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Interleukin 17A and IL-17F Expression and Functional Responses in Rheumatoid Arthritis and Peripheral Spondyloarthritis

Sijia Chen[1], Iris C. Blijdorp[2], Leonieke J.J. van Mens[3], Rowann Bowcutt[4], Talia E. Latuhihin[2], Marleen G.H. van de Sande[3], Stevan Shaw[4], Nataliya G. Yeremenko[2], and Dominique L.P. Baeten[5]

ABSTRACT. ***Objective.*** Targeting the interleukin 17 (IL-17) axis is efficacious in psoriasis and spondyloarthritis (SpA), but not in rheumatoid arthritis (RA). We investigated potential differences in tissue expression and function of IL-17A and IL-17F in these conditions.

Methods. mRNA expression of cytokines and their receptors was assessed by quantitative PCR in psoriasis skin samples, in SpA and RA synovial tissue (ST) samples and in fibroblast-like synoviocytes (FLS). Cytokines were measured in synovial fluid (SF) and FLS supernatants by ELISA. FLS were stimulated with IL-17A or IL-17F cytokines supplemented with tumor necrosis factor (TNF), or with pooled SF from patients with SpA or RA.

Results. Levels of *IL-17A* (P = 0.031) and *IL-17F* (P = 0.017) mRNA were lower in psoriatic arthritis ST compared to paired psoriasis skin samples. The level of *IL-17A* mRNA was 2.7-fold lower than that of IL-17F in skin (P = 0.0078), but 17.3-fold higher in ST (P < 0.0001). In SF, the level of IL-17A protein was 37.4-fold higher than that of IL-17F [median 292.4 (IQR 81.4–464.2) vs median 7.8 (IQR 7.7–8.7) pg/mL; P < 0.0001]. IL-17A and IL-17F mRNA and protein levels did not differ in SpA compared to RA synovitis samples, and neither were the IL-17 receptors *IL-17RA* and *IL-17RC*, or the TNF receptors *TNFR1* and *TNR2*, differentially expressed between SpA and RA ST, nor between SpA and RA FLS. SpA and RA FLS produced similar amounts of IL-6 and IL-8 protein upon stimulation with IL-17A or IL-17F cytokines, supplemented with 1 ng/ml TNF. Pooled SpA or RA SF samples similarly enhanced the inflammatory response to IL-17A and IL-17F simulation in FLS.

Conclusion. The IL-17A/IL-17F expression ratio is higher in SpA synovitis compared to psoriasis skin. Expression of IL-17A and IL-17F, and the functional response to these cytokines, appear to be similar in SpA and RA synovitis.

Key Indexing Terms: interleukin 17A, interleukin 17F, therapeutic targeting, rheumatoid arthritis, spondyloarthritis, synovial fibroblasts

Spondyloarthritis (SpA) and rheumatoid arthritis (RA) are the 2 most common forms of chronic inflammatory arthritis with tumor necrosis factor (TNF) as a common disease-modulating cytokine[1,2,3]. Interleukin 17A (IL-17A) has been hypothesized to be a key driver of the immunopathology of both diseases because it plays an essential role in animal models of joint inflammation and bone remodeling[1,3,4]. However, targeting the IL-17 axis was not efficacious in patients with RA[5,6,7,8,9,10]. In the two phase III trials in RA patients with the IL-17A inhibitor secukinumab, the American College of Rheumatology 20% (ACR20) response

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[1]S. Chen, MD, PhD, Department of Rheumatology and Clinical Immunology, Amsterdam Rheumatology and Immunology Center, University of Amsterdam, Amsterdam, Department of Experimental Immunology, Amsterdam University Medical Center, University of Amsterdam, Amsterdam, the Netherlands, and Division of Rheumatology, Department of Medicine, University of Massachusetts Medical School, Worcester, Massachusetts, USA; [2]I.C. Blijdorp, BSc, T.E. Latuhihin, BSc, N.G. Yeremenko, PhD, Department of Rheumatology and Clinical Immunology, Amsterdam Rheumatology and Immunology Center, University of Amsterdam, Amsterdam, and Department of Experimental Immunology, Amsterdam University Medical Center, University of Amsterdam, Amsterdam, the Netherlands; [3]L.J. van Mens, MD, PhD, M.G. van de Sande, MD, PhD, Department of Rheumatology and Clinical Immunology, Amsterdam Rheumatology and Immunology Center, University of Amsterdam, Amsterdam, the Netherlands; [4]R. Bowcutt, PhD, S. Shaw, PhD, New Medicines, UCB Pharma, Slough, UK; [5]D.L. Baeten, MD, PhD, Department of Rheumatology and Clinical Immunology, Amsterdam Rheumatology and Immunology Center, University of Amsterdam, Amsterdam, Department of Experimental Immunology, Amsterdam University Medical Center, University of Amsterdam, Amsterdam, the Netherlands, and New Medicines, UCB Pharma, Slough, UK.

