant correlations are observed between IL-23 producing DCs and ASDAS-CRP in nr-axSpA.

Data are presented as mean and SEM, with *p*-value calculated using an unpaired *t*-test or Mann-Whitney *U*-test for panels **A-D**. **p*<0.05, ***p*<0.01, ****p*<0.001, n.s.: not significant.

age of the nr-axSpA group was 41.1 (12.1) years, compared to 45.5 (12.4) years for the r-axSpA group ($p=0.243$). Most patients in both groups were male (85.0% in the nr-axSpA group and 79.2% in the r-axSpA group, $p=0.617$). The r-axSpA group had a significantly longer disease duration than the nr-axSpA group (13.9 [10.1] years vs. 5.2 [4.4] years, $p=0.001$). HLA-B27 positivity rates were comparable between the two groups (90.0% in the nr-axSpA group and 95.8% in the r-axSpA group, $p=0.445$). Disease activity measures, including Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI), and Ankylosing Spondylitis Disease Activity Score (ASDAS), did not differ significantly between the groups (BASDAI: 3.9 [2.5] vs. 3.0 [2.1], $p=0.175$; BASFI: 1.5 [1.9] vs. 1.4 [1.4], $p=0.861$; ASDAS: 2.6 [1.3] vs. 2.0 [0.8], $p=0.085$).

Serum IL-23 is higher in patients with nr-axSpA compared to those with r-axSpA

We first assessed differences in serum IL-23 levels and other inflammatory cytokines in patients with axSpA depending on the disease stage. Serum IL-23, IL-17A, TNF- α , GM-CSF, and IL-22 were measured using ELISA in patients with nr-axSpA ($n=10$) and r-axSpA ($n=17$) (Fig. 1A). Serum IL-23 levels were higher in patients with nr-axSpA than in those with r-axSpA (49.6 [17.2] vs. 16.7 [6.1], $p=0.014$, $q=0.069$) (Suppl. Table S2). No significant differences were observed for serum IL-17A between the two groups (3.1 [1.8] vs. 4.4 [2.0], $p=0.912$), TNF- α 10.7 [2.1] vs. 13.8 [4.1], $p=0.792$). However, GM-CSF (2.6 [0.5] vs. 5.1 [0.7], $p=0.025$; $q=0.069$) and IL-22 (45.4 [14.8] vs. 134.6 [25.1], $p=0.027$; $q=0.069$) were higher in patients with r-axSpA than in those with nr-axSpA. After adjustment for disease duration and extra-spinal manifestations, serum IL-23 levels remained significantly higher in nr-axSpA compared with r-axSpA ($\beta=45.811$, 95% CI 10.136 to 81.485, $p=0.014$), while differences in other cytokines were not significant (Suppl. Table S3). In addition, a multiplex CBA assay was

performed to measure IL-6, IL-1 β , and IL-18 in patients with nr-axSpA ($n=13$) and r-axSpA ($n=17$). IL-1 β (2.7 [0.6] vs. 3.0 [1.3], $p=0.257$) and IL-18 (98.6 [13.3] vs. 91.5 [14.2], $p=0.563$) levels were comparable between groups, and IL-6 (24.8 [8.0] vs. 37.3 [21.6], $p=0.658$) tended to be lower in nr-axSpA (Fig. 1B).

IL-23 protein expression is higher in PBMCs from patients with nr-axSpA compared to those with r-axSpA

To identify the cell types responsible for cytokine production, we measured monocytes (CD14 $^{+}$ HLA-DR $^{+}$), DCs (CD11c $^{+}$ HLA-DR $^{+}$), and Th17 cells in the PBMCs of patients with nr-axSpA ($n=8$) and r-axSpA ($n=8$). The proportion of IL-23-producing monocytes and DCs was higher in the nr-axSpA group than in the r-axSpA group ($p=0.028$; $q=0.098$ for monocytes and $p=0.010$; $q=0.073$ for DCs) (Fig. 1C and Suppl. Table S2). No significant differences were observed in the frequency of Th17 cells (CD4 $^{+}$ IL-17 $^{+}$) or Th17 cells producing TNF- α (CD4 $^{+}$ IL-17 $^{+}$ TNF- α $^{+}$), GM-CSF (CD4 $^{+}$ IL-17 $^{+}$ GM-CSF $^{+}$), and IL-22 (CD4 $^{+}$ IL-17 $^{+}$ IL-22 $^{+}$) between the two groups ($p>0.05$ for all comparisons) (Fig. 1D). Consistent with these findings, IL-23-producing DCs showed a positive correlation with disease activity in nr-axSpA ($\rho=0.714$, $p=0.047$), whereas no significant association was observed in r-axSpA ($\rho=0.024$, $p=0.955$) (Fig. 1E). Conversely, inverse relationships were observed between IL-17 $^{+}$, TNF- α $^{+}$, and GM-CSF $^{+}$ CD4 $^{+}$ T-cell frequencies and disease activity indices (ASDAS-CRP and ESR) in r-axSpA ($p<0.05$). (Fig. 1E and Suppl. Fig. S4).

