IL-17A and IL-17F expression and functional responses in rheumatoid arthritis and peripheral spondyloarthritis

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Abstract

Objectives

Targeting the IL-17-axis is efficacious in psoriasis (PsO) 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 qPCR in psoriasis skin, in SpA and RA synovial tissue (ST) and 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 TNF, or with pooled SF from SpA or RA patients.

Results

IL-17A (p=0.031) and *IL-17F* (p=0.017) mRNA were lower expressed in psoriatic ST versus paired psoriasis skin. *IL-17A* mRNA was 2.7-fold lower expressed than *IL-17F* in skin (p=0.0078) but 17.3-fold higher in ST (p<0.0001). In SF, IL-17A protein was 37.4-fold higher than IL-17F (292.4(81.4-464.2) vs 7.8(7.7-8.7) pg/ml, p<0.0001).

IL-17A and IL-17F mRNA and protein levels did not differ in SpA versus RA synovitis. Neither were *IL-17RA, IL-17RC* (IL-17-receptors), *TNFRI* and *TNRII* (TNF-receptors) differentially expressed between SpA and RA ST, nor between SpA and RA FLS. SpA and RA FLS produced similar amount of IL-6 and IL-8 protein upon stimulation with IL-17A or IL-17F, supplemented with 1 ng/ml TNF. Pooled SpA or RA SF enhanced FLS inflammatory response to IL-17A and IL-17F similarly.

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, however, appear to be similar in SpA and RA synovitis.

Keywords:

IL-17A, IL-17F, IL-17-axis therapeutic targeting, Spondyloarthritis, Rheumatoid arthritis, synovial fibroblasts

Key Messages:

1. The IL-17A/IL-17F expression ratio is higher in SpA synovitis compared to psoriatic skin.
2. Expression of and response to IL-17A and IL-17F are similar in SpA and RA synovitis.
3. SpA and RA SF similarly enhance FLS inflammatory response.

Background

Spondyloarthritis (SpA) and rheumatoid arthritis (RA) are the two most common forms of chronic inflammatory arthritis with TNF as a common disease-modulating cytokine (1-3). For long, interleukin-17A (IL-17A) has been hypothesized to be a key driver of the immunopathology of both diseases as IL-17A 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 RA patients (5-10). In phase III trials, the ACR20 response rate to the IL-17A inhibitor secukinumab (150 mg) versus placebo was 30.7% versus 18.1% (8) and 38.3% versus 27.2%(11). In contrast, the same dose of secukinumab is clinically effective in subtypes of SpA (12-14). The ACR20 response rate to secukinumab (150 mg) was 51% versus 15%(15) and 50% versus 17.3% in psoriatic arthritis (PsA) (16), 61% versus 29% and 61% versus 28% in ankylosing spondylitis (AS)(12). In line with this, the ACR20 response rate for ixekinumab, another IL-17A specific monoclonal antibody, was 62.1% versus 30% in PsA(17) and 52% versus 18% in AS.

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 two diseases which remains to be elucidated (2, 18). Although IL-17A protein has been reported to be elevated in the serum of SpA (19, 20) and RA patients (21) relative to healthy subjects, systematic comparisons of IL-17A expression in the inflamed target tissues in SpA and RA are still lacking.

In addition to IL-17A, IL-17F is another proinflammatory IL-17 family member which also contributes to chronic tissue inflammation (22, 23). 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 (22). 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 synovial cells (FLS) (22-24). Interestingly, IL-17F protein levels were reported to be approximately 30-fold higher than IL-17A in psoriatic dermal fluid (20) while IL-17F is 100-times less potent than IL-17A in human. More recently, IL-17F has been demonstrated to play a non-redundant role in addition to IL-17A in psoriasis (23, 25) and in psoriatic arthritis (PsA), a peripheral form of SpA (23), indicating that investigating IL-17F levels and function could be important in chronic arthritis as well. So far, potential differences in IL-17F's expression and function in RA and SpA synovitis remain understudied.

