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Arthritis in systemic lupus erythematosus is characterized by local IL-17A and IL-6 expression in synovial fluid

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Summary

Arthritis is a common clinical feature of systemic lupus erythematosus (SLE) and is usually non-erosive, as opposed to rheumatoid arthritis (RA). While RA synovial pathology has been extensively studied, little is known about the pathophysiology of lupus arthritis. Here, we aimed to explore the cytokine and cellular compartments in synovial fluids of SLE patients with arthritic manifestations. Acellular synovial fluid and paired serum samples from SLE patients (n = 17) were analyzed with cytokine bead array for T helper-associated cytokines. From two SLE patients, synovial fluid mononuclear cells (SFMC) could also be captured and were analyzed by multiparameter flow cytometry to dissect T cell, B cell, monocyte and dendritic cell phenotypes. SLE-derived SFMC were further stimulated *in vitro* to measure their capacity for producing interferon (IFN)-γ and interleukin (IL)-17A. All patients fulfilled the ACR 1982 classification criteria for SLE. Clinical records were reviewed to exclude the presence of co-morbidities such as osteoarthritis or overlap with RA. IL-17A and IL-6 levels were high in SLE synovial fluid. A clear subset of the synovial CD4$^+$ T cells expressed CCR6+, a marker associated with T helper type 17 (Th17) cells. IL-17A-production was validated among CD4+CCR6+ T cells following *in-vitro* stimulation. Furthermore, a strong IFN-γ production was observed in both CD4+ and CD8+ cells. Our study shows high IL-17A and IL-6 levels in synovial fluids of patients with lupus arthritis. The Th17 pathway has been implicated in several aspects of SLE disease pathogenesis and our data also point to Th17 involvement for lupus arthritis.

Keywords: arthritis, cytokines, synovial fluid, systemic lupus erythematosus, T cells

Introduction

Arthritis is a common manifestation in systemic lupus erythematosus (SLE), observed in up to 90% of patients [1]. It often occurs at disease onset and is part of the classification criteria [2,3], but it is usually non-erosive, although advanced imaging has revealed that chronic synovitis [4] and erosions [5] are more common than previously estimated.

Despite being frequent and causing a significant disease burden by influencing quality of life [6], arthritis has received less attention than other SLE manifestations, such as nephritis, that directly impact morbidity and survival. While several studies have investigated possible associations of arthritis with genetic risk factors, immunological features and autoantibodies [6], little is known of the local inflammatory cell phenotypes and cytokines.

In the 1970s and 1990s, descriptive studies of SLE synovial biopsies demonstrated microvascular changes and moderate proliferation of the synovial lining layer, as well as mononuclear cell infiltrates and fibrin deposits [7] in affected tissue. The synovial fluid was found to typically contain fewer than 2000 leukocytes per microliter and to be characterized by lymphocytes and so-called lupus erythematosus (LE) cells, i.e. the presence of nucleic acids in the cytoplasm of neutrophils and macrophages [8]. The presence of anti-nuclear antibodies (ANA) was also reported in synovial fluid of SLE patients [9]. In 2007, Toukap *et al.* [10] analyzed the gene expression of synovial

biopsies and found that SLE patients display a distinct molecular signature, with up-regulation of interferon-inducible genes and down-regulation of genes involved in extracellular matrix homeostasis. However, the involvement of specific cell types or cytokines in lupus arthritis still remains unknown.

In order to more clearly understand SLE joint pathology, we analyzed cytokines associated with T cell responses and the cellular composition (when present) of synovial fluid (SF) samples from SLE patients compared to paired peripheral blood samples, and found an up-regulation of interleukin (IL)-6 and IL-17A in synovial fluid of SLE patients, as well as potential pathogenic T cell subsets.

Materials and methods

Patient material

SF was obtained from routine large joint aspiration from SLE, spondyloarthritis (SpA) and rheumatoid arthritis (RA) patients fulfilling the 1982 American Rheumatology Association/American College of Rheumatology (ARA/ACR) [2], Assessment of SpondyloArthritis international Society (ASAS) [11] and ACR classification criteria [12], respectively. Informed consent was given prior to SF and blood sampling, as approved by the local ethics committee.

Once collected, SF was centrifuged and stored as acellular at −80°C. In parallel, paired serum samples were also processed. The mononuclear cell fractions from SF (when present) and peripheral blood were collected following gradient density centrifugation over Ficoll-Hypaque and were cryopreserved in dimethylsulfoxide/fetal calf serum (DMSO/FCS) in N_2(l) until use.

SLE synovial fluid samples, once retrieved, were selected for inclusion in the present study after revision of the electronic clinical charts, in order to exclude biological material from patients with evidence of joint involvement not primarily dependent upon SLE. We therefore revised clinical information to exclude primary osteoarthritis, overlap with RA, crystal-related arthritis and other co-morbidities which could entail potential confounders.

