Interactions between human osteoarthritic chondrocytes and synovial fibroblasts in co-culture

K. Huch, J. Stöve, K.P. Günther, W. Puhl

Department of Orthopaedic Surgery, University of Ulm, Ulm, Germany

Abstract

Objective

To imitate the in vivo joint situation and to allow cell interactions, a co-culture system of human osteoarthritic chondrocytes and synovial fibroblasts from a single joint was established and characterized with or without stimulation by IL-1ß.

Methods

Culture settings included chondrocytes in alginate alone, synovial fibroblasts in monolayer alone and a co-culture of both. Proteoglycan (PG) synthesis was measured by ^{35}S-incorporation, PG content by a dimethylmethylene blue assay, DNA content by a fluorometric assay, and prostaglandin-E_2 and IL-1ß release by ELISA.

Results

In co-culture PG synthesis by chondrocytes was significantly reduced in the presence of IL-1ß (1 ng/ml) compared to controls. PG content of chondrocyte cultures was reduced for controls and IL-1ß treated co-cultures. Synovial fibroblasts in co-culture did not show significant change of PG synthesis or content when compared to cells in mono-cell culture. PG release into the medium was relatively high in co-cultures. IL-1ß significantly decreased the proliferation rate of chondrocytes in co-cultures and slightly increased prostaglandin-E_2 release.

Conclusions

Co-culturing of osteoarthritic chondrocytes and synovial fibroblasts from a single human joint allows interactions between both entities and may offer a useful tool to study the effects of mediators or new drugs under more in vivo like conditions compared to mono-cell cultures.

Key words

Co-culture, chondrocyte, synovial fibroblast, interleukin-1ß.

K. Huch, MD; J. Stöve, MD; K.P. Günther, MD; W. Puhl, MD.

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Please address correspondence and reprint requests to: Klaus Huch, MD, Department of Orthopaedic Surgery, University of Ulm, Oberer Eselsberg 45, 89081 Ulm, Germany.
E-mail: klaus.huch@medizin.uni-ulm.de

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Introduction

Not only rheumatoid arthritis (RA) but also advanced osteoarthritis (OA) is characterized by cartilage degradation and inflammatory changes of synovial tissue (1, 2). The junction of synovial tissue and articular cartilage is the origin of pannus in RA and of osteophytes in OA. Routinely, *in vitro* studies use models to examine the metabolism of articular chondrocytes in monocell culture, whereas *in vivo* synovial fluid serves as a medium for cytokines, mediators etc. and allows chondrocytes and synovial lining cells to interact with each other. Up to now only a few reports have described the interactions of articular chondrocytes and synovial fibroblasts in co-culture under various conditions (3-10). Recently, D'Andrea *et al*. (10) reported on intercellular calcium signaling between articular chondrocytes from rabbits and HIG-82 (cell line) synoviocytes, both cultured in monolayer. Biochemical changes of synovial fibroblasts in co-cultures with articular chondrocytes have not been tested yet.

The aim of our study was to simulate the *in vivo* interactions of cartilage and synovial tissue *in vitro*. To maintain their phenotype, freshly isolated osteoarthritic chondrocytes were cultured in alginate and synovial fibroblasts in monolayer. In co-culture, these systems allow the cultivation of both cell types in the same well without direct cell contact. The cell activity in co-culture was compared to monocell cultures of chondrocytes and synovial fibroblasts from the same osteoarthritic knee joints to investigate the effect of cell interactions via the culture medium. IL-1ß, one of the key cytokines in OA pathogenesis, was employed to imitate acute inflammation *in vitro*.

Materials and methods

Reagents

All chemicals were of regular grade and purchased from Sigma (Deisenhofen/Munich, Germany) unless otherwise noted. Pronase was purchased from Sigma (Deisenhofen/Munich, Germany) and collagenase P (Clostridium histolyticum) from Boehringer Mannheim (Indianapolis, IN). Low viscosity alginate (Keltone LV) was a gift from Kelco (Chicago, IL). Ham's F-12 medium (F12), Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were from Biochrom (Berlin, Germany). Ciprofloxacin was obtained from Bayer (Leverkusen, Germany). ^{35}S-sulfate (25-40 Ci/mg, 0.9-1.5 TBq/mg) was purchased from Amersham Corporation (Arlington Heights, IL). Hionicfluor was purchased from Packard Instruments BV (Groningen, The Netherlands). Alcian blue was obtained from Roth (Karlsruhe, Germany). 96-well filter plates, the vacuum manifold and punch tip assemblies were purchased from Millipore (Bedford, MA). A competitive enzyme immunoassay (EIA) kit for prostaglandin E_2 (TiterZyme®) was purchased from PerSeptive Biosystems, Framingham, MA. IL-1ß was determined using an ELISA (enzyme linked immunosorbent assay) kit (Milenia®) from Diagnostic Products Corporation / Biermann (Bad Nauheim, Germany). Bisbenzimidazole fluorescent dye (Hoechst dye # 33258) was obtained from Polysciences (Warrington, PA).

