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Responses to the Proinflammatory Cytokines Interleukin-1 and Tumor Necrosis Factor α in Cells Derived From Rheumatoid Synovium and Other Joint Tissues Involve Nuclear Factor κB–Mediated Induction of the Ets Transcription Factor ESE-1

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Objective. **To investigate the expression of the novel Ets transcription factor ESE-1 in rheumatoid synovium and in cells derived from joint tissues, and to analyze the role of nuclear factor κB (NF-κB) as one of the central downstream targets in mediating the induction of ESE-1 by proinflammatory cytokines.**

Methods. **ESE-1 protein expression was analyzed by immunohistochemistry using antibodies in synovial tissues from patients with rheumatoid arthritis (RA) and osteoarthritis (OA). ESE-1 messenger RNA (mRNA) levels were analyzed by reverse transcriptase–polymerase chain reaction or Northern blotting in human chondrocytes, synovial fibroblasts, osteoblasts, and macrophages, before and after exposure to interleukin-1β (IL-1β), tumor necrosis factor α (TNFα), or lipopolysaccharide (LPS) with or without prior infection with an adenovirus encoding the inhibitor of nuclear factor κB (IκB). The wild-type ESE-1 promoter and the ESE-1 promoter mutated in the NF-κB site were cloned into a luciferase reporter vector and analyzed in transient transfections. Electrophoretic mobility shift assays (EMSAs) and supershift assays with antibodies against members of the NF-κB family were conducted using the NF-κB site from the ESE-1 promoter as a probe.**

Results. **Immunohistochemical analysis showed specific expression of ESE-1 in cells of the synovial lining layer and in some mononuclear and endothelial cells in RA and OA synovial tissues. ESE-1 mRNA expression could be induced by IL-1β and TNFα in cells such as synovial fibroblasts, chondrocytes, osteoblasts, and monocytes. Transient transfection experiments and EMSAs showed that induction of ESE-1 gene expression by IL-1β requires activation of NF-κB and binding of p50 and p65 family members to the NF-κB site in the ESE-1 promoter. Overexpression of IκB using an adenoviral vector blocked IL-1β–induced ESE-1 mRNA expression. Chromatin immunoprecipitation further confirmed that NF-κB binds to the ESE-1 promoter in vivo.**

Conclusion. **ESE-1 is expressed in synovial tissues in RA and, to a variable extent, in OA, and is specifically induced in synovial fibroblasts, chondro-**

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cytes, osteoblasts, and monocyte/macrophages by IL-1β, TNFα, or LPS. This induction relies on the translocation of the NF-κB family members p50 and p65 to the nucleus and transactivation of the ESE-1 promoter via a high-affinity NF-κB binding site. ESE-1 may play a role in mediating some effects of proinflammatory stimuli in cells at sites of inflammation.

Inflammatory processes contribute to the pathologic events that lead to tissue injury and destruction in autoimmune diseases and other inflammatory conditions. Rheumatoid arthritis (RA) is a prototypical immune-mediated disease characterized by chronic inflammation in the synovium and destruction of joint tissues. In RA and other joint diseases such as osteoarthritis (OA), a central role for interleukin-1 (IL-1) and tumor necrosis factor α (TNFα) has been established (1,2). These cytokines, similar to bacterial endotoxins, have major roles in inflammatory responses via the activation of a variety of transcription factors, including nuclear factor κB (NF-κB), activator protein 1 (AP-1), and CAAT enhancer binder protein (C/EBP) family members (1–6). One of the major transcriptional circuits implicated in inflammation is the NF-κB/inhibitor of NK-κB (IκB) pathway. NF-κB is rapidly activated by proinflammatory cytokines such as IL-1 and TNFα and by endotoxins, and is involved in the regulation of a large set of inflammatory response genes, including various cytokines and chemokines, acute-phase proteins, cell adhesion proteins, and immunoglobulins (3). Although many of these genes are regulated directly by NF-κB via high-affinity binding sites within their respective promoter regions, this mechanism does not account exclusively for the regulation of a significant number of cytokine-responsive genes. Thus, additional pathways play critical roles in the transcriptional regulation of these genes.