From an initial list of 27 patients, we therefore selected 17 SLE patients with joint involvement, excluding 10 patients (one with SLE RA overlap, one with onset of arthritis after the occurrence of a hematological malignancy, eight for evidence of primary OA or crystal-related arthritis). The clinical characteristics are listed in Table 1 (SLE patients used for the cytokine analysis) and Supporting information, Table S1 (RA patients used for the cytokine analysis) and S2 (patient samples for cell analysis).

Table 1. Characteristics of the systemic lupus erythematosus (SLE) patients analyzed with the cytokine bead array

Clinical characteristic	*n* = 17
Gender (females *n*, %)	16 (94·1)
Age at disease onset (years, M, IQR)	31 (23–42·5)
Age at SF sampling (years, M, IQR)	57 (48·5–63·2)
Disease duration (years, M, IQR)	16 (6–34)
Active smoking at sampling (*n*, %)	6 (35·3)
Organ involvement *	
Lupus nephritis (*n*, %)	3 (17·6)
Cutaneous manifestations (*n*, %)	6 (35·3)
Neurolupus (*n*, %)	3 (17·6)
Serositis (*n*, %)	2 (11·8)
Secondary APS (*n*, %)	3 (17·6)
Treatment at sampling	
Glucocorticoids (*n*, %)	10 (58·8)
Anti-malarial (*n*, %)	10 (58·8)
Methotrexate (*n*, %)	3 (17·6)
Combined therapy‡ (*n*, %)	6 (35·3)
No active treatment (*n*, %)	2 (11·8)
Autoantibodies	
ANA (*n*, %)	17 (100)
Anti-dsDNA (*n*, %)	6 (35·3)
RF (*n*, %)	2 (11·8)
Anti-SSA/SSB (*n*, %)	4 (23·5)
Anti-RNP (*n*, %)	3 (17·6)
Anti-PL† (*n*, %)	9 (52·9)

M = median; IQR = interquartile range; GC = glucocorticoids; MTX = methotrexate; ANA = anti-nuclear antibodies; anti-dsDNA = anti-double-stranded DNA; RF = rheumatoid factor; anti-SSA/SSB = anti-Sjögren's syndrome-related antibody A, B; anti-RNP = anti-ribonucleoprotein; aPL = anti-phospholipid antibodies, †mostly anti-cardiolipin; SF = synovial fluid.

*Manifested during the course of the disease; †dose range 2·5–15 mg daily; ‡combination of either GC and anti-malarial, GC and MTX, GC-MTX and anti-malarial.

Autoantibodies

Immunoglobulin (Ig)A and IgM rheumatoid factor (RF) (Elia, Thermo Fisher Scientific, Uppsala, Sweden) and anti-citrullinated peptide antibodies (ACPA; CCPlus, EuroDiagnostica, Malmö, Sweden) were analyzed in sera and SF from SLE patients according to the manufacturers' instructions.

Cytokine bead array

SF samples (n = 17), matched serum samples (n = 15) from SLE patients (Table 1) and SF samples from RA patients (n = 10, Supporting information, Table S1) matched for storage time were analyzed with cytokine bead array (CBA) for T helper-associated cytokines (BD Biosciences, San Jose, CA, USA), following the manufacturer's instructions. In brief, thawed serum samples were diluted 1 : 2 with assay diluent and run-in duplicates in

a 96-well plate. Cytokines tested were IL-17A, interferon (IFN)-γ, IL-10, IL-6, IL-4, IL-2 and tumor necrosis factor (TNF). The assay was run on a fluorescence activated cell sorter (FACS)Verse flow cytometer and analyzed using the CBA software (BD Biosciences). Limits of detection are displayed in Supporting information, Fig. S2c.

Lymphocyte isolation and cell assays

SF mononuclear cells (SFMC) and paired peripheral blood mononuclear cell (PBMC) samples from two SLE, three SpA and two seronegative RA patients (matched for age and sex, Supporting information, Table S2) were analyzed by flow cytometry panels for T cells, and when possible for B cells, monocytes and dendritic cells (Supporting information, Table S4). Cryopreserved PBMC and SFMC were thawed and stained with the corresponding antibody mixtures and viability dye (Supporting information, Tables S3 and S4). For functional studies, duplicates of 0·5 × 10^6 SFMC were stimulated with anti-CD3/CD28 beads (one bead/cell, Dynabeads; Thermo-Fisher, Waltham, MA, USA) for 16 h in a 96-well flat-bottomed plate. At the last 4 h, brefeldin-A (5 μg/ml; Sigma Aldrich, St Louis, MO, USA) was added. Analyses were run on a Fortessa LSR cell analyzer (BD Biosciences) and data were processed using FlowJo software (BD Biosciences). Gating strategies are displayed in Supporting information, Fig. S1.

Statistical analysis

Continuous variables are expressed as median and interquartile range (IQR), categorical variables as numbers and percentages. Non-parametric statistics was used to explore differences and correlations as appropriate. *P*-values < 0·05 were deemed statistically significant. Prism version 7 software (GraphPad, San Diego, CA, USA) was used.

Ethical statement

Our study complies with the Declaration of Helsinki and was approved by the locally appointed ethical committee.

