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JAK/STAT but Not ERK1/ERK2 Pathway Mediates Interleukin (IL)-6/Soluble IL-6R Down-regulation of Type II Collagen, Aggrecan Core, and Link Protein Transcription in Articular Chondrocytes

ASSOCIATION WITH A DOWN-REGULATION OF SOX9 EXPRESSION*

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Signal transducers and activators of transcription (STAT) factors are cytoplasmic proteins that can be activated by Janus kinases (JAK) and that modulate gene expression in response to cytokine receptor stimulation. STAT proteins dimerize, translocate into the nucleus, and activate specific target genes. In the present study, we show for the first time that interleukin-6 (IL), in the presence of its soluble receptor (sIL-6R), induces activation of JAK1, JAK2, and STAT1/STAT3 proteins in bovine articular chondrocytes. Western blotting and mobility shift assays demonstrated that this effect is accompanied by the DNA binding of the STAT proteins. The mitogen-activated protein kinase pathway was also activated in response to IL-6/sIL-6R association, as reflected by phosphorylation of ERK1 and ERK2 proteins. In these conditions, the expression of cartilage-specific matrix genes, type II collagen, aggrecan core, and link proteins was found to be markedly down-regulated. This negative effect was abolished by addition of parthenolide, an inhibitor of the STAT activation, whereas blockade of the MAP kinases with PD098059 was without significant effect. Thus, activation of the STAT signaling pathways, but not ERK-dependent pathways, is essential for down-regulation of the major cartilage-specific matrix genes by IL-6. In addition, a parallel reduction of Sox9 expression, a key factor of chondrocyte phenotype, was found in these experimental conditions. These IL-6 effects might contribute to the phenotype loss of chondrocytes in joint diseases and the alteration of articular cartilage associated with this pathology.

phic factor, leukemia inhibitory factor, interleukin-11, oncostatin M, and cardiotrophin-1, and which share a common signal transducing molecule, gp130, in their respective complexes (1). IL-6 family members have been shown to induce the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway in several systems (2). JAKs are cytoplasmic tyrosine kinase effectors that are activated by ligand-receptor interactions, leading to tyrosine phosphorylation and activation of the cytoplasmic transcription factors, STATs, which then translocate to the nucleus and act on target gene transcription. Four kinases of the JAK family and seven mammalian STATs have been identified, which may be activated individually or in combination (3).

IL-6 initiates its action by binding to its receptor, which is composed of the 80-kDa ligand-binding subunit (IL-6R) and the signal-transducing subunit, gp130 (4). After ligand binding, the gp130 subunits aggregate into a complex consisting of the IL-6R, two gp130 molecules, and one IL-6 molecule (5). This aggregation activates the gp130-associated protein-tyrosine kinases JAK1, JAK2, and TYK2, which tyrosine phosphorylate gp130, themselves, and STAT3 and -1. Tyrosine phosphorylation of STAT3/1 occurs at a single residue (STAT3-Tyr⁷⁰⁵; STAT1-Tyr⁷⁰¹) which is located in the conserved SH2 allowing homo- as well as heterodimerization (6). During translocation to the nucleus, STAT3 and STAT1 are specifically phosphorylated on a serine residue, a prerequisite for being fully active on gene transcription (7). However, cellular responses to IL-6 can also be implemented via gp130-mediated signaling through interaction of IL-6 with a soluble form of the IL-6R (sIL-6R), which is released by either differential IL-6R mRNA splicing or proteolytic shedding from the cell membrane (review in Ref. 8). This naturally occurring sIL-6R is present in several body fluids of patients with various diseases as well as in healthy subjects (9–11). IL-6 regulates several functions, including immunological reactions in host defense, inflammation, hematopoiesis, and oncogenesis (12–13). Accumulating evidence suggest that IL-6, as well as the other IL-6 family member oncostatin, is implicated in both inflammatory and degenerative joint diseases (rheumatoid arthritis (RA) and osteoarthritis). IL-6 has been shown to increase the amount of inflammatory cells in synovial tissue (14) and to amplify the IL-1 effects on the increased metalloprotease synthesis and inhibition of proteoglycan production (15). The levels of IL-6 in synovial

Interleukin-6 (IL-6)¹ is a pleiotropic factor that belongs to a cytokine subfamily, whose members include ciliary neurotro-

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¹ The abbreviations used are: IL, interleukin; BAC, bovine articular chondrocytes; Me₂SO, dimethyl sulfoxide; ECM, extracellular matrix; ERK, extracellular signal-regulated kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IκB, inhibitor of NFκB; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; MBP, myelin basic protein; MOPS, 3-(N-morpholino)propanesulfonic acid; NFκB, nuclear factor for κ light chain in B cells; PD 098059, Park Davis 098059; sIL-6R, soluble

interleukin-6 receptor; Sox9, Sry-type HMG box-9; STAT, signal transducer and activator of transcription; TYK, tyrosine kinase; gp, glycoprotein; EMSA, electrophoretic mobility shift assay; DTT, dithiothreitol; RA, rheumatoid arthritis.

fluid and serum are significantly higher in RA and osteoarthritis patients relative to controls (16–20). Furthermore, transgenic mice overexpressing IL-6 display abnormal features, including symptoms observed in human RA (21, 22). Today, IL-6 blocking strategy has become a potential approach to treat the IL-6-related immune inflammatory diseases of humans such as Castleman's disease and RA (22). Indeed, inactivation of the IL-6 gene completely protected DBA/1J mice from collagen-induced arthritis (23) or it delayed the onset of the pathological process and also reduced its severity (24), indicating that IL-6 seems to be required for the development of arthritis. Anti-mouse IL-6R antibody treatment also suppressed the development of arthritis and protected knee joints from destructive change in DBA/1J mice immunized with bovine type II collagen (25). Taken together with the findings that treatment of the collagen-induced arthritis with anti-tumor necrosis factor- α and anti-IL-1 antibodies has been shown to be effective on this established murine model (26, 27), IL-6 may even act earlier in the course of the disease than those of tumor necrosis factor- α and IL-1 (22). First treatment of human RA patients with mouse monoclonal anti-IL-6 antibody or with humanized anti-IL-6R antibody have resulted in improvement of symptoms and laboratory findings (28, 29).

Although IL-6 may contribute to the destructive changes of bone and cartilage accompanying joint diseases, its mechanism of action on the cartilage and bone cells and the pathways whereby it controls their gene expression are unknown. Only a limited number of *in vitro* findings have been reported. IL-6 in the presence of soluble IL-6R has been shown to activate osteoclasts to induce bone resorption *in vitro*, suggesting that IL-6 may be involved in osteoporosis (30). A combination of IL-6 and soluble IL-6R was found to activate the JAK/STAT and MAP kinase pathways in MG-63 human osteoblastic cells (31). In articular chondrocytes, contradictory results have been reported on the effects of IL-6 on proteoglycan synthesis. It must be noted that in most of the related studies, the IL-6 effect was investigated in connection with that of other cytokines, such as IGF-1 and IL-1. For example, very high doses of IL-6 were found to decrease the enhancing effect of IGF-1 on proteoglycan synthesis (32). It was also shown that IL-6 was required for the inhibition of proteoglycan synthesis by IL-1 in human articular chondrocytes (15) but the latter results were not reproduced by others (33). The contradictory results may be because of the fact that IL-6 effects were investigated in the absence of soluble IL-6 receptor. Indeed, more recent work from Guerne *et al.* (34) indicates that levels of membrane-anchored IL-6 receptor on chondrocytes are lower compared with those on other cell types, such as hepatocytes, and that *in vitro* addition of sIL-6R to IL-6 is required to observe the full effect of the cytokine. However, until recently, the IL-6 signaling pathway for these cells has not been identified nor has the functional significance been investigated on their effects on the major extracellular matrix components.

The aim of the present study was therefore to analyze the cytoplasmic signaling pathways that were activated in IL-6/sIL-6R-treated bovine articular chondrocytes, focusing on the Janus kinases (JAKs) and signal transducers and activators of transcription (STATs), which are known to be involved in IL-6 signaling, and the mitogen-activated protein (MAP) kinase pathway, which may play a role in IL-6 signaling (35–37). In addition, we determined the relationship between the activation of these IL-6 signaling pathways and the control of type II collagen, aggrecan core and link protein gene expression.