S. Chen, I.C. Blijdorp, N.G. Yeremenko, and D.L. Baeten contributed equally to this report.

Address correspondence to Dr. D. Baeten, Clinical Immunology and Rheumatology, F4-105, Amsterdam University Medical Center/University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands. Email: d.l.baeten@amc.uva.nl.

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rate to secukinumab (compared to placebo) was 30.7% (vs 18.1%) (ref 8) and 38.3% (vs 27.2%)[9]. In contrast, the same dose of secukinumab resulted in significant clinical improvement in SpA subtypes, including psoriatic arthritis (PsA) and ankylosing spondylitis (AS)[11,12,13,14,15]. In PsA, the ACR20 response rate to secukinumab 150 mg (vs placebo) was 51% (vs 15%)[14], and 50% (vs 17.3%)[15]. In patients with AS, the 20% improvement rate in the Assessment of Spondyloarthritis international Society response was 61% (vs 29%) and 61% (vs 28%) after secukinumab treatment (compared to placebo controls)[11]. Similarly, ixekizumab treatment, another IL-17A–specific monoclonal antibody, was also clinically effective in SpA[12,16].

The discrepancy in the clinical response to IL-17A blockade in RA and SpA patients indicates that there are differences in the pathogenesis of these 2 diseases that have yet to be elucidated[2,17]. Although IL-17A protein has been reported to be relatively high in the serum of patients with SpA and RA[20] in comparison to healthy subjects[18,19,20], systematic comparisons are still needed for IL-17A expression in the inflamed target tissues in SpA and RA.

In addition to IL-17A, the IL-17 family cytokine IL-17F also contributes to chronic tissue inflammation[21,22]. IL-17A and IL-17F share 55% homology at the amino acid level, and signal through the same heterodimeric receptor consisting of IL-17RC and IL-17RA[21]. Both IL-17A and IL-17F synergize with other inflammatory mediators such as TNF to enhance activation of tissue-resident target cells such as keratinocytes and fibroblast-like synoviocytes (FLS)[21,22,23]. Interestingly, protein levels of IL-17F were reported to be about 30-fold higher than those of IL-17A in psoriatic dermal fluid[19], while the proinflammatory activity of IL-17F is 100 times weaker than IL-17A in human biology. Recently, IL-17F was shown to play a nonredundant role in addition to IL-17A in psoriasis and in PsA[22,24], a peripheral form of SpA[22], indicating that IL-17F contributes to chronic arthritis as well. To date, potential differences in IL-17F expression and function in RA and SpA synovitis remain understudied.

To improve our understanding of the discrepancy in therapeutic responses to IL-17 blockade in SpA and RA, we hypothesized that IL-17 function or expression may differ in SpA and RA synovitis. We assessed synovial expression of IL-17A, IL-17F, and their receptors, as well as the functional responses to these cytokines in one of the target tissue cells, the FLS.

MATERIALS AND METHODS

Patient materials. Patients with SpA fulfilled the Assessment of Spondyloarthritis international Society criteria[25] for peripheral SpA (total n = 70), among whom 31 were diagnosed with PsA according to the Classification Criteria for Psoriatic Arthritis (CASPAR)[26,27]. Patients with RA (n = 47) fulfilled the ACR classification criteria[28].

Cohort 1 includes PsA patients with active psoriasis from whom we obtained skin lesion biopsies (n = 8; Table 1) and paired synovial tissue (ST) biopsies (n = 6). Cohort 2 includes ST biopsies from patients with SpA (n = 34) and RA (n = 32; Table 1). ST biopsies were obtained by arthroscopy from inflamed joints of patients with clinical arthritis as described[29]. Cohort 3 includes synovial fluid (SF) samples obtained from inflamed joints of patients with clinical arthritis (SpA, n = 24, and RA, n = 19; Table 1).

FLS were derived from ST samples of patients with SpA (n = 14) and RA (n = 8), and were used between passage 3 to 8[30]. The pooled SF samples used for FLS stimulation were obtained from SpA (n = 20) or RA (n = 19) SF samples from Cohort 3.

All patients provided written informed consent before enrollment, and the study was approved by the Ethics Committee of the Amsterdam University Medical Center/University of Amsterdam [METC 2013_051 (NL45246.018.13), METC 2013_057 (NL44031.018.13), and METC 2013_069 (NL44195.018.13)].