Results

Clinical characteristics

Clinical characteristics of the 17 SLE patients at the time of SF sampling are summarized in Table 1. The patients were fairly representative of a typical SLE population, with a prevalence of a subsets dominated by arthro-cutaneous manifestations. Samples were collected at a time of long disease history, including long-standing arthritis, often polyarticular, and which had occurred among the first disease manifestations. Six of the patients (age at onset = 28 years, IQR = 17–38) had developed secondary knee osteoarthritis over time.

IL-17A and IL-6 are highly expressed in SLE SF

First, we wanted to investigate T cell-associated cytokines in the SF of SLE patients. Thus, SF from SLE patients (n = 17) were tested for cytokine content in comparison to paired serum samples (n = 14) and SF from RA patients (n = 10), matched for storage time (Supporting information, Table S2). In SLE, IL-17A was detected in SF [44·9 pg/ml (10·7–405·2)] and matched serum [37·5 pg/ml (22·4–94·2)] (Fig. 1a), although no statistically significant difference was shown (P = 0·4).

IL-6 was significantly higher in SLE SF [771 pg/ml (108·5–2135)] compared to corresponding serum samples [1·7 pg/ml (1·2–4·9), P = 0·0006]. Low concentrations of IL-10 could be detected in 41% of SLE SF samples [0 pg/ml (0–3·4)] and were higher in serum [0·29 pg/ml (0·1–0·4)]. Overall, the RA SF samples displayed similar ranges of cytokines as SLE SF, with higher median values of IL-6 [3627 pg/ml (334·6–5000), P = 0·11] and IL-10 [1·9 pg/ml (0–7·9), P = 0·19] and less IL-17A [0 pg/ml (0–172), P = 0·11], although without statistical significance (Fig. 1a). The cytokine levels did not differ between SLE patients with and without secondary osteoarthritis (data not shown).

IL-17A levels in the SF seemed to separate into two clusters (Fig. 1a), with a subgroup of patients exhibiting low levels (< 80 pg/ml) and one exhibiting high levels (> 200 pg/ml). We examined whether this biological difference could correspond to a clinical difference; however, no significant differences in terms of age, disease duration, clinical manifestations, treatments or serological features could be found (data not shown).

Next, we explored the relationship of cytokine levels, in and between compartments (Fig. 1b,c). In SLE SF, the levels of IL-6 correlated with those of IL-10 [r = 0·84, $P \leq$ 0·0001, confidence interval (CI) = 0·69-0·92], and, weakly with IL-17A (r = 0·39, P = 0·03, CI = 0·02–0·66) (Fig. 1b). Also, IL-10 and IL-17A correlated weakly (r = 0·43, P = 0·01, CI = 0·07–0·69). When comparing serum and SF, levels of IL-6 correlated (r = 0·7, P = 0·0001) but not IL-17A or IL-10 (Fig. 1c).

The cytokine analyses also included IFN-γ, TNF, IL-2 and IL-4. In sera, IL-4 and IL-2 were higher compared to SF; however, their concentrations were consistently low (Supporting information, Fig. 2a). Very low levels of IFN-γ could be detected in SF of four SLE patients. TNF levels were below the detection limit in all samples (Supporting information, Fig. 2b).

Subsequently, we investigated the presence of IgA and IgM RF in serum and SF. These did not correlate with the cytokine levels or with other autoantibodies (data not