The high mobility group box containing transcription factor Sox9 has been shown to be required for chondrocyte differentiation and for expression of cartilage-specific marker genes

including type II collagen, collagen IX, collagen XI, and aggrecan (38). Sox9 was found to bind and activate a specific enhancer element in both the type II collagen (39) and aggrecan gene (40), and its expression profile was correlated with that of collagen II during the chondrogenesis process (41). Therefore, it was interesting to examine Sox9 expression in the present experimental protocol to investigate the potential implication of this transcription factor in the IL-6-induced effect on matrix gene expression by chondrocytes.

EXPERIMENTAL PROCEDURES

Reagents—Human recombinant IL-6 was purchased from Upstate Biotechnology, Inc. (Lake Placid, NY), human recombinant sIL-6R was from R & D Systems (Abingdon, NY), and IL-1 β was a generous gift from Dr. Soichiro Sato (Shizuoka, Japan). Mouse monoclonal anti-phosphotyrosine antibody (4G10), mouse monoclonal anti-phospho-MAP kinase (specific for threonine and tyrosine-phosphorylated residues of ERK1/ERK2), rabbit anti-MAP kinase 1/2 (ERK1/2-CT), and rabbit polyclonal anti-human TYK2 antibodies were purchased from Upstate Biotechnology, Inc. Rabbit polyclonal anti-JAK1 (HR-785) and anti-JAK2 (C-20), mouse monoclonal anti-STAT1 p84/p91 (C-136), anti-STAT3 (F-2 or C-20), anti-phosphospecific STAT1-Tyr⁷⁰¹ (A-2), STAT3-Tyr⁷⁰⁵ (B-7), anti- β -tubulin (H-235), and nonimmune serum were obtained from Santa Cruz Biotechnology, Inc. (Heidelberg, Germany). Rabbit polyclonal anti-I κ B α was obtained from Cell Signaling Technology. Rabbit polyclonal anti-Sox9 antibody was kindly provided by B. de Crombrughe (Department of Molecular Genetics, University of Texas M.D. Anderson Cancer Center, Houston, TX). Primary antibodies were revealed with anti-rabbit or anti-mouse horseradish peroxidase-labeled secondary antibodies (Santa Cruz Biotechnology, Inc.), using an ECL+Plus Western blot detection kit (Amersham Biosciences, Orsay, France). The oligonucleotide probes: STAT1 (5'-CATGTTATGCATATTCCTGTAAGTG-3'), mutant STAT1 (5'-CATGTTATGCATATTTGGGTAAGTG-3'), STAT3 (5'-GATCCTTCTGGGAATTCCTA-3'), mutant STAT3 (5'-GATCCTTCTGGGCCGCTCTA-3'), and NF κ B (5'-AGTTGAGGGGACTTTCAGGC-3') were supplied by Invitrogen (Cergy Pontoise, France). All other chemicals were of the highest purity available and were from Sigma.

Culture and Treatment of Articular Chondrocytes—Normal bovine articular cartilage was obtained from the knee joints of freshly slaughtered calves through a local slaughterhouse and chondrocytes were cultured as previously described (42). Slices of cartilage were dissected out and kept in Earle's balanced salt solution. Chondrocytes were released by digestion with type XIV protease (4 mg/ml) for 1.5 h and type I collagenase (1 mg/ml) overnight in Dulbecco's modified Eagle's medium at 37 °C. The cells were centrifuged, washed three times, and seeded at high density (3×10^7 cells/55-cm² flask) in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% heat-inactivated fetal calf serum and antibiotics: penicillin (100 IU/ml), streptomycin (100 μ g/ml), and Fungizone (0.25 μ g/ml). The cells were allowed to recover for 48 h at 37 °C in a humidified atmosphere supplemented with 5% CO₂. Then, cells were serum-starved for 16 h before IL-6 and/or sIL-6R treatments as indicated in the figure legends. Experiments were performed three times and data from a representative experiment are shown.

Use of Parthenolide and PD 098059—In some cases, parthenolide (Sigma), an inhibitor of phosphorylation and activation of STAT (43), at 50 μ M and PD 098059 (Sigma), an inhibitor of MAPK pathway (44, 45), at 25 μ M were added after serum deprivation and 2 h before the cytokine treatment. These inhibitors were dissolved in dimethyl sulfoxide (Me₂SO) to give a concentration of 50 mM, and diluted in Dulbecco's modified Eagle's medium immediately prior use. Control incubations contained the same amount of the vehicle. The maximal final concentration of Me₂SO in the cultures was 0.5% (w/v).

Preparation of Cytoplasmic and Nuclear Extracts—Following treatment, chondrocytes were rinsed once with ice-cold phosphate-buffered saline, and lysed in RIPA buffer to prepare cellular extracts for immunoprecipitation, kinase assays, and for Sox9 Western blot. RIPA buffer consisted of 150 mM NaCl, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1 mM NaF, 1% Nonidet P-40, leupeptin, pepstatin A, and aprotinin at 1 μ g/ml, and 10 μ g/ml phenylmethylsulfonyl fluoride, 1 mM Na₃VO₄. Cells were scraped off the plates, incubated on ice for 30 min, and centrifuged at 14,000 $\times g$ for 15 min. Alternatively, chondrocytes were also lysed in hypotonic buffer to prepare nuclear extracts (46, 47). Hypotonic buffer contained 10 mM Hepes (pH 7.9), 0.1% Nonidet P-40, 1.5 mM MgCl₂, 10 mM KCl, 1 mM EGTA, 1 mM EDTA, 5 mM NaF, 0.5 mM DTT, 0.5 mM

phenylmethylsulfonyl fluoride, leupeptin, pepstatin A, and aprotinin at 10 µg/ml, 1 mM Na₃VO₄. Hypertonic buffer was composed of 20 mM Hepes (pH 7.6), 25% glycerol, 1 mM MgCl₂, 420 mM NaCl, 0.2 mM EDTA, 0.25 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride, leupeptin, pepstatin A, and aprotinin at 10 µg/ml, 1 mM Na₃VO₄. Cellular extracts from HepG2 cell line were likewise lysed in RIPA buffer and used as positive controls for TYK2 expression. The protein amount was determined by the Bradford colorimetric procedure (Bio-Rad).

Immunoprecipitation and Western Blot Analysis—Cellular extracts were preincubated with protein A-Sepharose beads to reduce nonspe-

cific binding. Protein levels were determined on the supernatants before immunoprecipitation to have equal amounts of immunoprecipitated proteins. The samples (1–1.5 mg of protein) were incubated with JAK1, JAK2, or TYK2 antibodies overnight at 4 °C, followed by immunoprecipitation with protein A-Sepharose beads. Immunoprecipitates were washed twice with 0.14 M NaCl, 0.01 M Tris-HCl (pH 8.0), 1% Nonidet P-40, 0.1 mM Na₃VO₄, twice with 0.05 M Tris-HCl (pH 6.8), 0.1 mM Na₃VO₄, and boiled for 5 min in Laemmli buffer with 1 mM Na₃VO₄ (48). The supernatants were then analyzed by immunoblotting with JAK1, JAK2, TYK2, or Tyr(P) antibodies as follows. Immunoprecipitated proteins, cytoplasmic extracts (15–30 µg of protein), or nuclear extracts (20 µg of protein) were subjected to SDS-PAGE under reducing conditions and electrophoretically transferred to polyvinylidene difluoride transfer membrane (PerkinElmer Life Sciences, Zaventem, Belgium). Membranes were blocked for 1 h at room temperature in Tris-buffered saline (pH 7.6) with 0.1% Tween 20 (TBS-T) and 10% nonfat dry milk. Then, they were rinsed twice in TBS-T and incubated overnight at 4 °C with primary antibody. After washing with TBS-T, they were incubated for 1 h with the appropriate secondary antibody. The membranes were developed with the ECL+Plus chemiluminescence detection kit. To check for the presence of equal amounts of protein or to analyze the expression of several proteins, blots were stripped (100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl (pH 6.7)) for 30 min at 50 °C with stirring, and reprobed.

In Vitro Kinase Assay of MAP Kinase Activity—MAP kinase activity was determined using myelin basic protein (MBP) as a substrate. To 10 µg of cellular extract were added 20 µl of kinase buffer (25 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 2 mM MnCl₂, 1 mM DTT, 500 µM EDTA, and 40 µM ATP), 1 µg of MBP, and 1 µCi of [³²P]ATP (PerkinElmer Life Sciences). After 30 min at 30 °C, the reaction was stopped with 5 µl of Laemmli buffer and the sample was submitted to SDS-PAGE. The gel was then dried and subjected to autoradiography. The intensity of ³²P-labeled phosphorylated MBP bands was measured by scanning x-ray films (Kodak, X-Omat) and quantified by the ImageQuant program (Amersham Biosciences).

