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Interleukin-1ß-induced expression of the urokinase-type plasminogen activator receptor and its co-localization with MMPs in human articular chondrocytes

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Summary. The urokinase-type plasminogen activator receptor (uPAR) plays a critical role in cartilage degradation during osteoarthritis as it regulates pericellular proteolysis mediated by serine proteinases. Another important family of proteinases responsible for ECM destruction in arthritis are the matrix metalloproteinases (MMPs). MMPs are regulated by IL-1ß, a cytokine that plays a pivotal role in pathogenesis of osteoarthritis. This study was undertaken to address two questions: 1. Is uPAR-expression regulated by proinflammatory cytokines such as IL-1ß? 2. Does a functional co-localization exist between uPAR and MMPs?

By immunohistochemical analysis we observed enhanced expression of uPAR on chondrocytes derived from osteoarthritic human cartilage compared to non-osteoarthritic controls. We found an IL-1ß-mediated expression of uPAR by immunoelectron microscopy. Western blot analysis demonstrated that IL-1ß-stimulated expression of uPAR on chondrocytes *in vitro* increased in a dose-dependent manner. Furthermore, we found a functional co-localization between uPAR and MMP-9 on IL-1ß-stimulated chondrocytes by means of a co-immunoprecipitation assay.

Expression of uPAR in osteoarthritic cartilage but not in healthy cartilage suggests that uPAR plays a role in cartilage breakdown. We propose that uPAR-mediated effects e.g. pericellular proteolysis are one of other cytokine (IL-1ß)-mediated events that contribute to the pathogenesis of osteoarthritis. Furthermore, we found that MMPs and uPAR were part of the same cell surface complexes in chondrocytes. This finding underlines a functional interaction between MMPs and the serine proteinase system in the fine regulation of pericellular proteolysis. Interfering with uPAR signaling may present a novel target in arthritis therapy to prevent excessive proteolytic degradation.

Key words: Chondrocyte, Osteoarthritis, IL-1ß, uPAR, MMP-9

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Introduction

Osteoarthritis (OA) is the most common form of arthritis of weight bearing joints in humans and animals. Pathogenesis of OA is complex, initiated by biomechanical stress and biochemical changes in chondrocytes leading to distinct catabolic and reparative events in cartilage. OA changes involve not only the articular cartilage, but also the synovial membrane and subchondral bone of the joint (Martel-Pelletier, 1998); they are characterized by a breakdown of the extracellular cartilage matrix, revealed by cartilage fibrillation, fissures, ulcerations, and the loss of full thickness surface of articular cartilage (Martel-Pelletier, 1998; Goggs et al., 2003). OA changes are mainly the result of the altered capacity of chondrocytes to synthesize extracellular matrix macromolecules, leading to a mechanically inferior extracellular matrix that cannot withstand mechanical load and enhanced extracellular matrix degradation that arises from an imbalance between various proteinases and their tissue inhibitors as a sign of disequilibrium of cartilage homeostasis. Chondrocyte apoptosis also contributes to OA and this is recognizable by hypocellularity of osteoarthritic cartilage (Goggs et al., 2003). Cytokines such as interleukin-1, play a pivotal role in disturbance of chondrocyte synthesis program and induction of proteinases (Gowen et al., 1984; Martel-Pelletier, 1998; Vincenti and Brinckerhoff, 2001). Two important families of proteinases are responsible for enhanced pericellular proteolysis during OA: serine proteinases such as plasmin, urokinase plasmin activator (uPA) (Martel-Pelletier et al., 1991; Serni et al., 1995; Walter et al., 1998; Del Rosso et al., 1999) and the matrix

metalloproteinases (MMPs) (Gowen et al., 1984; Martel-Pelletier, 1998). Serine proteinases particularly uPA are activated after binding their specific receptor, urokinase-type plasminogen activator receptor (uPAR) initiating a cascade of extracellular proteinases that includes plasmin and various MMPs (Martel-Pelletier et al., 1991; Oleksyszyn and Augustine, 1996).