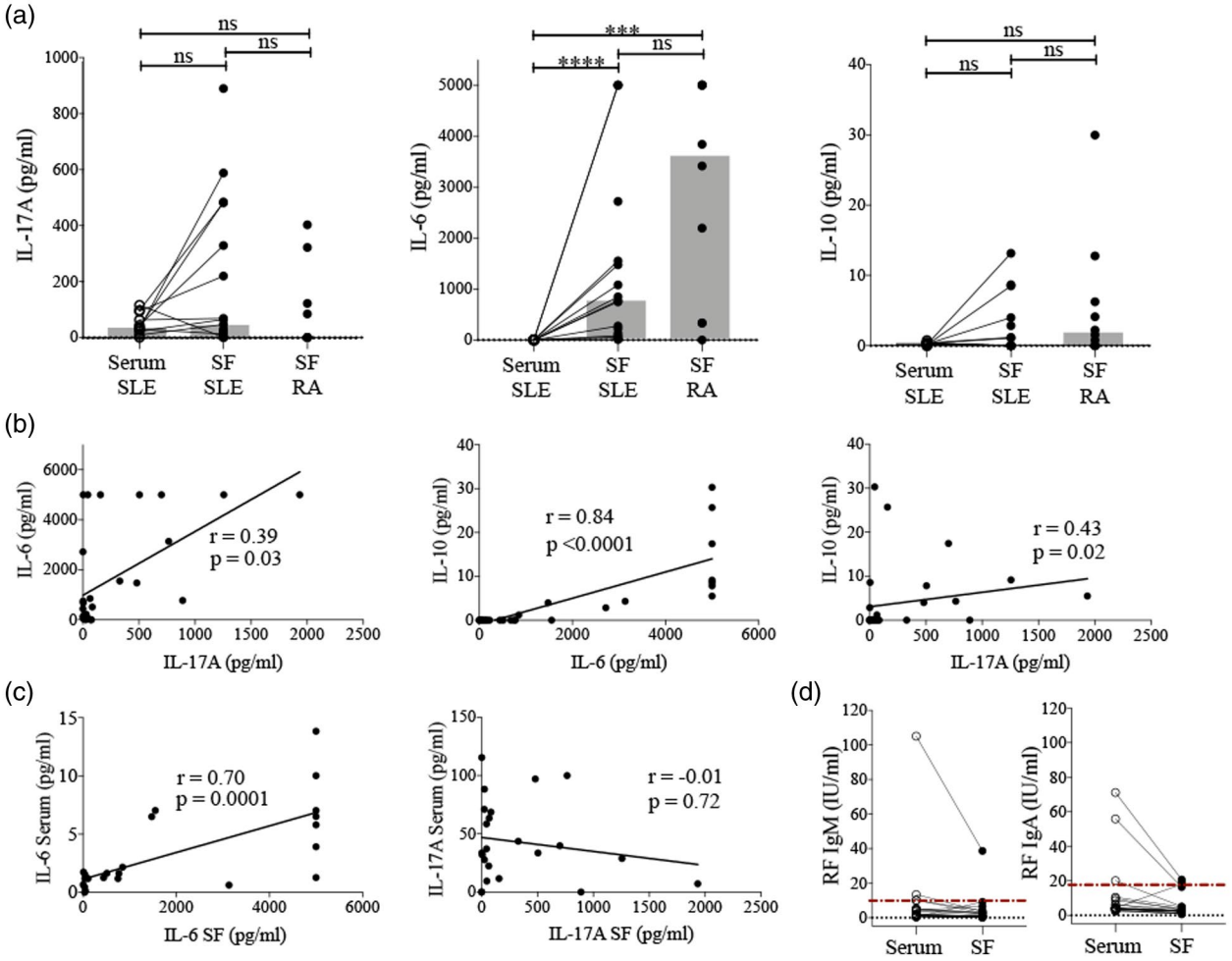

Fig. 1. Cytokine analysis of systemic lupus erythematosus synovial fluid (SLE SF) samples. (a) Interleukin (IL)-17A, IL-6 and IL-10 cytokine levels were measured in SLE SF samples (n = 17), matched serum samples (n = 14, combined with lines) and SF RA samples (n = 10). Significant differences between groups were analyzed using the Mann–Whitney test. Bars indicate median. (b) Spearman's correlations between IL-17A, IL-6 and IL-10 levels in SF are displayed. (c) Spearman's correlation between IL-17A and IL-6 levels in serum *versus* SF. (d) Immunoglobulin (Ig)A and IgM RF levels in serum and SF are shown. Red line indicates cut-off: IgA RF = 18 IU/ml; IgM RF = 10 IU/ml).

shown). Interestingly, the concentration of RF, when present, was mostly higher in serum compared to SF (Fig. 1d).

For one SLE patient, SF samples from six time-points were available, the first obtained approximately 15 years after disease onset. This patient presented with a recurrent polyarthritis, but no evidence of secondary OA could be tracked in the clinical records. High IL-6 levels were repeatedly measured during a 10-year period, while IL-17A levels fluctuated and IL-10 concentrations were low (Fig. 2a).

Cellular composition of SLE synovial fluid

Subsequently, we were interested in the cellular composition found in SF of SLE patients. All SF samples were processed to retrieve the mononuclear cell fraction. Such SFMC samples were available from two SLE patients, one from the patient described above and another patient with cells from two time-points. These samples were compared with SFMC from three SpA and two age- and sex-matched seronegative RA patients. All samples were investigated for T cell phenotypes and when possible also for B cell, monocyte and dendritic cell subsets (Supporting information, Table S4).

In SLE SF, lymphocytes consisted mainly of $CD3^+$ T cells, with $CD8^+$ cells being more common in SF than in peripheral blood (PB) (Fig. 2b, Supporting information, Fig. 3a). B cells, which were rare (< 1%), displayed primarly a memory phenotype for one patient including age-associated $CD11c^+CD27^+$ B cells (Fig. 2b).

Examining the monocyte and dendritic cell (DC) subsets in SLE SF, 64–67% of the $HLA\text{-}DR^+$ cells expressed CD14 without expressing CD16, a sign for classical monocytes (Fig. 2b, Supporting information, Fig. S3c). Non-monocyte cells represented ~30% of $HLA\text{-}DR^+$ cells in SF, and were mostly classical DC ($CD11c^+$, cDC) in one patient, mainly