Electrophoretic Mobility Shift Assays (EMSA)—Nuclear extracts (7.5 µg of protein) were incubated in binding buffer for 30 min at 25 °C with the cDNA probes, radiolabeled with [³²P]ATP (25 fmol) using T4 polynucleotide kinase (Invitrogen). Final binding reactions were performed in 13 mM Hepes (pH 7.9), 65 mM NaCl, 0.15 mM EDTA, 8% glycerol, 0.02% Nonidet P-40, 1 mM DTT, and 0.05 µg/µl poly(dI-dC) for STAT1 and STAT3, and in 20 mM Hepes (pH 7.5), 50 mM KCl, 4 mM MgCl₂, 0.2 mM EDTA, 0.5 mM DTT, 0.05% Nonidet P-40, 20% glycerol, 1 mg/ml bovine serum albumin, 0.025 mM poly(dI-dC) for NFκB. Super-shift experiments were performed by incubating the nuclear extracts with nonimmune IgG, anti-STAT1 antibody, or anti-STAT3 antibody for 2 h at room temperature before addition of the labeled probe. The samples were then submitted to a 6 or 8% PAGE in 0.5× TBE (45 mM

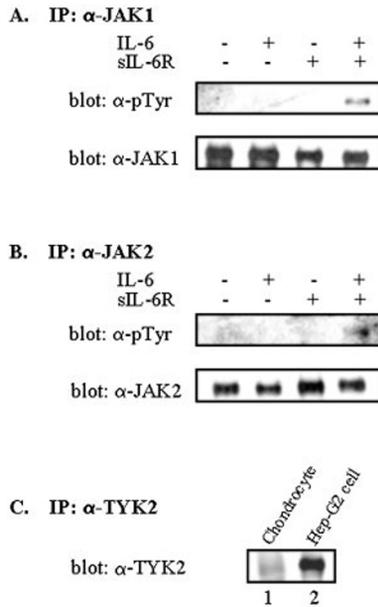


FIG. 1. Effect of IL-6 and/or sIL-6R on tyrosine phosphorylation of JAKs. A and B, after a 16-h preincubation in serum-free medium, chondrocytes were stimulated with or without IL-6 (500 ng/ml)/sIL-6R (500 ng/ml) for 10 min. Then, they were lysed, and the extracts were immunoprecipitated (IP) with anti-JAK1 or anti-JAK2 antibodies and immunoblotted (blot) with anti-Tyr(P) antibody, as described under “Experimental Procedures.” The same membranes were then reblotted with anti-JAK1 or -JAK2 antibodies, respectively. C, after a 16-h preincubation in serum-free medium, chondrocyte (lane 1) or control HepG2 cell (lane 2) extracts were lysed, immunoprecipitated (IP) with anti-TYK2 antibody, and immunoblotted (blot) with the same antibody, as described under “Experimental Procedures.”

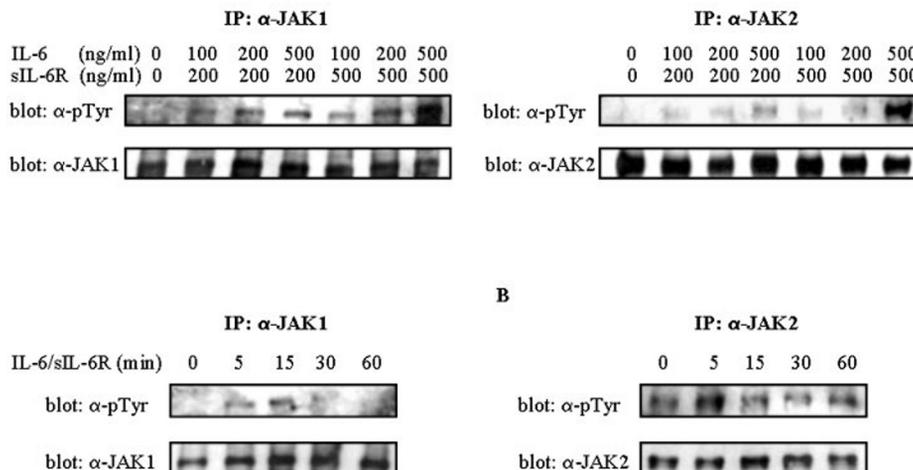


FIG. 2. Effect of IL-6/sIL-6R on tyrosine phosphorylation of JAK1 and JAK2. A, dose-response of JAK1 and JAK2 tyrosine phosphorylation to IL-6/sIL-6R treatment. Serum-deprived chondrocytes were stimulated for 10 min with increasing concentrations of IL-6 (0, 100, 200, or 500 ng/ml) and sIL-6R (0, 200, or 500 ng/ml). Cell extracts were prepared and immunoprecipitated (IP) with anti-JAK1 or -JAK2 antibodies. Immunoprecipitated proteins were analyzed by SDS-PAGE, followed by Western blotting (blot), using antibody to phosphotyrosine. Then, the membranes were stripped and probed again with antibodies to JAK1 or JAK2, respectively. B, time course of JAK1 and JAK2 tyrosine phosphorylation by IL-6/sIL-6R. Serum-deprived chondrocytes were stimulated with IL-6 (500 ng/ml)/sIL-6R (500 ng/ml) for 5, 15, 30, and 60 min. Immunoprecipitation and Western blotting were performed as described above. See “Experimental Procedures” for details.

Tris (pH 7.8), 45 mM boric acid, and 1 mM EDTA) and visualized by autoradiography (46).

RNA Extraction and Northern Blot—Total RNA was extracted from the chondrocyte cultures by the guanidium isothiocyanate-phenol-chloroform procedure, with an additional precipitation step in 6 M LiCl to remove any residual trace of genomic DNA (49). RNA was fractionated by electrophoresis on 1% agarose/MOPS/formaldehyde gel and transferred to a nylon membrane (Bio-dyne B, PerkinElmer Life Sciences). RNA samples were fixed on the membrane by UV exposure. Type II collagen, aggrecan core and link protein, and GAPDH probes were generated by reverse transcription-polymerase chain reaction, using the following primers: type II collagen, forward primer, GACCCATG-CAGTACATG; reverse primer, GACCGTCTTGCCCCACTT; aggrecan core protein, forward primer, CCCTGGACTTTGACAGGGC, reverse primer, AGGAACTCGTCCTTGTCTCC; link protein, forward primer, CTCACCTGGAAGATTATGGG, reverse primer, CACAGCATCCTG-GTCCAG; GAPDH, forward primer, TGGTATCGTGAAGGACTCAT-GAC, reverse primer, ATGCCAGTGAGCTTCCCGTTCAGC.

The probes were 32 P-labeled by random priming using the Strip-EZ DNATM kit (Ambion, Austin, TX). Prehybridization (1 h) and hybridization (18 h) were performed at 42 °C in ULTRAhybTM hybridization buffer (Ambion). Blots were washed several times in 2× SSC (standard saline citrate) and 0.1% SDS at 42 °C. Final washes were in 0.1× SSC plus 0.1% SDS at 42 °C. To detect several mRNA signals, filters were stripped according to the manufacturer's instructions. The 32 P-labeled cDNA-mRNA hybrids were visualized by autoradiography.

Reverse Transcription-Polymerase Chain Reaction—Two-microgram samples of total RNA were reverse transcribed into cDNA in a volume of 50 μ l containing 10 μ l of 5× First Strand buffer, 20 μ M oligo(dT)16, 1 μ l of recombinant ribonuclease inhibitor RNaseOUT (40 units/ μ l), 3 μ l of dNTPs (10 mM each), and 1 μ l of Moloney murine leukemia virus (200 units/ μ l) (Invitrogen) for 15 min at 42 °C and 5 min at 99 °C. Amplification of generated cDNA was performed in a thermocycler using the Invitrogen PCR kit, in the presence of both sense and antisense primers: Sox9, sense 5'-ACAACCCGTCTACACACAGC-3', antisense 5'-ACGATTCATCATCTCC-3'; β -actin, sense 5'-GTGGGG-CGCCAGGCACCA-3', antisense 5'-CTCCTTAATGTACG CACGA-TTTC-3'. The following amplification protocol was used: 1 min at 95 °C, 1 min at 55 °C, 1 min at 72 °C. PCR products were analyzed in 2% agarose gels after staining with ethidium bromide. Densities of the band were quantified using ImageQuant software (Amersham Biosciences) and the relative expression of messengers studied was calculated as relative ratio to β -actin. The procedure was repeated on three different experiments with always the same findings.