In order to understand the discrepancy in therapeutic response to IL-17-blockade in SpA and RA, we aimed to assess the synovial expression of IL-17A, IL-17F and their receptors, as well as the functional responses to these cytokines in the target tissue cells: the fibroblast-like synoviocytes (FLS).

Methods

Patient material

The SpA patients included fulfilled the Assessment of Spondyloarthritis International Society (ASAS) criteria (26) for peripheral SpA (n=70 in total), among whom n=31 were diagnosed with psoriatic arthritis (PsA) according to the ClASsification criteria for Psoriatic ARthritis (CASPAR) (27, 28)). The RA patients (n=47) included fulfilled the American College of Rheumatology classification criteria (29). Cohort 1 included PsA patients with active psoriasis from whom we obtained lesional skin biopsies (n=8, table 1) and paired synovial tissue (ST) biopsies (n=6). Cohort 2 included ST biopsies from SpA (n=34) and RA (n=32) patients (table 1). ST biopsies were obtained by arthroscopy from inflamed joints of patients with clinical arthritis as described before (30). Cohort 3 included 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 of SpA (n=14) and RA (n=8) patients according to standardized protocol (31). The pooled SF used for FLS stimulation was obtained from SpA (n=20) or RA (n=19) SF from cohort 3. All patients provided written informed consent before enrolment in the study, approved by the Ethics Committee of the Amsterdam University Medical Center/University of Amsterdam with the ethics approval numbers METC 2013_051 (NL45246.018.13), METC 2013_057 (NL44031.018.13) and METC 2013_069 (NL44195.018.13).

mRNA, cDNA and qPCR

Total RNA was extracted from synovial and skin biopsies by homogenization of biopsies in STAT60 (Tel-Test) according to manufacturer's instruction, treated with DNAse, and purified using RNeasy mini columns (Qiagen). RNA from FLS was isolated with RNeasy micro columns (Qiagen) according to manufacturer's protocol. RNA was processed for cDNA synthesis (Fermentas). Analysis of cDNA by qPCR (Applied Biosystems) was performed with TaqMan gene expression assays (Thermo Fisher Scientific) for IL-17A (Hs00174383_m1), IL-17F (Hs00369400_m1), IL-17RA (Hs01064648_m1), IL-17RC (Hs00994305_m1), TNFRI (Hs01042313_m1), TNFRII (Hs00961749_m1). Expressions of all genes were normalized to expression of GAPDH (4310884E) as housekeeping gene. All samples with GAPDH Ct values > 25 were excluded from analysis. *IL-17A* and *IL-17F* 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 using Single Molecule Counting technology (SMC™, Singulex) using IL-17F antibodies (BAF1335 and AF1335, R&D). The samples were assayed according to the manufacturer's instructions and analysed using the Erenna® Immunoassay System.

In vitro FLS stimulation

SpA FLS (n=8) and RA FLS (n=8) were starved overnight in DMEM with 1% FCS and subsequently stimulated for 24 hours with IL-17A (50 ng/ml, R&D) or IL-17F (50 ng/ml, R&D) 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 (20%), supplemented with IL-17A (50 ng/ml, R&D) or IL-17F (50 ng/ml, R&D). The supernatants were harvest after 24 hours for protein analyses.

Data analysis and statistics

Prism version 7 software (GraphPad) and SPSS 24.0 (IBM) were used for statistical testing. Data are presented as median (interquartile range (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 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 less than 0.05 were considered statistically significant.

Results

The IL-17A/IL17F expression ratio is higher in the joint compartment compared to skin in SpA

As IL-17A and IL-17F expression have been well described in psoriatic skin (PsO) (16), we first assessed the expression of both cytokines in psoriasis skin samples and paired synovial tissue samples from patients with PsA, the prototypical form of peripheral SpA (the characteristics of these patients were summarized in Table 1, cohort 1). *IL-17A* and *IL-17F* were both significantly lower expressed in ST when compared to matched PsO skin biopsies (p=0.031 and p=0.017) (Figure 1A and 1B).

