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Responses to Cytokine Inhibitors Associated with Cellular Composition in Models of Immune-Mediated Inflammatory Arthritis

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Objective. Immune-mediated inflammatory arthritis (IMIA) is a heterogeneous group of diseases including rheumatoid arthritis (RA), psoriatic arthritis (PsA), and spondyloarthritis (SpA). Disease-modifying antirheumatic drugs (DMARDs) target very different cellular components of the disease processes. Characterization of the pathobiological subtypes of IMIA could provide more specific treatment approaches for each disease. For example, RA has been proposed to consist of at least three synovial pathotypes (lymphoid, myeloid, and fibroid), and only a subgroup of RA patients have erosive disease. The objective of this study was to evaluate the effects of various DMARDs on different synovial cell subsets using human ex vivo models of IMIA.

Methods. Synovial fluid and blood samples were obtained from a study population consisting of patients with RA, PsA, or peripheral SpA with at least one swollen joint (n = 18). The DMARDs used in this study were methotrexate, adalimumab, etanercept, tocilizumab, anakinra, ustekinumab, secukinumab, tofacitinib, and baricitinib. Paired synovial fluid mononuclear cells (SFMCs), peripheral blood mononuclear cells (PBMCs), and fibroblast-like synovial cells (FLSs) were used in three different previously optimized ex vivo models.

Results. In SFMCs cultured for 48 hours, all DMARDs except anakinra decreased the production of monocyte chemoattractant protein (MCP)-1. In SFMCs cultured for 21 days, only the two tumor necrosis factor alpha (TNFα) inhibitors adalimumab and etanercept decreased the secretion of tartrate-resistant acid phosphatase ($P < 0.01$, $P < 0.001$). In the FLS and PBMC 48-hour co-cultures, only tocilizumab ($P < 0.001$) and the two Janus kinase inhibitors tofacitinib and baricitinib (both $P < 0.05$) decreased the production of MCP-1 by around 50%.

Conclusion. TNFα inhibition was effective in preventing inflammatory osteoclastogenesis, whereas tocilizumab, tofacitinib, and baricitinib had superior efficacy in cultures dominated by FLSs. Taken together, this study reveals that responses to cytokine inhibitors associate with cellular composition in models of IMIA. In particular, this study provides new evidence on the differential effect of DMARDs on leukocytes compared with stromal cells.

INTRODUCTION

Immune-mediated inflammatory arthritis (IMIA), including rheumatoid arthritis (RA), psoriatic arthritis (PsA), and spondyloarthritis (SpA), encompasses a group of immune-mediated inflammatory diseases characterized by synovitis and cartilage and bone damage. Early intervention with disease-modifying antirheumatic drugs (DMARDs) and the development of therapies targeting specific components of the disease pathogenesis has radically improved the treatment of these diseases (1). However, despite general improvements in treatment options, some patients still do not respond to treatment (2).

Tumor necrosis factor alpha (TNFα) plays a central role in the pathogenesis of all of the IMIA diseases. Thus, TNFα inhibitors have

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shown efficacy in patients suffering from RA, PsA, and SpA. In contrast, other proinflammatory cytokines are considered to play a central role in only some of these diseases; for example, interleukin (IL)-6 is important in RA, whereas IL-17 and IL-23 play more prominent roles in the pathogenesis of SpA and PsA (3,4). However, there is still lack of tailored therapy for patients within each disease subgroup. Currently, the first choice of DMARD in RA is mostly dependent on local policies including market pricing, administration route, and side effects. This is perpetuated by the rather similar efficacy profile of the biological DMARDs in the clinical trials (5,6).

Cytokine profiling (4) and synovial phenotyping (7) holds promise for the future stratification of patients with immune-mediated inflammatory diseases. The RA synovium can histologically be divided in the three synovial pathotypes: 1) lymphoid, 2) myeloid, and 3) fibroid (8). The fibroid pathotype is believed to include a large proportion of the nonresponders to biologic DMARDs (9,10). In addition, erosive disease can be seen in patients with combinations of RA, PsA, and SpA (11,12). There are also some links between pathobiology and DMARD-specific treatment responses. Thus, IL-6 inhibition seems to be more efficacious in RA patients with a high C-reactive protein level (13) and inhibition of lymphocytes with either rituximab or abatacept is more efficacious in anticitrullinated protein antibody–positive RA patients (14). Furthermore, TNFα inhibitors seem to be superior in patients with a CD68-positive macrophage-dominated synovium (9) and are most effective in reducing erosive joint damage in RA (15). In PsA, treatment with different DMARDs based on T cell phenotyping was recently shown to be beneficial (16).