moDC from patients with nr-axSpA potently stimulates CD4 $^{+}$ T cells from healthy controls

Since DCs in PBMCs from patients with nr-axSpA produced higher levels of IL-23 than those from patients with r-axSpA, we examined the capacity of moDCs from each group to stimulate inflammatory cytokine production in CD4 $^{+}$ T cells from healthy controls. moDCs from patients with nr-axSpA ($n=5$) and r-axSpA ($n=5$) were differ-

entiated from monocytes over 7 days in the presence of GM-CSF and IL-4, then activated with LPS for 24 h. Subsequently, allogeneic co-cultures of LPS-treated moDCs from patients with axSpA and CD4 $^{+}$ T cells from healthy controls ($n=10$) were incubated in a 1:20 ratio for 4 days. CD4 $^{+}$ T cells were stimulated to produce a higher proportion of Th17 cells ($p=0.028$; $q=0.056$), as well as IFN- γ ($p=0.047$; $q=0.056$), TNF- α ($p=0.009$; $q=0.032$), and GM-CSF ($p=0.047$; $q=0.056$) producing Th17 cells when in contact with DCs from patients with nr-axSpA compared to those from patients with r-axSpA (Fig. 2A-B, and Suppl. Fig. S5). Additionally, proliferation analysis using CellTrace Violet staining showed that CD4 $^{+}$ T cells from healthy controls producing IFN- γ , TNF- α , and GM-CSF proliferated significantly more when stimulated by allogeneic moDCs from patients with nr-axSpA than by those from patients with r-axSpA ($p<0.05$ for all comparisons; $q=0.074$ – 0.095 after FDR correction). (Fig. 2C and Suppl. Table S2).

Tr1 cells are lower in PBMCs from patients with nr-axSpA compared to those from patients with r-axSpA

To explore whether IL-23 production by moDCs is differentially influenced by Tr1 cells as regulatory counterparts depending on the disease stage, we measured IL-10-producing Tr1 cells (CD4 $^{+}$ CD49b $^{+}$ LAG3 $^{+}$ FoxP3 $^{+}$ IL-10 $^{+}$) in PBMCs from patients with nr-axSpA ($n=17$) and r-axSpA ($n=18$), as well as healthy controls ($n=9$) using flow cytometry (Fig. 3A). The proportion of IL-10-producing Tr1 cells was lower in patients with nr-axSpA than in those with r-axSpA ($p=0.025$; $q=0.099$) as well as in healthy controls ($p=0.575$).

Tr1 cells show reduced suppressive effects on moDCs cytokine production in nr-axSpA

Given the reduced proportion of circulating Tr1 cells in patients with nr-axSpA, we next examined whether Tr1 cells from the two axSpA subsets differed in their ability to regulate moDCs inflammatory responses. FACS-purified Tr1 cells (CD4 $^{+}$ CD49b $^{+}$ PD-1 $^{+}$ LAG-3 $^{+}$) from patients with nr-axSpA ($n=4$) or r-axS-