When comparing *IL-17A* and *IL-17F* within the same tissue compartment, the IL-17A/IL-17F ratio was inversed in synovium compared to skin. In lesional skin, the relative expression ratio of *IL-17A* and *IL-17F* to *GAPDH* were 7.76 (3.46-11.3, median (IQR)) and 23.6 (13.1-35.6) (Figure 1C), versus 0.685 (0-3.95) and 0 (0-0.200) in ST (Figure 1D). *IL-17A* and *IL-17F* were below detection threshold in respectively n=17 and n=28 of total n=41 ST samples. In PsO lesional skin, mRNA levels of *IL-17F* were 2.68 (1.01-23.2, median (confidence interval (CI)) fold higher than *IL-17A* (p=0.0078, Figure 1C), which is in line with previously reported protein data demonstrating higher IL-17F than IL-17A levels in psoriasis (16). The IL-17A/IL-17F expression ratio in skin was in sharp contrast to their relative expression levels in ST. In ST of patients with both *IL-17A* and *IL-17F* mRNA detectable above threshold, *IL-17A* mRNA levels were 17.3 (3.61-31.4, median (CI)) fold higher than *IL-17F* (p<0.0001, Figure 1D). In addition, the protein levels measured in the SF were in line with the ST mRNA expression: the IL-17A protein concentration was 37.4-fold higher than IL-17F in SpA SF (292.4 (81.4-464.2) versus 7.8 (7.7-8.7) pg/ml, p<0.0001, Figure 1E).

Collectively, these data indicate that IL-17A and IL-17F expression are lower in the SpA joint compartment compared to PsO skin, with a striking inverse IL-17A/IL-17F expression ratio in the joint compared to the skin compartment in SpA.

IL-17A, IL-17F, IL-17RA, and IL-17RC expression levels do not differ between SpA and RA synovitis

In order to determine if the expression of IL-17 cytokines and their receptors may be responsible for the differential response to IL-17A blocking therapy in SpA and RA patients, we first set out to compare IL-17A and IL-17F cytokine expression in SpA versus RA peripheral synovitis (cohort 2,

Table 1). The *IL-17A* mRNA expression was similar in SpA and RA ST (Figure 2A). Accordingly, SF IL-17A protein levels were similar in SpA and RA ((292.4 (81.4-464.2) versus 228.3 (76.1-322.4) pg/ml, Figure 2B). For IL-17F, there was no difference in mRNA expression in SpA and RA ST (Figure 2A) and IL-17F SF protein levels were also similar in SpA and RA (7.8 (7.7-8.7) versus 6.8 (4.6-14.4) pg/ml, Figure 2B).

We next assessed the expression of the common IL-17A and IL-17F receptor, consisting of IL-17RA and IL-17RC, as this may influence response to IL-17A or IL-17F locally in the inflamed target tissue. qPCR analysis revealed comparable expression of *IL-17RA* and *IL-17RC* in the synovial tissue of SpA and RA patients (Figure 2C) as well as in SpA and RA FLS (Figure 2D).

As IL-17A and IL-17F are not potent inflammatory cytokines on their own but rather synergize with other pro-inflammatory mediators such as TNF, we additionally investigated if TNF receptors are differentially expressed in ST and FLS from SpA and RA patients. mRNA levels of *TNFRI* and *TNFRII* were similar in SpA and RA ST (Figure 2E) as well as in SpA and RA FLS (Figure 2F). These data indicate that mRNA expression levels of the IL-17 and TNF receptors do not differ *ex vivo* and *in vitro* between SpA and RA synovitis.

The expression levels of IL-17A, IL-17F, IL-17RA, IL-17RC, TNFRI and TNFRII were not significantly different in patients taking disease-modifying anti-rheumatic drugs (DMARDs) compared to patients without medication (Supplemental figure 1), nor in sub-analyses stratifying the patients for their disease duration (data not shown).

