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Gender-related differences observed among immune cells in synovial fluid in knee osteoarthritis

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SUMMARY

Objective: There is no existing comprehensive report on the cellular composition of synovial fluids (SFs) from knee osteoarthritis (OA). We therefore aimed to characterise the immune cell composition in SFs from knee OA (KOA) and in subgroups according to gender.
Design: The immunophenotyping of monocyte/macrophage lineage cells, T and B cells, NK cells, neutrophils, dendritic and mast cells (MC) present in SFs from 53 patients (24 males/29 females) with KOA was performed using 6-colour flow cytometry.
Results: SFs from patients with OA contained 90% hematopoietic cells. Lymphocytes were the predominant cell population (44.8%) in the SFs of OA patients, with $CD4^+$ T lymphocytes being more prevalent than $CD8^+$ T cells ($CD4^+/CD8^+$ ratio = 1.3). Within the monocyte/macrophage lineage gating, monocytes accounted for 33.9%, macrophages 14.8%, myeloid dendritic cells 16.4%. The rest of the hematopoietic cells were comprised of neutrophils (8%), NK cells (3.8%), T regulatory cells (1.2%), plasmacytoid dendritic cells (1.1%), mast cells (0.3%). In OA females, a higher percentage of $CD4^+$ T cells ($P = 0.023$), macrophages ($P = 0.012$), and a lower percentage of monocytes ($P = 0.008$) and $CD8^+$ T cells ($P = 0.002$) were detected in comparison to OA males.
Conclusions: Based on the immune cell composition of SFs, data mining analysis revealed distinct phenotypes (monocyte- and lymphocyte-predominant) within each gender group. This first study on the cellular complexity of SFs in KOA showed marked differences between male and female patients. The findings give a rational starting point for patient stratification according to their phenotypes, as is required for phenotype-specific treatment strategies.
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Introduction

Osteoarthritis (OA) is increasingly recognised as a highly heterogeneous group of diseases characterised by variable clinical phenotypes[1,2]. OA heterogeneity underlines the various responses to current therapies for individual patients and inconsistency of outcomes observed in clinical practice[1,2]. There is, therefore, an urgent need to differentiate between distinct clinical phenotypes, especially in the early stages of the disease and progressive phenotypes.

There is currently a growing body of evidence concerning the crucial role of immune cells in OA disease progression and chronicity[3]. Although immune cells may significantly influence the disease development in OA and may contribute to the development

Abbreviations: ACL, anterior cruciate ligament; ASC, arthroscopy; BSA, bovine serum; DAF, decay-accelerating factor; FSC, forward scatter; FSC-A, forward scatter area; KL, Kellgren-Lawrence; KOA, knee osteoarthritis; LPS, Lipopolysaccharide; Mϕ, macrophage; mDC, myeloid dendritic cell; MC, mast cell; MFI, median fluorescence intensity; MMP, matrix metalloproteinase; OA, osteoarthritis; PBS, Phosphate-buffered saline; pDC, plasmacytoid dendritic cells; RA, rheumatoid arthritis; SSC, side scatter; SSC-a, side scatter area; SF, synovial fluid; TIMP-1, tissue inhibitor of metalloproteinase-1; TKA, total knee arthroplasty; Treg, T regulatory.

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of a particular disease phenotype, the complex cellular composition of synovial fluid (SF) in knee OA (KOA) has not yet been characterised. Current knowledge regarding immune and non-immune cells in OA comes mainly from the histopathological assessment of the synovial membranes, which shows the prevalence of macrophages and T cells, followed by mast cells (MC)[4–6]. Regarding the cells in synovial fluids (SFs) in OA, only a few studies exist, particularly on cultured cells[7,8] or selected subpopulations such as MC[9], plasmacytoid dendritic cells (pDC)[10], T cells[11], and T regulatory cells (Treg)[12]. Thus, there remains a need to characterise the cellular composition of SFs, in order to gain the ability to search for individual phenotypes associated with OA development and progression based on immune cell composition and in turn link this to a particular treatment.

We, therefore, aimed to study the cellular complexity of freshly collected SFs in a sizable cohort of KOA patients as well as in subgroups according to gender, using flow cytometry. The comprehensive immunophenotyping of immune cells in SFs may advance our understanding of a particular phenotype/stage of OA and provide an eligible rationale for suggesting an appropriate phenotype-based treatment.