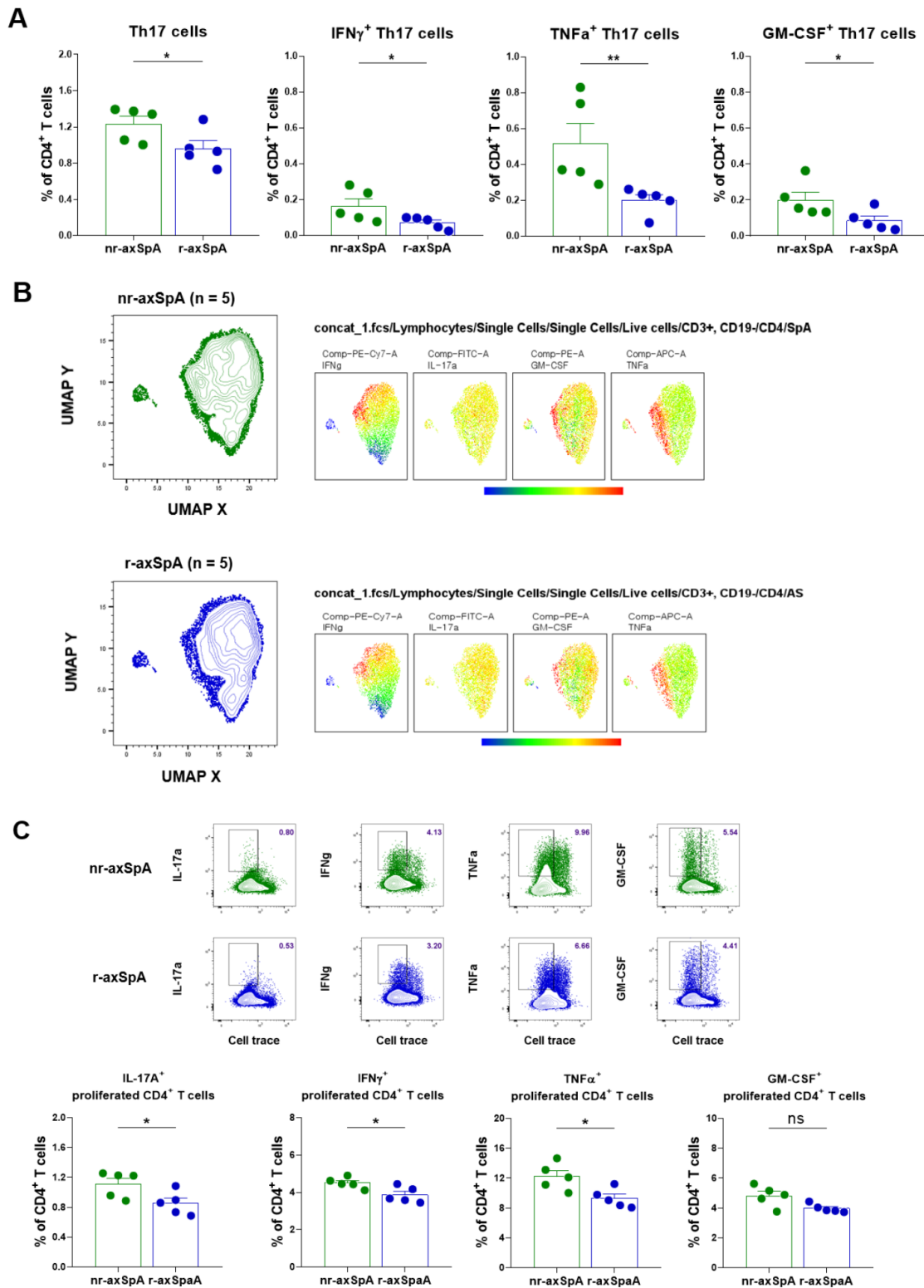


Fig. 2. Co-culture of healthy CD4⁺ T cells with moDC from patients with non-radiographic and radiographic axial spondyloarthritis.

A: moDC from patients with nr-axSpA (n=5) and r-axSpA (n=5) are differentiated over 7 days and then activated with LPS for 24 h. These moDCs are then co-cultured with CD4⁺ T cells from healthy controls (n=10) at a 1:20 ratio for 4 days. The proportion of Th17 cells (CD4⁺IL-17⁺) and IFN- γ , TNF- α , and GM-CSF producing Th17 cells is higher when healthy CD4⁺ T cells are co-cultured with moDC from patients with nr-axSpA compared to those with r-axSpA.

B: UMAP visualisations show CD4⁺ T cell response by co-culturing with moDCs from patients with nr-axSpA (upper panel) and r-axSpA (lower panel).

C: Healthy CD4⁺ T cells (n=10) are labelled with cell trace violet and co-cultured with LPS-activated moDC from patients with nr-axSpA and r-axSpA. IL-17-, IFN- γ -, and TNF- α -producing CD4⁺ T cells are higher when CD4⁺ T cells are co-cultured with moDC from patients with nr-axSpA compared to those with r-axSpA.

Data are presented as mean and SEM, with *p*-value calculated using an unpaired t-test or Mann-Whitney U-test. **p*<0.05, ***p*<0.01, ****p*<0.001, n.s.: not significant.