The increase in treatment options now requires more definitive studies on how to optimize patient-tailored therapy in IMIA. Therefore, we used in vitro models that mimic different pathotypes of IMIA to study potential associations between the treatment effect of different cytokine inhibitors and the cellular composition of the cultures. The first model used was synovial fluid mononuclear cell (SFMC) 48-hour cultures. SFMCs have previously been shown to consist of primarily lymphocytes and cells of the monocyte and macrophage lineage (17). Cultures of SFMCs have been shown to preserve functional characteristics, such as stimulated proliferation and spontaneous production of autoantibodies (18,19). Therefore, this culture was used as a model of a lymphocyte- and monocyte-dominated environment (20). SFMCs cultured for 21 days can differentiate into osteoclasts without the addition of any inducers in vitro. The cells could differentiate into osteoclasts only because they were primed in vivo in the inflamed joint (21,22). This model was used to mimic inflammatory osteoclastogenesis. The fibroblast-like synovial cell (FLS) and peripheral blood mononuclear cell (PBMC) co-cultures have previously been used to evaluate the contribution of fibroblast-leukocyte interactions and treatment response of immunosuppressive compounds in IMIA (23–25). Therefore, this model was used to study immune reactions in an environment dominated by fibroblasts. The model was further optimized by using autologous PBMCs to prevent any contribution of graft versus host reactions to the model. DMARDs included in this study were methotrexate, adalimumab, etanercept, tocilizumab, anakinra, ustekinumab, secukinumab, tofacitinib, and baricitinib. Collectively, the objective of this study was to examine the effect of different DMARDs on different synovial cell subsets using three human ex vivo models of IMIA.

MATERIALS AND METHODS

Patients and samples. A cross-sectional, paired set of PBMCs and SFMCs were obtained from patients with chronic RA (n = 10), peripheral SpA (n = 5), or PsA (n = 3) with at least one swollen joint (from where synovial fluid was acquired) (n = 18) at the outpatient clinic at Aarhus University Hospital at the time of therapeutic arthrocentesis (Table 1). Not all patient samples were used in all experiments. The exact number of patients in each experiment is stated in the figure legends. We did not have access to data on treatment for all patients.

Ethics. All samples were obtained after informed written consent was provided according to the Declaration of Helsinki and approved by the Local Ethics Committee (the Central Denmark Region committee on health research ethics, project 20121329) and the Danish Data Protection Agency.

Culture conditions. Human PBMCs, SFMCs, and FLSs were cultured with the DMARDs shown in Table 2. In all experiments, untreated cells or cells treated with dimethyl sulfoxide (DMSO) were used as matched controls, and cells cultured with lipopolysaccharide at 100 ng/ml were included as a positive control. Supernatants were harvested after centrifugation of the culture plates at 1200 rpm for 5 minutes, and the cell-free supernatant

Table 1. Patient characteristics[a]

Diagnosis	RA (n = 10)	SpA (n = 5)	PsA (n = 3)
Disease activity			
VAS (0-100)	58.5 (40-84)	74.5 (37-86)	40.0 (-)
Swollen joint count (0-28)	2.0 (1–7)	1.5 (1-2)	1.0 (1-2)
HAQ (0-3)	0.3 (0-1)	0.5 (0.1-1.4)	0.8 (-)
CRP (mg/L)	31.5 (19-105)	29.0 (5-62)	36.0 (5-67)
DAS28CRP (0-10)	4.2 (4-6)	4.0 (3-5)	3.0 (-)
Treatment			
MTX (n)	4	1	1
TNFα inhibitor (n)	0	2	2
Salazopyrin (n)	1	1	1

Abbreviation: CI, confidence interval; CRP, C-reactive protein; DAS-28CRP, disease activity score 28 based on CRP; HAQ, Health Assessment Questionnaire; MTX, methotrexate, PsA, psoriatic arthritis; RA, rheumatoid arthritis; SpA, spondyloarthritis; TNF, tumor necrosis factor; VAS, Visual Analogue Scale.
[a]Data are expressed as the mean (CI) or percentage were indicated. A few patients had missing treatment data or were treated with multiple compounds. No patients received other biological DMARDs besides TNFi.