UPAR is a glycoprotein with a molecular weight (MW) of 45-60 kDa that is anchored by a glycosyl phosphatidylinositol (GPI)-moiety to the plasma membrane (Preissner et al., 2000). UPAR is composed of three homologous domains (DI, DII and DIII). The DI domain contains the urokinase-type plasminogen activator (uPA)-binding site for urokinase-type plasminogen activator (uPA) (Behrendt et al., 1991), whereas the DII/DIII domains bind the extracellular matrix protein vitronectin (Wei et al., 1994). Binding of uPA to uPAR results in activation of pro-uPA and plasminogen initiating pericellular proteolysis. On the other hand, ligand-receptor binding stimulates plasmin generation-independent events including cell proliferation (Fischer et al., 1998), chemotactic cell migration (Herbert et al., 1997) as well as cellular adhesion (Waltz et al., 1993) in various cell types (for review see Del Rosso et al., 1999). In chondrocytes it has been shown that uPA/uPAR regulates chondrocyte proliferation (Fibbi et al., 1998).

Beside plasmin-independent events, the regulation of pericellular proteolysis is a key mechanism of uPAR in cartilage matrix degradation in OA (Del Rosso et al., 1990; Walter et al., 1998). Moreover, it seems that uPAR acts additionally as a direct regulator of MMP activity (Mazzieri et al., 1997). In arthritic conditions enhanced levels of uPA, the ligand for uPAR, have been demonstrated (Hamilton et al., 1991). Furthermore, it has been shown that cytokines such as IL-1ß, induce increased uPA-expression in chondrocytes *in vitro* (Hamilton et al., 1991). Regulation by IL-1ß is also a common feature for various MMPs (Saito et al., 1998; Schulze-Tanzil et al., 2002). Several stimuli, such as growth factors (Siren et al., 1999), proinflammatory cytokines (Wu et al., 2002), lipopolysaccharides (Ogura et al., 1999) and the phorbol ester phorbol 12-myristate 13-acetate (PMA) (Lund et al., 1995) induce the expression of uPA and its receptor uPAR in other cell systems. Therefore the question arises as to whether the specific receptor for uPA, uPAR is also stimulated by IL-1ß in chondrocytes, in this way enhancing catabolic pathways during arthritis.

In a previous study a specific co-localization and functional interaction has been shown between various MMPs (MMP-1, -3 and -9) and ß1-integrins in chondrocytes by immunoprecipitation assays (Schulze-Tanzil et al., 2001). Another related study demonstrated a tripartitate complex between caveolin, ß1-integrin and uPAR in human chondrocytes (Schwab et al., 2001). These findings support the concept that multifunctional receptor complexes are expressed and aggregate on chondrocyte surface in response to IL-1ß. Thus, ß1-integrin is a central component which may coordinate and modify intracellular signaling pathways in chondrocytes in arthritis. Another question arises as to whether uPAR is mediated by IL-1ß, and whether MMPs interact directly with uPAR in chondrocytes.

Taken together the aims of the present study were as follows: (i) to examine by means of immuno-cytochemical methods the distribution pattern of uPAR in OA cartilage, and (ii) whether cytokine IL-1ß regulates the expression of uPAR in chondrocytes and (iii) whether uPAR functionally co-localizes and interacts with MMPs.

Materials and methods

Antibodies

The mAb against uPAR was generated by immunizing mice with non-glycosylated human recombinant uPAR produced in *E. coli* (Luther et al., 1997). MAb (IID7) recognizes an epitope located in the domain II of uPAR. Monoclonal anti-MMP-9 antibody was obtained from R&D (Heidelberg, Germany). The secondary gold-labeled antibodies were purchased from Amersham (Brunswick, Germany). The secondary antibody conjugated to alkaline phosphatase was purchased from Roche (Mannheim, Germany).