Analysis procedures. Total RNA was extracted from synovial and skin biopsies by homogenization of biopsies in STAT60 (Tel-Test) according to manufacturer's instructions, treated with DNAse, and purified using RNeasy minicolumns (Qiagen). RNA from FLS was isolated with RNeasy microcolumns (Qiagen) according to manufacturer's protocol. RNA was processed for cDNA synthesis (Fermentas). Analysis of cDNA by quantitative PCR (qPCR; Applied Biosystems) was performed with TaqMan gene expression assays (ThermoFisher Scientific) for *IL-17A* (Hs00174383_m1), *IL-17F* (Hs00369400_m1), *IL-17RA* (Hs01064648_m1), *IL-17RC* (Hs00994305_m1), *TNFR1* (Hs01042313_m1), and *TNFR2* (Hs00961749_m1). Expressions of all genes were normalized to expression of *GAPDH* (4310884E) as reference gene. Data were represented as relative expression (according to the 2^{-ddCt} method). All samples with *GAPDH* Ct values > 25 were excluded from analysis. *IL-17A* and *IL-17F* mRNA expression with Ct > 40 were considered undetectable. Patients with detectable *IL-17A* and *IL-17F* were included in the *IL-17A*/*IL-17F* ratio calculation in ST and skin.

IL-17A and IL-17F protein measurements. IL-17A protein was measured in SF by ELISA (eBio64CAP17 and eBio64Dec17; eBioscience).

IL-17F protein was measured with Single Molecule Counting technology (SMC; Singulex) using IL-17F antibodies (BAF1335 and AF1335; R&D Systems). Samples were assayed according to the manufacturer's instructions and analyzed using the Erenna Immunoassay System.

In vitro *FLS stimulation.* SpA FLS (n = 8) and RA FLS (n = 8) samples were starved overnight in Dulbecco modified Eagle's medium with 1% fetal calf serum and subsequently stimulated for 24 h with IL-17A (50 ng/mL; R&D Systems) or IL-17F (50 ng/mL; R&D Systems) in the presence or absence of TNF (1 ng/mL; Biosource). IL-6 and IL-8 protein levels were assessed in the supernatant by ELISA (Ucytech).

SpA FLS (n = 6) were also stimulated with pooled SF from RA or SpA patients (20% of final volume), supplemented with IL-17A (50 ng/mL; R&D Systems) or IL-17F (50 ng/mL R&D Systems). Supernatants were harvested after 24 h for protein analysis.

Data analysis and statistics. Prism version 7 software (GraphPad) and SPSS 24.0 (IBM) were used for statistical testing. Data are presented as median (IQR) if not otherwise stated. Statistics were calculated with paired *t* test when the data were normally distributed in paired ST and skin samples. Wilcoxon signed-rank test was performed for paired non-normal distributed samples. For unpaired samples, the Mann-Whitney *U* test was performed. One-way ANOVA test with Bonferroni corrections was applied for multiple comparisons. *P* values < 0.05 were considered statistically significant.

RESULTS

IL-17A/IL-17F expression ratio is higher in the joint compartment compared to skin in SpA. Because the expression of IL-17A and IL-17F has been well described in psoriatic skin[19], we first assessed the expression of both cytokines in psoriasis skin samples and paired synovial tissue samples from patients with PsA, a form of peripheral SpA (characteristics of these patients are summarized in Table 1, Cohort 1). The mRNA expression of IL-17A and IL-17F were both significantly lower in PsA ST

Table 1. Demographic and clinical characteristics of patients in 3 cohorts.

	Cohort 1*: Psoriasis Skin Biopsies	Cohort 2: Synovial Tissue		Cohort 3: Synovial Fluid	
	PsA, n = 8	SpA, n = 34	RA, n = 32	SpA, n = 24	RA, n = 19
Age, yrs	43.5 (37.0–48.0)	44.0 (34.8–54.3)	59.5 (51.5–65.8)	48.0 (42.0–56.0)	57.0 (50.0–67.0)
Male, %	87.5	67.6	34.4	75.5	26.3
Disease duration, yrs	1.3 (1.0–17.3)	4.0 (0.5–11.0)	2.0 (0.1–11.5)	14.0 (5.5–25.5)	2.5 (0.5–10.8)
Swollen joint count	2.0 (1.0–3.0)	2.0 (1.0–2.0)	4.0 (1.0–11.0)	1.0 (1.0–2.0)	2.0 (1.0–3.0)
Tender joint count	6.5 (1.3–10.0)	2.5 (1.0–7.0)	6.0 (2.0–19.8)	1.0 (1.0–2.0)	4.0 (1.0–5.0)
CRP, mg/l	3.9 (1.5–15.7)	10.2 (1.0–24.1)	15.8 (2.1–43.7)	18.0 (1.3–60.0)	11.0 (2.5–25.4)
ESR, mm/h	9.5 (2.0–35.0)	9.0 (5.0–36.0)	32.5 (11.0–46.5)	24.0 (9.0–43.0)	29.0 (7.0–43.5)
Patients taking DMARD, %	25.0	51.8	65.7	37.5	68.4
Patients taking anti–tumor necrosis factor, %	0	0	3.1	29.2	5.3
Axial involvement, %	NA	64.3	NA	16.7	NA
Presence of psoriasis, %	100	35.7	NA	54.2	NA

Data are median (IQR) unless otherwise indicated. * In Cohort 1, paired synovial tissue biopsy samples were obtained from 6 (out of 8) patients with psoriatic arthritis (PsA). SpA: spondyloarthritis; RA: rheumatoid arthritis; DMARD: disease-modifying antirheumatic drugs; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; NA: not applicable.