Table 2. List of disease-modifying antirheumatic drugs used in the ex vivo cultures

Category	Drug Names								
Generic name	Adalimumab	Etanercept	Tocilizumab	Anakinra	Ustekinumab	Secukinumab	Tofacitinib	Baricitinib	Methotrexate
Commercial name	Humira	Enbrel	RoActemra	Kineret	Stelara	Cosentyx	Tofacitinib citrate	Baricitinib citrate	Ebetrex
Source	AbbVie	Pfizer	Roche	Swedish Orphan Biovitrum	Janssen	Novartis	Selleckchem	Selleckchem	Sandoz
Concentration	5 μg/ml	5 μg/ml	5 μg/ml	10 μg/ml	5 μg/ml	5 μg/ml	200 nM	200 nM	0.5 μg/ml
Target	TNFα	TNFα	IL-6R	IL-1R	IL-12/23	IL-17A	JAK1/JAK2	JAK1/JAK3	Not known

Abbreviation: IL, interleukin; JAK, Janus kinase; TNF, tumor necrosis factor.

was thereafter stored at –80°C for later analysis. Monocyte chemoattractant protein 1 (MCP-1) was chosen as a readout because this chemokine is highly increased in both RA and SpA (26,27). Furthermore, MCP-1 has been extensively studied in a variety of inflammatory diseases and is known to be produced by many cells, including monocytes and fibroblasts. Finally, we had to choose a readout not directly affected by any of the included drugs (28).

SFMC 48-hour ex vivo model. The SFMC 48-hour culture is an in vitro model of IMIA dominated by lymphocytes and monocytes (20,29). SFMCs were isolated by conventional Ficoll-Paque (GE Healthcare) density-gradient centrifugation and cryopreserved at –150°C. The cells were then thawed and seeded in Dulbecco's modified Eagle's medium, 10% fetal calf serum, penicillin, streptomycin, and glutamine (culture medium) for 48 hours at a concentration of 1×10^6 cells/ml (n = 14) with or without the DMARDs in Table 2 and were kept in a humidified incubator at 37°C and 5% CO_2 as described previously (30). After 48 hours, the cell-free culture supernatants were analyzed for the concentration of MCP-1.

SFMC 21-day ex vivo model. The 21-day culture is an in vitro model of low-grade inflammatory osteoclastogenesis containing macrophage-like synovial cells (21). Long-term cultures were performed as described for the SFMC 48-hour model, changing the medium every 2 to 3 days as previously described for a total of 21 days with or without the DMARDs in Table 2 (21,31) (n = 10). Culture supernatants were analyzed for the enzyme activity of tartrate-resistant acid phosphatase (TRAP).

FLS 48-hour ex vivo model. The 48-hour co-culture with FLSs and PBMCs is an in vitro model of IMIA dominated by FLSs (23). FLSs were grown from SFMCs, and the medium was changed every 2 to 3 days. When the cell layer was 70% confluent, the FLSs were passaged by trypsin/EDTA treatment and used for analyses at passage 4 to 5 as previously described (32,33). The FLSs were then cultured at 2×10^4 cells/ml with either allogenic PBMCs from a healthy donor (n = 6) or paired autologous PBMCs (n = 6) at 1 to 2×10^6 cells/ml in culture medium for 48 hours. Initially, FLS and PBMC monocultures were used as controls. Culture supernatants were analyzed for the concentration of multiple cytokines (interferon-gamma [IFN-γ], MCP-1, TNFα, interleukin [IL]-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, and IL-13). The co-cultures with FLSs and autologous PBMCs were then grown in culture medium for 48 hours (n = 6) with or without the DMARDs in Table 2. The culture supernatants were analyzed for the concentration of MCP-1.

MCP-1 enzyme-linked immunosorbent assay and TRAP measurement. The concentration of MCP-1 was analyzed by a commercially available enzyme-linked immunosorbent assay (Biolegend) according to the manufacturer's instructions. The concentration of TRAP was analyzed by an enzymatic assay (B-bridge International) according to the manufacturer's instructions.

Electrochemoluminescence-based sandwich immunoassay. The concentrations of IFN-γ, TNFα, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, and IL-13 were analyzed using multiplex electrochemoluminescence-based sandwich immunoassay (V-PLEX Pro-inflammatory Panel 1, Meso Scale Discovery) according to the manufacturer's instructions.

Statistics. Statistical analyses and graphs were done using GraphPad Prism 7 for Mac (GraphPad Software). All data were transformed to ratios by dividing the value of the samples with the value of either untreated cultures (biological DMARDs and methotrexate) or DMSO-treated cultures (tofacitinib and baricitinib). Data were analyzed with paired or unpaired *t* test. A two-sided P value < 0.05 was considered statistically significant.