Growth medium (Ham`s F-12/Dulbecco`s modified Eagle`s medium [50/50] containing 10% fetal calf serum, 25 μg/ml ascorbic acid, 50 IU/ml streptomycin, 50 IU/ml penicillin, 1% essential amino acids, 1% glutamine and 2.5 μg/ml amphotericin B) was purchased from Seromed (Munich, Germany). Collagenase and trypsin/EDTA (EG 3.4.21.4) were obtained from Sigma (Munich, Germany), pronase was purchased from Roche (Mannheim, Germany). Interleukin-1ß was obtained from Strathman Biotech GmbH (Hannover, Germany). LR-White and Epon were obtained from Plano (Marburg, Germany).

Immunocytochemistry of tissue samples

Osteochondral specimens were harvested during routine knee replacement from patients with OA of the knee (n=10). Specimens of normal human femoral knee joint cartilage originate from routine autopsies not later than 24 h post mortem. The grading of cartilage samples was performed histologically (Mankin et al., 1971).

Sections were cut (5 μm) and mounted on silane-coated slides. The sections were dewaxed and antigen retrieval was performed by microwave irradiation in 0.01 M sodium citrate buffer (pH 6.0), 2x5 min at 850 W. After washing in phosphate-buffered saline (PBS; pH 7.4), the sections were treated with 0.3% hydrogen peroxide for 30 min, incubated for 1 h at 37 °C with primary antibody against uPAR (monoclonal, final IgG concentration 5 μg/ml). The primary antibody was detected with biotinylated secondary antibodies, followed by incubation with streptavidin/biotin-

peroxidase complex (Vectastain Elite, Vector, Burlingame, CA, USA). The peroxidase activity was visualized with 3,3'-diaminobenzidine. In control sections the primary antibodies were replaced by either PBS, or non-immune monoclonal IgG's (same species, same isotype; mouse isotype control (IgG1) from Sigma, Germany).

Preparations were examined and photographed with a Nikon microscope (Optiphot-2; Nikon Corporation, Japan) or with a Leica confocal laser scanning microscope (TCS 4D; Leica, Bensheim, Germany).

Immunoelectron microscopy (Pre-embedding technique)

A detailed description of this procedure has been previously published (Shakibaei et al., 1993). Briefly, after stimulation with 10 ng IL-1ß for 1 h, the chondrocytes were treated with hyaluronidase (5000 U/ml) for 15 min at RT to unmask epitopes. After washing with PBS/BSA the cells were incubated in medium with mAb to uPAR (final IgG concentration 50 μg/ml in PBS/BSA) for 5 min at 37 °C. The cells were washed and then incubated with the secondary antibody (GAM-10 nm, 1:30 in PBS/BSA) for 5 min at 37 °C. Subsequently, they were fixed with 2% glutaraldehyde, 1% tannic acid in 0.1 M phosphate buffer, pH 7.4 and post-fixed in a 2% OsO_4 solution. After dehydration in ascending alcohol series, the specimens were embedded in Epon. Ultrathin sections were contrasted with 2% uranyl acetate and lead citrate and investigated under a transmission electron microscope (TEM 10, Zeiss, Germany).

Chondrocyte isolation, immunoblotting and immunoprecipitation of uPAR and MMP-9 in IL-1ß-stimulated chondrocytes

