Effect of Inflammatory Signaling on Human Articular Chondrocyte Hypertrophy: Potential Involvement of Tissue Repair Macrophages

Mauricio N. Ferrao Blanco, MSc, Yvonne M. Bastiaansen-Jenniskens, PhD, Mark G. Chambers, PhD, Andrew A. Pitsillides, PhD, Roberto Narcisi, PhD, and Gerjo J.V.M. van Osch, PhD

Abstract

Objective. In osteoarthritis, chondrocytes tend to acquire a hypertrophic phenotype, which contributes to the modification of the extracellular matrix, resulting in permanent cartilage changes. In mouse chondrocytes, pro-inflammatory macrophages and pro-inflammatory cytokines have been shown to stimulate hypertrophy via the activation of the nuclear factor kappa B (NF-κB) pathway. Whether or not this also occurs in human chondrocytes remains unclear. We therefore aimed to investigate whether hypertrophy-like responses in human cartilage are driven mainly by intrinsic inflammatory signaling or shaped by specific macrophage populations. Design. Human articular chondrocytes were cultured with pro-inflammatory cytokines or medium conditioned by defined macrophage subsets. Furthermore, the effect of inhibition of NF-κB-dependent gene expression was evaluated using the NF-κB inhibitor SC-514. Hypertrophy was assessed by measuring the transcription level of alkaline phosphatase (ALPL), type X collagen (COL10A1), Indian hedgehog (IHH), and runt-related transcription factor 2 (RUNX2). Results. The expression of hypertrophic genes was not promoted in human chondrocytes by pro-inflammatory cytokines neither pro-inflammatory M(iFNγ + tNFα) macrophages. Inhibition of the NF-κB-dependent gene expression did not affect human articular chondrocyte hypertrophy. However, tissue repair M(il4) macrophages induced hypertrophy by promoting the expression of COL10A1, RUNX2, and IHH. Conclusion. Intrinsic inflammatory signaling activation is not involved in the hypertrophic shift observed in human articular chondrocytes cultured in vitro. However, tissue repair macrophages may contribute to the onset of this detrimental phenotype in human osteoarthritic cartilage, given the effect observed in our experimental models.

Keywords

osteoarthritis, articular chondrocytes, hypertrophy, inflammation, macrophages

Introduction

Osteoarthritis (OA) is characterized by progressive loss of articular cartilage, formation of osteophytes, degeneration of the ligaments, and inflammation of the synovium. Even though significant progress has been made in OA research in recent years, advances are still needed to understand the molecular mechanisms of OA in order to develop therapeutic strategies. Articular chondrocytes are responsible for maintaining the balance between catabolic and anabolic processes in the cartilage. In OA, this homeostatic state is lost and chondrocytes acquire an altered phenotype, promoting the degradation of the cartilage and vascularization. These hypertrophic-like chondrocytes are characterized by the expression of alkaline phosphatase (ALPL), type X collagen (COL10A1), Indian hedgehog (IHH), matrix...
metalloproteinase 13 (MMP13), and runt-related transcription factor 2 (RUNX2).\(^4\) Increased attention has been paid to the inflammatory process in OA, not only in the symptomatology but also in the pathophysiology of disease initiation and progression.\(^5\) Interestingly, inflammatory signaling activation can direct mouse chondrocytes toward hypertrophic differentiation through the nuclear factor kappa B (NF-\(\kappa\)B) pathway, which has a major role in the progression of OA in mice models.\(^6,7\) Macrophages play a prominent role in the progression of OA and are the dominant leukocyte population in inflamed osteoarthritic synovium.\(^8,9\) Macrophages are plastic cells that can acquire a pro- or anti-inflammatory phenotype, depending on environmental cues.\(^10\) Pro-inflammatory macrophages induced the upregulation of catabolic enzymes in human articular chondrocytes\(^14\) and have been shown to promote hypertrophy in mouse chondrocytes.\(^15\) Here we sought to understand whether chondrocyte hypertrophic-like responses in human cartilage are driven mainly by intrinsic inflammatory signaling, as in mouse, or shaped by specific macrophage populations.