Tissue acquisition

Osteoarthritic articular cartilage and synovial tissue were obtained during total knee arthroplasty from 3 female patients (64, 73, and 78 years old, respectively) with their consent. The tisue was processed within 12 hours after harvesting. Cartilage and synovial tissue were resected from the entire joint.

Assessment of disease state

The knee joints of the 64- and 73-year-old patients showed grade IV osteoarthritis both radiographically (11) and macroscopically (12). Chondrocyte recruitment was 1.83 x 10^6/g and 1.66 x 10^6/g respectively. The knee joint of the 78-year old patient revealed radiographically grade II and macroscopically grade III osteoarthritis. Chondrocyte recruitment was 3.71x10^6/g. Microscopically chondrocytes from all 3 donors exhibited the characteristic shape.

Levels of IL-1ß and prostaglandin-E_2 were determined for the synovial fluids from the knees of the 64-year-old pa-

tient (IL-1ß and PG-E2 under the detection limit of 12 - 5.8 pg/ml) and the 78-year-old patient (IL-1ß under the detection limit; PG-E_2: 2609 ± 199 pg/ml) by ELISA (see reagents).

Cell culture

Synovial fibroblasts were cultured in monolayer; the synovial tissue was minced and digested in collagenase P for 1 hour at 37°C. The cell suspension was rinsed 3 times with PBS, resuspended in culture medium, and cultured in a culture flask to a density of 100,000/cm^2.

Articular chondrocytes were released by cartilage digestion with 0.4% pronase and 0.02% collagenase (12 ml/g cartilage) at 37°C overnight (13). After rinsing, filtering and counting, isolated chondrocytes were suspended in 1.2% alginate (Keltone LV from Kelco, Chicago, IL). By dropping the chondrocyte-suspension into isoosmotic calciumchloride solution (102 mM), alginate beads containing approximately 40,000 chondrocytes were formed (13-15).

Over a pre-culture period of 8 to 10 days, chondrocytes and synovial fibroblasts were cultured at 37°C, 95% humidity and 5% CO_2 in the presence of basic medium containing Dulbecco's modified Eagle's medium (DMEM; 45%), Ham's medium F12 (45%), 10% fetal bovine serum (FBS), 25 µg/ml ascorbic acid and 10 µg/ml ciprofloxacin to allow reconstitution of the chondrocyte matrix and confluence of the synovial fibroblasts. Beads were then distributed in a 24-well plate, plating 5-10 beads per well. The monolayer was trypsinized and the fibroblastic cells were also distributed on a 24-well plate at a density of 100,000 cells per cm^2. Starting 24 hours later, three different culture groups were created: (1) articular chondrocytes alone, (2) synovial fibroblasts alone, and (3) co-cultures of both cell types. Experiments were carried out in triplicate for each donor tissue and assay.

The mono- and co-cultures of both chondrocytes and synovial fibroblasts were treated every other day over a period of 2 weeks with the basic medium in the presence or absence of IL-1ß (1 ng/ml). At the end of the experiment, the alginate beads were dissolved in sodium citrate solution (55 mM sodium citrate, 30 mM EDTA, 90 mM sodium chloride, pH 7.45) for further analysis.

DNA content

To compare proliferation rates, the DNA content was measured in aliquots of the sodium citrate dissolved beads and of the synovial fibroblasts after papain digestion by a modified fluorometric assay (emission measured at 400-550 nm for an excitation wavelength of 365 nm) using bisbenzimidazole dye (Hoechst 33258) (16).

Proteoglycan synthesis, content and release

During the last 24 hours of cytokine treatment, cultures were labeled in the presence of ^{35}S-sulfate (20 µCi/ml) to evaluate proteoglycan synthesis. Beads, synovial fibroblasts and medium were processed separately to determine the distribution of proteoglycans in the extracellular matrix and in the medium. Beads were dissolved in sodium citrate. After papain digestion, the amount of all newly synthesized proteoglycans was measured by a rapid filtration assay using alcian blue to bind the proteoglycans as described by Masuda *et al.* (17). Dots per minute (dpm) were measured in a scintillation counter.

Proteoglycan content was determined for alginate and monolayer cultures by binding to dimethylmethylene blue (DMB) dye (16 mg/ml DMB, 0.03 M sodium formate, 0.2% formic acid, pH 6.8) in the presence of 0.24 M GuHCl which is effective in avoiding precipitation and interference of hyaluron acid and/or DNA (18). The results are reported as equivalents of a standard of purified bovine nasal aggrecan. Absorbency was measured at 530 and 595 nm using the plate reader MRX from Dynatech. Modifications of the method included the adaptation of the dilution buffer to the alginate system and the use of papain digested probes.

IL-1ß and PG-E_2 content in culture medium

Medium from the different cultures (last 24 hours) was rapidly frozen and stored at -80°C until determination of IL-1ß and PG-E_2 by ELISA (see reagents). The concentration of IL-1ß (detection limit 12 pg/ml) was measured at 450 nm,and the concentration of PG-E_2 (detection limit 5.8 pg/ml) at 405 nm. Pure FBS contained 53 ± 4 pg/ml IL-1ß and 7794 ± 686 pg/ml prostaglandin-E_2.