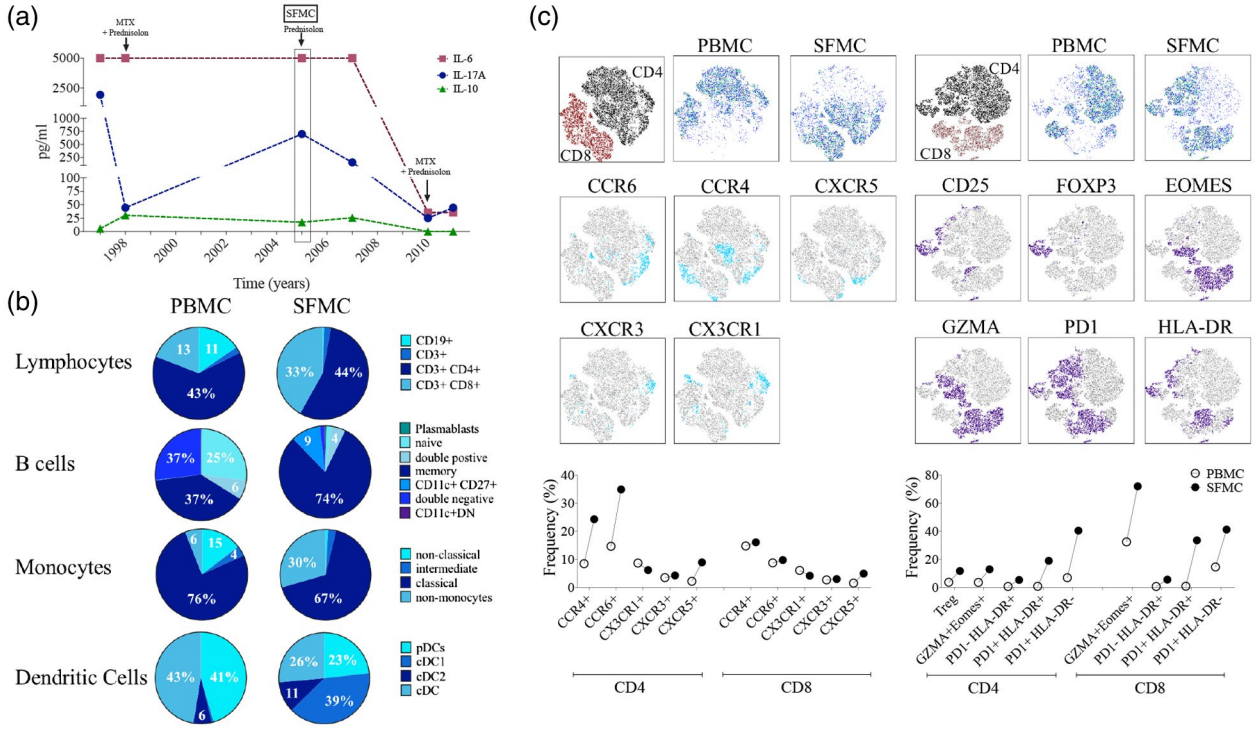

Fig. 2. Longitudinal cytokine analysis and cellular composition in systemic lupus erythematosus synovial fluid (SLE SF). (a) Longitudinal cytokine analysis of SLE SF in one patient with multiple available time-points. Change of medication and the time-point of SF mononuclear cells (SFMC) acquired are indicated. (b) Proportions of cell subsets in SF and matched peripheral blood (PB) from the same patient described in (a). B cell subsets: plasmablasts ($CD27^{++}CD38^{++}$); naive ($IgD^{+}CD27^{-}$), double-positive ($IgD^{+}CD27^{+}$), memory ($IgD^{-}CD27^{+}$), double-negative (DN, $IgD^{-}CD27^{-}$). Monocyte subsets: non-classical ($CD14^{-}C16^{+}$), intermediate ($CD14^{+}C16^{-,+}$), classical ($CD14^{+}C16^{-}$) and non-monocytes ($CD14^{-}CD16^{-}$). Dendritic cell subsets: classical DC (cDC, $CD11c^{+}$), cDC1 ($CD11c^{+}CD1c^{+}$), cDC2 ($CD11c^{+}CD141^{+}$) and pDC ($CD11c^{-}CD123^{+}CD303^{+}$). Frequencies < 4% are not displayed. (c) t-Distributed stochastic neighbor-embedding (tSNE) clustering of $CD3^{+}$ cells from the two T cell panels (Supporting information, Table S4) and $CD4^{+}$ and $CD8^{+}$ T cell subsets in paired PB and SF (n = 1).

cDC1, while the other exhibited equal proportions of cDC1 and cDC2. The frequency of plasmacytoid DC varied between patients from 7 to 26% (Fig. 2b, Supporting information, Fig. S3c).