Our interest has focused on the role of the Ets transcription factor family in epithelial cell differentiation, and in this regard we and others have isolated 4 novel epithelial cell–specific members of the Ets transcription factor family, ESE-1 (ESX/ELF3/ERT/JEN), ESE-2 (ELF5), ESE-3 (EHF), and prostate-derived Ets factor (PDEF) (PSE) (7–17). Under normal physiologic conditions, ESE-1 expression is restricted to epithelial cell types in a variety of tissues, with highest expression in the gastrointestinal tract (7,14,17). Although it is not expressed significantly under normal physiologic conditions, we recently demonstrated that ESE-1 expression is transiently induced by proinflammatory cytokines in endothelial and vascular smooth muscle cells, indicating a potential role of ESE-1 in the vasculature during inflammatory processes (18). We also have strong evidence that ESE-1 can activate the cyclooxygenase 2 (COX-2) gene (Grall F, et al: unpublished observations) and the inducible nitric oxide synthase (iNOS) gene (18), 2 genes central to inflammatory processes.

We now report that ESE-1 is expressed in RA synovial tissues and, to a variable extent, in OA and is rapidly and transiently induced in the major cell types of joint tissues by IL-1β or TNFα, and endotoxin via activation of NF-κB. Thus, ESE-1 may play a major role in mediating the effects of proinflammatory cytokines and endotoxin in nonepithelial cell types present at sites of inflammation.

MATERIALS AND METHODS

Patient samples and cell culture. Joint tissues from patients with RA and OA were obtained as discarded materials from total joint replacement surgery or arthroplasty. Tissue procurement was approved by the Institutional Review Board. RA synovial tissue samples were either fixed and embedded in paraffin, or cultured for the generation of adherent synovial fibroblasts. Dispersed synovial cells were prepared according to a previously published method (19). Briefly, synovial tissues were minced on tissue culture plates and treated with type 1 collagenase (4 mg/ml; Worthington, Lakewood, NJ) in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Grand Island, NY), incubated for 1 hour at 37°C, treated with 0.25% trypsin for 30 minutes, harvested, and centrifuged at 1,000 revolutions per minute for 10 minutes. Pellets were suspended in 0.05% trypsin–0.02% EDTA for 10 minutes, centrifuged, and resuspended in 50% phosphate buffered saline (PBS), 50% DMEM containing 10% fetal calf serum (FCS; Sigma, St. Louis, MO). Cells were then centrifuged and suspended in DMEM, 10% FCS, and plated at a density of 10×10^6 cells/10-cm plate. Cells were grown initially for 7–10 days and subsequently subjected to 2–4 passages. Passaged cells were stimulated with IL-1β (R&D Systems, Minneapolis, MN) at 10 ng/ml, indomethacin (Sigma) at 10 μM, or TNFα (R&D Systems) at 10 ng/ml.

The cell lines T/C-28a2, C-28/I2, and C-20/A4 (immortalized human chondrocytes), THP-1 (human monocytic), LB-12 (human osteoblast-like large T antigen immortalized bone marrow stromal cells), and U-138 MG and U-373 MG (human glioma) were grown and treated with cytokines as previously described (25–27). RAW 264-7 (murine monocytic) cells were cultured in DMEM containing 10% FCS and antibiotics (penicillin and streptomycin; Gibco BRL).

Tissue preparation and immunohistochemistry. RA and OA samples were fixed for 48 hours in 4% paraformaldehyde, and specimens containing bone were subsequently decalcified for at least 2 weeks in 14% EDTA. Specimens were processed for paraffin embedding (Citadel 1000; Shandon, Pittsburgh, PA), and 4-μm serial sections were cut for immunohistochemical staining using 2 alternate techniques. Briefly, sections were deparaffinized, followed by microwave antigen

retrieval (GE sensor convection microwave oven) in 10 m*M* EDTA pH 7.5, at 93°C for 10 minutes and allowed to cool for at least 2 hours. Sections were washed in Tris buffered saline (TBS) and incubated for 60 minutes in serum block (10% FCS plus 10% normal rabbit serum diluted in TBS). Sections were then incubated with an affinity-purified rabbit polyclonal anti-human ESE-1 antibody (1:1,000; Chemicon, Temecula, CA) or with an isotype-matched control antibody (polyclonal rabbit IgG; Santa Cruz Biotechnology, Santa Cruz, CA) for 60 minutes. All incubations were carried out at room temperature. Sections were washed between every subsequent step with TBS. Endogenous peroxidase activity was blocked by incubating the sections in 3% H_2O_2 (diluted in TBS) for 30 minutes. Sections were subsequently incubated for 30 minutes with biotinylated swine anti-rabbit $F(ab')_2$ (Dako, Carpinteria, CA), followed by horseradish peroxidase–conjugated streptavidin (Dako), and developed with diaminobenzidine (DAB; Dako) as the chromogen, according to the manufacturer's specifications.