Statistical analysis

Data are expressed as means ± standard error. For all studies, 3 chondrocyte preparations from different human donors were tested. Each experimental point was determined in triplicate. The statistical significance of differences between group means was determined by Student's t-test. $P < 0.05$ was considered statistically significant.

Results

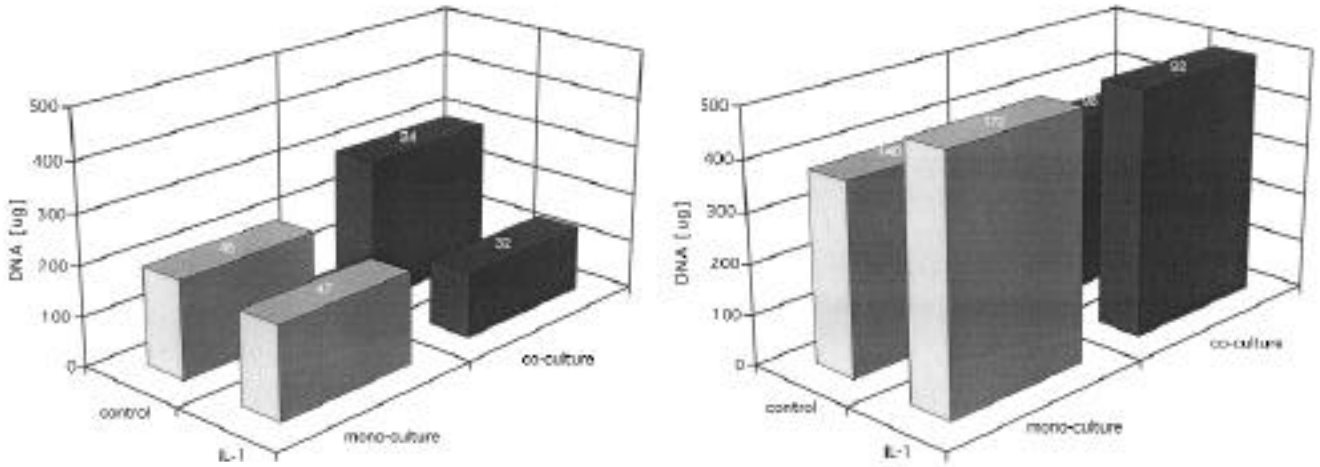
Proliferation rate

Monocell cultures of articular chondrocytes did not change their cell number in the presence of IL-1ß. However, co-cultures showed a relatively high DNA content for controls (1.5-fold), whereas IL-1ß significantly reduced the DNA content of chondrocytes in co-culture (1.4-fold) (Fig. 1a). The proliferation rate of synovial fibroblasts was slightly increased by IL-1ß in both monocell and co-cultures when compared to controls (Fig. 1b).

Proteoglycan synthesis, content and release

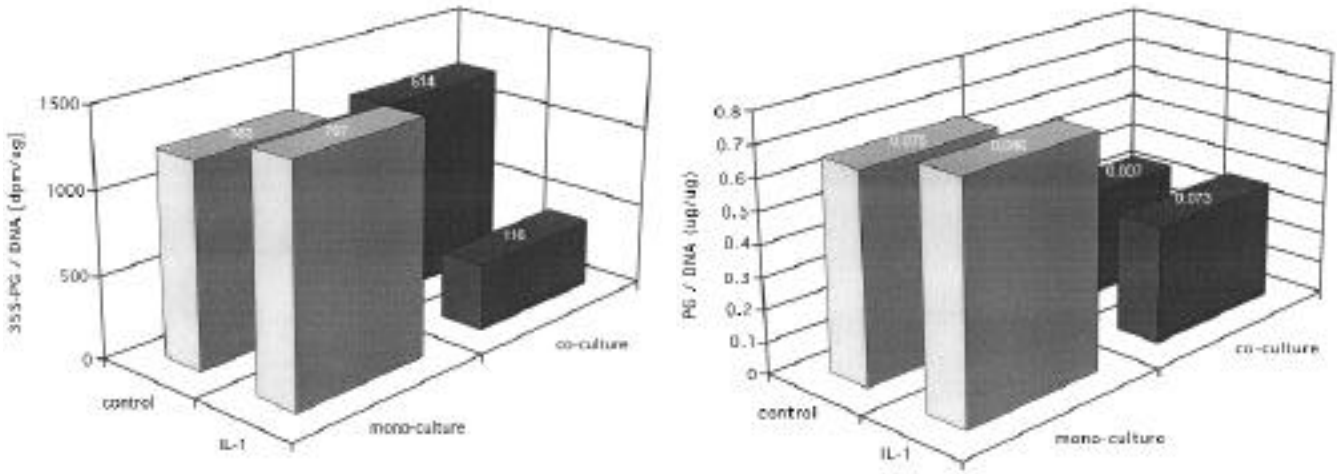
^{35}S-proteoglycan synthesis per DNA was nearly unchanged in control monocell and co-cultures of articular chondrocytes, whereas IL-1ß treated co-cultures revealed a significant decrease of PG synthesis compared to monocell cultures (Fig. 2a). PG content was significantly reduced in controls and IL-1ß co-cultures (Fig. 2b).