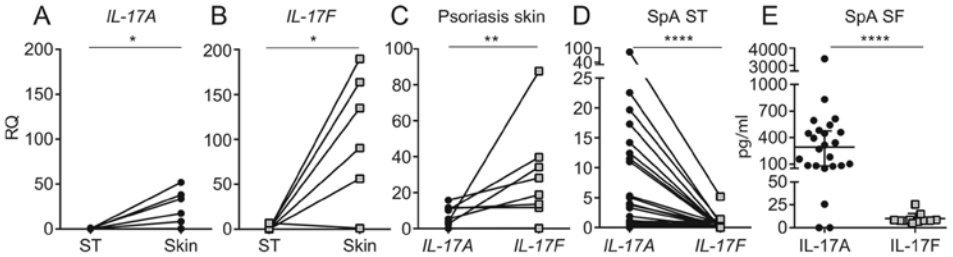

Figure 1. Expression of *IL-17A* and *IL-17F* in psoriasis skin and SpA synovitis. (A) *IL-17A* and (B) *IL-17F* mRNA expression was lower in PsA synovial tissue (ST) in comparison to paired psoriatic skin ($P = 0.031$, $P = 0.017$, respectively). (C) In psoriasis skin, *IL-17F* mRNA expression was 2.7-fold higher than *IL-17A*, whereas in SpA ST (D), mRNA expression of *IL-17A* was 17.3-fold higher than *IL-17F*. (E) In SpA synovial fluid (SF), IL-17A protein levels were 37.4-fold higher than IL-17F levels. $P > 0.05$ nonsignificant. *$P < 0.05$. **$P < 0.01$. ****$P < 0.0001$. IL: interleukin; PsA: psoriatic arthritis; RQ: relative quantification of mRNA expression; SpA: spondyloarthritis.

compared to matched psoriatic skin biopsies ($P = 0.031$ and $P = 0.017$, respectively; Figure 1A,B).

We next compared *IL-17A* and *IL-17F* expression within the same tissue compartment. The *IL-17A*/*IL-17F* ratio was inverted in the ST compared to psoriasis lesion skin biopsies. In these lesion biopsies, the relative expression of *IL-17A/GAPDH* was median 7.76 (IQR 3.46–11.3) and *IL-17F/GAPDH* was median 23.6 (IQR 13.1–35.6; Figure 1C). In psoriatic lesional skin, mRNA levels of *IL-17F* were a median 2.68-fold (95% CI 1.01–23.2) higher than levels of *IL-17A* ($P = 0.0078$; Figure 1C), which is in agreement with a previous publication describing higher levels of IL-17F than IL-17A in psoriasis dermal interstitial fluid[19]. The *IL-17A*/*IL-17F* expression ratio in skin samples was in sharp contrast to their relative expression levels in ST. In ST, *IL-17A* and *IL-17F* mRNA levels were below the detection threshold in, respectively, 17 and 28 of the total n = 42 ST samples. *IL-17A/GAPDH* was median versus 0.685 (IQR 0–3.95) and *IL-17F/GAPDH was* median 0 (IQR 0–0.200; Figure 1D). In ST of patients with both *IL-17A* and *IL-17F* mRNA detectable above threshold (< Ct = 40), *IL-17A* mRNA levels were median 17.3-fold (95% CI 3.61–31.4) higher than *IL-17F* levels ($P < 0.0001$; Figure 1D). We next measured protein levels of IL-17A and IL-17F cytokines in the SF: the IL-17A protein concentration was 37.4-fold higher than that of IL-17F in SpA SF [median 292.4 (IQR 81.4–464.2) vs median 7.8 (IQR 7.7-8.7) pg/mL; $P < 0.0001$; Figure 1E].

Collectively, these data indicate that levels of *IL-17A* and *IL-17F* are lower in the SpA joint compartment compared to psoriatic skin, with a striking inverse IL-17A/IL-17F ratio in the synovial tissue and fluid compared to the psoriasis skin biopsies in SpA patients.