Material and methods

Patients

A total of 53 patients with KOA were enrolled in our study. The inclusion criteria were as follows: aged between 40 and 85 years, no evidence of inflammatory KOA, gout/pseudogout and endocrine disorders at the time of sampling, and no injections of hyaluronic acid and/or steroids or knee surgery within the previous 3 months. Only patients with synovial effusion were enrolled in the study. All patients had to fulfil the clinical and radiographic criteria for the diagnosis of KOA[13,14]. To grade KOA, the Kellgren-Lawrence (KL) radiographic classification was determined by an experienced orthopaedic surgeon for the knee joint on anteroposterior, lateral, and Merchant projections. In order to cover the full clinical spectrum of patients, KL grade I was also included. The extension of OA changes over a knee was expressed as follows: 0 = early stage of KOA (i.e., neither compartment is affected by KL grade 2 and higher); 1 = either medial or lateral tibiofemoral joint is affected with a KL grade 2 or higher; 2 = both the medial and lateral tibiofemoral compartments affected with a KL grade 2 or higher. The pain was scored at the time of SF sampling according to patient self-reports based on an 11-point scale (0–10) as follows: no pain (0 points), mild (1–2 points), moderate (3–4 points), severe (5–7 points), and very severe (>7 points). In enrolled patients, arthroscopic findings were collected in cases where an arthroscopy had been performed. Infections were excluded if there was (1) an absence of fever; (2) no local signs of infection; (3) CRP<10 mg/L, and (4) a synovial white cell count of less than 25 × 10^9/L. Patients were assessed for eligibility at the time of an SF aspiration visit or before knee surgery. A questionnaire was used to exclude the use of the common anti-inflammatory drugs at the time of SF sampling. Patient characteristics are summarised in Table I and Supplementary Table S1.

The samples were collected after obtaining informed consent of the patients and approval from the ethics committee of University Hospital and Palacky University Olomouc (No. VES16-31852A).

SF sampling and processing

SFs from the affected knee joints were collected by needle aspiration during (1) outpatient examination requiring SF aspiration; (2) arthroscopy or (3) primary surgery for total knee arthroplasty (TKA) (Table I, Table S1). In patients treated arthroscopically for suspected meniscus rupture, anterior cruciate ligament (ALC) rupture or focal cartilage defects, SF was collected at the beginning of the arthroscopy. In patients operated on due to TKA, SF was sampled just before knee joint capsule incision.

SFs were carefully cross-examined (volume, colour, opacity and viscosity) (Table I) and processed within 2 h of fluid collection. The total number of immune cells in SFs (mean 0.424 × 10^9/L, 95% confidence interval 0.132–0.715 × 10^9/L) was assessed by $CD45^+$ positivity using flow cytometry. For immunophenotyping, the cell debris was removed by double-filtering of the SFs through 70 μm pore size filter (Miltenyi Biotec), followed by a wash in PBS and sequential centrifugation steps at 4°C to remove remaining SF. The resulting cell pellet was further aliquoted and stained with monoclonal fluorescent antibodies. The microbial culture and synovial white cell count were assessed as reported previously[15] when an appropriate amount of SF was available.

Table I
Summarized clinical and demographic data for enrolled osteoarthritis (OA) patients Q2

Demographic characteristics of patients	
No. of patients	53
Gender (male/female)	24/29
Mean age (yr) range (min–max)	64.5 (46–83)
Mean BMI range (kg/m2)	30.4 (21.6–47.8)
KL grade of KOA 1/2/3/4	4/17/27/5
Extension of joint damage* 0/1/2	4/4/45
SF sampling as a part of (arthroscopy/TKA/outpatient examination)	24/9/20
Reason for arthroscopy (meniscal tear or rupture of ACL/cartilage defects)	9/15
Pain level† no pain/mild/moderate/severe/very severe	5/15/28/5/0
Fluid characteristics	
Fluid volume‡ low/intermediate/high/n.a.	6/28/17/2
Fluid appearance transparent/yellow/yellow + viscous/cloudy/orange/unknown	35/3/2/7/5/1

n.a.: not available/appropriate; ACL: anterior cruciate ligament; TKA: total knee arthroplasty; KL grade of KOA: classified according to Kellgren-Lawrence Grading Scale.

* The involvement of a particular joint space was estimated from radiography and scored as follows: 0 = early stage of OA; 1 = either medial or lateral tibiofemoral joint is clearly affected with a KL grade 2 and higher; 2 = both the medial and lateral tibiofemoral compartments affected with a KL grade 2 and higher.

† No pain (0), mild (1–2 points), moderate (3–4 points), severe (5–7 points), and very severe (>7 points) on the sampling day.

‡ Fluid volume: (+) low <5 mL, (++) intermediate 5–25 mL, (+++) high >25 mL.

Immunophenotyping of cells present in synovial fluids

Flow cytometry was used to analyse the percentage and activation status of immune cell populations (CD4+ and CD8+ T lymphocytes, T regulatory cells, B lymphocytes, NK cells, monocytes, macrophages, neutrophils, plasmacytoid and myeloid dendritic cells and mast cells) in SFs. The markers for immunophenotyping and the staining procedure are reported in the Supplementary file (Table S1). The main cell populations were identified using a sequential gating strategy after the exclusion of doublets (Fig. 1).

A six-colour flow cytometry analysis was performed on a BD FACSCanto II (Becton Dickinson). Flow cytometry data was analysed using FlowJo vX0.7 software (FlowJo, LLC). Results are expressed as the percentage of positive cells and median fluorescence intensity (MFI), defined as the difference between the MFI of the tested cells for each examined marker and the MFI of the background staining.

Statistics and data mining approach

Statistical tests (non-parametric Mann-Whitney-Wilcoxon test, Spearman's Rank–Order Correlations and the Shapiro-Wilk test for

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