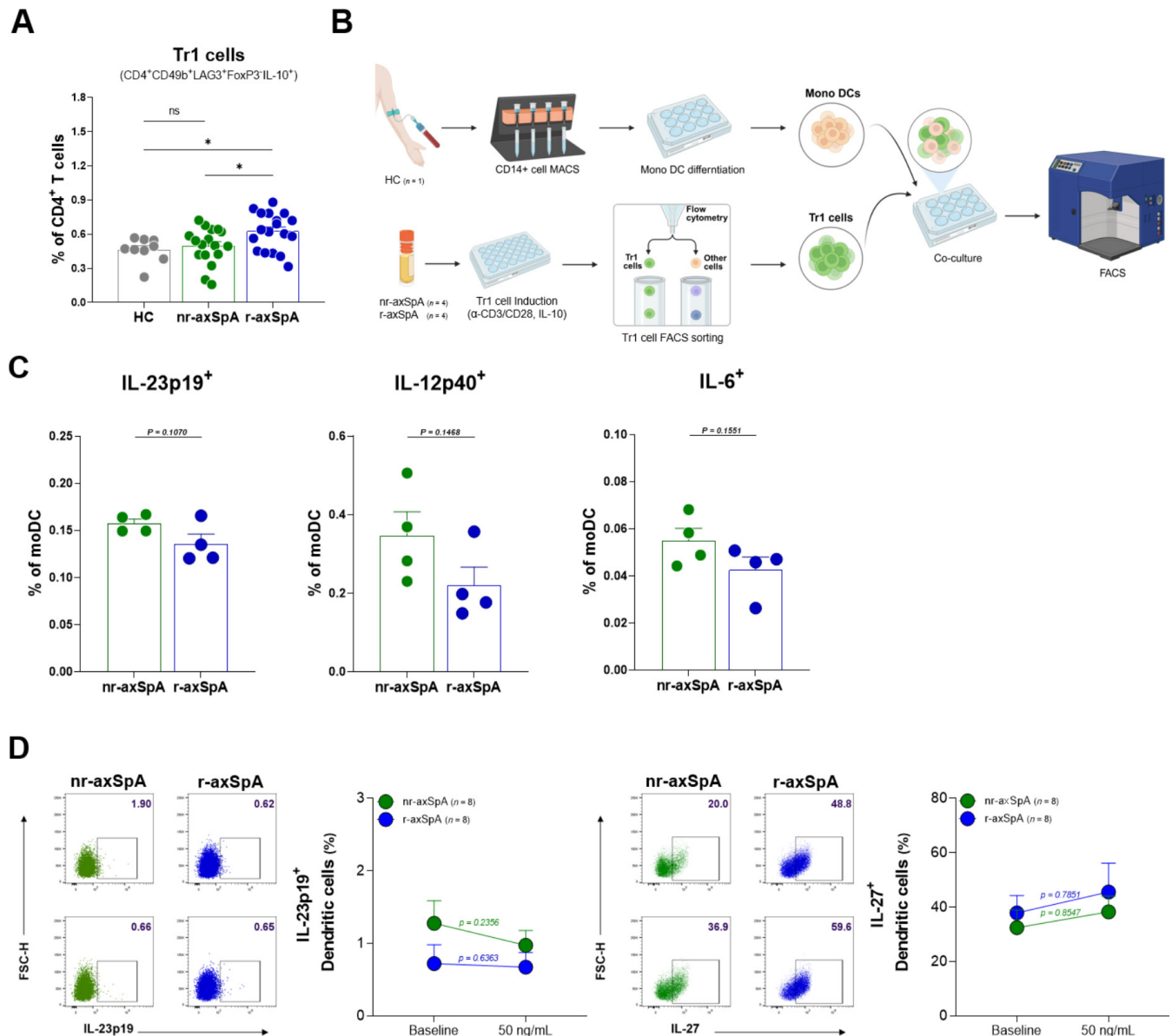


Fig. 3. Tr1 cell frequency and IL-10 effect on IL-23 and IL-27 production by moDCs from patients with non-radiographic and radiographic axial spondyloarthritis. **A:** The proportion of IL-10-producing Tr1 cells (CD4⁺CD49b⁺LAG3⁺FoxP3⁺IL-10⁺) is measured in PBMCs from healthy controls (n=9), as well as patients with nr-axSpA (n=17) and r-axSpA (n=18) using flow cytometry. Tr1 cells are lower in patients with nr-axSpA compared to those with r-axSpA. **B:** Tr1 cells from axSpA patients are isolated and cocultured with healthy donor moDCs. CD14⁺ monocytes from a healthy control are differentiated into moDCs, and FACS-sorted Tr1 cells from nr-axSpA and r-axSpA patients are co-cultured for 48 h before cytokine analysis. **C:** Tr1 cells from nr-axSpA show reduced suppression of moDCs cytokine production. The protein expression of IL-23p19, IL-12p40, and IL-6 in moDCs after co-culture with Tr1 cells from each group is shown. **D:** moDCs from patients with nr-axSpA (n=8) and r-axSpA (n=8) are cultured with or without IL-10 (50 ng/mL) for 7 days and then activated with LPS for 24 h. IL-23 production is decreased in moDCs from patients with nr-axSpA treated with IL-10 but not in moDCs from patients with r-axSpA. IL-27 production increases in the presence of IL-10 in both groups. Data are presented as mean and SEM, with p-value calculated using an unpaired t-test or Mann-Whitney U-test. *p<0.05, **p<0.01, ***p<0.001, n.s.: not significant.

pa (n=4) were co-cultured with moDCs generated from a healthy control donor. After 48 hours, intracellular cytokine production by moDCs was assessed by flow cytometry (Fig. 3B). Tr1 cells from nr-axSpA exhibited reduced suppressive effects on moDCs, leading to higher frequencies of IL-23p19⁺, IL-12p40⁺, and

IL-6⁺ moDCs compared with Tr1 cells from r-axSpA (p=ns for all comparisons) (Fig. 3C). These findings suggest a potential reduction in the regulatory capacity of Tr1 cells in nr-axSpA, which may contribute to less effective modulation of moDC-derived proinflammatory cytokines, including IL-23.