Isolation and cultivation of chondrocytes were performed as described previously (Shakibaei et al., 1999). Briefly, human articular cartilage slices (from femoral heads obtained during joint-replacement surgery for femoral neck fractures) were collected in Ham's F-12 medium. Cartilage slices were rinsed with Ham's F-12 medium and digested with 1% pronase (from *Streptomyces griseus*, 7000 units/g) in Ham`s F-12 medium containing 2.5% (v/v) fetal calf serum for 2 h at 37 °C and then with 0.2% (v/v) collagenase (from *Clostridium histolyticum*, 0.15 unit/mg) in the same solution for 4 h at 37 °C. After rinsing in growth medium, a single-cell suspension was obtained by repeated pipetting and separation from undissolved tissue fragments using a nylon mesh with a pore width of 80 μm. Cells were sedimented by centrifugation at 6000 g and rinsed twice in growth medium. After counting, the cells were diluted to $1.5\text{-}10^6$/ml in serum-free medium, plated on dishes coated with collagen type II. Then, cells were treated with IL-1ß (0.1, 1, 5, 10, 50 ng/ml) for 1 h or left untreated. After rinsing with PBS, cells were extracted with lysis buffer [50 mM Tris/HCl, pH 7.2/150 mM NaCl/1% (v/v) Triton X-100/1 mM sodium orthovanadate/50 mM sodium pyrophosphate/100 mM sodium fluoride/0.01% (v/v) aprotinin/4 μg/ml pepstatin A/10 μg/ml leupeptin/1 mM PMSF] on ice for 30 min. Insoluble material was removed by centrifugation at 10000 g for 30 min. Lysates were stored at –70 °C until use. For immunoprecipitation, the extracts were precleared by incubation with 25 μl of normal rabbit IgG serum or normal mouse IgG serum and *S. aureus* cells, incubated with primary antibodies diluted in wash buffer (0.1% Tween 20/150 mM NaCl/50 mM Tris/HCl, pH 7.2/1 mM $CaCl_2$/1 mM $MgCl_2$/1 mM PMSF) for 2 h at 4 °C, followed by *S. aureus* cells for 1 h at 4 °C. Control immunoprecipitations were performed by incubating the samples with rabbit anti-mouse IgG alone. *S. aureus* cells were washed five times with wash buffer and once with 50 mM Tris/HCl, pH 7.2, followed by boiling in SDS/PAGE sample buffer. For immunoblotting, equal amounts of total proteins were separated on 10% or 7.5% polyacrylamide gels by SDS/PAGE under reducing conditions. Membranes were blocked with 5% (w/v) skimmed-milk powder in PBS/0.1% Tween 20 overnight at 4 °C and incubated with the primary anti-uPAR or -MMP-9 antibodies diluted in blocking buffer for 1 h at room temperature. After five washes in blocking buffer, membranes were incubated with alkaline-phosphatase-conjugated secondary antibody diluted in blocking buffer for 30 min at room temperature. Membranes were finally washed twice in blocking buffer, three times in 0.1 M Tris, pH 9.5, containing 0.05 M $MgCl_2$ and 0.1 M NaCl; specific binding was detected using Nitro Blue Tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate (p-toluidine salt; Pierce, Rockford, IL, USA) as substrates and quantified by densitometry. Protein determination was done with the bicinchoninic acid system (Pierce) using BSA as a standard.

Statistical analysis

The results are expressed as the means ±SD of a representative experiment performed in triplicate. The means were compared using student's *t*-test assuming equal variances. $p<0.05$ was considered statistically significant.

Results

Immunocytochemical detection of uPAR in healthy and osteoarthritic cartilage

Normal articular cartilage showed no signs of surface fibrillation or cartilage erosion (Mankin's grade 0-1). In moderately damaged OA cartilage samples, the surface showed fibrillation, clustering of chondrocytes, but the preserved overall thickness of cartilage (Mankin's grade 2-6). Severely damaged OA cartilage showed extensive fissuring and destruction of the extracellular matrix (Mankin's grade 7-13).

In all specimens of severely damaged OA cartilage analyzed, we found chondrocytes intensively immunostained with the anti-uPAR antibody. The uPAR-specific staining was predominantly detectable in outer layers of severely degenerated OA (Fig. 1b,c). In areas of moderately damaged cartilage, chondrocytes of the superficial layer of articular cartilage showed only a moderate immunoreactivity (IR) for uPAR. In samples of non-arthritic cartilage, the majority of chondrocytes did not display any uPAR-specific staining (Fig. 1a).

UPAR expression in IL-1ß-stimulated chondrocytes revealed by immunoelectron microscopy

Immunoelectron microscopy using the "pre-embedding technique" was performed to show the particular distribution of uPAR on chondrocytes cultured in monolayer culture on collagen type II. Immunogold labelled uPAR was detected at the cell membrane and cytoplasmic processes of IL-1ß-stimulated chondrocytes (Fig. 2B), whereas unstimulated control cultures remained unlabelled (Fig. 2A).