RESULTS

The IL-6-soluble Receptor Is Required for JAK1 and JAK2 Tyrosine Phosphorylation by IL-6 in Articular Chondrocytes—To examine whether chondrocytes from normal bovine articular cartilage (BAC) respond to IL-6, primary cultures of bovine articular chondrocytes kept in serum-deprived medium for 16 h were subjected to a 10-min treatment with IL-6 (500 ng/ml), in the presence or absence of sIL-6R (500 ng/ml). Because IL-6 was previously found to activate JAK1, JAK2, and TYK2 in several cell types, we wanted to determine whether this JAK family kinases were also involved in IL-6 signaling in chondrocytes. Cellular extracts were immunoprecipitated with anti-JAK1, anti-JAK2, and anti-TYK2 antibodies and immunoblotted with anti-Tyr(P) antibody. The same membranes were reblotted with JAK1, JAK2, and TYK2 antibodies. The results revealed that BAC express JAK1 and JAK2 in normal conditions (Fig. 1, A and B). In contrast, barely detectable amounts of TYK2 could be detected in these cells, either in controls (Fig. 1C) or in IL-6/sIL-6R-treated cultures (not shown). As a control, cellular extracts from human hepatoma HepG2 cells clearly displayed a significant level of TYK2, validating the antibody efficiency. Phosphorylation of JAK1 and JAK2 was only found in the case of treatment with associated IL-6 and sIL-6R, each element being separately inactive (Fig. 1, A and B). The dose of 500 ng/ml for IL-6 and sIL-6R was used here to obtain maximum effect but lower concentrations were also found to be active (see Fig. 2) and alternatively used for the subsequent study.

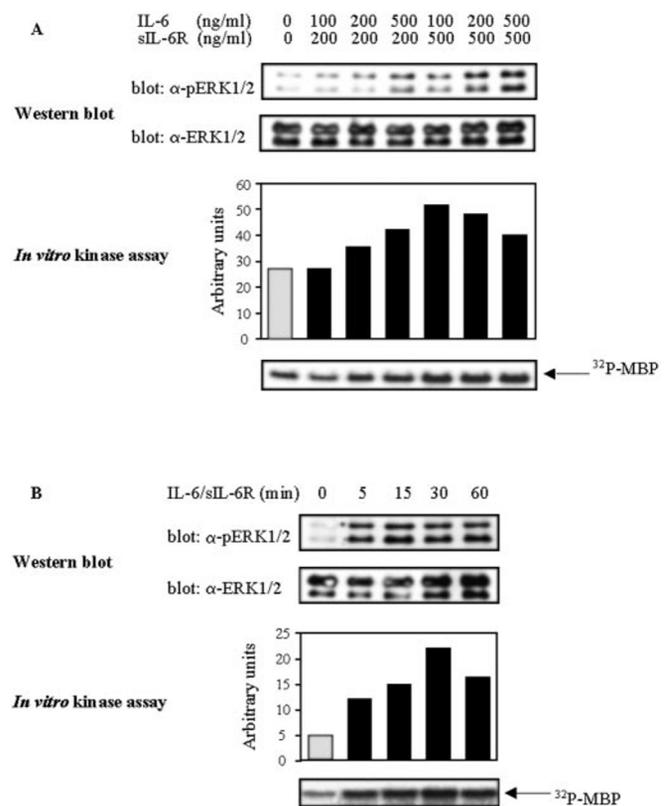


FIG. 3. Effect of IL-6/sIL-6R on MAP kinase activation. A, dose response of MAP kinase activation to IL-6/sIL-6R treatment. Serum-deprived chondrocytes were stimulated for 10 min with increasing concentrations of IL-6 (0, 100, 200, or 500 ng/ml) and sIL-6R (0, 200, or 500 ng/ml). For Western blot (upper panel), cytoplasmic extracts, obtained with hypotonic buffer, were subjected to SDS-PAGE. They were successively immunoblotted with anti-phospho-ERK1/ERK2 (blot: α -pERK1/2) and anti-ERK1/ERK2 (blot: α -ERK1/2) antibodies to verify equal loading. For *in vitro* kinase assay (lower panel), cellular extracts obtained with RIPA buffer were subjected to MAP kinase assay using MBP as a substrate. The histograms represent the levels of phosphorylated MBP, obtained by quantifying the intensity of the MBP-phosphorylated bands (indicated by an arrow) in the autoradiography. B, time course of MAP kinase activation by IL-6/sIL-6R. Cultures of chondrocytes were exposed to IL-6 (500 ng/ml)/sIL-6R (500 ng/ml) for 5, 15, 30, and 60 min in the same conditions as previously. Western blot (upper panel) and *in vitro* MAP kinase assay (lower panel) were performed as described above. See "Experimental Procedures" for details.

IL-6/sIL-6R Induces Dose- and Time-dependent Phosphorylation of JAK1 and JAK2 in Articular Chondrocytes—To investigate the mechanisms of IL-6/sIL-6R effects on chondrocytes, IL-6/sIL-6R-treated cells were analyzed for activation of JAK1 and JAK2 in a dose- and time-dependent manner. BAC were first exposed for 10 min to increasing concentrations of IL-6 (100, 200, and 500 ng/ml), in the presence of either 200 or 500 ng/ml sIL-6R, and protein extracts were analyzed by Western immunoblotting. Activation of JAK1 and JAK2 reached a maximum for the combination IL-6/sIL-6R at 500 ng/ml of each component, although the phosphorylation was already detectable for association of lower levels, such as 100 ng/ml IL-6 and 200 ng/ml sIL-6R, particularly for JAK1 (Fig. 2A). The amounts of respective total proteins remained generally unaffected (lower panels of each blot). Kinetic studies were then undertaken, using the maximum concentration, *i.e.* 500 ng/ml of both IL-6 and sIL-6R, and different time periods (5–60 min). As seen in Fig. 2B, IL-6/sIL-6R-stimulated phosphorylation of JAK1 occurred within 5 min, peaked at 15 min, and rapidly declined to a nondetectable level. JAK2 activation was more sustained in BAC, showing a low level of phosphorylation in controls that suggested the presence of some amount of constitutively active