Methods

Cartilage Explant and Chondrocyte Isolation

Human articular cartilage was obtained with implicit consent as waste material from patients undergoing total knee replacement surgery (9 females, 5 males, \(67 \pm 11\) years). This protocol was approved by the Medical Ethical Committee of the Erasmus MC, University Medical Center, Rotterdam, protocol number MEC-2004-322. Full thickness cartilage explants (\(\varnothing = 5\) mm) were harvested from macroscopically intact areas and washed twice with 0.9% NaCl (Sigma Aldrich, St. Louis, MO, USA). To isolate chondrocytes, cartilage chips were subjected to protease (2 mg/mL, Sigma Aldrich) for 2 hours followed by overnight digestion with 1.5 mg/mL collagenase B (Roche Diagnostics, Basel, Switzerland) in Dulbecco’s modified Eagle’s medium (DMEM) high glucose supplemented with 10% fetal bovine serum. Single cell suspension was obtained by filtering the cellular solution by a 100 \(\mu\)m filter. The isolated chondrocytes were expanded in monolayer at a seeding density of 7,500 cells/cm\(^2\) in DMEM high glucose supplemented with 10% fetal bovine serum, 50 \(\mu\)g/mL gentamicin, and 1.5 \(\mu\)g/mL fungizone (Gibco, Grand Island, NY, USA). Approximately 80% conflueny cells were trypsinized and reseeded at 7,500 cells/cm\(^2\). Cells were used for experiments after 3 passages.

Preparation of Macrophage Conditioned Medium

Monocytes were isolated from 2 buffy coats (males, 54 and 58 years, Sanquin, Amsterdam, the Netherlands) using Ficoll (GE Healthcare, Little Chalfont, UK) density gradient separation and cluster of differentiation (CD)14 magnetic-activated cell sorting microbeads (MACS; Miltenyi, Bergisch Gladbach, Germany). To prepare macrophage conditioned medium (MCM), monocytes were seeded in culture flasks at 500,000 monocytes/cm\(^2\) and cultured in X-VIVO TM-15 (Lonza, Verviers, Belgium) containing 20% heat-inactivated fetal calf serum (FCS; Lonza), 50 \(\mu\)g/mL gentamicin (Gibco), and 1.5 \(\mu\)g/mL fungizone (Gibco) at 37 °C and 5% CO\(_2\). Monocytes were stimulated with 10 ng/mL interferon-\(\gamma\) (IFN\(\gamma\); PeproTech, Rocky Hill, NJ, USA) and 10 ng/mL tumor necrosis factor-\(\alpha\) (TNF\(\alpha\), PeproTech) to obtain pro-inflammatory M(IFN\(\gamma\) + TNF\(\alpha\)) macrophages. Tissue repair M(IL-4) macrophages were obtained after stimulation with 10 ng/mL interleukin-4 (IL-4; PeproTech) and anti-inflammatory M(IL-10) macrophages were acquired by stimulation with 10 ng/mL IL-10 (PeproTech). After 72 hours, medium and stimuli were renewed and after 24 hours the medium was removed, the macrophages were washed twice with 0.9% NaCl and subsequently cultured in serum-free DMEM low glucose supplemented with 1% insulin-transferrin-selenium (ITS premix, BD Biosciences, San Jose, CA, United States), 50 \(\mu\)g/mL gentamicin, 1.5 \(\mu\)g/mL fungizone, and 25 \(\mu\)g/mL \(L\)-ascorbic acid 2-phosphate (Sigma Aldrich) to obtain MCM. After 24 hours, the MCM was harvested, centrifuged at 200 \(\times\) g and stored at −80 °C until use. The media conditioned by M(IFN\(\gamma\) + TNF\(\alpha\)), M(IL4), and M(IL10) macrophages were confirmed to contain a higher concentration of IL-6, CCL18, and sCD163, respectively (Supplementary Figure 1), in accordance with our previous work.\(^16^-19\) Nonconditioned DMEM low glucose supplemented with 1% ITS premix, 50 \(\mu\)g/mL gentamicin, 1.5 \(\mu\)g/mL fungizone, and 25 \(\mu\)g/mL \(L\)-ascorbic acid 2-phosphate was also incubated, centrifuged, and frozen to serve as control medium. Cells were harvested for DNA quantification with a modified CyQUANT assay (Invitrogen, Carlsbad, CA, USA). All MCM used for culture and analysis were frozen and thawed once. For further experiments 50% of MCM or 50% nonconditioned medium was mixed with 50% fresh medium to replenish potentially depleted nutrients.