Expression of uPAR in IL-1ß-stimulated chondrocytes revealed by Western blotting

To investigate whether IL-1ß stimulates the expression of uPAR, human chondrocytes were incubated with increasing concentrations of IL-1ß (0.1, 1, 5, 10, 50 ng/ml). Western blot experiments indicated that IL-1ß stimulated the expression of uPAR in a dose-dependent manner in chondrocytes *in vitro* (Fig. 3a,b). High levels of uPAR protein expression were detected in experiments with 10 and 50 ng/ml IL-1ß, whereas unstimulated chondrocytes expressed uPAR only in trace quantities.

Densitometry

Densitometric evaluation of a representative experiment performed in triplicate Western blot to detect expression of uPAR by unstimulated (0) human chondrocytes cultured on collagen type II, or chondrocytes exposed to IL-1ß with increasing concentrations (0.1, 1, 5, 10, 50 ng/ml) showed that the relative uPAR expression increased compared to chondrocytes cultured on collagen type II and in absence of IL-1ß (Fig. 3b).

Co-immunoprecipitation assay of uPAR and MMP-9 in IL-1ß-stimulated chondrocytes

Co-immunoprecipitation assay was performed to investigate IL-1ß-stimulated and unstimulated chondrocytes for co-localization and a functional interaction between uPAR and MMP-9. Immunoprecipitation with uPAR antibody or normal IgG serum followed by immunoblotting with anti-MMP-9 antibody in chondrocytes stimulated with IL-1ß (10 ng for 1 h) revealed a protein band of 92 kDa according to

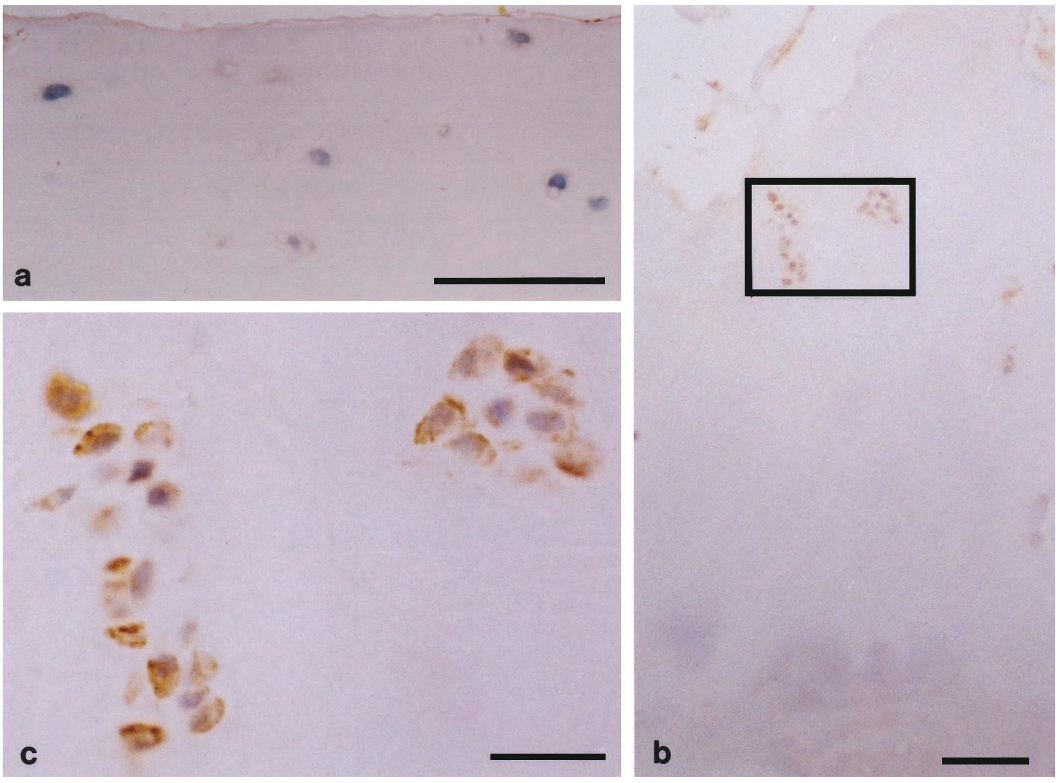

Fig. 1. Detection of uPAR immunoreactivity (IR) in healthy **(a)** and osteoarthritic (OA) cartilage **(b, c)** by the ABC technique. **a.** Chondrocytes of the superficial layer were negative for uPAR staining. **b.** Immunodetection of uPAR in a representative OA cartilage sample. UPAR was detected in a chondrocyte cell cluster in fissured cartilage. **c.** Higher magnification of the frame in 1b. UPAR immunoreactivity was detected at the cell membrane and in the cytoplasm of chondrocytes. Bar: 50 μm.