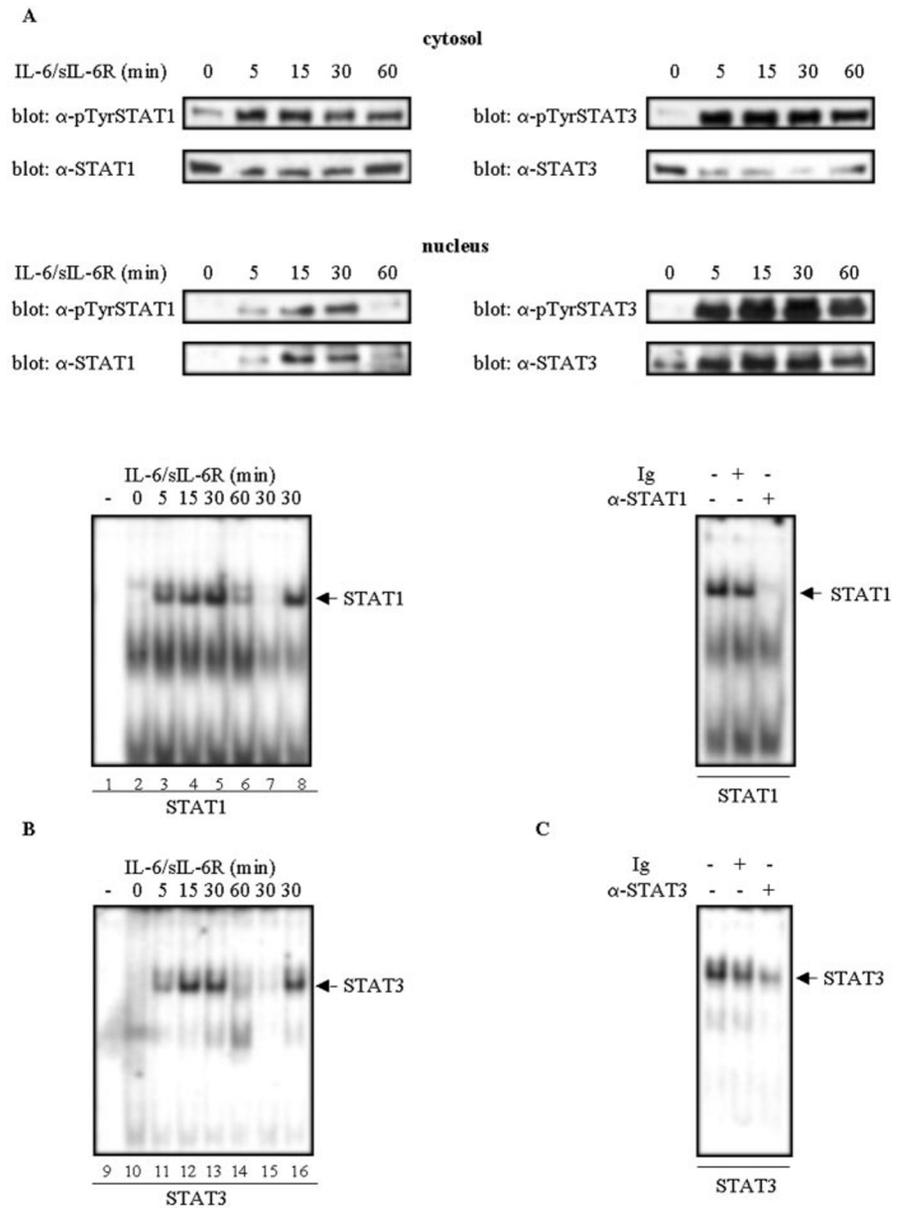


FIG. 4. Effect of IL-6/sIL-6R on STAT activation. Serum-deprived chondrocytes were stimulated with IL-6 (500 ng/ml)/sIL-6R (500 ng/ml) for 5, 15, 30, and 60 min. Cytoplasmic and nuclear extracts were obtained with hypotonic and hypertonic buffer as described under "Experimental Procedures." **A**, STAT1 and STAT3 tyrosine phosphorylation and translocation from cytosol to the nucleus in chondrocytes treated with IL-6/sIL-6R. Cytoplasmic (*upper panel*) and nuclear (*lower panel*) fractions were resolved by SDS-PAGE and successively immunoblotted with anti-phospho-STAT1 (blot: α -pTyrSTAT1), anti-STAT1 (blot: α -STAT1), anti-phosphoSTAT3 (blot: α -pTyrSTAT3), and anti-STAT3 (blot: α -STAT3). **B**, STAT1 and STAT3 binding activity in chondrocytes treated with IL-6/sIL-6R. Nuclear extracts of control (-) (lanes 2 and 10) and stimulated chondrocytes (lanes 3-8 and 11-16) were subjected to EMSA using γ -³²P-labeled DNA probes of STAT1 (*upper panel*) and STAT3 (*lower panel*). Fifty-fold excess of corresponding unlabeled probes (lanes 7 and 15) or mutant unlabeled probes (lanes 8 and 16) were used for competitive inhibition assay. Lanes 1 and 9 were loaded with only radio-labeled probes. Arrows indicate complexes of nuclear extracts with STAT1 or STAT3. **C**, nuclear extracts of stimulated cells (30 min) were preincubated with a nonimmune serum, with anti-STAT1 or anti-STAT3 antibodies for 2 h and subjected to EMSA using γ -³²P-labeled DNA probes of STAT1 (*upper panel*) and STAT3 (*lower panel*). Arrows indicate complexes of nuclear extracts with STAT1 or STAT3. See "Experimental Procedures" for details.

form. A maximum is reached at 5 min, thus earlier than for JAK1.

IL-6/sIL-6R Induces Activation of the MAP Kinase Pathway in Articular Chondrocytes—In addition to activating JAKs, stimulation of BAC with IL-6/sIL-6R elicited the MAPK pathway (Fig. 3). Indeed, IL-6/sIL-6R treatment caused specific threonine/tyrosine phosphorylation of ERK1 and ERK2 in a dose-dependent manner (Fig. 3A, *upper panel*), a maximum being reached with addition of 500 ng/ml of both IL-6 and sIL-6R. This effect was also evidenced by the ability of cell lysates to phosphorylate MBP, a known substrate of *in vitro* MAPK assay (Fig. 3A, *lower panel*). There was no absolute correlation between Western blotting and MBP assay, regarding the peak of activity, probably because other MBP kinases beside ERK1 and ERK2 are contributing to MBP phosphorylation (50). The time course of the effects of IL-6/sIL-6R on ERK1/ERK2 phosphorylation and total MAPK activity is shown in Fig. 3B, *upper and lower panels*, respectively. The phosphorylation of ERK1 and ERK2 showed a plateau of maximum stimulation within 5-15 min, which was maintained for up to 60 min.

IL-6/sIL-6R Induces STAT1 and STAT3 Activation in Artic-

ular Chondrocytes—To gain insight into the mechanism of IL-6/sIL-6R signaling in BAC, cells were treated for different time periods (5-60 min) with 500 ng/ml of both IL-6 and sIL-6R, and the STAT1 and STAT3 phosphorylation status in the cytosol and in the nucleus was analyzed by Western blotting of the related extracts. After 5 min of treatment, STAT1 and STAT3 levels decreased in the cytosol, whereas their phosphorylation increased in parallel (Fig. 4A, *upper panel*). This IL-6/sIL-6R-induced tyrosine phosphorylation of STAT1/STAT3 in the cytosol was accompanied by its translocation to the nucleus (Fig. 4A, *lower panel*). Comparison between the time course of the cytosolic and the nuclear extracts suggests that nuclear translocation reached maximum by ~30 min. It must be noted that levels of STAT1/STAT3 reincreased in the cytosolic extracts after 60 min, more probably reflecting a redistribution of the proteins to this cellular compartment. In some of these experiments, a low level of STAT3 was detected in the nucleus extracts of untreated cultures, attributable to small basal activation.

To determine whether IL-6/sIL-6R induced the DNA binding activity of STAT1 and STAT3, we examined the kinetics of STAT1/STAT3 binding activity in treated BAC. Nuclear ex-

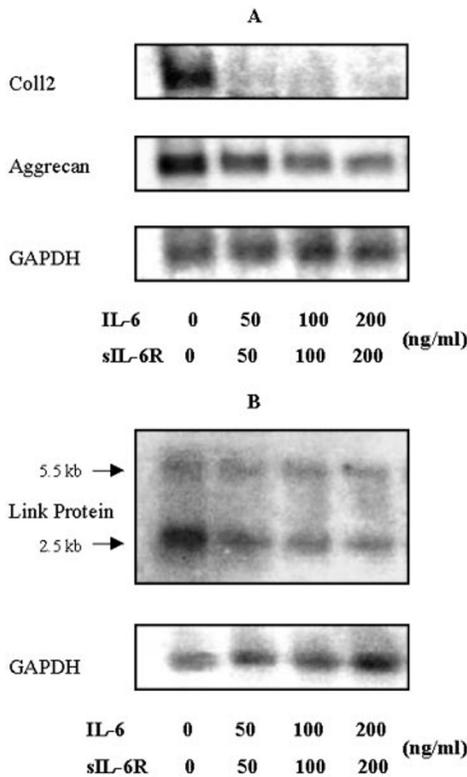


FIG. 5. Effect of IL-6/sIL-6R on type II collagen, aggrecan and link protein mRNA expression. Serum-deprived chondrocytes were stimulated for 24 h with or without IL-6 (50, 100, and 200 ng/ml)/sIL-6R (50, 100, and 200 ng/ml). Total RNA was isolated from the chondrocyte cultures and analyzed by Northern blotting. The blot was hybridized with specific α - 32 P-labeled probes for type II collagen (coll2), aggrecan, and GAPDH mRNA, which served as a control for sample loading (A), and for link protein and GAPDH mRNA successively (B). See "Experimental Procedures" for details.

tracts were prepared from cells exposed to IL-6/sIL-6R for different time periods, in the same conditions as above, and were subjected to EMSA analysis, using 32 P-labeled oligonucleotides containing the STAT1 and STAT3 consensus sequences as respective probes. STAT1 binding activity was significantly increased over the control level, within 5–30 min, which correspond to the same time period where nuclear translocation was observed in the Western blots (Fig. 4B, upper panel). This binding was efficiently competed with a 50-fold molar excess of the same unlabeled oligonucleotide (Fig. 4B, upper panel, lane 7), but not with 50-fold excess of a mutant unlabeled probe (Fig. 4B, upper panel, lane 8). Similar results were found for STAT3 binding activity (Fig. 4B, lower panel). As shown in Fig. 4C, supershift analysis confirmed the identity of the complexes revealed by the previous EMSA study.