Synovial fibroblasts showed in co-culture a relative decrease in PG synthesis. IL-1ß reduced PG synthesis in mono- and co-culture (Fig. 3a). PG content was significantly increased in co-cultured controls of synovial fibroblasts, whereas IL-1ß treated synovial fibroblasts did not reveal culture-associated differences (Fig. 3b).



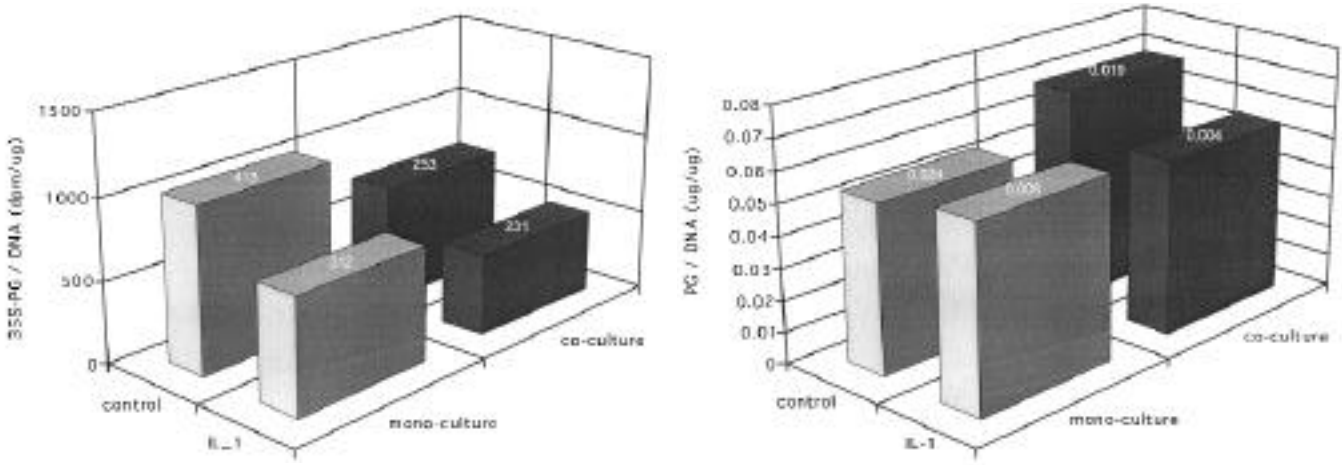
(a) **(b)**

Fig. 1. DNA content of chondrocytes (**a**) (control versus IL-1ß in co-culture $p < 0.05$; control of mono- versus co-culture $p < 0.06$) and synovial fibroblasts (**b**) after 14 days in mono- and co-culture in the absence and presence of 1 ng/ml IL-1ß (means as bars, standard error as numbers).



(a) **(b)**

Fig. 2. ^{35}S-PG synthesis (**a**) (IL-1ß of mono- versus co-culture $p < 0.05$; control mono- versus IL-1ß co-culture $p < 0.05$) and PG content (**b**) (controls / IL-1ß of mono- versus co-culture $p < 0.05$) of chondrocytes in mono- and co-culture in the absence and presence of 1 ng/ml IL-1ß (means as bars,standard error as numbers).



(a) **(b)**

Fig. 3. ^{35}S-PG synthesis (**a**) (control versus IL-1ß in co-culture $p < 0.05$; control mono- versus IL-1ß co-culture $p < 0.05$) and PG content (**b**) (controls of mono- versus co-culture $p < 0.05$) of synovial fibroblasts in mono- and co-culture in the presence and absence of 1 ng/ml IL-1ß (mean as bars,standard error as numbers).