Alternatively, sections were deparaffinized, and endogenous peroxidases were blocked prior to antigen retrieval in 10 m*M* citrate buffer (pH 6.0) as described above. Sections were then blocked with 10% goat serum for 30 minutes, followed by overnight incubation at 4°C with primary antibody (anti–ESE-1; Chemicon) or a rabbit polyclonal anti–ESE-1 antibody raised against a glutathione S-transferase fusion protein of the amino terminus of human ESE-1 (1:1,000; East Acres Biologicals, Southbridge MA). Sections were then incubated with goat anti-rabbit biotinylated secondary antibodies (Vector, Burlingame, CA), followed by ABC reagent (Vectastain; Vector), and DAB was used as the chromogen. Sections were washed between every subsequent step with PBS–Brij-35 (Fisher Scientific, Fair Lawn, NJ). All sections were counterstained with hematoxylin (Sigma). Slides were examined and photographed using a transmitted light microscope and digital camera (AxioCam; Zeiss, Wetzlar, Germany).

Northern blot and reverse transcriptase–polymerase chain reaction (RT-PCR) analysis. Total RNA was harvested using a QIAshredder (Qiagen, Chatsworth, CA) and the RNeasy Mini Kit (Qiagen) or TRIzol (Gibco BRL). Poly(A+) RNA was prepared with MicroPoly(A)Pure (Ambion, Austin, TX). Northern blots (5 μg total RNA) were hybridized using a full-length ESE-1 complementary DNA (cDNA) probe as previously described (18). The cDNA was generated from 1 μg total RNA using Ready-To-Go You-Prime First-Strand Beads (Amersham Pharmacia Biotech, Piscataway, NJ). RT-PCR amplifications of 0.1 μg cDNA were carried out using the PTC-100 thermal cycler (MJ Research, Waltham, MA) as follows: 5 minutes at 94°C, 20–37 cycles of 30 seconds at 94°C, 30 seconds at 56°C, and 30 seconds at 72°C, followed by 5 minutes at 72°C (19). The sequences of the human ESE-1 primers and the primers for human GAPDH were as previously described (16).

Real-time quantitative PCR. SYBR Green I–based real-time PCR was carried out on the Opticon monitor (MJ Research). All PCR mixtures contained PCR buffer (final concentration 10 m*M* Tris HCl, pH 9.0, 50 m*M* KCl, 2 m*M* $MgCl_2$, and 0.1% Triton X-100), 250 μM dNTP (Roche Molecular Biochemicals, Indianapolis, IN), 0.5 μM of each PCR primer, 0.5× SYBR Green I, 5% DMSO, and 1 unit *Taq* DNA polymerase (Promega, Madison, WI) with 2 μl cDNA in a final volume of 25 μl reaction mix. The samples were loaded into wells of low-profile 96-well microplates. After an initial denaturation step at 95°C for 2 minutes, conditions for cycling were 38 cycles of denaturation (95°C for 30 seconds), annealing (54°C for 30 seconds), and extension (72°C for 1 minute). Then, the fluorescence signal was measured right after incubation at 78°C for 5 seconds following each extension step, which eliminates possible primer dimer detection. At the end of the PCR cycles, a melting curve was generated to identify the specificity of the PCR product.

For each run, serial dilutions of human GAPDH (hGAPDH) plasmids were used as standards for quantitative measurement of the amount of amplified cDNA. Also, for normalization of each sample, hGAPDH primers were used to measure the amount of hGAPDH cDNA. All samples were run as duplicates, and the data were presented as ratios of ESE-1:hGAPDH. The primers used for real-time PCR were as follows: for hGAPDH, forward 5′-CAAAGTTGTCATGGATGACC, reverse 5′-CCATGGAGAAGGCTGGGG, which will amplify 195 bp of human GAPDH; for ESE-1, forward 5′-ACCTGGATCCCACTGATGGCAAGCTC, reverse 5′-CCGACTCTGGAGAACCTCTTCCTCC.