RESULTS

Most DMARDs reduced the MCP-1 production from IMIA SFMCs cultured for 48 hours. We first tested whether the different DMARDs affected production of MCP-1 by SFMCs. SFMCs were cultured for 48 hours with or without DMARDs (Table 2). All DMARDs except anakinra resulted in a decrease in MCP-1 production in most cultures. The reduction of MCP-1 was significantly different from controls in cultures with methotrexate (0.93 mean [confidence interval (CI), 0.86-1.00;

$P < 0.05$]), adalimumab (0.74 mean [CI, 0.55-94; $P < 0.05$]), etanercept (0.77 mean [CI, 0.60-93; $P < 0.01$]), ustekinumab (0.80 mean [CI, 0.70-0.91; $P < 0.01$]), secukinumab (0.81 mean [CI, 0.72-0.90; $P < 0.001$]), and baricitinib (0.75 mean [CI, 0.60-0.90; $P < 0.05$]). The decrease seen with tocilizumab and tofacitinib did not reach statistical significance (Figure 1). Furthermore, the response to the different DMARDs was analyzed separately for RA, PsA, and SpA. There were no obvious differences in treatment responses in the three disease subgroups. However, the low sample size in the PsA and SpA subgroups makes it impossible to do statistical analyses (Supplementary Figure 1).

TNFα inhibitors were the only DMARDs to inhibit TRAP-positive osteoclastogenesis from adherent synovial cells. We used SFMCs cultured for 21 days as an inflammatory osteoclastogenesis model. Here, adalimumab and etanercept robustly reduced the secretion of TRAP (0.78 mean [CI, 0.63-0.92]) and (0.67 mean [CI, 0.58-0.76; $P < 0.01$ and $P < 0.0001$]). Anakinra showed a significant but very modest reduction in the secretion of TRAP (0.93 mean [CI, 0.87-0.99; $P < 0.05$]). In the SFMCs cultured for 21 days, methotrexate, ustekinumab, secukinumab, tocilizumab, tofacitinib, and baricitinib did not significantly decrease the secretion of TRAP (Figure 2). The responses to the different DMARDs were again analyzed separately for RA, PsA, and SpA without obvious differences (Supplementary Figure 2).

Co-cultures with FLSs and PBMCs produced higher levels of MCP-1 than monocultures. We next evaluated the effect of the DMARDs in an inflammatory environment dominated by fibroblasts. First, we addressed whether untreated co-cultures of FLSs with PBMCs were different from monocultures measured as the production of MCP-1. In co-cultures with FLSs and PBMCs, there was much higher production of MCP-1 (3890.0 pg/ml mean) compared with monocultures of either FLSs (7.8 pg/ml mean [cutoff]) or PBMCs (124.0 pg/ml mean) measured after 48 hours. However, none of these results were statistically significant because of the small number of cultures ($n \leq 2$) (Supplementary Figure 3A).

Cytokine production in FLS co-cultures with autologous PBMCs and FLS co-cultures with allogenic PBMCs was not statistically different. We then investigated whether co-culture of FLSs and PBMCs was influenced by the PBMCs being either autologous or allogenic. This difference was evaluated by measuring IFN-γ, TNFα, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and MCP-1 in the co-culture supernatants. Overall, production from co-cultures of FLSs with allogenic PBMCs was 1.8 (1.8 mean [CI 1.5-2.1]) times higher than the production by co-cultures of FLSs with autologous PBMCs. The amount of IL-12p70, IL-1β, IL-2, and TNFα on average more than doubled in co-cultures with allogenic PBMCs compared with autologous PBMCs. None of these differences reached statistical significance (Supplementary Figure 3B).