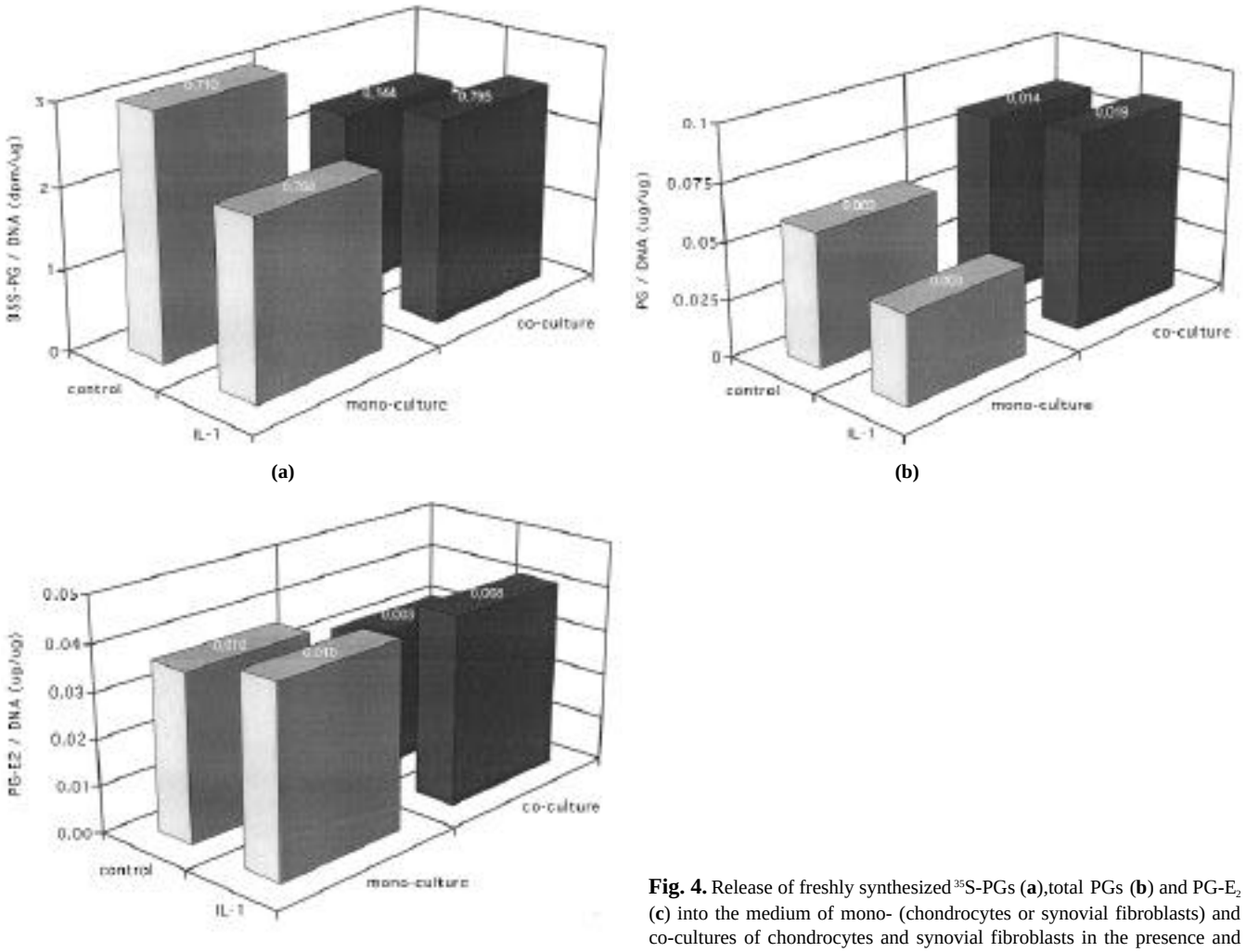


Fig. 4. Release of freshly synthesized ^{35}S-PGs (**a**),total PGs (**b**) and PG-E_2 (**c**) into the medium of mono- (chondrocytes or synovial fibroblasts) and co-cultures of chondrocytes and synovial fibroblasts in the presence and absence of IL-1ß (1 ng/ml) (mean as bars, standard error as numbers).

^{35}S-proteoglycan release into the medium was relatively high in control mono-cell cultures and demonstrated only slight differences for the other groups (Fig. 4a). The medium of co-cultures showed a relatively high PG content and IL-1ß reduced the PG content in monocell cultures (Fig. 4b).

IL-1ß and PG-E_2 levels

IL-1ß was released by all control mono-cultures since the control medium (10% FBS) contained only about 5.3 ± 0.4 pg/ml IL-1ß before exposure to the cell culture (see above). In control cultures chondrocytes released 77.7 ± 19.2 pg/ml IL-1ß, synovial fibroblasts 60.2 ± 44.1 pg/ml and co-cultures 95.4 ± 22.6 pg/ml, concentrations which are similar to that found in the synovial fluids of osteoarthritic joints (from less than 1 pg/ml up to over 500 pg/ml) (19-26). Basal PG-E_2 release per DNA was slightly higher in IL-1ß treated cultures. This effect was more pronounced in the co-cultures (Fig. 4c).

Discussion

We used a new culture set-up to mimic the natural articular environment and to demonstrate that co-cultured human chondrocytes and synovial cells interact with each other, leading to a change in their proliferation and metabolism. This system uses the 3-dimensional culture of chondrocytes in alginate to maintain their phenotype over several months (13, 27, 28). Synovial cells in monolayer at the bottom of the well and chondrocytes floating in the form of beads in the medium avoid direct cell contact (as *in vivo*) and offer separate analysis of both cell types and the medium in mono- and in co-culture.

An increased proliferation rate of co-cultured chondrocytes suggests that the coexistence of synovial fibroblasts and articular chondrocytes might be advantageous. However, IL-1ß suppresses the proliferation rate of co-cultured chondrocytes and increases that of co-cultured synovial cells. This mimics the *in vivo* situation, where osteoarthritic joints are characterized by increased IL-1ß levels, a loss of cartilage and a hypertrophy of synovial tissue.