Plasmid constructs and DNA transfection assays. The human ESE-1 promoter sequences spanning from −1,541 to +29 were cloned into the pXP2 luciferase vector (pXP2/ESE-1). Mutation in the ESE-1 promoter NF-κB site was generated by site-directed mutagenesis with the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) using the primer 5′-CTAAAGGCCAGGAAATCGGATCCATCCAATGAGACAC-3′, and was confirmed by sequencing. The pCI-ESE1 expression vector was constructed as previously described (12). Cotransfections of 3–8 × 10^5 cells were carried out with 600 ng of reporter gene construct DNA and 200 ng of expression vector DNA using LipofectAMINE Plus (Gibco BRL) for 16 hours, as previously described (12). In transfections with pCI/ESE-1 alone, cells were treated with IL-1β or lipopolysaccharide (LPS) for 16 hours. Transfections were performed independently in duplicate and repeated 3–4 times with different plasmid preparations, yielding similar results. Cotransfection of a second plasmid for determination of transfection efficiency was omitted because potential artifacts with this technique have been reported (20) and many commonly used viral promoters contain binding sites for Ets factors.

Electrophoretic mobility shift assays (EMSAs). EMSAs and supershift assays were performed using 3 μl of whole cell extract and ^{32}P-labeled double-stranded oligonucleotide probes in the presence or absence of competitor oligonucleotides (1, 10, or 100 ng) or antibodies as previously described (21). Whole cell extracts were made from U-138 Mg cells after incubation with IL-1β for 0, 3, or 8 hours using as lysis buffer 1% Triton X-100, 25 m*M* glycylglycine (pH 7.8), 15 m*M* $MgSO_4$, 4 m*M* EGTA, 1 m*M* dithiothreitol (DTT), phenylmethylsulfonyl fluoride, aprotinin, and pepstatin. All antibodies for supershift assays (2 μl per assay) were obtained from Santa Cruz Biotechnology (22). Oligonucleotides used as probes and for competition studies were as follows: ESE-1 promoter wild-type NF-κB 5′-AGGCCAGGAAATCCCCTCCATC-3′ and 3′-TCCGGTCCTTTAGGGGAGGTAG-5′; ESE-1 promoter mutant NF-κB 5′-AGGCCAGGAAATCggaTCCATC-3′ and 3′-TCCGGTCCTTTAGcctAGGTAG-

5′; IL-6 promoter NF-κB 5′-TCGACATGTGGGATTTTCC-CATGAC-3′ and 3′-AGCTGTACACCCTAAAAGGGTAC-TG-5′.

Adenovirus infection. Infections with the IκB adenovirus (kindly provided by Dr. Fionula Brennan) (23) were performed for 1 hour in serum-free medium using a multiple of infection of 1,000. After infection, the cells were washed with medium and incubated for 16 hours in the absence or presence of IL-1β in DMEM containing 10% FCS.

Chromatin immunoprecipitation (ChIP). THP-1 human monocytic cells were grown in RPMI 1640 medium containing 10% FCS (low LPS; Hyclone, Logan, UT), 0.05 m*M* β-mercaptoethanol, and 1% penicillin/streptomycin (Gibco BRL Life Technologies, Grand Island, NY) in a 5% CO_2 incubator. Cells (8×10^6) were plated on 100-mm dishes and then stimulated with LPS (1 μg/ml) for 1 hour. Crosslinking was performed by adding formaldehyde directly to the tissue culture medium to a final concentration of 1% and incubating for 10 minutes at room temperature. Cells were rinsed with ice-cold PBS twice, incubated with 100 m*M* Tris HCl (pH 9.4) and 10 m*M* DTT for 15 minutes at 30°C, and centrifuged for 5 minutes at 2,000*g*. Cells were washed sequentially with 1 ml of ice-cold PBS, buffer A (0.25% Triton X-100, 10 m*M* EDTA, 0.5 m*M* EGTA, 10 m*M* HEPES, pH 6.5), and buffer B (200 m*M* NaCl, 1 m*M* EDTA, 0.5 m*M* EGTA, 10 m*M* HEPES, pH 6.5). Cells were then resuspended in 0.3 ml of lysis buffer (1% sodium dodecyl sulfate [SDS], 5 m*M* EDTA, 50 m*M* Tris HCl, pH 8.1, 1× protease inhibitor cocktail [Roche Molecular Biochemicals]), incubated on ice for 10 minutes, and sonicated 3 times for 30 seconds each with a 1 minute interval at the maximum setting to make 400 bp to 1 kb DNA fragmentation, followed by centrifugation for 10 minutes at 14,000*g* at 4°C. Supernatants were collected and 100 μl of chromatin preparation was aliquoted as the input fraction.