IL-6/sIL-6R Decreases Type II Collagen, Aggrecan Core and Link Protein mRNA Expression in Articular Chondrocytes—To determine the effects of IL-6/sIL-6R on type II collagen, aggrecan core and link protein gene expression, we examined the steady-state levels of the corresponding mRNAs in BAC, which were serum-deprived and treated for 24 h with increasing concentrations of IL-6/sIL-6R (50, 100, 200 ng/ml each). The level of type II collagen, aggrecan core and link protein mRNAs was determined by Northern blotting and normalized to the level of GAPDH mRNA. This treatment resulted in a marked decrease of steady-state levels of these three cartilage-specific mRNAs (Fig. 5). Thus, 50/50 ng/ml IL-6/sIL-6R already decreased type II collagen mRNA levels and the effect was so marked for greater concentrations (100/100 and 200/200 ng/ml) that the signal was almost undetectable. Likewise, but to a

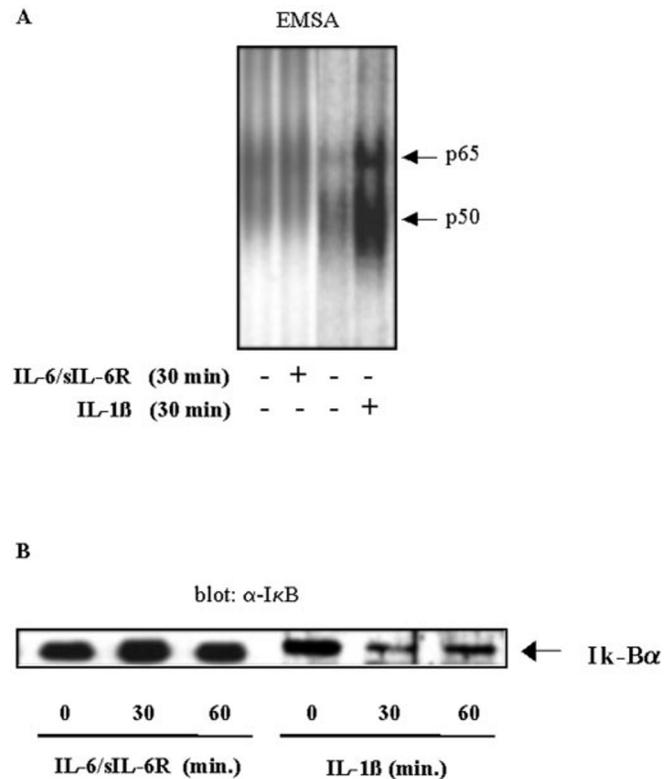


FIG. 6. Effect of IL-6/sIL-6R on NF κ B activation. Serum-deprived chondrocytes were stimulated for 30 and 60 min with IL-6 (100 ng/ml)/sIL-6R (100 ng/ml) or with IL-1 β (10 ng/ml) as a positive control. Cytoplasmic and nuclear extracts were obtained with hypotonic and hypertonic buffer as described under "Experimental Procedures." A, effect on NF κ B DNA binding. Nuclear extracts of control (0) and stimulated chondrocytes (30 min) were subjected to EMSA using γ - 32 P-labeled DNA probes of NF κ B. p65 and p50 indicate the subunits of the NF κ B system. B, effect on I κ B degradation. Cytoplasmic extracts were subjected to SDS-PAGE and immunoblotted with anti-I κ B α antibody.

lower extent, aggrecan core protein mRNA levels were dose dependently reduced after incubation with 50/50, 100/100, and 200/200 ng/ml IL-6/sIL-6R. Similar results were observed for the link protein mRNA, albeit with smaller effect.

Parthenolide and PD098059 Inhibit JAK/STAT and MAPK Pathways, Respectively, in Articular Chondrocytes—To further investigate the potential role of IL-6/sIL-6R-induced activation of JAK/STAT and MAPK pathways in the control of type II collagen and aggrecan core and link protein gene expression in articular chondrocytes, we used inhibitors, parthenolide and PD098059, to selectively block these signaling pathways. Because parthenolide was shown to also block NF κ B activation (51), we wanted to determine whether IL-6/sIL-6R could activate NF κ B in our experimental conditions (Fig. 6). Effects of IL-6/sIL-6R (100/100 ng/ml) on NF κ B activity were analyzed by EMSA and by I κ B Western blotting after 30 or 60 min exposure. As shown in Fig. 6, both EMSA and Western blot analyses did not reveal any significant change between controls and IL-6/sIL-6R-treated chondrocytes, attesting that IL-6/sIL-6R did not activate NF κ B in the present conditions, whereas IL-1 β , which is well known for its ability to stimulate NF κ B, was found efficient in the same conditions (positive control).

Next, we wanted to verify that these inhibitors were efficient in the experimental conditions used here on articular chondrocytes. Thus, serum-deprived BAC were incubated for 2 h with or without 50 μ M parthenolide or 25 μ M PD098059, or with 0.5% Me $_2$ SO as the control vehicle of these compounds. They were then subjected to IL-6/sIL-6R (100/100 ng/ml) for 30 min. Effects on STAT1 and STAT3 DNA binding activity were ana-