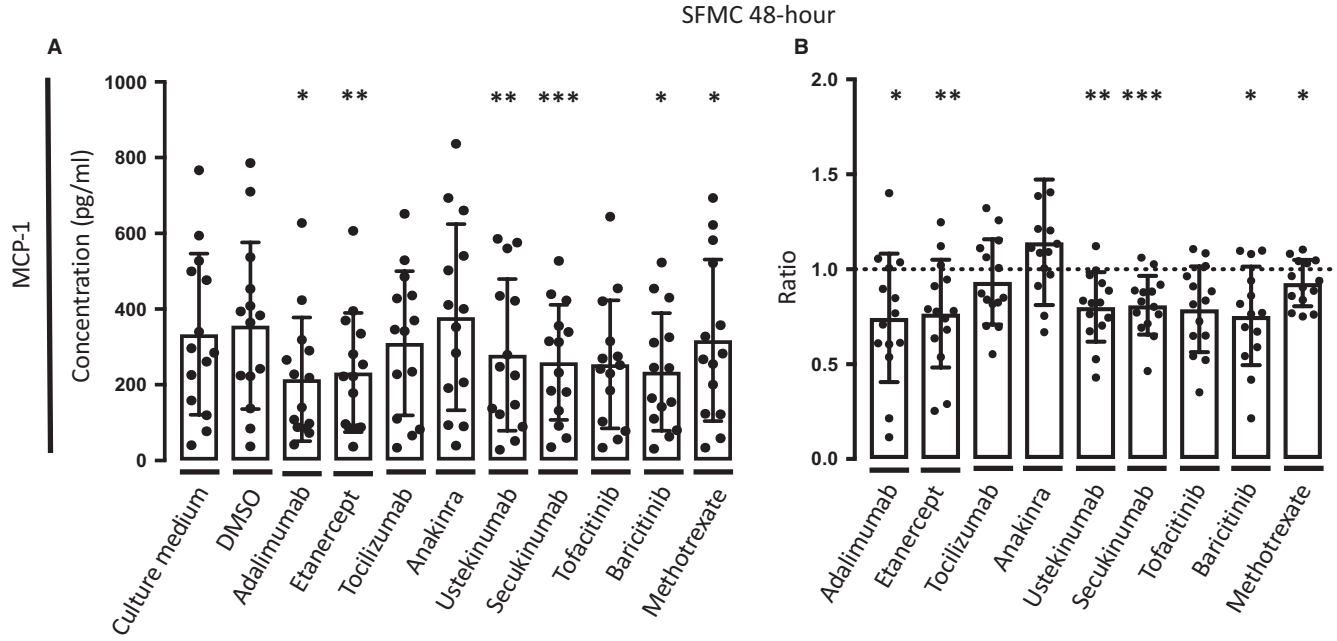

Figure 1. Secretion of monocyte chemoattractant protein (MCP-1; n = 14) by synovial fluid mononuclear cells (SFMCs) cultured for 48 hours in culture medium, treated with dimethyl sulfoxide (DMSO), or treated with the panel of different disease-modifying antirheumatic drugs (DMARDs) in Table 2. **A**, Data are expressed as concentrations. **B**, Data are expressed as ratios of the concentration of MCP-1 in DMARD-treated cultures divided by the concentration in untreated cultures (biological DMARDs and methotrexate) or DMSO-treated cultures (tofacitinib and baricitinib). Differences were analyzed using the paired *t* tests to compare two groups. Bars indicate mean and SD. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