IL-1ß leads to an impressive decrease of ^{35}S-proteoglycan synthesis by co-cultured chondrocytes, suggesting a strong influence on the interactions between chondrocytes and fibroblasts. A reduced proteoglycan content in co-cultured chondrocytes and an increased content in their medium corresponds to the *in vivo* situation of osteoarthritic joints.

Steinberg *et al*. (5) also observed increased proteoglycan release in co-cultures of cartilage (bovine nasal) and

synovium (human or rabbit arthritic). Fell *et al*. (4) assumed that synovium has a direct (presumably enzymatic) and an indirect (via chondrocytes) effect on proteoglycan and collagen release from young porcine cartilage. Dogterom *et al*. (7) found that synovium causes proteoglycan release in organ cultures of human and porcine cartilage. They also demonstrated the increase of proteoglycan release by synovium in dead cartilage and state that chondrocyte-mediated cartilage breakdown plays a minor role. Alternatively, one could argue that mediators and proteases are being released from dead chondrocytes, stimulating the synovial tissue and degrading the cartilage. Their experiments with human tissue reinforced this hypothesis by showing a higher release from dead cartilage compared to live cartilage in the presence and absence of synovium. Retinol, on the other hand, changed proteoglycan release by live cartilage but not by dead cartilage, which indicates the stimulation of chondrocyte mediators or proteases.

Examining proteoglycan contents, Ismaiel *et al*. (29) described inter-individual differences in the response of various human organ cultures (cartilage) to IL-1ß (10 ng/ml). Only 38% of the cultures from different patients showed a decrease in proteoglycan content (range 11-20%) compared to the controls. In our 3 experiments we did not observe a significant decrease of proteoglycan content after IL-1ß stimulation.

IL-1ß levels in control co-culture medium (originally containing 5.3 ± 0.4 pg/ml) were between 80 and 121 pg/ml, representing a range which has been observed by several authors in the synovial fluids of osteoarthritic knee joints (19-26) and by Elson *et al.* (30) *in vitro*. This indicates that osteoarthritic cells are able to synthesize or to release IL-1ß in the presence of low IL-1ß concentrations (about 5 pg/ml). Co-culturing did not significantly change IL-1ß release by osteoarthritic chondrocytes or synovial fibroblasts.

$PG\text{-}E_2$ is known to be released by and synovial cells and IL-1ß is known to be a potent stimulator of $PG\text{-}E_2$ release (31-33). However, Arner *et al*. (34) suggest an independent effect of IL-1ß on proteoglycan breakdown,proteoglycan synthesis, and $PG\text{-}E_2$ release. This, and a similar release of $PG\text{-}E_2$ from human synovial cells and chondrocytes in the presence of IL-1 and ß as shown by Knott *et al*. (35), might explain the mild increase of the $PG\text{-}E_2$ release in the presence of IL-1ß in contrast to different responses concerning other parameters in our study.

In general, interactions of chondrocytes and synovial fibroblasts seem to play an important role *in vitro*. Studies by Hamerman *et al*. (3) (human organ culture from different donors), Fell and Jubb (4) (porcine), Steinberg *et al*. (5) (bovine nasal cartilage and human rheumatoid synovium), and Panagides *et al*. (8) (rabbit) used animal tissue or a mixture of tissues of different origin and show that synovial tissue increases the release of proteoglycans into the medium.

D'Andrea *et al*. (10) describe intercellular signaling between chondrocytes and synovial fibroblasts in co-culture using a mixed monolayer of rabbit chondrocytes and HIG-82 synovial fibroblasts. However, *in vivo* articular chondrocytes and synovial fibroblasts are in direct contact with each other only at the junction. This and the apparent loss of phenotype of chondrocytes in mixed monolayers have to be kept in mind when interpreting their results. Recklies *et al*. (36) showed recently that chondrocytes and synovial fibroblasts synthesize cartilage oligomeric matrix protein (COMP) but neither skin nor fetal lung fibroblasts, thus indicating a special differentiation of synovial fibroblasts. Saklatvala *et al*. (37) and Dogterom *et al*. (7) demonstrated that supernatants from human synovial cell culture lead to a higher proteoglycan release than co-cultures with synovial cells. Lately, Webb (38) showed that synovial fluid and synovium supernatants up-regulate tumor necrosis factor receptors on human articular chondrocytes. However, their procedure did not allow for any feedback between chondrocytes and synovial fibroblasts.

To date only organ culture systems or monolayer cultures have been used for the co-culturing of articular and non-articular cartilage in the presence of synovial fibroblasts. We consider the system presented here to be advantageous because cells from the cartilage and synovium of one single human joint are freshly isolated and cultured under established conditions in alginate (chondrocytes) and monolayer (synovial fibroblasts, respectively. Therefore, both entities are separated (as *in vivo*) and can communicate via diffusible mediators in the tissue culture medium. Keeping in mind the enormous inter-individual differences and differences between species, co-culturing cells from the same joint appears to be crucial and would allow one to imitate the *in vivo* situation with interactions of articular chondrocytes and synovial cells.