Exposure of Chondrocytes and Cartilage Explants to Inflammatory Cytokines and Macrophage Conditioned Medium

In order to select an inflammatory stimulus, passage three chondrocytes cultured in a 6-well plate (BD Falcon, Bedford, MA, USA) at a seeding density of 20,000 cells/cm\(^2\) were exposed to pro-inflammatory cytokines (IL-1\(\beta\), TNF-\(\alpha\), or IFN-\(\gamma\)) at 1 ng/mL and, alternatively, to a combination of the
3 cytokines, each at 0.1 ng/mL. The combination of pro-inflammatory cytokines was selected based on a pilot experiment where nitric oxide (NO) was measured in the media as measurement of induction of inflammation (Supplementary Figure 2). Pro-inflammatory cytokines alone at 1 ng/mL did not significantly increase NO in the medium, which might be due to the basal NO production in OA chondrocytes.

To accommodate the likelihood that the acquisition of more fibroblast-like phenotype by these passage 3 chondrocytes may modify these responses, we examined behavior following redifferentiation. Briefly, redifferentiation of articular chondrocytes was performed using the well-established 3-dimensional alginate bead culture model,\(^{20}\) and confirmed by COL2A1 expression. Moreover, our data show that OA chondrocytes express the hypertrophic markers COL10A1 and RUNX2, being a suitable model to study chondrocyte hypertrophy. For preparation of alginate beads, chondrocytes after 3 passages in monolayer were resuspended in 1.2% (w/v) low-viscosity alginate (Kelton LV alginate, Kelko Co, San Diego, CA, USA) in 0.9% NaCl at a concentration of 4 × 10^6 cells/mL. Beads were made by drip-ping the cell-alginate suspension in 105 mM CaCl\(_2\) (Sigma Aldrich) through a 22-gauge needle. Beads were washed with 0.9% NaCl and DMEM low glucose. Beads with a size that deviated from the average after a visual inspection were not included in the experiment. Redifferentiation of chondrocytes was performed in a 24-well plate (BD Falcon) for 2 weeks in 100 µL/bead DMEM low glucose supplemented with 1% ITS fetal (Biosciences), 10 ng/mL transforming growth factor beta 1 (TGF-\(\beta\)1, recombinant human, R&D systems) 25 µg/mL 1-ascorbic acid 2-phosphate (Sigma Aldrich), 50 µg/mL gentamicin, and 1.5 µg/mL fungizone (both Gibco). After 2 weeks, TGF-\(\beta\)1 was no longer added to the medium and cells were either cultured with 10 µM of the NFKB inhibitor SC-514 (Cayman Chemicals, Ann Arbor, MI, USA) for 24 hours or with the combination of pro-inflammatory cytokines TNF\(\alpha\), IFN\(\gamma\), IL-1\(\beta\) at 0.1 ng/mL for 1 week, refreshing the medium twice.

Cartilage explants were cultured in DMEM low glucose supplemented with 1% ITS premix, 50 µg/mL gentamicin, and 1.5 µg/mL fungizone and either a combination of pro-inflammatory cytokines TNF\(\alpha\), IFN\(\gamma\), IL-1\(\beta\) at 0.1 ng/mL each or medium conditioned by macrophages during 1 week, refreshing the medium twice.

**Nitric Oxide Assay**

NO production was measured in the medium of OA chondrocytes by determining the content of nitrite using the Griess reagent (Sigma Aldrich). The reaction was monitored at 540 nm using a spectrophotometer (VersaMax; Molecular Devices, Sunnyvale, CA, USA). Sodium nitrite (NaNO\(_2\); Chemlab, Zedelgem, Belgium) was used as standard.