IL-17A, IL-17F, IL-17RA, and IL-17RC levels do not differ between SpA and RA synovitis. We hypothesized that the expression of IL-17 cytokines and their receptors may be responsible for the differential response to IL-17A–blocking therapy in patients with SpA and RA. We first compared *IL-17A* and *IL-17F* mRNA expression in inflamed SpA and RA (peripheral) ST (Table 1, Cohort 2). The *IL-17A* mRNA expression was similar

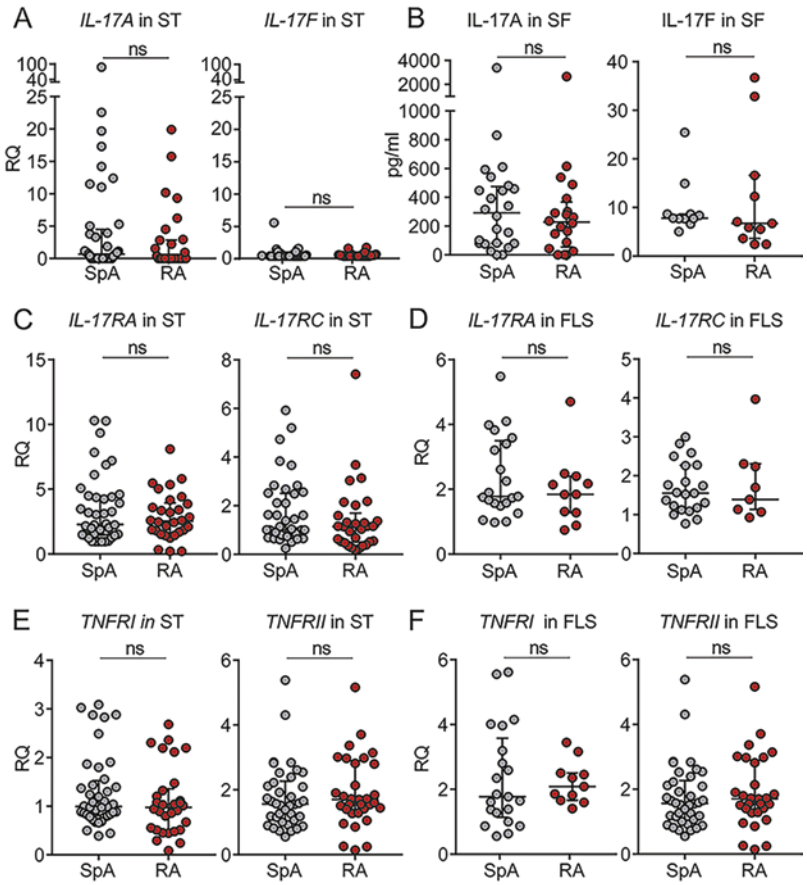

Figure 2. The expression of IL-17A, IL-17F, IL-17 receptors, and TNF receptors in SpA and RA synovitis. (A) *IL-17A* and *IL-17F* mRNA were not expressed differently in ST of SpA and RA patients. (B) Levels of IL-17A protein and IL-17F protein were similar in SF from patients with SpA and RA. (C) *IL-17RA* and *IL-17RC* were expressed similarly in ST from patients with SpA and RA, and (D) in SpA and RA FLS. (E) *TNFR1* and *TNFR2* did not differ in expression in ST from patients with SpA and RA, nor (F) in SpA and RA FLS. $P > 0.05$ nonsignificant. FLS: fibroblast-like synoviocytes; IL: interleukin; RA: rheumatoid arthritis; RQ: relative quantification of mRNA expression; SpA: spondyloarthritis; SF: synovial fluid; ST: synovial tissue; TNF: tumor necrosis factor.

in SpA and RA ST samples (Figure 2A). The SF IL-17A protein levels were also similar in SpA and RA [median 292.4 (IQR 81.4–464.2) vs median 228.3 (IQR 76.1–322.4) pg/mL; Figure 2B]. With regard to the expression of IL-17F, *IL-17F* mRNA expression did not differ between SpA and RA ST (Figure 2A), and the IL-17F protein concentration was also similar in SpA and RA SF [median 7.8 (IQR 7.7–8.7) vs median 6.8 (IQR 4.6–14.4) pg/mL; Figure 2B].

We next assessed the expression of the canonical receptor used by IL-17A and IL-17F, which is a heterodimer consisting of IL-17RA and IL-17RC. We hypothesized that the expression of IL-17RA and IL-17RC may influence the response to IL-17A or IL-17F locally in the inflamed target tissue. A qPCR analysis revealed comparable expression of *IL-17RA* and *IL-17RC* in the ST of patients with SpA and RA (Figure 2C). We also tested for the expression of *IL-17RA* and *IL-17RC* in SpA and RA FLS, which are considered important target cells of IL-17A and IL-17F in the ST[23]. We did not observe differences in the expression of *IL-17RA* and *IL-17RC* in SpA and RA FLS (Figure 2D).

Because IL-17A and IL-17F are not potent inflammatory cytokines on their own, but instead synergize with other proinflammatory mediators such as TNF, we additionally investigated if TNF receptors are differentially expressed in ST and FLS from patients with SpA and RA. mRNA levels of *TNFR1* and *TNFR2* were similar in SpA and RA ST (Figure 2E) and also in SpA and RA FLS (Figure 2F).