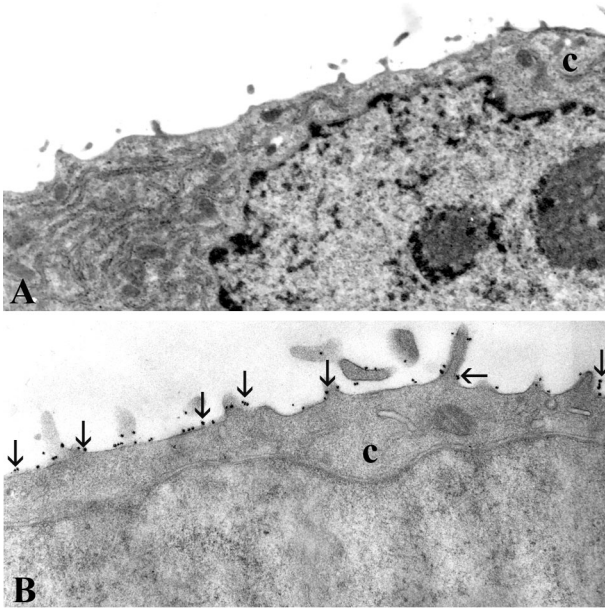

Fig. 2. A, B. Immunoelectron microscopic demonstration of uPAR in monolayer cultures of IL-1ß-stimulated human chondrocytes (c) by immunogold labeling. Gold particles (10 nm) are detectable at the plasma membrane of IL-1ß-stimulated **(B)** chondrocytes with antibodies against uPAR (arrows) but not in unstimulated chondrocytes **(A)**. X 50,000

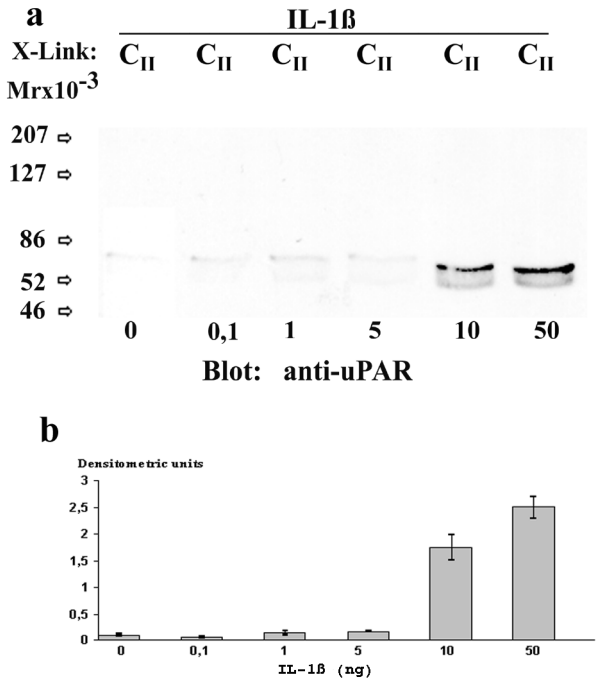

Fig. 3 Dose-dependence of IL-1ß-mediated increase in the expression of uPAR. **a.** Immunochemical detection of uPAR by Western blot analysis in chondrocytes stimulated with varying concentrations of IL-1ß (0, 0.1, 1, 5, 10, 50 ng/ml) for 1 h. Molecular weight markers are indicated in kDa. **b.** Densitometric quantification of uPAR synthesis revealed by Western blot analysis in chondrocytes. Values are means ± SD of a representative experiment performed in triplicate.

the molecular weight of MMP-9. In contrast no MMP-9 band could be shown by immunoblotting in unstimulated chondrocytes, precipitated with uPAR (Fig. 4).