**Gene Expression**

Alginate beads were dissolved using citrate buffer, centrifuged at 200 × g and the pellet was resuspended in RLT (Qiagen, Hilden, Germany) buffer containing 1% \(\beta\)-mercaptoethanol for RNA isolation. RNA was isolated from the cartilage explants by snap freezing in liquid nitrogen followed by pulverization using a Mikro-Dismembrator (B. Braun Biotech International GmbH, Melsungen, Germany) at 2800 rpm. The tissue was homogenized with 18 µL/mg sample RNA-Beet TM (Tel-Test Inc., Friendswood, TX, USA) and 20% chloroform. mRNA isolation was performed according to manufacturer’s protocol utilizing the RNeasy column system (Qiagen, Hilden, Germany). The RNA concentration was determined using a NanoDrop spectrophotometer (Isogen Life Science, Utrecht, the Netherlands). 0.5 µg RNA was used for cDNA synthesis following the protocol of the manufacturer of the RevertAid First Strand cDNA kit (Thermo Fisher Scientific, Waltham, MA, United States). Quantitative polymerase chain reaction (qPCR) was performed on a Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad) to assess gene expression, alkaline phosphatase (ALPL, Fw: GACCCCTTGACCCCCCAACAT; Rev: CTCGTACTGATG TCCCCCT; Probe: TGAGCTACTTATGGTCTTCTGCA GCCA), Collagen type 2 (COL2A1; Fw: GGCAATAGCAG GTTCAGCTAC; Rev: CGATAACAGTCTGCTCCACCTT; Probe: CCGGTATGTTTTCGTGCAGCCATCTC), Collagen type 10 (COL10A1; Fw: CAAGGCCACCATCTCCAGGAA; Rev: AAGGGTTATTTGTGGCAGCATT; Probe: TCCA GCAGGCAGAAATCCATCTGA), matrix metalloproteinase-13 (MMP13; Fw: AAGGAGCATGCGGAGCATCTCT; Rev: TGG CCCAGGGAAAGGC; Probe: CCGTCTGGGCTTGGC GCTCA), Runx-related transcription factor 2 (RUNX2; Fw: AGCTTCGGGGTCCATCCA; Rev: TGGCAGTGTCTCAT CTGAAATG; Probe: ACTGGGCTCTTCGTCCATCCAGA), Tumor Necrosis Factor-a (TNF\(\alpha\); Fw: CGCCGATCTGCGTCTCTACT; Rev: AGCCGCTAGTCCGTCCACTAC), Indian hedgehog (IHH) primer was purchased as assays-on-demand from BioRad. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Fw: ATGGGGAAGTGGAAGTGTCG; Rev: TAAAAAGCAGCCCCGGTGGAC; Probe: CGCCCAA TACGACAAGATCCCTTGAC) was found stable and therefore used as reference gene. Data were analyzed by the ΔΔCt method and normalized to the expression of GAPDH of each condition and compared to the corresponding gene expression in the control groups. Articular cartilage explants were divided in hypertrophic and nonhypertrophic donors based on the expression of the hypertrophic markers COL10A1, RUNX2, and IHH in the basal – control condition, by using a cycle cutoff of 36. Donors with Cq of 36 or higher were classified as nonhypertrophic.

**Statistics**

Each experiment included at least 3 technical replicates and was repeated with cells/explants derived from at
least 3 OA donors. Statistical evaluation was performed using IBM SPSS 22.0. The normal distribution of the data was confirmed using the Kolmogorov-Smirnov test. The linear mixed model was applied using the different conditions as fixed parameters and the donors as random factors.

**Results**

**Pro-inflammatory Cytokines Did Not Promote Hypertrophy in Human Chondrocytes In Vitro**

To study the effect of pro-inflammatory signaling activation on chondrocyte hypertrophy, we used a combination of inflammatory cytokines that are secreted by macrophages, IL1β, TNFα, and IFNγ and used 2 different models, human articular cartilage explants and human articular chondrocytes in alginate. On inflammatory stimulation, the expression of the catabolic enzyme MMP13 was increased in cartilage explants and chondrocytes in alginate (Fig. 1A and B). Interestingly, the expression of the hypertrophic marker COL10A1 was significantly decreased in both models. RUNX2 was downregulated in alginate on cytokine addition and not detectable in explants. IHH and ALPL were not detectable in either of the models. To evaluate whether endogenous inflammation present in osteoarthritic chondrocytes influenced hypertrophy, the NF-κB inhibitor SC-514 was added to alginate cultures. SC-514 significantly decreased mRNA expression of the NFκB-dependent gene TNEA, confirming its efficacy (Fig. 1C). However, NFκB inhibition did not modify COL10A1, RUNX2, or MMP13 expression, indicating that hypertrophy is not affected in osteoarthritic chondrocytes when the main inflammatory pathway is inhibited (Fig. 1D). The expression of MMP13 is related to hypertrophy and to inflammatory signaling in chondrocytes. The absence of an effect of SC-514 on MMP13 expression thus suggests that MMP13 in these osteoarthritic chondrocytes is regulated by other transcription factors such as β-catenin.21,22 Summarizing, these data indicate that pro-inflammatory signaling did not stimulate a hypertrophic phenotype in human articular chondrocytes in vitro.