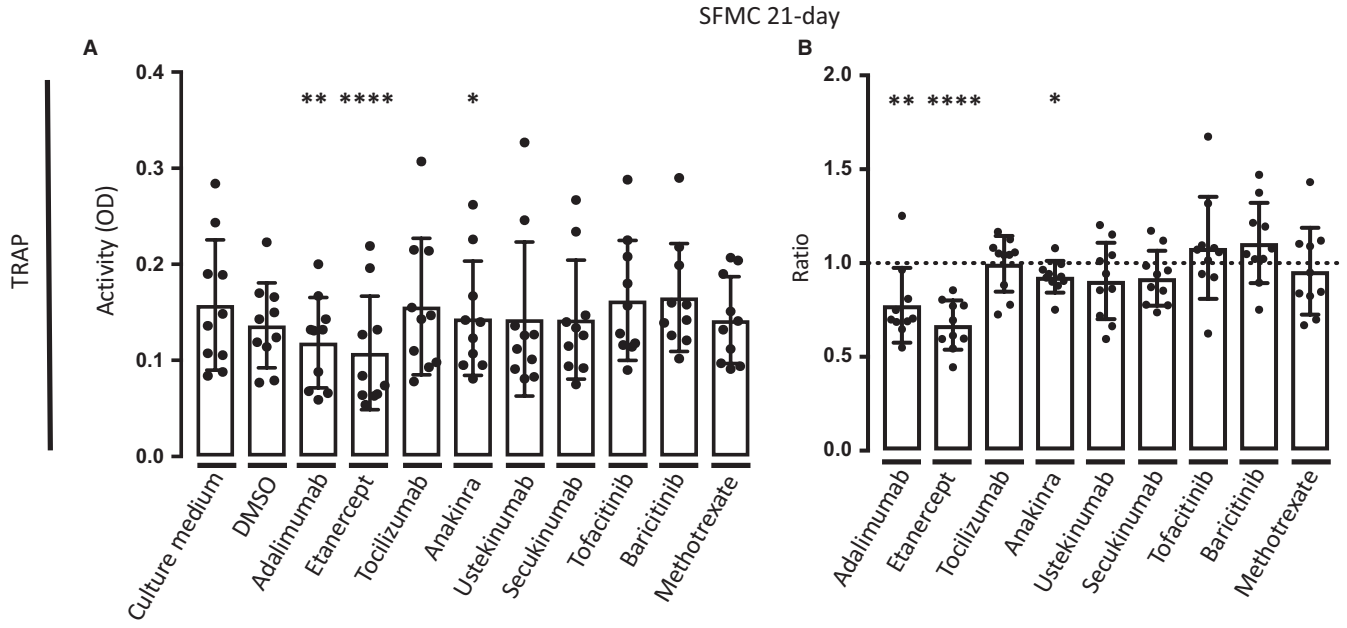

Figure 2. Production of tartrate-resistant acid phosphatase (TRAP; n = 10) by synovial fluid mononuclear cells (SFMCs) cultured for 21 days in culture medium, treated with dimethyl sulfoxide (DMSO) control or treated with the panel of different disease-modifying antirheumatic drugs (DMARDs) in Table 2. **A**, Data are expressed as TRAP activity. **B**, Data are expressed as ratios of the activity of TRAP in DMARD-treated cultures divided by the concentration in untreated cultures (biological DMARDs and methotrexate) or DMSO-treated cultures (tofacitinib and baricitinib). Differences were analyzed using the paired *t* tests to compare two groups. Bars indicate mean and SD. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

Tocilizumab considerably reduced the production of MCP-1 from co-cultures with FLSs and autologous PBMCs. Next, the effects of different DMARDs were evaluated in the co-cultures of FLSs and autologous PBMCs. Tocilizumab (0.449 mean [CI, 0.30-0.59; $P < 0.001$]) and the two Janus kinase (JAK) inhibitors tofacitinib (0.49 mean [CI, 0.34-64; $P < 0.05$]) and baricitinib, (0.38 mean [CI, 0.27-49; $P < 0.05$]) were exclusive in lowering the production of MCP-1 by roughly 50%. A significant decrease was also observed in cultures with ustekinumab (0.82 mean [CI, 0.70-0.94; $P < 0.05$]), secukinumab (0.78 mean [CI, 0.58-0.97; $P < 0.05$]), and methotrexate (0.68 mean [CI, 0.58-79; $P < 0.001$]), though to a much lesser extent. Furthermore, the level of MCP-1 was significantly lower in tocilizumab-treated co-cultures compared with co-cultures treated with either of the TNFα inhibitors adalimumab (0.80 mean [CI, 0.51-1.1; $P < 0.05$]) or etanercept (0.91 mean [CI, 0.62-1.2; $P < 0.01$]) (Figure 3). The response to the different DMARDs was again analyzed separately for RA, SpA, and PsA without obvious differences (Supplementary Figure 4).

DISCUSSION

One of the big challenges in the management and treatment of IMIA is to choose the right treatment to the right patient (34,35). Recent findings indicate that it might be possible to stratify patients based on synovial pathobiology and cellular subsets found in the peripheral blood (7,16,36,37). Subtyping these diseases based on phenotyping and pathobiology may provide important insights into the heterogeneity of RA, SpA, and PsA, opening the possibility of treatment matched to the dominating pathogenic pathways or immunological mechanism (38). This approach is already being implemented in oncology and hematology.

Our study reveals a fairly diverse response signature between various DMARDs in different ex vivo models. First, SFMC 48-hour cultures were used. This model has been extensively used following the identification of the in vitro effect of TNFα inhibition 3 decades ago (29). In the SFMC 48-hour cultures, all DMARDs except anakinra effectively decreased MCP-1 production. This is in line with the literature showing that IL-1 receptor blockade is less effective in the treatment of RA compared with other biological DMARDs such as IL-6 or TNFα inhibition (39). Anti-TNFα treatment was found to most effectively decrease the MCP-1 production. The decrease in MCP-1 could be caused by either a decrease in the production of MCP-1 per monocyte or a change in the number or subsets of monocytes.