T helper type 17 (Th17) signatures in SF of SLE patients

Focusing upon T cell phenotypes, we visualized the distribution by t-distributed stochastic neighbor-embedding (tSNE) analysis of the $CD3^{+}$ T cells. As depicted in Fig. 2c, PBMC and SFMC T cell cluster in different directions. Within the $CD4^{+}$ compartment, we observed higher frequencies of $CCR4^{+}$, $CXCR5^{+}$ and $CCR6^{+}$ and similar levels of $CXCR3^{+}$ in SF compared to PB. The most prominent T helper subset in SF was $CCR6^{+}$ cells, a marker for Th17 cells; 35–55% of $CD4^{+}$ T cells expressed CCR6 (Fig. 2c, Supplementary Fig. 3d), with ~40% co-expressing CCR4. Moreover, T_{regs} [$CD25^{+}$forkhead box protein 3 ($FoxP3^{+}$)], eomesodermin ($Eomes^{+}$) and granzyme A^{+} (GZMA) were also enriched in SF compared to PB (Fig. 2c). programmed cell death 1 $(PD\text{-}1)^{+}$human leukocyte antigen D-related ($HLA\text{-}DR^{+}$) cells were also abundant, and we identified a distinct population of T peripheral helper (Tph; $PD\text{-}1^{high}HLA\text{-}DR^{high}$) cells in SF, of which ~30% co-expressed CCR6 (Fig. 3). In SF, ~70–80% of $CD8^{+}$ T cells expressed Eomes and GZMA and a high proportion of $CD8^{+}PD\text{-}1^{+}HLA\text{-}DR^{+/-}$ cells were detected in all SF samples (Fig. 2c, Supporting information, Fig. S3d). CX3CR1 was not expressed on $CD4^{+}$ or $CD8^{+}$ T cells in SF. SF T cell phenotypes in SLE, RA and SpA presented similar patterns.

From one SLE patient, SFMC from two time-points were available (Supporting information, Fig.S3e). For most $CD8^{+}$ and $CD4^{+}$ subsets, similar frequencies were detected with the exception of $GZMA^{+}Eomes^{-}$ T cells which were more prominent at the first time-point. In line with the other SLE patient, $CCR6^{+}CD4^{+}$ T cells was one of the most prominent subsets in SF.

As we observed both IL-17A and increased $CCR6^{+}CD4^{+}$ T cells in SF of SLE patients, we investigated the capacity of SF-derived T cells to produce IL-17A. Following *in-vitro* stimulation of SFMCs from one SLE and one

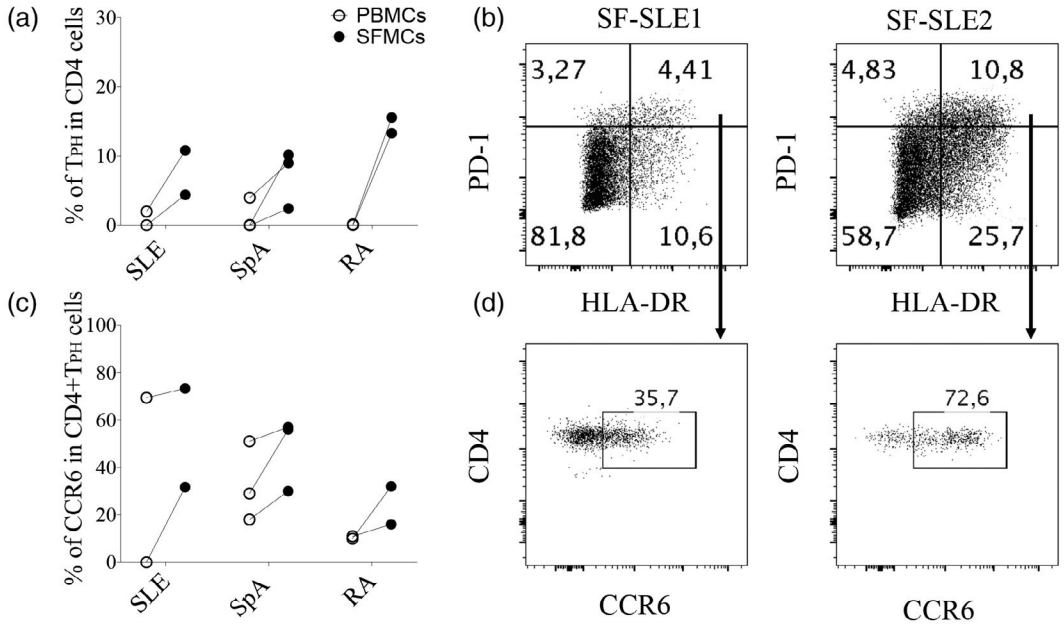

Fig. 3. T peripheral helper (Tph) cells in synovial fluid (SF) of systemic lupus erythematosus (SLE) patients. (a) Frequency of Tph cells in SF and serum samples in SLE ($n = 2$), spondyloarthritis (SpA) ($n = 3$) and age- and sex-matched RA ($n = 2$) patients. (b) Gating plots of the SF samples of the two SLE patients. Tph cells are defined as $CD4^+$ programmed cell death 1 ($PD\text{-}1^{high}$) human leukocyte antigen D-related ($HLA\text{-}DR^{high}$) cells gated by quadrant gating. (c) Frequency of $CCR6^+$ expression in Tph cells in the three different diseases. (d) SLE SF plots showing chemokine receptor (CCR6) gates after gating for $CD4^+$ $PD\text{-}1^{high}$ $HLA\text{-}DR^{high}$ cells.

SpA sample, intracellular IL-17A and IFN-γ production was analyzed. In both samples, IL-17A production was induced and confined to the $CCR6^+CD4^+$ cells (Fig. 4, Supporting information, Fig. S4). We also observed abundant IFN-γ production in IL-17A-negative $CD4^+$ T cells. Of note, $CD8^+$ T cells also produced IFN-γ but not IL-17A.