SpA and RA ST-derived FLS are equally sensitive to IL-17A and IL-17F

Considering the comparable expression levels of IL-17A, IL-17F, and their receptor in SpA and RA synovitis, we next tested the hypothesis that the SpA and RA inflamed target tissue cells, the FLS, may respond differently to IL-17A and IL-17F cytokine simulation. In line with published literature (23, 24), neither SpA FLS, nor RA FLS produced significant amounts of IL-6 (Figure 3A) or IL-8 (Figure 3B) upon stimulation with either only IL-17A (50 ng/ml) or IL-17F (50 ng/ml) cytokine. Compared to conditions stimulated with low concentration of TNF (1 ng/ml), SpA and RA FLS both increased the production of IL-6 (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) when stimulated with both IL-17A and TNF cytokines.

Similarly, in the presence of TNF, stimulation with 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, in the presence of TNF. However, there were no differences between SpA and RA FLS in IL-6 (Figure 3A) or IL-8 (Figure 3B) production, indicating that SpA and RA FLS do not intrinsically differ in their sensitivity to IL-17A and IL-17F stimulation.

SF from RA or SpA patients equally enhance pro-inflammatory response to IL-17A and IL-17F

Both IL-17A and IL-17F are weak pro-inflammatory cytokines alone and depend on their synergy with other pro-inflammatory mediators like TNF to enhance proinflammatory responses in target cells such as FLS (22-24). We next hypothesized that there may be additional pro-inflammatory mediators synergizing with IL-17 present in SpA SF, and to a lesser extent in RA SF, resulting in an increased pro-inflammatory response in SpA. It has been previously shown that SpA and RA SF differ in composition (32, 33), which could support our hypothesis.

To test this hypothesis, we pooled SF from SpA or RA patients and stimulated SpA FLS with 20% SF supplemented with IL-17A or IL-17F. Pooled SpA and RASF both elicited increased production of IL-6 (p=0.029 and p=0.001, respectively, *p-values not shown in Figure 4A and 4B*) by SpA FLS, without differences between SpA and RA SF conditions (Figure 4A). Similar trend was 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 and 4B). Collectively, these data indicate that SpA and RA SF similarly enhance the proinflammatory response of FLS to IL-17A and IL-17F.

Discussion

As recent clinical trials revealed that the IL-17-axis plays a central role in SpA (3, 4, 12-16, 34) but not in RA, contrary to prior expectations based on animal data (1, 3, 4), we hypothesized that differential expression of IL-17 cytokines, their receptors and/or differences in functional response to IL-17 cytokines in the target tissues may be explain the discrepancy in the clinical response to IL-17 targeting in SpA and RA.

Until now, human expression data comparing IL-17A and IL-17F levels in SpA and RA synovial fluid and synovitis have been lacking. Most previous studies focused on comparing circulating IL-17A (19-21) and IL-17F (35, 36) cytokines in SpA or RA patients to healthy controls, and reported higher IL-17A in either SpA (19, 20) or RA patients serum (21), and higher IL-17F in in SpA serum compared to healthy controls (35, 36). One study reported higher IL-17 cytokine levels in SF of undifferentiated SpA patients than in RA SF (37). Within the same patient, IL-17A protein levels are higher in SF than in serum (37, 38), indicating the importance to study the cytokine levels in close proximity to the inflamed target tissue.

In the current study, we systematically studied 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 versus RA using *ex vivo* and *in vitro* approaches. 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 SpA and RA patients to IL-17 inhibition.

We did not detect differences in IL-17A and IL-17F mRNA and protein levels in SpA and RA synovitis. In a previous multi-omics comparison of cytokines in SF, we systematically compared pro-inflammatory mediators in SpA versus RA SF and reported a decrease in IL-17A levels in SpA based on a Luminex assay (32), which was, however, not confirmed by classical ELISA (32). In contrast, we previously could confirm a decrease in soluble TNF protein in SpA SF compared to RA SF, which led to the finding of an increased transmembrane TNF over soluble TNF ratio in SpA versus RA (32, 39). Whereas clear differences in expression of pro-inflammatory 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 also do not find differences in the expression of the relevant receptors, IL-17RA and IL-17RC or TNFRI and TNFRII, in ST or in FLS.