Discussion

The major findings of the present study are as follows: (1) In arthritic samples of human cartilage, we detected an enhanced expression of uPAR compared to non-arthritic controls. (2) In addition, we could demonstrate the IL-1ß-mediated uPAR synthesis in human chondrocytes *in vitro*. (3) Furthermore, we found that uPAR and MMP-9 co-localize and functionally interact in chondrocytes stimulated with IL-1ß (as demonstrated by co-immunoprecipitation assay).

IL-1ß, is a proinflammatory cytokine with a central role in tissue destruction in OA (Vincenti and Brinckerhoff, 2001). Many of the catabolic events that occur in arthritic cartilage are mediated by this cytokine such as MMP-expression, decrease in synthesis of cartilage-specific matrix proteins and COX-1/-2 production (Cook et al., 2000; Robbins et al., 2000). Stimulation of chondrocytes with IL-1ß is commonly used as an *in vitro* model for OA. The present study shows an IL-1ß-stimulated expression of uPAR in human chondrocytes which is in line with a growing body of evidence that components of the plasminogen activator system were induced by inflammatory cytokines. IL-1ß affects uPA in rat granulosa and mesangial cells (Hurwitz et al., 1995), induces the coordinated increase in uPA and uPAR in keratinocytes (Bechtel et al., 1996), in lung fibroblasts (Shetty et al., 1996; Hasegawa et al., 1997) and increases the production of uPA and PAI-1 (inhibitor of plasminogen activator-1) in colon carcinoma cells (Tran-Thang et al., 1996). These findings correlate with IL-1ß,-induced uPAR gene expression in SW 1353 chondrosarcoma

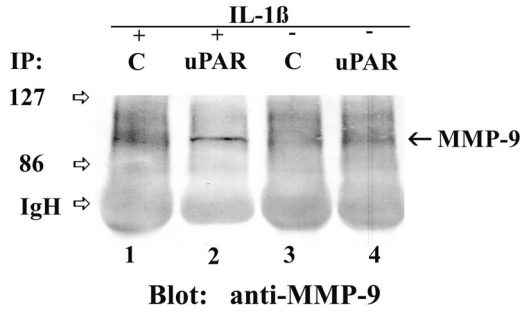

Fig. 4. Co-immunoprecipitation assays and immunoblotting of uPAR and MMP-9 in IL-1ß-stimulated chondrocytes and unstimulated chondrocytes. Immunoprecipitation with uPAR antibody or normal IgG serum (C) followed by immunoblotting with anti-MMP-9 antibody in chondrocytes stimulated with IL-1ß (10 ng for 1h) revealed a protein band of 92 kDa according to the molecular weight of MMP-9. In contrast no MMP-9 band could be shown by immunoblotting in unstimulated chondrocytes, precipitated with uPAR. Molecular weight markers are indicated in kDa.