Discussion

We found that local joint inflammation in SLE is dominated by IL-6 and IL-17A. IL-6, a pleiotropic cytokine, can influence the function of several immune cells such as B and T cells, orientating their fate towards Th17 and T follicular helper phenotypes [13]. Its local expression in SLE-inflamed large joints might also suggest a more acute joint pathology and an effect on recruiting neutrophils. Indeed, most of the SLE SF samples contained only neutrophils and became acellular following the cell purification step. Previous studies investigating IL-6 plasma concentration in relation to SLE arthritis have shown that elevated IL-6 was associated with active ongoing arthritis but not with CRP levels [14,15] and also a correlation between IL-6 plasma level and joint involvement [15]. In our study, IL-6 SF levels also correlated with IL-6 serum levels, although IL-6 concentration was much lower in serum, suggesting a more relevant local role for this cytokine.

IL-17A, which is known to trigger the recruitment of neutrophils and monocytes to the site of inflammation [16], was abundant in SLE SF samples, similar to what has been observed in ankylosing spondylitis [17]. In recent years, several studies have highlighted the possible implications of the IL-17 axis in SLE pathogenesis [18]. IL-17A has been found to be increased in sera of SLE patients compared to healthy controls, expressed in kidney tissue of patients with lupus nephritis and to predict an unfavorable histopathological response to immunosuppressive treatment [19]. Current clinical trials are evaluating IL-17A inhibition in refractory lupus nephritis (https://www.clinicaltrials.gov/ct2/show/NCT04181762).

This is the first study, to our knowledge, that investigates the cellular composition of SF in SLE. In most cases, the numbers of SF mononuclear cells were low, thereby only the fluid could be preserved. However, we were able to analyze mononuclear cells from two synovial fluids from SLE patients. Similar to other inflammatory arthritides [20,21], an increase of T_{regs} could be observed in SLE SF. We could also identify a $PD\text{-}1^{high}HLA\text{-}DR^{high}$ Tph population in the SF of SLE patients. This T cell subset has been described in $ACPA^+$ RA patients and functions by promoting B cell responses and antibody production [22]. Similarly, $PD\text{-}1^{high}CXCR5^-$ Tph have been described in SLE, where they appear to drive the

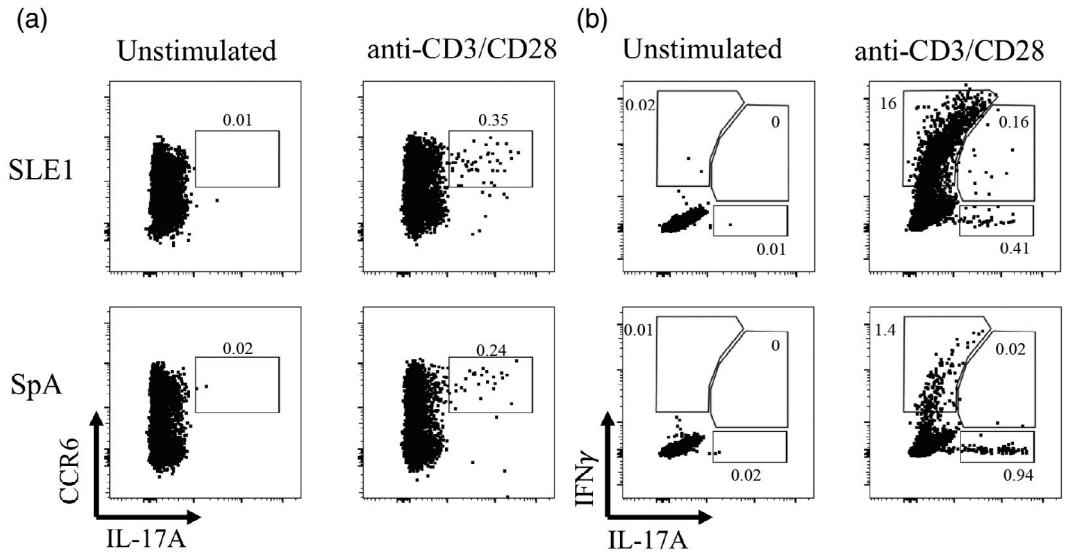

Fig. 4. T cell stimulation of synovial fluid mononuclear cells (SFMC) from systemic lupus erythematosus (SLE) and spondyloarthritis (SpA). Intracellular flow cytometry dot-plots of (a) CCR6 *versus* IL-17A and (b) interferon (IFN)-γ *versus* interleukin (IL)-17A in stimulated SFMC (one SLE and one SpA). The cells were stimulated overnight with anti-CD3/CD28 beads and then stained for the intracellular cytokines IL-17A and IFN-γ. Gating strategies are shown in Supporting information, Fig. S4.

dynamics of pathological B cell responses, correlate with disease activity and are expressed in kidney tissue in lupus nephritis [23].