Next, SFMC 21-day cultures were studied. This is a simplistic model of inflammatory osteoclastogenesis without the addition of macrophage colony-stimulating factor or receptor activator of nuclear factor kappa-B ligand (22). In this model, only anti-TNFα treatment reduced the production of TRAP and was thus superior to all the other DMARDs we tested. This is in line with the finding that anti-TNFα treatment reduces erosive joint damage in RA (15) even though the literature on this matter is contradictory. Our SFMC 21-day culture model has specific limitations because the osteoclast formation in our model is only modest and potentially not effective enough to detect small treatment effects. This implies

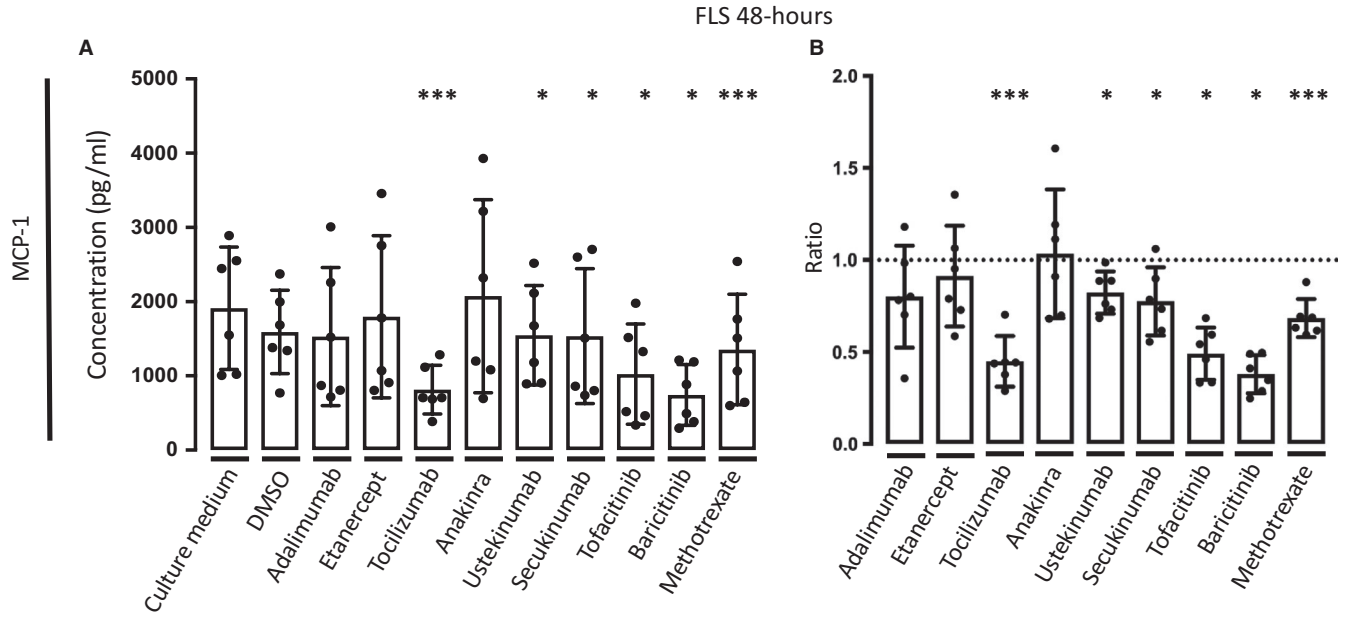

Figure 3. Secretion of monocyte chemoattractant protein (MCP-1; n = 6) by co-cultures of fibroblast-like synovial cells (FLSs) and autologous peripheral blood mononuclear cells (PBMCs) cultured for 48 hours in culture medium, treated with dimethyl sulfoxide (DMSO) control, or treated with the panel of different disease-modifying antirheumatic drugs (DMARDs) in Table 2. **A**, Data are expressed as concentrations. **B**, Data are expressed as ratios of the concentration of MCP-1 in DMARD-treated cultures divided by the concentration in untreated cultures (biological DMARDs and methotrexate) or DMSO-treated cultures (tofacitinib and baricitinib). Differences were analyzed using the paired *t* tests to compare two groups. Bars indicate mean and SD. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

that some of the DMARDs could potentially have an effect in models with more pronounced osteoclastogenesis.