We moreover tested if patient characteristics including medication status or disease duration could influence IL-17 cytokines and receptors. We compared the levels of *IL-17A*, *IL-17F*, *IL-17RA*, *IL-17RC*, *TNFR1*, and *TNFR2* between patients taking disease-modifying antirheumatic drugs (DMARD) and patients without medication and did not find significant differences (Supplementary Figure 1, available with the online version of this article). Further, *IL-17A*, *IL-17F*, *IL-17RA*, *IL-17RC*,

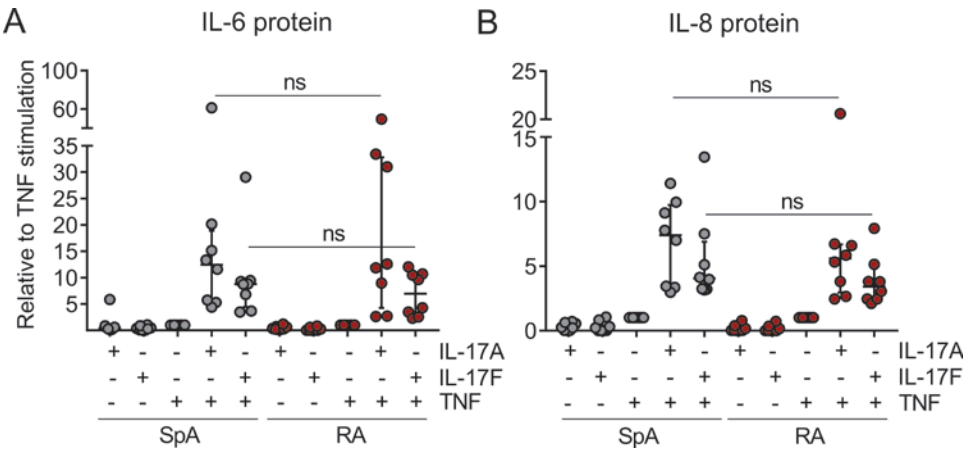

Figure 3. SpA and RA fibroblast-like synoviocytes (FLS) did not differ in their response to IL-17A and IL-17F stimulation. SpA and RA FLS responded similarly to IL-17A and IL-17F stimulation in the presence and absence of TNF, as shown by their production of IL-6 protein (A) and IL-8 protein (B). $P > 0.05$ nonsignificant. IL: interleukin; ns: nonsignificant; RA: rheumatoid arthritis; SpA: spondyloarthritis; TNF: tumor necrosis factor.

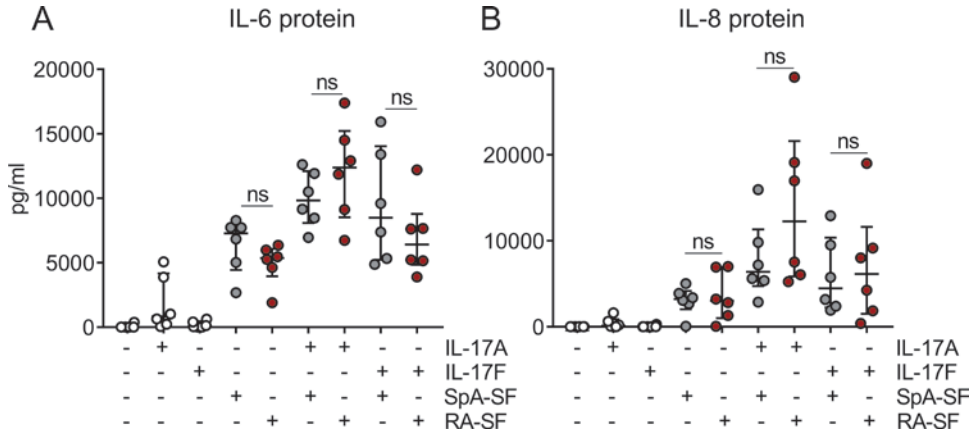

Figure 4. SF samples from patients with RA or SpA similarly enhanced the proinflammatory response to IL-17A and IL-17F stimulation. (A) IL-6 protein and (B) IL-8 protein secretion by SpA FLS was similar after stimulation with pooled SpA SF compared to RA SF, in the presence or absence of IL-17A or IL-17F. $P > 0.05$ nonsignificant. FLS: fibroblast-like synoviocytes; IL: interleukin; ns: nonsignificant; RA: rheumatoid arthritis; SF: synovial fluid; SpA: spondyloarthritis.

TNFR1, and *TNFR2* did not differ in patient groups stratified for disease duration (data not shown).