**Tissue Repair M(IL4) Macrophages Are Associated with the Onset of Human Chondrocyte Hypertrophy In Vitro**

To better mimic the complex combination of inflammatory factors in the joint, the medium conditioned by different macrophage phenotypes was evaluated for its capacity to modulate chondrocyte hypertrophy. Medium conditioned by pro-inflammatory M(IFNγ + TNFα) or anti-inflammatory M(IL10) macrophages had no effect on expression of COL10A1, RUNX2, IHH, or ALPL in cartilage explants (Fig. 2). Medium conditioned by tissue repair M(IL4) macrophages, however, significantly upregulated the expression of

---

**Figure 1.** Effect of pro-inflammatory signal activation in chondrocytes hypertrophy. (A) Osteoarthritic (OA) human cartilage explants and (B) chondrocytes encapsulated in alginate stimulated with the combination of the inflammatory cytokines at 0.1 ng/mL for 1 week (n = 3 donors, 3 samples per donor). (C and D) OA human chondrocytes encapsulated in alginate cultured with the NFκB inhibitor, SC-514 at 10 µM for 24 hours (n = 3 donors, 3 samples per donor). UD = undetectable. Data are shown as minimum to maximum.
**Discussion**

Inflammation-induced hypertrophy is a process that has been suggested to play role in the progression of osteoarthritis, but current studies mainly focused on mouse chondrocytes. The findings of our study demonstrate that pro-inflammatory cytokines do not mediate the hypertrophic differentiation of human articular chondrocytes in vitro. Inflammatory processes in OA are mainly driven by macrophages, which generate a broad spectrum of cytokines and immune factors. Previous studies in mouse have shown that pro-inflammatory macrophages induce hypertrophy. However, here we show that pro-inflammatory macrophages do not increase hypertrophy of human chondrocytes. In addition, although previous studies in mice chondrocytes have shown that the NF-κB pathway is responsible for inflammation-induced hypertrophy, here we showed that inhibition of NF-κB did not reduce hypertrophy in human osteoarthritic chondrocytes. These results indicate that, differently from murine chondrocytes, pro-inflammatory signaling cues do not unavoidably induce hypertrophy in human osteoarthritic chondrocytes that have been isolated from osteoarthritic knee joints and subsequently maintained in vitro.

Macrophages can acquire different phenotypes depending on the environmental stimuli, hence secreting cytokines that lead to various responses in the tissue. Our data suggest that tissue repair macrophages can induce a phenotypic shift in articular chondrocytes toward a hypertrophy state. A limitation of our study is that the number of nonhypertrophic OA donors was low, probably due to the late stage of disease in the majority of OA donors that undergo total knee replacement. These donors had a higher basal MMP13 expression compared with the hypertrophic donors, which might suggest that they had a higher basal inflammatory state. Even though the numbers are low, this demonstrates a proof of principle that macrophages with tissue repair phenotype have the capacity to induce hypertrophy. Interestingly, this macrophage subset secretes the cytokine TGFβ, which has been associated to hypertrophy in aging cartilage as well as in articular chondrocytes in culture.

Current literature in the field suggests that M1, also known as pro-inflammatory macrophages are detrimental for the disease while M2, also known as anti-inflammatory and tissue repair macrophages might have a protective role, driving the joint to homeostasis. Moreover, it has been suggested that drugs that alter macrophage phenotype from M1 to M2 would be an effective treatment for OA. However, our findings suggest that not only pro-inflammatory but also tissue repair macrophages contribute to chondrocyte catabolism.