IL-10 decreases IL-23 production by moDCs in patients with nr-axSpA
Because Tr1 cells are a major source of IL-10 and exhibited impaired regulatory function in nr-axSpA, we next investigated whether restoring IL-10 signalling could modulate DCs cytokine production in axSpA patients. moDCs

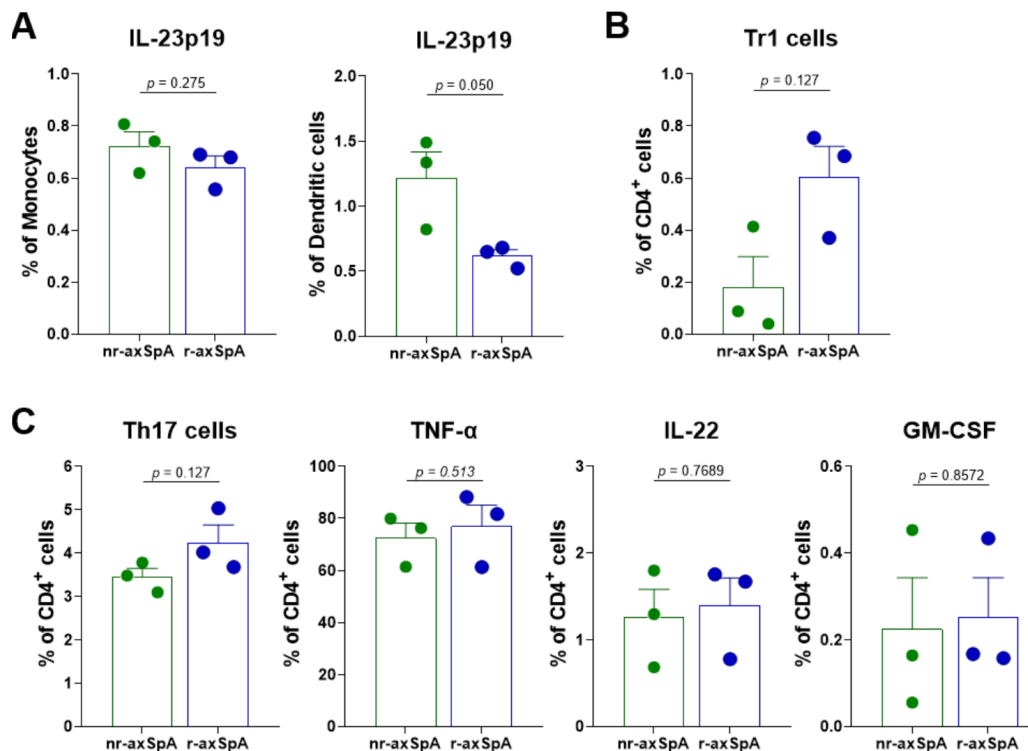


Fig. 4. Synovial fluid mononuclear cell (SFMC) analysis of myeloid and CD4⁺ T-cell subsets in non-radiographic (n=3) and radiographic (n=3) axial spondyloarthritis.

A: IL-23p19 protein expression shows a higher trend in monocytes (CD14⁺HLA-DR⁺) and dendritic cells (DCs, CD11c⁺HLA-DR⁺) from nr-axSpA.

B: Tr1 cells (CD4⁺CD49b⁺LAG-3⁺) frequency shows a lower trend in nr-axSpA compared with r-axSpA.

C: Frequencies of cytokine-producing CD4⁺ T-cell subsets are generally comparable between groups, with modest increase observed in r-axSpA.

Data are presented as mean and SEM, with p -value calculated using a Mann-Whitney U-test.

from patients with nr-axSpA (n=8) and r-axSpA (n=8) were cultured for 7 days with GM-CSF and IL-4, either with or without IL-10 (50 ng/mL) and then activated with LPS for 24 h. The changes in IL-23 and IL-27 production by moDCs are shown in Fig. 3D. In the absence of IL-10, moDCs from nr-axSpA showed a higher trend in IL-23 production compared with those from r-axSpA. IL-10 supplementation appeared to reduce IL-23 levels in moDCs from nr-axSpA, whereas its effect was minimal in r-axSpA (p =ns for all comparisons). IL-27 production increased in both groups following IL-10 treatment (p =ns for all comparisons). Thus, IL-10 may differentially modulate IL-23 responses in nr-axSpA, raising the possibility of altered IL-10-mediated regulatory mechanisms in this subset.