Finally, co-cultures of FLSs and PBMCs were used. The differences observed between the co-cultures with FLSs and autologous PBMCs compared with allogenic PBMCs points to an increased activation in cultures with allogenic PBMCs in which T cells encounter foreign FLSs. This could be due to a degree of graft-versus-host response. However, of note, the autologous and allogenic responses were comparable without statistically significant differences. IL-6 receptor inhibition with tocilizumab showed a selective effect on co-cultures of FLSs and autologous PBMCs, whereas anti-TNFα treatment had no or very limited effect. These findings are supported by a recent study showing that IL-6 is central for the transformation of FLSs in early RA from an immunosuppressive to an inflammatory phenotype (40). The IL-6 pathway has been further shown to be important in FLSs, evaluated by joint-specific DNA methylation in RA (41). Furthermore, IL-6 knockout mice have been shown to be protected from developing arthritis (42). IL-6 is primarily produced by FLSs and mediates the recruitment and activation of T cells at sites of chronic inflammation (43,44). Taken together, this study underlines the central role of IL-6 in synovial microenvironments dominated by FLSs.

Methotrexate induced a significant reduction of MCP-1 production by both SFMC 48-hour cultures and co-cultures of FLSs and PBMCs. This finding is in line with the actions of methotrexate in arthritis being both immunosuppressive and anti-proliferative. Methotrexate is known to lower both IL-6 and TNFα production (45).

It is interesting that anti-TNFα had the most pronounced effect in the SFMC 21-day cultures, whereas tocilizumab showed the most robust decrease in MCP-1 production in the FLS and PBMC co-cultures. This finding indicates that differences in cellular subsets in the inflammatory microenvironment are likely to determine the effectiveness of DMARDs with differences in mode of action. This is in line with studies showing that targeting the IL-6 receptor is beneficial in RA anti-TNFα nonresponders (46). The different response signatures between anti-TNFα and tocilizumab observed in this study are also supported by the fact that the primary downstream targets are different. Tocilizumab affects the JAK/signal transducer and activator of transcription proteins pathway. In contrast, anti-TNFα primarily affects the NF-κB pathway (8). The two JAK inhibitors tofacitinib and baricitinib were also found to profoundly decrease the production of MCP-1 in the FLS and PBMC co-cultures. These JAK inhibitors modulate IL-6 production by inhibition of JAK1/JAK2 (47). This could be the mechanism for the treatment effect in the FLS-dominated ex vivo model. However, JAK inhibition also modulated signaling through many other cytokine receptors, preventing signaling from IFN-γ, IL-2, IL-7, and IL-23. Taken together, the present study supports the hypothesis that different pathotypes of IMIA respond differently to different DMARD treatments. Specifically, this study substantiates that patients with the fibroid pathotype could probably benefit from a treatment targeting the IL-6 axis

rather than anti-TNFα treatment, as has been suggested previously (41).

Our study has some notable limitations. First, the ex vivo study findings cannot be translated directly into an in vivo setting. It is unlikely that these models truly reflect disease pathotypes of IMIA. Furthermore, the cellular composition and activation state of specific signaling pathways could be very different in comparing the patient samples. This might explain some of the variation in the treatment response for each of the DMARDs in each of the ex vivo models. In line with this, the patients received different treatments at the time of sampling (Table 1). This could also influence the results. However, patients were primarily receiving methotrexate or a TNFα inhibitor, and these compounds still showed significant treatment effects in the SFMC 48-hour cultures (even though the patients could be classified as at least partial nonresponders to these drugs). Drug concentrations were chosen in order to simulate in vivo plasma concentrations as well as possible (Table 2). Our in vitro models also have some strengths. First, the in vitro design allowed paralleled assessment of different DMARDs on cells activated in vivo with no further ex vivo stimulation. Furthermore, the in vitro experimental design offers us the ability to isolate the different components of arthritis pathobiology and evaluate them separately.

This study reveals a fairly sharp distinction between the efficacy profiles of the different DMARDs on different cellular subsets derived from the synovium of patients with IMIA. Tocilizumab showed a clear reduction in an FLS-dominated environment, whereas anti-TNFα treatment had the most pronounced effects in the SFMC 21-day culture. This study herby points to a different efficacy profile of the different DMARDs, depending on the cellular subsets present in the in vitro culture. This could be of great interest in the future when synovial biopsies or other stratification tools are further established in the clinic and holds promises for future studies on treatments based on the composition of the synovial environment.

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AUTHOR CONTRIBUTIONS

Drs. Nielsen, Buckley, and Kragstrup drafted the manuscript. All authors were involved in revising the manuscript and read and approved the final manuscript.

Study conception and design. Nielsen, Kragstrup.
Acquisition of data. Nielsen, Lomholt, Mellemkjær, Andersen, Kragstrup.
Analysis and interpretation of data. Nielsen, Buckley, Kragstrup.

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