Here we report that chondrocyte hypertrophy is not necessarily promoted in cultured human chondrocytes by pro-inflammatory signaling cues, as was observed in mice. Attention should be paid to the difference between human and murine chondrocytes when looking for disease modifying drugs, as hypertrophic differentiation might be differently regulated. Our data suggest that targeting tissue repair macrophages might be used as a therapy to inhibit hypertrophy of human chondrocytes.
Author Contributions
MNFB designed the study, performed the experiments, analyzed and interpreted the data, and wrote the manuscript. YMBJ and GJVMvO designed the study, interpreted the data and edited the manuscript. RN, MGC and AAP interpreted the data and edited the manuscript. All authors approved the final version of the manuscript.

Acknowledgments and Funding
We are grateful to Wendy Koevoet for the technical assistance. This work was performed within the framework of the Erasmus Postgraduate School Molecular Medicine and the Medical Delta RegMed4D program. This project has received funding from the European Union’s Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie grant agreement no. 721432 CarBon.

Declaration of Conflicting Interests
The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: M.G. Chambers is an employee of Eli Lilly but they did not fund this research.

Ethical Approval
The use of human osteoarthritic cartilage was approved by the Medical Ethical Committee of the Erasmus MC, University Medical Center, Rotterdam, protocol number MEC-2004-322.

ORCID iD
Mauricio N. Ferrao Blanco https://orcid.org/0000-0003-2639-0724

References
1. Ripmeester EJG, Timur UT, Caron MMJ, Welting TJM. Recent insights into the contribution of the changing hypertrophic chondrocyte phenotype in the development and progression of osteoarthritis. Front Bioeng Biotechnol. 2018;6:18.
2. Pesesse L, Sanchez C, Delcour JP, Bellahcène A, Baudouin C, Msika P, et al. Consequences of chondrocyte hypertrophy on osteoarthritic cartilage: potential effect on angiogenesis. Osteoarthritis Cartilage. 2013;21(12):1913-23.
3. He Y, Siebua AS, Brandt-Hansen NU, Wang J, Su D, Zheng Q, et al. Type X collagen levels are elevated in serum from human osteoarthritic patients and associated with biomarkers of cartilage degradation and inflammation. BMC Musculoskeletal Disorders. 2014;15:309.
4. von der Mark K, Kirsch T, Nerlich A, Kuss A, Weseloh G, Glückert K, et al. Type X collagen synthesis in human osteoarthritic cartilage. Indication of chondrocyte hypertrophy. Arthritis Rheum. 1992;35:806-11.
5. Aigner T, Reichenberger E, Bertling W, Kirsch T, Stöss H, von der Mark K. Type X collagen expression in osteoarthritic and rheumatoid articular cartilage. Archivs Arch B Cell Pathol Incl Mol Pathol. 1993;63(4):205-11.
6. Orita S, Koshi T, Mitsuka T, Miyagi M, Inoue G, Arai G, et al. Associations between proinflammatory cytokines in the synovial fluid and radiographic grading and pain-related scores in 47 consecutive patients with osteoarthritis of the knee. BMC Musculoskeletal Disorders. 2011;12:144.
7. Spector TD, Hart DJ, Nandra D, Doyle DV, Mackillop N, Gallimore JR, et al. Low-level increases in serum C-reactive protein are present in early osteoarthritis of the knee and predict progressive disease. Arthritis Rheum. 1997;40(4):723-7.
8. Saito T, Fukai A, Mabuchi A, Ikeda T, Yano F, Obha S, et al. Transcriptional regulation of endochondral ossification by HIF-2alpha during skeletal growth and osteoarthritis development. Nat Med. 2010;16(6):678-86.
9. Yang S, Kim J, Ryu JH, Oh H, Chun CH, Kim BJ, et al. Hypoxia-inducible factor-2alpha is a catabolic regulator of osteoarthritic cartilage destruction. Nat Med. 2010;16(6):678-93.
10. Daghestani HN, Pieper CF, Kraus VB. Soluble macrophage biomarkers indicate inflammatory phenotypes in patients with knee osteoarthritis. Arthritis Rheumatol. 2015;67(4):956-65.
11. Kraus VB, McDaniel G, Huebner JL, Stabler TV, Pieper CV, Shipes SW, et al. Direct in vivo evidence of activated macrophages in human osteoarthritis. Osteoarthritis Cartilage. 2016;24(9):1613-21.
12. Klein-Wieringa IR, de Lange-Brokkaar BJ, Yusuf E, Andersen SN, Kwekkeboom JC, Kroon HM, et al. Inflammatory cells in patients with endstage knee osteoarthritis: a comparison between the synovium and the infrapatellar fat pad. J Rheumatol. 2016;43(4):771-8.
13. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. Immunity. 2014;41(1):14-20.
14. Utomo L, Bastiaansen-Jenniskens YM, Verhaar JAN, van Osch GJVM. Cartilage inflammation and degeneration is enhanced by pro-inflammatory (M1) macrophages in vitro, but not inhibited directly by anti-inflammatory (M2) macrophages. Osteoarthritis Cartilage. 2016;24(12):2162-70.
15. Zhang H, Lin C, Zeng C, Wang Z, Wang H, Lu J, et al. Synovial macrophage M1 polarisation exacerbates experimental osteoarthritis partially through R-spondin-2. Ann Rheum Dis. 2018;77(10):1524-34.
16. Lopa S, Leijts MJC, Moretti M, Lubberts E, van Osch GJVM. Cartilage inflammation and degeneration is enhanced by pro-inflammatory (M1) macrophages in vitro, but not inhibited directly by anti-inflammatory (M2) macrophages. Osteoarthritis Cartilage. 2016;24(12):2162-70.
17. Grotenhuis N, Bayon Y, Lange JF, Van Osch GJVM, Bastiaanssen-Jenniskens YM. Arthritic and non-arthritic synovial fluids modulate IL10 and IL1RA gene expression in differentially activated primary human monocytes. Osteoarthritis Cartilage. 2015;23(11):1853-7.
18. Fahy N, de Vries-van Melle ML, Lehmann J, Wei W, Grotenhuis N, Farrell E, et al. Human osteoarthritic synovium impacts chondrogenic differentiation of mesenchymal stem cells via macrophage polarisation state. Osteoarthritis Cartilage. 2014;22(8):1167-75.
19. Utomo L, van Osch GJVM, Bayon Y, Verhaar JAN, Bastiaanssen-Jenniskens YM. Guiding synovial inflammation by macrophage phenotype modulation: an in vitro study towards a therapy for osteoarthritis. Osteoarthritis Cartilage. 2016;24(9):1629-38.
20. Yaeger PC, Masi TL, de Ortiz JL, Binette F, Tubo R, McPherson JM. Synergistic action of transforming growth factor-beta and insulin-like growth factor-I induces expression of type II collagen and aggrecan genes in adult human articular chondrocytes. Exp Cell Res. 1997;237(2):318-25.