To account for potential clinical parameters which could confound the lack of differences found between RA and SpA, we performed sub-analysis stratified for the use of DMARDs (supplemental figure 1). It is worth mentioning that all patients were present with active arthritis upon inclusion, despite of the medication status. It remains to be formally studied if long-time use of a specific medication could influence the expression of IL-17 cytokines, their receptors or additional cytokines relevant for the synergy of the proinflammatory response to IL-17.

Due to the scarcity of human ST samples, the expression of these receptors was compared at the mRNA level, which is a limitation of our current study. We previously demonstrated the presence of some of these receptors by immunohistochemistry (data not shown), but this approach does not allow a reliable assessment of quantitative differences between SpA and RA. Therefore, we rather checked the functional outcome of the IL-17RA/RC receptor engagement by assessing FLS activation. Also in this *in vitro* assay, 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 also 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 which may synergize with IL-17A or IL-17F.

Albeit the data we have obtained from these different approaches to compare SpA to RA in the context of IL-17A and IL-17F are consistent, one should consider a number of limitations related to the design of our translational study. First, as we investigated the ST, we cannot exclude that the main local source of IL-17A and IL-17F in peripheral arthritis could be another tissue of the peripheral joint such as the enthesis or the bone (40) as the mRNA expression level of IL-17A is relatively low in a ST samples, whereas IL-17A protein is clearly detectable in SF. This discrepancy is likely not due to technical errors as we only included samples with GAPDH Ct<25 and within the same experiment, the mRNA expression of IL-17A and IL-17F cytokines were high in our psoriasis skin samples. The other joint tissues such as enthesis or bone remain, difficult to sample in human pathology. A second limitation is that we only tested FLS as responder cells and can thus not exclude that other cell types, including other subsets of stromal cells or leukocytes, may show differences in response to IL-17A and IL-17F which are intrinsic to SpA or RA. Thirdly, we used SF as a proxy for the inflammatory milieu in peripheral synovitis and hypothesized that the SpA SF contains unique factors which may synergize

with IL-17A and IL-17F in SpA synovitis, and to a lesser extent in RA. It is possible that SF incompletely mimics the inflammatory milieu of different tissue compartments (synovium, enthesis, bone) in the peripheral joint as the SF only contains soluble factors. This is supported by our finding that tmTNF contributes to SpA pathology (39). Finally, our study focused on peripheral disease in SpA and did not assess axial disease. It remains to be established to what extend the pathobiology of peripheral and axial disease may or may not overlap in SpA, especially in view of the discrepancy in therapeutic efficacy reported: p19 and p40 blockade are effective in PsA (41, 42), but not in AS (43).

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 in the pathobiology of joint versus skin inflammation. We found that the mRNA levels of both IL-17A and IL-17F were significantly lower in inflamed ST than in lesional skin. The IL-17A/IL-17F expression ratio is high in ST due to higher relative IL-17A expression, whereas in skin, IL-17F is higher expressed. This inverse ratio is also observed in protein levels: IL-17F protein concentration has been reported to be higher than IL-17A (317.0 pg/ml vs 9.8 pg/ml) in dermal interstitial fluid (20) and we have shown that IL-17A protein concentration (292.4 pg/ml) is higher than IL-17F (7.8 pg/ml) in SpA SF.

The implications of our data could be twofold. As IL-17A mRNA expression is low in ST but IL-17A protein levels relatively high in SF, this striking discrepancy suggests that the IL-17A protein found in the inflamed joint may originate from a different tissue compartment than the synovial tissue. This hypothesis is supported by our previous studies in which we could not identify IL-17A-producing cells in SpA synovitis except for low numbers of T-cells (44), whereas we did observe large quantities of IL-17A protein in ST colocalizing with mast cells (45-47). Second, the relative expression of IL-17A versus IL-17F is inversed in inflamed joint and skin compartments with IL-17F levels being approximately 30-fold higher than IL-17A levels in the skin (20) but IL-17A being more than 30-fold higher than IL-17F in the joint. Together with the higher potency of IL-17A compared to IL-17F, these data suggest that the relative contribution of IL-17F in chronic tissue inflammation may be more prominent in skin than in joints.