Conclusions

Co-culturing of osteoarthritic chondrocytes and synovial fibroblasts might reflect the *in vivo* situation better than monocell culturing by allowing interactions of both cell types. The advantages of our newly arranged culture system are the phenotypic stability of articular chondrocytes in alginate and the separation of chondrocytes and synovial fibroblasts by using two different culture systems. Both systems can interact via cell culture medium.

However, the co-culturing of articular chondrocytes and synovial fibroblasts from the same osteoarthritic joint changed not only their proliferation rate, but also their biochemical responses in all individuals. Co-culture showed accentuated IL-1ß effects by osteoarthritic chondrocytes and synovial fibroblasts from one single human joint, indicating that this system might be a useful tool to study the effects of other mediators or new drugs under more *in vivo* like conditions compared to classical monocell procedures.

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References

1. KENNEDY RD, PLATER-ZYBERK C, PARTRIDGE TA, WOODROW DF, MUIR RH: Morphometric comparison of synovium from patients with osteoarthritis and rheumatoid arthritis. *J Clin Pathol* 1988; 41: 847-52.
2. REVELL PA, MAYSTON V, LALOR P, MAPP P: The synovial membrane in osteoarthritis: A histological study including the characterization of the cellular infiltrate present in inflammatory osteoarthritis using monoclonal antibodies. *Ann Rheum Dis* 1988; 47: 300-7.
3. HAMERMAN D, JANIS R, SMITH C: Cartilage matrix depletion by rheumatoid synovial cells in tissue culture. *J Exp Med* 1967; 126:1005-12.
4. FELL HB, JUBB RW: The effect of synovial tissue on the breakdown of articular cartilage in organ culture. *Arthritis Rheum* 1977; 20: 1359-71.
5. STEINBERG J, SLEDGE CB, NOBLE J, STIRRAT CR: A tissue-culture model of cartilage breakdown in rheumatoid arthritis. *Biochem J* 1979; 180: 403-12.
6. JACOBY RK: Effect of homologous synovial membrane on adult human articular cartilage in organ culture and failure to influence it with D-penicillamine. *Ann Rheum Dis* 1980; 39: 53-8.
7. DOGTEROM AA, HUBER-BRUNING O, VERNOOY JE, WILBRINK B, DEN OTTER W, HUBER J: Matrix depletion of young and old human articular cartilage by cultured autologous synovium fragments: A chondrocyte-independent effect. *Rheumatol Int* 1985; 169-73.
8. PANAGIDES J, LANDES MJ, SLOBODA AE: Destruction of articular cartilage by arthritic synovium *in vitro*: Mechanism of breakdown and effect of indomethacin and prednisolone. *Agents Actions* 1980; 10: 22-30.
9. RAMACHANDRULA A, TIKU K, TIKU ML: Tripeptide RGD-dependent adhesion of articular chondrocytes to synovial fibroblasts. *J Cell Sci* 1992; 101: 859-71.
10. D'ANDREA P, CALABRESE A, GRANDOLFO M: Intercellular calcium signaling between chondrocytes and synovial cells in co-culture. *Biochem J* 1998; 329: 681-7.
11. KELLGREN JH, LAWRENCE JS: Radiological assessment of osteoarthritis. *Ann Rheum Dis* 1957; 16: 494-501.
12. COLLINS DH: *The Pathology of Articular and Spinal Diseases*. London,Edward Arnold and Co. 1949: 76-9.
13. HÄUSELMANN HJ, AYDELOTTE MB, SCHUMACHER BL, KUETTNER KE, GITELIS SH, THONAR EJ-MA: Synthesis and turnover of proteoglycans by human and bovine adult articular chondrocytes cultured in alginate beads. *Matrix* 1992; 12: 116-29.
14. KUPCHIK HZ,LANGER RS, HABERERN C, EL DERINY S, O'BRIEN M: A new method for three-dimensional *in vitro* growth of human cancer cells. *Exp Cell Res* 1983; 147: 454-60.
15. GUO J, JOURDIAN GW, MACCALLUM DK: Culture and growth characteristics of chondrocytes encapsulated in alginate beads. *Connec Tissue Res* 1989; 19: 277-97.
16. KIM Y-J, SAH RLY, DOONG J-YH, GRODZINSKY AJ: Fluorometric assay of DNA in cartilage explants using Hoechst 33258. *Anal Biochem* 1988; 174: 168-76.
17. MASUDA K, SHIROTA H, THONAR EJ-MA: Quantification of ^{35}S-labeled proteoglycans complexed to alcian blue by rapid filtration in multiwell plates. *Anal Biochem* 1994; 217: 167-75.
18. CHANDRASEKHAR S, ESTERMAN MA, HOFFMAN HA: Microdetermination of proteoglycans and glycosaminoglycans in the presence of guanidine hydrochloride. *Anal Biochem* 1987; 161: 103-8.