cells (Vincenti and Brinckerhoff, 2001). In cultured chondrocytes, several findings demonstrate the expression of uPA by IL-1ß and TNF (Bunning et al., 1987; Campbell et al., 1988), respectively. In this study we have demonstrated that IL-1ß stimulates the expression of uPAR, the receptor for uPA in human chondrocytes. In contrast to findings of Fibbi et al. (1994) we could not detect any uPAR immunoreactivity on chondrocytes of healthy cartilage, whereas osteoarthritic cartilage samples expressed a strong immunoreactivity for uPAR in superficial chondrocytes. In healthy cartilage IL-1ß expression is probably too low to induce a significant degree of uPAR. Because IL-1ß simultaneously stimulates the expression of ligand (uPA) (Bunning et al., 1987; Campbell et al., 1988) and the specific receptor (uPAR) in chondrocytes we can assume that pericellular proteolysis must be considerably enhanced by this proinflammatory cytokine. The binding properties of uPAR include the active two-chain uPA and its single-chain pro-enzyme (pro-uPA). Binding of pro-uPA facilitates its activation to uPA, and therefore plasmin generation, which thereby focuses proteolytic activity to the cell surface (Ploug and Ellis, 1994). The broad spectrum proteinase activity can directly mediate breakdown of extracellular matrix constituents, including fibronectin, laminin, and vitronectin (reviewed by Saksela and Rifkin, 1988) and activates latent forms of other proteinases, such as collagenases and stromelysin (Mazzieri et al., 1997). Plasminogen activator inhibitor type 1 (PAI-1) forms a covalent uPA-PAI-1 complex which inhibits uPA activity and induces the internalization and degradation of uPAR-bound uPA (Nykjaer et al., 1992). In addition, cell-bound plasmin has additional functional activities. Plasmin activates growth factors, which are secreted as inactive precursors, such as basic FGF (Falcone et al., 1993) and TGF-ß (Lyons et al., 1990; Chu and Kawinski, 1998; Pedrozo et al., 1999). Studies by Guo et al. (2002) demonstrated that the uPA/plasminogen/plasmin pathway is involved in the cathepsin B-mediated activation of the latent TGF-ß. It has been shown that plasmin is able to induce expression of cytokines such as IL-1ß, and TNF-ß in monocytes. Plasmin induced cytokine expression in monocytes is mediated by the activation of the common transcription factors NF-κB and AP-1 (Syrovets et al., 2001).

In addition to its role in the regulation of pericellular proteolysis, the localization of uPAR in caveolae and its association with the ß1-integrin receptor (Schwab et al., 2001) may point to further involvement of uPAR in signaling pathways of human chondrocytes. Interaction of uPAR with integrins and vitronectin was suggested to regulate cancer cell invasion (Del Rosso et al., 1999). The binding of uPA transforms uPAR from a receptor for uPA into "activated uPAR" which causes cytoskeletal changes, activation of kinases and cell migration (Blasi, 1999). It has been demonstrated that ß1-integrins co-localize and interact with MMPs on chondrocytes by co-immunoprecipitation assay (Schulze-Tanzil et al., 2001). On the other hand it has previously been reported that uPAR co-localizes with ß1-integrins and caveolae on chondrocytes (Schwab et al., 2001). Moreover, we can now show that uPAR co-localizes and functionally interact with MMP-9 on the surface of IL-1ß-stimulated chondrocyte. At present we can only speculate about this functional interaction. One may assume that these cell surface proteinase/receptor complexes may regulate proteinase activity and direct pericellular proteolysis in chondrocytes. We suggest that there may be direct cross-talk between the serine proteinase and matrix metalloproteinase systems in cartilage cultures by uPAR and ß1-integrins that seem to be a central component of these proteinase receptor complexes on chondrocyte surface. Furthermore, more recent investigations revealed another receptor VEGFR-3 that is regulated by IL-1ß and co-localizes also with ß1-integrins (Shakibaei and coworkers, in preparation). These apparently cytokine-sensitive proteinase-receptor complexes may influence via integrin signaling intracellular signal transduction pathways and gene expression in chondrocytes. Previously, we found ß1-integrins in the cartilage extracellular matrix, the function of these extracellular integrins remains obscure, but may be influenced by these proteinase-associated receptor complexes and play a role in directing pericellular proteolysis (Shakibaei and Merker, 1999; Schulze-Tanzil et al., 2001).

Taken together, it appears that in arthritic conditions cytokines induce the expression and interaction of new surface receptors on the chondrocyte surface. These receptors form functional complexes and interact with cell matrix receptors such as ß1-integrins and caveolins initiating arthritis associated signaling pathways. These receptor complexes may represent focal regulation points of pericellular proteolysis on the chondrocyte surface. Inhibition of uPAR signaling in arthritis via antagonists of the uPAI/uPAR system may be a very promising novel approach in arthritis therapy to inhibit degradative proteinases. IL-1ß-mediated expression of uPAR and other arthritis associated receptors (e.g. VEGFR-3) further supports the concept that IL-1ß-signaling represents a major catabolic system in the pathogenesis of osteoarticular disorders such as OA and related rheumatic conditions.

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