SFMC analysis shows distinct immunological patterns across disease stages

Analysis of SFMCs further demonstrated distinct immunological patterns

across disease stages (nr-AxSpA, n=3; r-axSpA, n=3). Monocytes (CD14⁺HLA-DR⁺) and dendritic cells (CD11c⁺HLA-DR⁺) from nr-axSpA showed higher trends of IL-23p19 protein expression compared with r-axSpA ($p=0.275$ for monocytes; $p=0.050$ for DCs) in SFMC (Fig. 4A). This pattern is compatible with increased IL-23-associated myeloid activity profile observed in PBMCs during early disease. Tr1-cell proportions were lower in nr-axSpA compared with r-axSpA ($p=0.127$), in line with the PBMC data and differences in features of Tr1 cells observed in early disease (Fig. 4B). Cytokine-producing CD4⁺ T-cell subsets, including IL-17A⁺ Th17 cells, TNF- α ⁺ cells, IL-22⁺ cells, and GM-CSF⁺ cells, were generally comparable between groups, with a slight increase observed in r-axSpA (p =ns) (Fig. 4C).

Discussion

In this study, we explored the different roles of the IL-23/IL-17 axis across different stages of axSpA using a co-cul-

ture system of moDCs and CD4⁺ T-cells. Our findings suggest a more prominent involvement of IL-23 in the early, non-radiographic stages of axSpA, with this association appearing less pronounced in advanced radiographic stages. Furthermore, Tr1 cells were lower in patients with nr-axSpA than in those with r-axSpA, and IL-10 treatment was associated with a reduction in IL-23 production by moDCs from patients with nr-axSpA but not from those with r-axSpA. These stage-dependent differences in IL-23-related protein expression and Tr1 cell-associated functional responses provide contextual insights into immune alterations across axSpA stages, and may help to inform future mechanistic and therapeutic investigations.

Recent experimental research in mouse models has shown that IL-23 is crucial in the pre-clinical stages of axSpA, but its influence wanes over time (13, 14). However, studies examining the effect of IL-23 inhibitors on the early stages of axSpA are limited. In line with observations from murine models, our

study demonstrated higher serum levels of IL-23 in patients with nr-axSpA than in those with r-axSpA (Fig. 1A), in contrast to other DC-derived cytokines such as IL-6, IL-1 β , and IL-18, which were similar or lower in nr-axSpA (Fig. 1B). Importantly, this difference in serum IL-23 levels remained after adjustment for extra-spinal manifestations in multi-variable analyses. This was further supported by the higher expression of IL-23 protein in monocytes and DCs derived from patients with nr-axSpA than in those from patients with r-axSpA (Fig. 1C). In addition, IL-23-producing DCs correlated with ASDAS-CRP and ESR in nr-axSpA but not in r-axSpA, suggesting that early-stage disease may be more linked to IL-23-driven pathways (Fig. 1E).

In contrast, we observed no significant differences in serum IL-17A levels between patients with nr-axSpA and those with r-axSpA (Fig. 1D). This finding indicates that IL-17A production by CD4⁺ T cells may be consistent across the different stages of axSpA (15, 16). However, moDCs from patients with nr-axSpA were more effective at stimulating CD4⁺ T cells from healthy controls to produce Th17 cells and pro-inflammatory cytokines, such as IFN- γ , TNF- α , and GM-CSF, than moDCs from patients with r-axSpA (Fig. 2). This suggests that although IL-17A remains a crucial effector cytokine throughout axSpA progression, the cellular sources driving its production may differ between the early and advanced stages of the disease (17, 18). The negative correlations observed between IL-17⁺, TNF- α ⁺, GM-CSF⁺, CD4⁺ T cell frequencies and disease activity indices (ASDAS-CRP and ESR) in r-axSpA may reflect a divergence between peripheral immune signatures and clinical inflammation in later disease stages (Fig. 1E). Previous studies have suggested that IL-17 production in established axial disease may predominantly originate from tissue-resident immune populations rather than circulating CD4⁺ T cells (19, 20). In this context, we additionally analysed SFMCs and observed higher trends of IL-23p19 protein expression in monocytes and DCs from nr-axSpA compared with r-axSpA, while cytokine-producing CD4⁺