SpA and RA ST-derived FLS are equally sensitive to IL-17A and IL-17F. We next tested the hypothesis that the inflamed SpA and RA target tissue cells, the FLS, may respond differently to IL-17A and IL-17F cytokine stimulation. IL-17A and IL-17F are weak proinflammatory cytokines alone and depend on their synergy with other mediators like TNF to enhance proinflammatory responses in target cells such as FLS[21,22,23]. In agreement with the literature[22,23], neither SpA FLS nor RA FLS produced significant amounts of IL-6 (Figure 3A) or IL-8 (Figure 3B) upon stimulation with IL-17A (50 ng/mL) or IL-17F (50 ng/mL) cytokine. When stimulated with IL-17A in the presence of low concentration of TNF (1 ng/mL), both SpA and RA FLS significantly increased the production of IL-6 protein ($P = 0.078$ and $P = 0.039$; P values not shown in Figure 3A) and IL-8 protein ($P = 0.003$ and $P = 0.054$; P values not shown in Figure 3B).

Similarly, in the presence of TNF, addition of IL-17F cytokine enhanced IL-6 ($P = 0.031$ and $P = 0.009$; P values not shown in Figure 3A) and IL-8 protein ($P = 0.018$ and $P = 0.009$; P values not shown in Figure 3B) production by SpA and RA FLS. However, there were no differences between SpA and RA FLS in IL-6 (Figure 3A) or IL-8 (Figure 3B) production, indicating that it is highly unlikely that SpA and RA FLS differ intrinsically in their sensitivity to IL-17A and IL-17F stimulation, in the presence or absence of TNF cytokine.

SF from patients with RA or SpA equally enhance proinflammatory responses to IL-17A and IL-17F in FLS. As SpA and RA SF differ in composition[31,32], we hypothesized that there may be proinflammatory mediators present in SpA SF, which could

synergize with IL-17, or IL-17F. These proinflammatory mediators may be present to a lesser extent in RA.

To test this hypothesis, we pooled SF from patients with SpA or RA and stimulated SpA FLS with media containing 20% SF supplemented with IL-17A or IL-17F. Pooled SpA and RA SF both elicited an increased production of IL-6 ($P = 0.029$, $P = 0.001$, respectively; P values not shown in Figure 4A,B) by SpA FLS, with no differences between SpA and RA SF conditions (Figure 4A). Similar trends were observed for IL-8 production (Figure 4B). Combinations of SF with IL-17A or IL-17F further enhanced the production of IL-6 and IL-8 compared to SF alone. However, SpA and RA SF did not differ in their capacity to induce IL-6 and IL-8 production in FLS (Figure 4A,B). Collectively, these data indicate that pooled SpA and RA SF similarly enhance the proinflammatory response of FLS to IL-17A and IL-17F to a similar extent.

DISCUSSION

Recent clinical trials revealed that the IL-17 axis plays a central role in SpA patients[11,12,13,14,15,33], but not in RA[5,6,7,8,9,10], contrary to expectations based on preclinical findings[1,3,4]. We hypothesized that this discrepancy in the clinical response to IL-17 targeting in SpA and RA could be explained by differential expression of IL-17 cytokines and their receptors and/or differences in functional responses to IL-17 cytokines in the target tissues.

To date, there are no systematic comparisons of IL-17A and IL-17F expression between SpA and RA synovitis, to our knowledge. Most studies focused on comparing circulating IL-17A[18,19,20,34,35], and reported higher IL-17A levels in serum of patients with either SpA[18,19] or RA[20], and higher IL-17F levels in SpA serum compared to healthy controls[34,35]. One study reported higher IL-17 cytokine levels in the SF of patients with reactive arthritis, than in RA SF[36]. Within the same patient, IL-17A protein levels were higher in SF than in serum[36,37], indicating the importance to measure cytokine levels in SF, which is close to the inflamed ST.

We systematically studied the expression and function of the IL-17 axis (in particular IL-17A, IL-17F, and their common receptor consisting of IL-17RA and IL-17RC) in peripheral arthritis in SpA compared to RA. Overall, our data do not support the hypothesis that differential expression and/or function of the IL-17 axis in the synovial compartment contributes to the differential responsiveness of patients with SpA and RA to IL-17 inhibition.

We did not detect differences in IL-17A and IL-17F mRNA and protein levels in SpA and RA synovitis. Previously, we compared proinflammatory mediators in SF from SpA patients to SF from RA patients. In that study, we reported lower IL-17A levels in SpA compared to RA SF as measured by a Luminex assay[31]; however, this was not confirmed by classic ELISA. In contrast, we could confirm that SpA SF contains lower levels of soluble TNF protein compared to RA SF, which led to the finding of an increased transmembrane TNF to soluble TNF ratio in SpA versus RA[31,38]. Whereas clear differences in expression of proinflammatory mediators can thus be detected between different types of peripheral synovitis, this does not appear to be the case for IL-17A and IL-17F in our current study. In addition, we found no differences in expression of the relevant receptors, *IL-17RA* and *IL-17RC* or *TNFR1* and *TNFR2*, in ST or in FLS.