Conclusion

The expression of and functional response to IL-17A and IL-17F appear to be similar in SpA and RA synovitis, failing to explain the differential response to IL-17A inhibition in these two diseases. Strikingly, IL-17A/IL-17F expression ratio is markedly higher in SpA ST 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.

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Disclosure

R.B, S.S and D.B are employees of UCB Pharma.

Figure legends

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

Figure 2: 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 differently expressed in ST of SpA and RA. B) Levels of IL-17A protein and IL-17F protein were similar in SF from SpA and RA patients. C) *IL-17RA* and *IL-17RC* were similarly expressed in ST from SpA and RA patients and in D) SpA and RA FLS. E) *TNRI* and *TNRII* did not differ in expression in ST from SpA and RA patients, nor in F) SpA and RA FLS. ns = P>0.05. RQ: Relative quantification of mRNA expression.

Figure 3: SpA and RA FLS do not differ in their response to IL-17A and IL-17F. A) SpA and RA FLS respond similarly to IL-17A and IL-17F stimulation in the presence and absence of TNF as demonstrated by their A) IL-6 protein and B) IL-8 protein production. ns = P>0.05.

Figure 4: SF from RA or SpA patients similarly enhance pro-inflammatory response to IL-17A and IL-17F. A) IL-6 protein and B) IL-8 protein production by SpA FLS were similar after stimulation with pooled SpA SF compared to RA SF, in the presence or absence of IL-17A, or IL-17F. ns = P>0.05.

Supplemental Figure 1: Expression of IL-17A, IL-17F and IL-17 receptors in SpA and RA after stratification for usage of disease-modifying anti-rheumatic drugs (DMARDs). mRNA of A) *IL-17A* mRNA in ST. B) *IL-17F* mRNA in ST. C) IL-17A protein in SF. D) IL-17F protein in SF. E) *IL-17RA* mRNA in ST. F) *IL-17RC* mRNA in ST. G) TNFRI mRNA and H) TNFRII mRNA in ST. ns= P>0.05.

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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, years	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, years	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/liter	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/hour	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 DMARDs, %	25,0	51,8	65,7	37,5	68,4
Patients taking anti-TNF, %	0	0	3,1	29,2	5,3
Axial involvement, %	n.a.	64,3	n.a.	16,7	n.a.
Presence of psoriasis, %	100	35,7	n.a	54,2	n.a.

Median (interquartile range) is represented, if not stated otherwise.

*In cohort 1, paired synovial tissue biopsy samples were obtained from n=6 PsA patients. CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; DMARDs = disease-modifying anti-rheumatic drugs. n.a. not applicable.

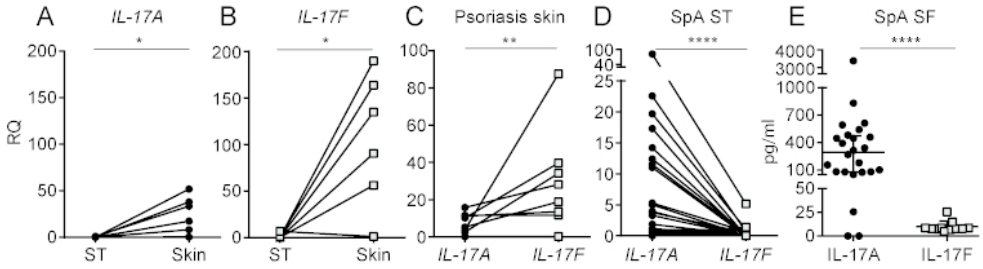

Figure 1: Expression of IL-17A and IL-17F in psoriasis skin and SpA synovitis. A) IL-17A and B) IL-17F mRNA expression were both lower in PsA ST in comparison to paired psoriatic skin (p=0.031 and p=0.017 respectively). C) In psoriasis skin, IL-17F mRNA expression was 2.7-fold higher than IL-17A whereas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