21. Zhu M, Tang D, Wu Q, Hao S, Chen M, Xie C, et al. Activation of beta-catenin signaling in articular chondrocytes leads to osteoarthritis-like phenotype in adult beta-catenin conditional activation mice. J Bone Miner Res. 2009;24(1):12-21.

22. Attur M, Yang Q, Shimada K, Tachida Y, Nagase H, Mignatti P, et al. Elevated expression of periostin in human osteoarthritic cartilage and its potential role in matrix degradation via matrix metalloproteinase-13. FASEB J. 2015;29(10):4107-21.

23. Wu D, Jin SS, Lin S, Chen S, Pan T, Kang X, et al. Sauchinone inhibits IL-1beta induced catabolism and hypertrophy in mouse chondrocytes to attenuate osteoarthritis via Nrf2/HO-1 and NF-κB pathways. Int Immunopharmacol. 2018;62:181-90.

24. van der Kraan PM, Goumans MJ, Davidson ES, ten Dijke P, et al. Age-dependent alteration of TGF-β signalling in osteoarthritis. Cell Tissue Res. 2012;347(1):257-65.

25. Narcisi R, Quarto R, Ulivi V, Muraglia A, Molfetta L, Giannoni P. TGF β-1 administration during ex vivo expansion of human articular chondrocytes in a serum-free medium redirects the cell phenotype toward hypertrophy. J Cell Physiol. 2012;227(9):3282-90.

26. Wu CL, Harasymowicz NS, Klimak MA, Collins KH, Guilak F. The role of macrophages in osteoarthritis and cartilage repair. Osteoarthritis Cartilage. 2020;28(5):544-54.

27. Zhang H, Cai C, Bai X. Macrophages regulate the progression of osteoarthritis. Osteoarthritis Cartilage. 2020;28(5):555-61.