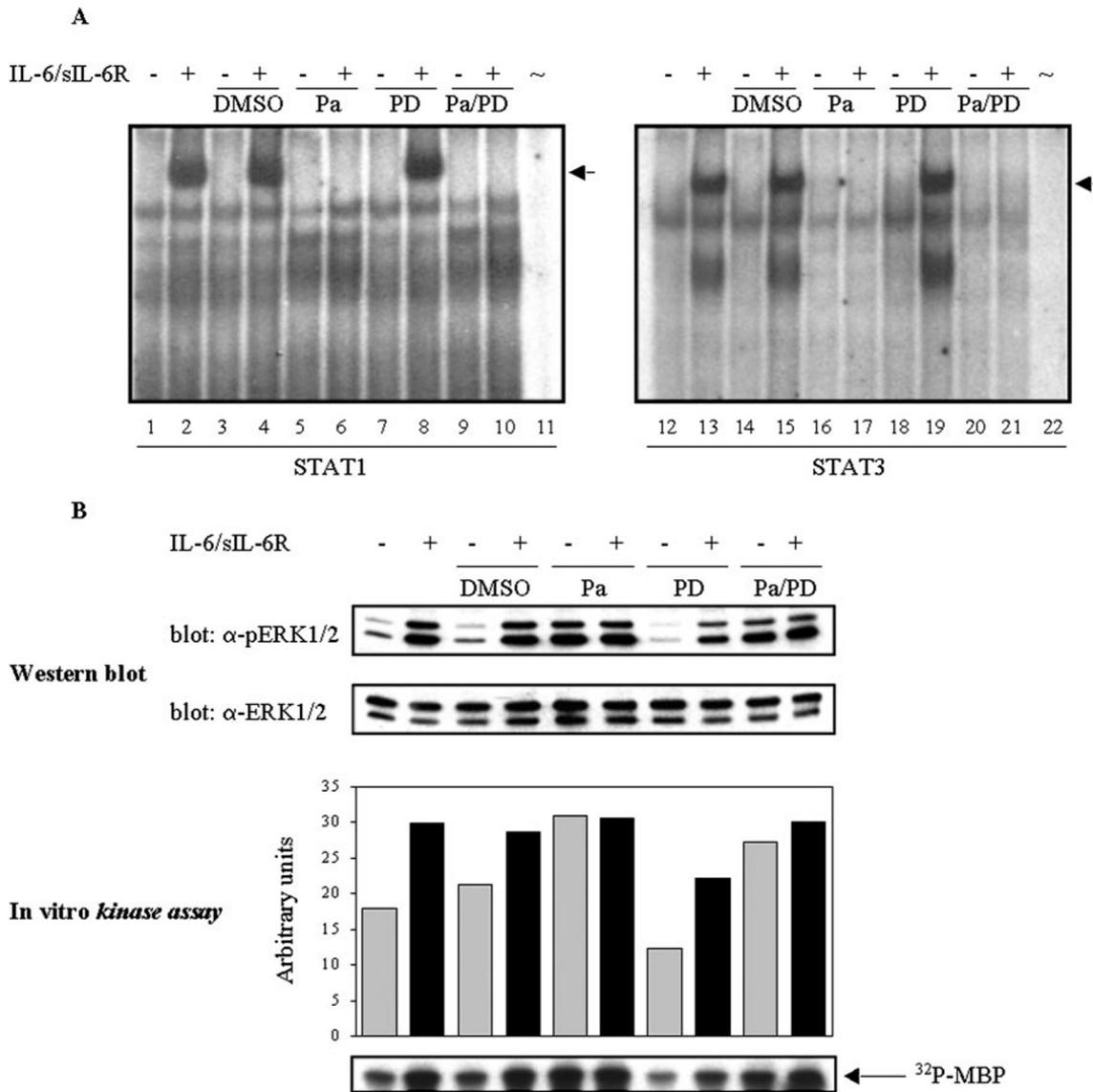


FIG. 7. Effect of parthenolide and PD098059 on JAK/STAT and MAP kinase pathways. Serum-deprived chondrocytes were incubated for 2 h with or without Me₂SO (0.5% (v/v) final concentration), parthenolide (Pa, 50 μ M) and PD098059 (PD, 25 μ M). Then, they were stimulated (+) or not (–) with IL-6 (100 ng/ml)/sIL-6R (100 ng/ml) for 30 min. Cytoplasmic and nuclear extracts were obtained with hypotonic and hypertonic buffers as described under “Experimental Procedures.” **A**, effects of parthenolide and PD098059 on STAT1 and STAT3 binding activity. Nuclear extracts of control (–) (lanes 1, 3, 5, 7, 9, 12, 14, 16, 18, and 20) and stimulated chondrocytes (+) (lanes 2, 4, 6, 8, 10, 13, 15, 17, 19, and 21) were subjected to EMSA using γ -³²P-labeled DNA probes of STAT1 (left) and STAT3 (right). Lanes 11 and 22 were loaded with only radiolabeled probes. Arrows indicate complexes of nuclear extracts with STAT1 or STAT3. **B**, effects of parthenolide and PD098059 on ERK1/ERK2 phosphorylation and MAP kinase activity. Cytoplasmic extracts were subjected to SDS-PAGE. They were successively immunoblotted with anti-phospho-ERK1/ERK2 (blot: α -pERK1/2) and anti-ERK1/ERK2 (blot: α -ERK1/2) antibodies to verify equal loading. For *in vitro* kinase assay (lower panel), cellular extracts were subjected to MAP kinase assay using MBP as a substrate. The histograms represent the levels of phosphorylated MBP, obtained by quantifying the intensity of the MBP-phosphorylated bands (indicated by an arrow) in the autoradiography. See “Experimental Procedures” for details.

lyzed by EMSA, whereas ERK1 and ERK2 activity was studied by Western blotting, together with determination of total MAPK activity by MBP *in vitro* assay.

As can be seen on Fig. 7A, STAT1 and STAT3 DNA binding was completely abrogated by the parthenolide treatment, whereas it was not affected by PD098059. We demonstrated likewise that parthenolide blocked STAT1 and STAT3 phosphorylation (data not shown). Additionally, parthenolide was still efficient in the presence of PD098059, suggesting that this latter does not interfere with the parthenolide effect on STAT DNA binding. Although not completely inhibited, ERK activity was markedly reduced at the PD098059 concentration used here, as revealed by Western blotting (Fig. 7B). It must be noted that concentrations greater than 25 μ M PD098059 were

difficult to handle because of low solubility in aqueous medium. On the other hand, parthenolide by itself was found to stimulate the basal MAPK activity. The data were also confirmed by the results obtained with MBP *in vitro* kinase assay (Fig. 7B).

Parthenolide, but Not PD098059, Abrogates IL-6/sIL-6R-induced Down-regulation of Type II Collagen, Aggrecan Core and Link Protein Transcription in Articular Chondrocytes—To further study the mechanism of IL-6/sIL-6R signaling leading to type II collagen, aggrecan core and link protein mRNA down-regulation, BAC were exposed for 24 h to IL-6/sIL-6R at the concentration of 100/100 ng/ml, alone or in combination with the STAT inhibitor parthenolide (50 μ M) or MAPK inhibitor PD098059 (25 μ M), and Northern blotting of RNA was performed as previously described.

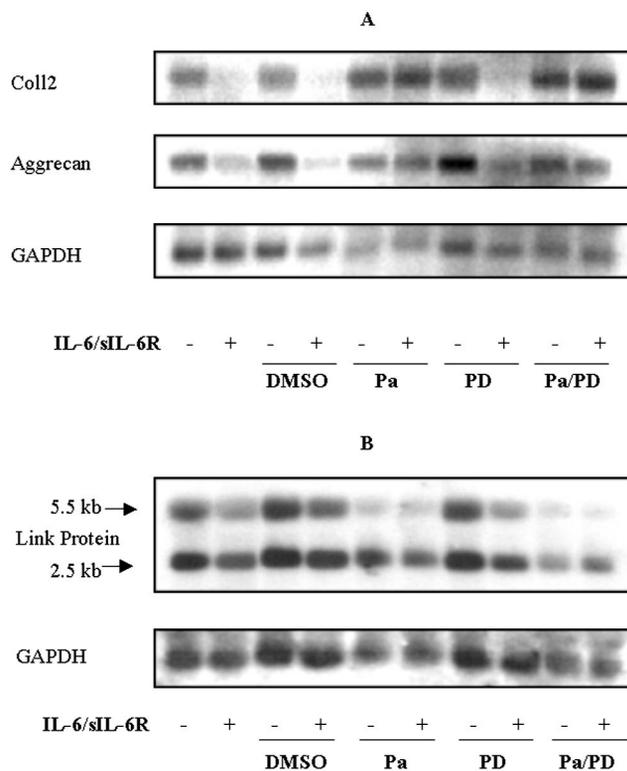


FIG. 8. Effect of parthenolide and PD098059 on IL-6/sIL-6R induced down-regulation of type II collagen, aggrecan and link protein mRNA expression. Serum-deprived chondrocytes were incubated for 2 h with or without Me₂SO (0.5% (v/v) final concentration), parthenolide (Pa, 50 μ M) and PD098059 (PD, 25 μ M). Then, they were stimulated (+) or not (-) with IL-6 (100 ng/ml)/sIL-6R (100 ng/ml) for 24 h. Total RNA was isolated from the chondrocyte cultures and analyzed by Northern blotting. The blot was hybridized with specific α -³²P-labeled probes for type II collagen (coll2), aggrecan, and GAPDH mRNA (A), and for link protein and GAPDH mRNA successively (B). See "Experimental Procedures" for details.

It was found that parthenolide alone was capable of inducing a significant increase of the basal level of type II collagen mRNA. Furthermore, it completely abrogated the IL-6/sIL-6R down-regulation of the collagen message (Fig. 8A). PD098059 alone was without significant effect on the basal collagen mRNA level, related to the controls (Me₂SO-containing cultures). This inhibitor was also unable to influence the IL-6/sIL-6R-induced inhibition. Similar results were obtained for aggrecan core and link protein mRNA steady-state levels (Fig. 8, A and B). When both inhibitors were combined, the IL-6/sIL-6R inhibitory effect was abolished for the three mRNA. These findings demonstrate that the IL-6/sIL-6R inhibitory effect on type II collagen, aggrecan core and link protein gene transcription in chondrocytes is mediated by the JAK/STAT pathway, whereas the MAPK signaling is not essential for these mechanisms.

Sox9 Expression Is Down-regulated by IL-6/sIL-6R—To determine a potential effect of IL-6/sIL-6R on the expression of Sox9, a key factor that could be implicated in the observed down-regulation of the cartilage-specific genes produced by chondrocytes (type II collagen, aggrecan), we analyzed the steady-state levels of Sox9 mRNA by semiquantitative reverse transcriptase-PCR and the protein amounts by Western blotting (Fig. 9, A and B). We found a parallel decrease of both mRNA and protein levels under the effect of IL-6/sIL-6R. As expected, IL-1 β also exerted an inhibition on Sox9 expression in the same experimental conditions (52).