Input diluted to 1:100 was used for PCR amplification as a control. The rest of the supernatants were diluted in buffer (1% Triton X-100, 2 m*M* EDTA, 150 m*M* NaCl, 20 m*M* Tris HCl, 1× protease inhibitor cocktail, pH 7.9) followed by immunoclearing with 2 μg sheared salmon sperm DNA, 20 μl normal rabbit serum, and protein A–Sepharose (45 μl of 50% slurry in 10 m*M* Tris HCl, pH 8.1, 1 m*M* EDTA; Amersham Pharmacia Biotech) for 2 hours at 4°C. Immunoprecipitation was performed overnight at 4°C with 0.5 μg of specific antibody, anti–NF-κB p65 (Santa Cruz Biotechnology), or with 0.5 μg of rabbit IgG as a negative control.

After immunoprecipitation, 45 μl protein A–Sepharose and 2 μg of sheared salmon sperm DNA were added and the incubation was continued for another 1 hour. Precipitates were washed sequentially for 10 minutes each in Tris-SDS-EDTA I (TSE I) (20 m*M* Tris HCl, pH 8.0, 0.1% SDS, 1% Triton X-100, 2 m*M* EDTA, 150 m*M* NaCl), TSE II (20 m*M* Tris HCl, pH 8.0, 0.1% SDS, 1% Triton X-100, 2 m*M* EDTA, 500 m*M* NaCl), and TSE III (0.25*M* lithium chloride, 1% Nonidet P40, 1% deoxycholate, 10 m*M* Tris HCl, pH 8.0, 1 m*M* EDTA). Precipitates were then washed 3 times with $T_{10}E_1$ buffer (pH 8.0) and extracted 3 times with 1% SDS and 0.1*M* $NaHCO_3$. Eluates were pooled and heated at 65°C overnight to reverse the formaldehyde crosslinking. DNA fragments were purified with QIAquick PCR purification kit (Qiagen). PCR was performed using 2 μl of a 50 μl DNA extraction in TE buffer with Hi-Fi *Taq* polymerase (Invitrogen, San Diego, CA). PCR mixtures were amplified for 1 cycle at 94°C for 2 minutes, and 35–38 cycles at 95°C for 30 seconds, annealing temperatures of 54°C for 30 seconds and 72°C for 1 minute, and then final elongation at 72°C for 8 minutes. The sequences of the primers used were as follows: hESE-1/P-F1 5′-GCCAAGTGGCACTGAATATG and hESE-1/P-R1 5′-GGTAGCGCTGAGGTATCTGG, which amplifies 200 bp of the hESE-1 promoter; TLR4/P-F1 5′-CATTGCAC-TTGCTACTTTCCA and TLR4/P-R1 5′-CGCATGTGT-TTTGAATTACTGAA, which amplifies 215 bp of hTLR4 promoter.

RESULTS

Expression of ESE-1 in inflamed joints. Previous studies by our group and others had indicated that under normal physiologic conditions, ESE-1 expression is restricted to epithelial cells and regulated by growth and differentiation factors (7,8,14). Nevertheless, we recently discovered that ESE-1 is inducible by proinflammatory cytokines in the vasculature (18). To determine whether ESE-1 could be a general marker of inflammation in cytokine-responsive tissues, we investigated the expression of ESE-1 in RA and OA synovial tissues by immunohistochemistry. The examination of synovial tissues from 5 patients with RA demonstrated localization of ESE-1 protein in cells within the synovial lining layer, as well as in scattered mononuclear cells deep to the lining layer. Occasional endothelial cell staining was also present, but no staining was observed in cells within cellular aggregates that morphologically resembled lymphocytes.

All RA samples showed a similar pattern of ESE-1 expression. ESE-1 protein expression was predominantly located in the cytoplasm of cells, with occasional nuclear localization. The staining pattern using 2 different anti–ESE-1 antibodies and using the same and alternative immunohistochemistry methods showed close correlation, supporting the specificity of this staining. Isotype control antibody or preimmune rabbit serum showed no staining. In samples from 5 patients with OA, staining for ESE-1 protein was also present, but the intensity of staining varied among samples. Two OA samples showed significantly weaker staining, while 3 showed staining levels similar to those seen in RA. In the latter 3 OA samples, histologic evidence of inflammation was present. The staining pattern in OA samples was similar to that seen in RA samples (Figure 1).