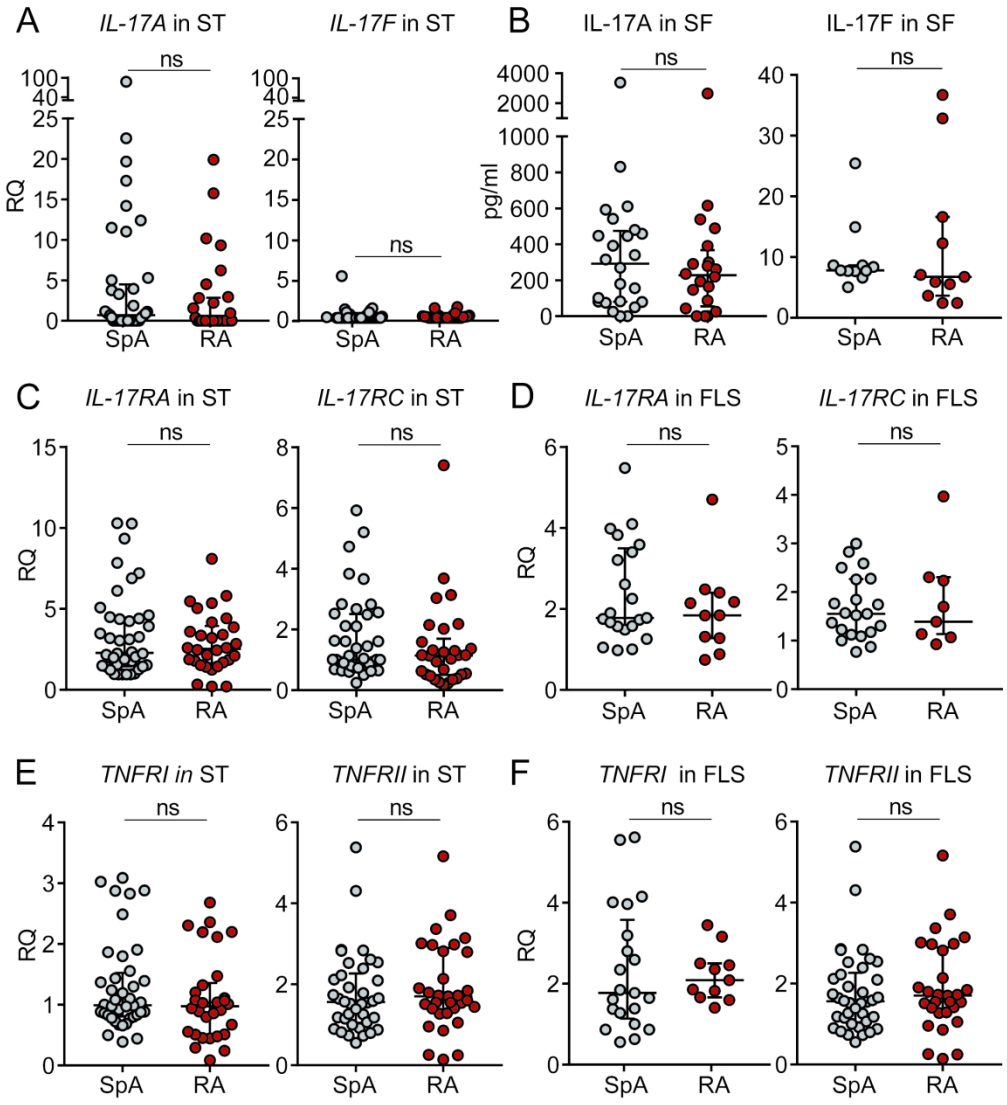

Figure 2: Expression of IL-17A, IL-17F, IL-17 receptors and TNF receptors in SpA and RA synovitis. A) IL-17A and IL-17F mRNA were not differently expressed in ST of SpA and RA. B) Levels of IL-17A protein and IL-17F protein were similar in SF from SpA and RA patients. C) IL-17RA and IL-17RC were similarly expressed in ST from SpA and RA patients and in D) SpA and RA FLS. E) TNRI and TNRII did not differ in expression in ST from SpA and RA patients, nor in F) SpA and RA FLS. ns = P>0.05. RQ: Relative quantification of mRNA expression.