In our study, $CCR6^+CD4^+$ T cell subsets were abundant in SLE SF. $CCR6^+$ is a chemokine receptor expressed on Th17 cells and Th17 differentiation is dependent upon the cytokines IL-6, IL-1β and TGF-β [24]. Th17 cells have previously been reported to increase during SLE flare and to be reduced after successful treatment [25]. Leipe *et al.* [26] reported an increase of Th17 cells in psoriatic arthritis and RA patients in peripheral blood and synovium, with an enrichment of Th17 in SF in the affected joint. Similarly, $CCR6^+$ cells were increased in SF of the two analyzed SLE patients compared to peripheral blood.

Due to the increase of IL-17A and $CCR6^+$ T cells in SF of SLE patients, we stimulated SFMC with anti-CD3/CD28 beads to study their capacity to produce cytokines. Thereby, we identified IL-17A-producing T cells, primarily represented by $CCR6^+CD4^+$ T cells. It is, however, possible that other cells also could contribute to IL-17A production, as both CD4 and CD8, as well as γδ T cells, innate lymphoid cells and mast cells, have been identified as IL-17A producers in psoriasis and psoriatic arthritis [27].

While $CCR6^+$ was one of the most prominent $CD4^+$ T cell subsets in SLE SF, the vast majority of SF $CD8^+$ T cells expressed the transcription factor Eomes and GZMA, similar to observations in RA [28]. GZMA induces pro-inflammatory cytokines such as IL-6, IL-8 and IL-1β. Altogether, our analysis suggests an involvement of T cells in the pathogenesis of lupus arthritis.

The main limitation of our study is that it was conducted retrospectively on historical material and on a relatively small-sized patient sample. Systematic assessment of joint involvement and SLE disease activity were not performed at the time of sampling; thus, clinical associations could not be further explored. Moreover, the sampling was performed only on large joints, which are technically more accessible but imply some limitations, as SLE arthritis more typically involves small joints.

In addition, the long disease duration and the exposure to treatments, both ongoing at the time of sampling and previously used in the patients, may potentially have influenced the composition of the SF. This intrinsic limitation, together with the small sample size, warrant further investigation and the attempt to replicate the findings in patients with shorter disease duration and more limited, if not absent, exposure to therapeutics.

Being SF obtained from SLE patients who are most often acellular or paucicellular, the availability of two cellular samples which we could analyze might even be regarded as a strength of our study. This highlighted, however, that even though our cell data align with the cytokine data, the sample size of cells is very limited. Our results are more exploratory and warrant exploration of larger biological material. Nevertheless, we suggest a role for IL-17A-related pathways and eagerly await replication and extension of our data by other researchers.

From a clinical perspective, lupus arthritis is already common from the time of diagnosis, but is a manifestation often responding to treatment. This may be a reason why arthritis has been given relatively less attention from a research perspective. Our study cohort is admittedly small, but still represents one of the most comprehensive collections of SF material in SLE. The patients included in the analysis belong to a historical SLE cohort and perhaps, in light of the long disease duration, the active manifestations at the time of sampling might give the impression of a sample that does not fully represent the clinical spectrum of SLE. However, previous manifestations included also major organ manifestations such as nephritis and central nervous system (CNS) involvement, although the patients may represent a subset with prevalent arthro-cutaneous clinical manifestations in which arthritis was somewhat aggressive.

It is tempting to speculate that lupus arthritis is less autoimmune in character compared to rheumatoid arthritis, and our data lend support to such a theory by suggesting that it is part of a systemic immune deviation towards Th17, and is not joint-specific. Indeed, Th17 responses implicate the involvement of innate immunity through neutrophils, cells that historically have been linked to SLE pathophysiology as LE cells [8].

In conclusion, our study provides new exploratory insights into the immunology of lupus arthritis. IL-17A and IL-6 levels were high in synovial fluid of SLE patients. The presence of $CCR6^+CD4^+$ T cells, $GZMA^+Eomes^+$ and $PD\text{-}1^+HLA\text{-}DR^+CD4^+$ and $CD8^+$ T cells suggest a pathogenic role of T cells in lupus arthritis. Our findings suggest that SLE patients with arthritis may improve if treated with IL-17A blockade for other clinical manifestations.

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Disclosures

The authors have no conflicts of interest to disclose.

Author contributions

This study was conceived, designed and coordinated by N. S., F. F., K. C. and I. G.; V. M., F. F. and S. T. contributed to data collection and clinical assessments. Experimental work was performed by N. S. in supervision of K. C. and V. M. Rheumatoid factor data were obtained by J. R. The data analysis and interpretation were conducted by N. S., F. F., K. C., V. M. and I. G. and the writing was drafted by F. F. and N. S.

Data Availability Statement

Data will be made available upon reasonable request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1. Flow cytometry gating strategies

Figure S2. Continued Data from the Cytokine Bead Array

Figure S3. Cell analysis of SLE patients and controls

Figure S4. Gating strategy from the T-cell stimulation assay.

Table S1. Characteristics of the Rheumatoid Arthritis (RA) patients analyzed with the cytokine bead array.

Table S2. Patient Characteristics for Cell Analysis.

Table S3. Flow Cytometry Antibody List.

Table S4. Panels for Flow Cytometry.