T-cell subsets were largely comparable, showing a modest increase in r-axSpA, consistent with the peripheral blood findings (Fig. 4). These stage-specific immune patterns may partly account for the divergent clinical responses to IL-23- and IL-17- targeted therapies reported in axSpA (5). Tr1 cells are crucial in maintaining long-term tolerance and restoring tissue homeostasis (7). Emerging evidence indicates that impaired function and reduced Tr1 cell numbers are involved in the immunopathogenesis of various immune-mediated diseases, including inflammatory bowel disease, psoriasis, and systemic lupus erythematosus (8, 21, 22). Notably, in our study, the levels of IL-10-producing Tr1 cells were lower in patients with nr-axSpA than in those with r-axSpA (Fig. 3A), suggesting a potential regulatory imbalance in early axSpA, which may contribute to a heightened inflammatory state and elevated IL-23 production. Our functional assays further indicated that Tr1 cells from nr-axSpA appeared to have reduced suppressive capacity, leading to less effective regulation of DCs-derived cytokines such as IL-23, IL-12p40, and IL-6 (Fig. 3C). This functional attenuation may help explain how altered Tr1 activity could contribute to enhanced IL-23 responses in nr-axSpA. Furthermore, our study demonstrated that exogenous IL-10 reduced IL-23 production by moDCs in nr-axSpA but not in r-axSpA, and increased IL-27, promoting regulatory responses in both groups (Fig. 3D). Taken together, these findings suggest the possibility of a disrupted Tr1/IL-10 regulatory axis in nr-axSpA and raise the potential that strategies aimed at supporting IL-10-mediated regulatory pathways may be beneficial, particularly in earlier disease stages when immune dysregulation might be more amenable to modulation. The limitations of this study include the following points. First, we did not use spinal tissue where the inflammation occurs directly. Although spinal tissue could not be examined due to limited feasibility, comparable trends in SFMCs, a tissue-proximal immune compartment, support the relevance of the observed peripheral immune signatures. Second, we could not assess changes in the

pre-clinical stage at which the disease begins. Like most human studies, we faced limitations in obtaining samples from the pre-clinical stage; therefore, we compared and analysed nr-axSpA and r-axSpA by classifying disease stages based on radiographic progression. Lastly, given the relatively small sample sizes and cross-sectional design limiting causal inference, our findings should be interpreted with caution, and validation in larger, independent cohorts will be important to establish their robustness and generalisability.

In conclusion, this study suggests that IL-23-associated immune activity may be more prominent in early axSpA, whereas IL-17 responses appear to become increasingly dissociated from IL-23 in advanced r-axSpA, accompanied by enhanced IL-10-producing Tr1 cells as a regulatory counterpart. This study provides preliminary evidence for stage dependent differences in the IL-23/IL-17 axis, supporting the concept that therapeutic strategies targeting this pathway may need to consider disease stage. Further longitudinal and interventional studies are warranted to validate stage-specific strategies.

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References

1. NAVARRO-COMPÁN V, SEPRIANO A, EL-ZOR-KANY B, VAN DER HEIJDE D: Axial spondyloarthritis. *Ann Rheum Dis* 2021; 80(12): 1511-21. <https://doi.org/10.1136/annrheumdis-2021-221035>
2. KIM SH, LEE SH: Updates on ankylosing spondylitis: pathogenesis and therapeutic agents. *J Rheum Dis* 2023; 30(4): 220-33. <https://doi.org/10.4078/jrd.2023.0041>
3. BAETEN D, OSTERGAARD M, WEI JC *et al.*: Risankizumab, an IL-23 inhibitor, for ankylosing spondylitis: results of a randomised, double-blind, placebo-controlled, proof-of-concept, dose-finding phase 2 study. *Ann Rheum Dis* 2018; 77(9): 1295-302. <https://doi.org/10.1136/annrheumdis-2018-213328>
4. DEODHAR A, GENSLER LS, SIEPER J *et al.*: Three multicenter, randomized, double-blind, placebo-controlled studies evaluating the efficacy and safety of ustekinumab in axial spondyloarthritis. *Arthritis Rheumatol* 2019; 71(2): 258-70. <https://doi.org/10.1002/art.40728>
5. SIEBERT S, MILLAR NL, MCINNES IB: Why did IL-23p19 inhibition fail in AS: a tale of