19. HOLT I, COOPER RG, DENTON J, MEAGER A, HOPKINS SJ: Cytokine inter-relationships and their association with disease activity in arthritis. *Br J Rheumatol* 1992; 31: 725-33.
20. HOPKINS SJ, HUMPHREYS M, JAYSON MIV: Cytokines in synovial fluid. I. The presence of biologically active and immunoreactive IL-1. *Clin Exp Immunol* 1988; 72: 422-7.
21. KAHLE P, SAAL JG, SCHAUDT K, ZACHER J, FRITZ P, PAWELEC G: Determination of cytokines in synovial fluids: Correlation with diagnosis and histomorphological characteristics of synovial tissue. *Ann Rheum Dis* 1992; 51: 731-4.
22. MANNAMI K, MITSUHASHI T, TAKESHITA H *et al.*: Concentration of interleukin-1ß in serum and synovial fluid in patients with rheumatoid arthritis and those with osteoarthritis. *J Jpn Orthop Assoc* 1989; 63: 1343-52.
23. NEIDEL J, SCHULZE M, SOVA L, LINDSCHAU J: Praktische Bedeutung der Zytokinbestimmung im Gelenkpunktat von Patienten mit Arthrosen oder rheumatischen Arthritiden. *Z Orthop* 1996; 134: 381-5.
24. VÄÄTÄINEN U, LOHMANDER S, THONAR E *et al.*: Markers of cartilage and synovial metabolism in joint fluid and serum of patients with chondromalacia of the patella. *Osteoarthritis and Cartilage* 1998; 6: 115-24.
25. VIGNON E, BALBLANC JC, MATHIEU P, LOUISOT P, RICHARD M: Metalloprotease activity, phospholipase A2 activity and cytokine concentration in osteoarthritis synovial fluids. *Osteoarthritis and Cartilage* 1993; 1: 115-20.
26. WESTACOTT CI, WHICHER JT, BARNES IC, THOMPSON D, SWAN AJ, DIEPPE PA: Synovial fluid concentration of five different cytokines in rheumatic disease. *Ann Rheum Dis* 1990; 49: 676-81.
27. HÄUSELMANN HJ, FERNANDES RJ, MOK SS *et al.*: Phenotypic stability of bovine articular chondrocytes after long-term culture in alginate beads. *J Cell Sci* 1994; 107: 17-27.
28. LEMARE F, STEIMBERG N, LE GRIEL C, DEMIGNOT S, ADOLPHE M: Dedifferentiated chondrocytes cultured in alginate beads: Restoration of the dedifferentiated phenotype and of the metabolic responses to interleukin-1 ß. *J Cell Physiol* 1998; 176: 303-13.
29. ISMAIEL S, ATKINS RM, PEARSE MF, DIEPPE PA, ELSON CJ: Susceptibility of normal and arthritic human articular cartilage to degradative stimuli. *Br J Rheumatol* 1992; 31: 369-73.
30. ELSON CJ, MORTUZA FY, PERRY MJ, WARNOCK MG, WEBB GR, WESTACOTT CI: Cytokines and focal loss of cartilage in osteoarthritis. *Br J Rheumatol* 1998; 37: 106-7.
31. BANDARA G, LIN CW, GEORGESCU HI, EVANS CH: The synovial activation of chondrocytes: Evidence for complex cytokine interactions involving a possible novel factor. *Biochim Biophys Acta* 1992; 1134: 309-18.
32. CAMPBELL IK, PICCOLI DS, HAMILTON JA: Stimulation of human chondrocyte prostaglandin E2 production by recombinant human interleukin-1 and tumor necrosis factor. *Biochim Biophys Acta* 1990; 1051: 310-18.
33. WITTENBERG RH, WILLBURGER RE, KLEEMEYER KS, PESKAR BA: *In vitro* release of prostaglandins and leukotrienes from synovial tissue, cartilage, and bone in degenerative joint diseases. *Arthritis Rheum* 1993; 10: 1444-50.
34. ARNER EC,PRATTA M: Independent effects of interleukin-1 on proteoglycan synthesis and prostaglandin E_2 release from cartilage in organ culture. *Arthritis Rheum* 1989; 32: 288-97.
35. KNOTT I, DIEU M, BURTON M, LECOMTE V, REMACLE J, RAES M: Differential effects of interleukin-1 and ß on the acid cascade in human synovial cells and chondrocytes in culture. *Agents Actions* 1993; 39: 126-31.
36. RECKLIES AD, BAILLARGEON L, WHITE C: Regulation of cartilage oligomeric matrix protein synthesis in human synovial cells and articular chondrocytes. *Arthrtis Rheum* 1998; 41: 997-1006.
37. SAKLATVALA J, SARSFIELD SJ, PILSWORTH LMC: Characterization of proteins from human synovium and mononuclear leukocytes that induce resorption of cartilage proteoglycan *in vitro*. *Biochem J* 1983; 209: 337-44.
38. WEBB GR, WESTACOTT CI, ELSON CJ: Osteoarthritic synovial fluid and synovium supernatants up-regulate tumor necrosis factor receptors on human articular chondrocytes. *Osteoarthritis Cartilage* 1998; 6: 167-76.