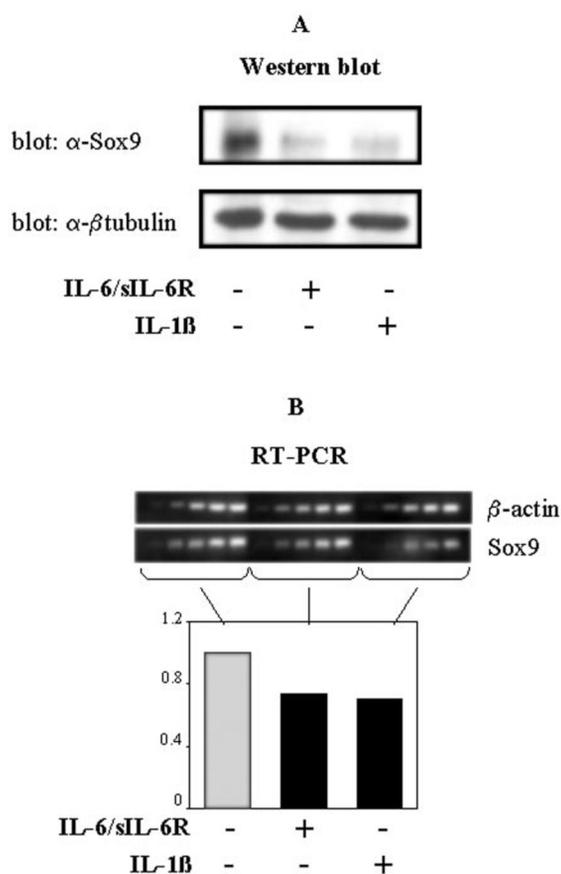


FIG. 9. Effect of IL-6/sIL-6R on Sox9 expression. Serum-deprived chondrocytes were stimulated with IL-6 (100 ng/ml)/sIL-6R (100 ng/ml) or with IL-1 β (10 ng/ml) for 24 h. A, cellular extracts (30 μ g of protein obtained with RIPA buffer) were subjected to SDS-PAGE and immunoblotted with anti-Sox9 and anti- β -tubulin as a control for protein loading. B, total RNA was used for reverse transcriptase (RT)-PCR assay of SOX9 and β -actin mRNA steady-state levels. PCR reactions were carried out at increasing numbers of cycles (20, 23, 26, 29, and 32 cycles for β -actin and 25, 28, 31, 34, and 37 cycles for SOX9) to check the linearity of amplification before analysis of transcript scanning. Results are expressed as a representative histogram of Sox9 relative expression versus β -actin.

DISCUSSION

We show here that IL-6 activates JAK/STAT and MAPK pathways in the primary bovine articular chondrocyte system. Furthermore, blockage of STAT phosphorylation by parthenolide results in the inhibition of IL-6-induced down-regulation of type II collagen, aggrecan core and link protein gene expression. On the other hand, inhibition of MAP kinase pathway by PD 098059 does not significantly alter the cytokine effect on transcriptional activity of these matricial genes. These results demonstrate for the first time that the JAK/STAT pathway is essential for IL-6 signaling responses in chondrocytes, including down-regulation of cartilage-specific ECM components.

Chondrocytes play an essential role in regulating the balance between ECM degradation and synthesis and their primary function to maintain cartilage ECM integrity is significantly altered in both inflammatory and degenerative joint diseases because of changed levels of local growth factors and cytokines (53, 54). IL-6 was initially considered as a proinflammatory cytokine-like tumor necrosis factor- α and IL-1, because of its IL-1-like effects on immune and hepatic cells. Recently, the cytokine has been shown to enhance the aggrecanase-mediated proteoglycan catabolism in articular cartilage (55). Elevated IL-6 levels in serum and synovial fluids correlate with inflammatory and erosive arthritides (11, 16–20). Furthermore, IL-1

and tumor necrosis factor- α can also stimulate the production of IL-6 by articular cartilage chondrocytes (56, 57). As previously reported by others (34, 55), we found that the effect of IL-6 requires the presence of its soluble receptor (sIL-6R) in the culture medium. This is probably because of the fact that levels of membrane-anchored IL-6 receptor (gp80) on chondrocytes are very low compared with those on other cell types such as hepatocytes (34). However, high amounts of sIL-6R are found in synovial fluid of arthritic patients (5–40 ng/ml) (11) and are likely to participate in the signaling of IL-6 in both synovial cells and articular chondrocytes. Although the concentrations used here, which were chosen to demonstrate more efficiently the IL-6 action, may appear greater than those found in the joint of arthritic patients, it was clear here that the effect could already be detected for equivalent doses.

IL-6, as other members of the IL-6-type cytokines, including oncostatin M, are known to induce the JAK/STAT signaling cascade in other systems (2) but this has not been shown in chondrocytes. Our demonstration that IL-6 triggers phosphorylation of JAK1, JAK2, STAT1, and STAT3 in primary cultures of articular chondrocytes suggest that these cells, together with the synoviocytes, could be a source of activated STAT1 and STAT3 found in the synovial fluid and fibroblasts of patients with RA (58, 59). Interestingly enough, we demonstrate here for the first time that the IL-6-induced activation of the JAK/STAT pathway results in down-regulation of three important specific genes of cartilage, type II collagen, aggrecan core and link proteins, suggesting that IL-6 not only contributes to cartilage erosion by inducing degradative enzymes but also via reduction of ECM synthesis. Indeed, parthenolide, a sesquiterpene lactone previously found as an effective inhibitor of IL-6-type cytokine by blocking STAT phosphorylation (43), was capable of abolishing the STAT DNA-binding and the subsequent IL-6 inhibition of the three ECM genes transcription. Although the inhibitor was also shown to block the activation of NF- κ B in other systems, we did not detect any significant effect of IL-6 on this transcription factor in our experimental conditions. Interestingly enough, putative STAT3 regulatory sites have been already reported in the promoter or first intron regions of the genes for rat type II collagen, human and rat link protein, and rat stromelysin (metalloprotease-3), suggesting that these are target genes for the JAK-STAT signaling pathway (60, 61). Similarly, a potential STAT sequence has been found in the promoter of the human aggrecan gene, which may confer responsiveness to cytokines of the IL-6 family (62). Work is in progress to determine whether these putative STAT binding sites are actually implicated in the expression control of these genes in articular chondrocytes. However, the fact that the promoters of these genes share similar STAT elements strongly suggests that they are coordinately regulated by IL-6, as observed in this study.

We found here that in the presence of parthenolide, the MAP kinase pathway was already activated in the controls (in the absence of IL-6/sIL-6R), as shown by increased phosphorylation of ERK1 and ERK2 on the Western blots. This clearly indicates that an interaction exists between the JAK/STAT and MAPK pathways in articular chondrocytes. To this regard, it has been demonstrated in a recent report that there is a balanced activation of the SHP-2/Ras/Erk and STAT signaling cascades emanating from gp130 (63). The authors have generated mice with a COOH-terminal gp130^{ΔSTAT} “knock-in” mutation that deletes all STAT-binding sites, but still permits activation of SHP-2/Ras/Erk pathway. Gp130^{ΔSTAT} mice phenocopied mice deficient for IL-6 (impaired humoral and mucosal immune and hepatic acute phase responses) and LIF (failure of blastocyst implantation). However, unlike mice with null

mutations in any of the components of the gp130 signaling pathway, gp130^{ΔSTAT} mice also displayed gastrointestinal ulceration and a severe joint disease with features of chronic synovitis and degradation of the articular cartilage. Interestingly enough, a mitogenic hyper-responsiveness of synovial cells was found, resulting from the sustained gp130-mediated SHP-2/Ras/Erk activation because of impaired STAT-mediated induction of suppressor of cytokine signaling proteins, which normally limits gp130 signaling. These data identified the importance of STAT signals in promoting inhibitory feedback signals on gp130 and the associated SHP-2/Ras/Erk pathway, thereby reinforcing the concept that the two pathways are under reciprocal negative control (64).

Our study indicates that Sox9, a key factor of type II collagen and aggrecan expression, is down-regulated by IL-6/sIL-6R association at both mRNA and protein levels. This suggests that Sox9 may play a role in the molecular mechanism whereby the cartilage-specific matrix gene expression is inhibited by IL-6 associated to its soluble receptor, through the JAK/STAT signaling pathway. The observed correlation between Sox9 inhibition and down-regulation of matrix gene expression under the IL-6/sIL-6R effect suggests a link between this transcription factor and the STAT proteins in the mechanisms controlling the transcriptional activity of these matricial genes. How Sox9 may cooperate with the STATs at the level of DNA-binding activity and subsequent modulation of type II collagen, aggrecan core and link protein gene transcription remains to be elucidated.

Finally, our data strongly suggest that the activation of the JAK/STAT pathway by inflammatory cytokines of the IL-6-type mediates a change in the chondrocyte phenotype and loss of cartilage matrix by inhibiting the expression of specific cartilage matrix molecules, including type II collagen, aggrecan core and link proteins, that maintain the differentiated phenotype of chondrocytes. Therefore, inhibiting this signaling pathway may be an effective approach in preventing cartilage alteration induced by IL-6-type cytokines in arthritides, as it has been recently proposed (65–69). However, because of the cross-talk between ERK and STAT pathways, already mentioned above, a combination of selective blockers may be necessary.

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