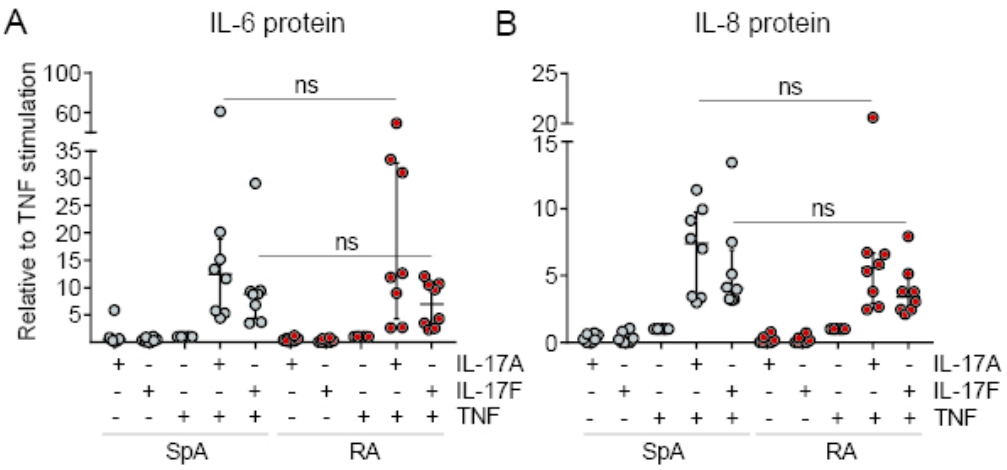

Figure 3: SpA and RA FLS do not differ in their response to IL-17A and IL-17F. A) SpA and RA FLS respond similarly to IL-17A and IL-17F stimulation in the presence and absence of TNF as demonstrated by their A) IL-6 protein and B) IL-8 protein production. ns = P>0.05.

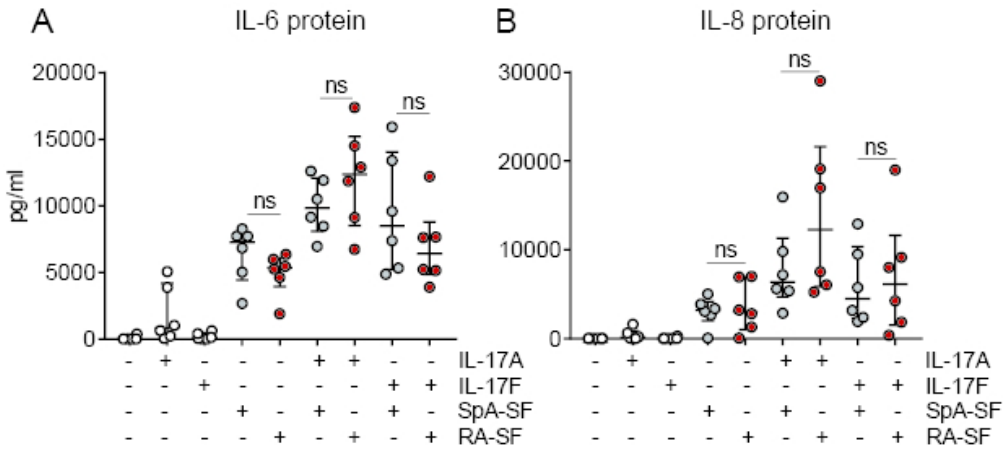

Figure 4: SF from RA or SpA patients similarly enhance pro-inflammatory response to IL-17A and IL-17F . A) IL-6 protein and B) IL-8 protein production by SpA FLS were similar after stimulation with pooled SpA SF compared to RA SF, in the presence or absence of IL-17A, or IL-17F. ns = P>0.05.