To account for potential clinical variables that could confound the lack of differences between RA and SpA synovitis, we performed a subanalysis stratifying for the use of DMARD (Supplementary Figure 1, available with the online version of this article). All patients had active arthritis upon inclusion, despite medication status. It remains to be determined whether the long-term use of a specific medication could influence the expression of IL-17 cytokines, their receptors, or additional cytokines that are relevant for the synergy of the proinflammatory response to IL-17.

Owing to the scarcity of human ST samples, we compared the expression IL-17A receptors at the mRNA level. We previously demonstrated the presence of these receptors by immunohistochemistry (data not shown), an approach that does not allow the quantitative assessment of the expression differences between SpA and RA ST. We further assessed the functional outcome of the IL-17RA/RC receptor engagement by assessing FLS activation by IL-17A or IL-17F. In these *in vitro* assays, there was no evidence for differential sensitivity of SpA and RA FLS to IL-17A and IL-17F, either in the absence or presence of TNF in low concentration. Finally, the functional response of FLS to IL-17A and IL-17F was similarly enhanced in the presence of SpA or RA SF, which argues against the potential presence of unique local mediators in SpA, but not RA SF, that may synergize with IL-17A or IL-17F.

Although the data we obtained from these different approaches to compare SpA to RA in the context of IL-17A and IL-17F consistently failed to demonstrate considerable differences, one should consider a number of limitations related to the design of our translational study. First, because we investigated the ST, we cannot exclude that the main local source of IL-17A and IL-17F in peripheral arthritis could be a different tissue of the peripheral joint, such as the enthesis or the bone[39], which remain difficult to sample in human pathology. It is notable that the mRNA level of IL-17A is relatively low in ST samples, whereas IL-17A protein is clearly detectable in SF. This discrepancy is likely not due to technical errors because we included only samples with *GAPDH* Ct values < 25, and within the same experiment, the mRNA expression of IL-17A and IL-17F cytokines was high in our psoriasis skin samples. A second limitation is that we tested only FLS as responder cells to IL-17A and IL-17F and thus cannot exclude that other cell types, including other subsets of stromal cells or leukocytes from SpA and RA patients, may respond differently to IL-17A and IL-17F. Third, we used SF as a proxy for the inflammatory milieu in peripheral synovitis and hypothesized that the SpA SF contains unique factors that may synergize with IL-17A and IL-17F in SpA synovitis. It is possible that the SF incompletely mimics

the inflammatory milieu of different tissue compartments (the synovium, enthesis, bone) in the peripheral joint because the SF contains only soluble factors. This is supported by our finding that transmembrane TNF, in addition to soluble TNF, contributes to SpA pathology[38]. Finally, our study focused on peripheral arthritis in SpA and did not assess the axial disease. It has yet to be established to what extent the pathobiology of peripheral and axial disease may or may not overlap in SpA, especially in view of the reported discrepancy in therapeutic efficacy: IL-23 (p19 and p40) blocking therapy are effective in PsA[40,41], but not in AS[33,42].

Although we did not find differences in IL-17A and IL-17F expression and function comparing SpA to RA, the analysis of paired ST and skin samples of patients with PsA yielded interesting new insights into the pathobiology of joint versus skin inflammation. We found that in the same patient, the mRNA levels of both IL-17A and IL-17F were significantly lower in inflamed ST than in psoriasis skin. We also observed that the relative expression of IL-17A versus IL-17F is inverted in inflamed joint and skin compartments. Our data are in line with protein levels measured in dermal interstitial fluid: IL-17F protein concentration is higher than IL-17A (317.0 pg/mL vs 9.8 pg/mL) in dermal interstitial fluid[19]. The *IL-17A*/*IL-17F* ratio is low in psoriasis skin because of the higher relative *IL-17F* expression, whereas in ST, *IL-17A* is more highly expressed. In line with that, we observed that the IL-17A protein concentration (292.4 pg/mL) is higher than IL-17F (7.8 pg/mL) in SpA SF.

Unexpectedly, we observed that the *IL-17A* mRNA expression is low in ST but IL-17A protein levels are relatively high in SF; this striking discrepancy suggests that the IL-17A protein in the inflamed joint may originate from a different source than the ST. This hypothesis is supported by previous studies in which we could not identify IL-17A–producing cells in SpA synovitis except for low numbers of T cells[43], whereas we did observe large quantities of IL-17A protein in ST colocalizing with mast cells that do not express *IL-17A* or *IL-17F*[44,45,46].

The expression of and functional response to IL-17A and IL-17F appear to be similar in SpA and RA synovitis. These findings fail to explain the differential response to IL-17A inhibition in these two diseases. Strikingly, the *IL-17A*/*IL-17F* expression ratio was markedly higher in SpA synovial tissue compared to skin inflammation, suggesting that the relative contribution of IL-17F to chronic tissue inflammation may be more prominent in skin than in joints.

ONLINE SUPPLEMENT

Supplementary material accompanies the online version of this article.

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