- tissues, trials or translation? *Ann Rheum Dis* 2019; 78(8): 1015-18. <https://doi.org/10.1136/annrheumdis-2018-213654>
6. DROSOS AA, PELECHAS E, VENETSANOP-OULOU AI, VOULGARIS PV: TNF- α inhibitors versus newer therapies in spondyloarthritis: where do we stand today? *Mediterr J Rheumatol* 2024; 35(Suppl 3): 519-27. <https://doi.org/10.31138/mjr.040224.tvn>
 7. ZENG HY, ZHANG R, JIN BQ, CHEN LH: Type 1 regulatory T cells: a new mechanism of peripheral immune tolerance. *Cell Mol Immunol* 2015; 12(5): 566-71. <https://doi.org/10.1038/cmi.2015.44>
 8. RONCAROLO MG, GREGORI S, BACCHETTA R, BATTAGLIA M, GAGLIANI N: The biology of T regulatory type 1 cells and their therapeutic application in immune-mediated diseases. *Immunity* 2018; 49(6): 1004-19. <https://doi.org/10.1016/j.immuni.2018.12.001>
 9. SHARIFINEJAD N, ZAKI-DIZAJI M, SEPAHVANDI R *et al.*: The clinical, molecular, and therapeutic features of patients with IL10/IL10R deficiency: a systematic review. *Clin Exp Immunol* 2022; 208(3): 281-91. <https://doi.org/10.1093/cei/uxac040>
 10. GIRYES S, WONG C, BRIDGEWOOD C *et al.*: Paradoxical activation of enthesal myeloid cells by JAK1 and Tyk2 inhibitors via interleukin-10 antagonism. *Arthritis Rheumatol* 2025; 77(10): 1373-86. <https://doi.org/10.1002/art.43210>
 11. RUDWALEIT M, VAN DER HEIJDE D, LANDEWÉ R *et al.*: The development of Assessment of SpondyloArthritis International Society classification criteria for Axial Spondyloarthritis (part II): validation and final selection. *Ann Rheum Dis* 2009; 68(6): 777-83. <https://doi.org/10.1136/ard.2009.108233>
 12. VAN DER LINDEN S, VALKENBURG HA, CATS A: Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. *Arthritis Rheum* 1984; 27(4): 361-68. <https://doi.org/10.1002/art.1780270401>
 13. BENHAM H, REHAUME LM, HASNAIN SZ *et al.*: Interleukin-23 mediates the intestinal response to microbial beta-1,3-glucan and the development of spondyloarthritis pathology in SKG mice. *Arthritis Rheumatol* 2014; 66(7): 1755-67. <https://doi.org/10.1002/art.38638>
 14. VAN TOK MN, NA S, LAO CR *et al.*: The initiation, but not the persistence, of experimental spondyloarthritis is dependent on interleukin-23 signaling. *Front Immunol* 2018; 9: 1550. <https://doi.org/10.3389/fimmu.2018.01550>
 15. AMATYA N, GARG AV, GAFFEN SL: IL-17 signaling: the Yin and the Yang. *Trends Immunol* 2017; 38(5): 310-22. <https://doi.org/10.1016/j.it.2017.01.006>
 16. TOUSSIROT É, LAHEURTE C, GAUGLER B, GABRIEL D, SAAS P: Increased IL-22 and IL-17A-producing mucosal-associated invariant T cells in the peripheral blood of patients with ankylosing spondylitis. *Front Immunol* 2018; 9. <https://doi.org/10.3389/fimmu.2018.01610>
 17. CICCIA F, GUGGINO G, RIZZO A *et al.*: Type 3 innate lymphoid cells producing IL-17 and IL-22 are expanded in the gut, in the peripheral blood, synovial fluid and bone marrow of patients with ankylosing spondylitis. *Ann Rheum Dis* 2015; 74(9): 1739-47. <https://doi.org/10.1136/annrheumdis-2014-206323>
 18. MAURO D, SIMONE D, BUCCI L, CICCIA F: Novel immune cell phenotypes in spondyloarthritis pathogenesis. *Semin Immunopathol* 2021; 43(2): 265-77. <https://doi.org/10.1007/s00281-021-00837-0>
 19. SCHETT G, MCINNES IB, NEURATH MF: Reframing immune-mediated inflammatory diseases through signature cytokine hubs. *New Engl J Med* 2021; 385(7): 628-39. <https://doi.org/10.1056/nejmra1909094>
 20. LEE JS, TATO CM, JOYCE-SHAIKH B *et al.*: Interleukin-23-independent IL-17 production regulates intestinal epithelial permeability. *Immunity* 2015; 43(4): 727-38. <https://doi.org/10.1016/j.immuni.2015.09.003>
 21. LE BUANE C H, GOUGEON ML, MATHIAN A *et al.*: IFN- α and CD46 stimulation are associated with active lupus and skew natural T regulatory cell differentiation to type 1 regulatory T (Tr1) cells. *Proc Natl Acad Sci USA* 2011; 108(47): 18995-9000. <https://doi.org/10.1073/pnas.1113301108>
 22. KIM J, LEE J, GONZALEZ J, FUENTES-DUCULAN J, GARCET S, KRUEGER JG: Proportion of CD4(+)CD49b(+)LAG-3(+) type 1 regulatory T cells in the blood of psoriasis patients inversely correlates with Psoriasis Area and Severity Index. *J Invest Dermatol* 2018; 138(12): 2669-72. <https://doi.org/10.1016/j.jid.2018.05.021>