Induction of ESE-1 by IL-1β, TNFα, and LPS in nonepithelial cells. To test the hypothesis that ESE-1 is a mediator of cytokine responses in inflammatory disorders such as RA, we analyzed ESE-1 expression in

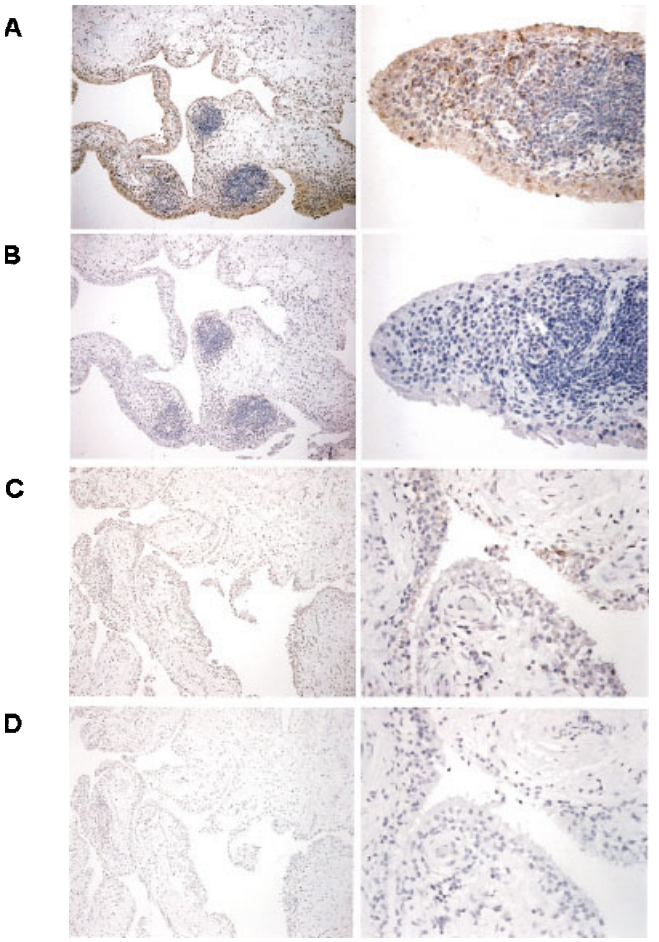

Figure 1. Immunohistochemical staining of human rheumatoid arthritis (RA) (**A** and **B**) and osteoarthritis (OA) (**C** and **D**) synovial tissue sections. The expression of ESE-1 protein was analyzed using an anti–ESE-1 antibody (Chemicon, Temecula, CA) in RA (**A**) and OA (**C**). Positive staining of ESE-1 protein appears as a brown color (**A** and **C**), and negative control antibody staining (**B** and **D**) demonstrates specificity of staining. (Original magnification × 25, left; × 100, right.)

several cell types that are resident in articular tissues and are targets for proinflammatory stimuli that also induce tissue destruction. The synovium in RA is composed of heterogeneous cell populations, including monocyte/macrophages, lymphocytes, and synovial fibroblasts. These cells are targets for proinflammatory stimuli, such as IL-1 and TNFα, which up-regulate products such as metalloproteinases, nitric oxide, and prostaglandins (24). Similarly, during infection, LPS acts on articular tissues and cells in the central nervous system to produce inflammatory and destructive tissue changes.

To evaluate ESE-1 expression in response to proinflammatory cytokines, RA synovial fibroblasts were stimulated for different lengths of time with IL-1β in the absence or presence of indomethacin. A conventional RT-PCR analysis revealed that unstimulated or indomethacin-treated cells did not express detectable ESE-1 mRNA (Figure 2a); however, IL-1β strongly induced ESE-1 expression in synovial fibroblasts within 6 hours (Figure 2a), which declined after 24 hours and was very low after 5 days. Real-time PCR using SYBR Green I (Figure 2b) confirmed the results quantitatively and revealed that in the presence of indomethacin, which inhibits basal or IL-1–induced prostaglandin synthesis (25), the mRNA levels of ESE-1 were superinduced, especially at 6 hours, and appeared stabilized over the 5-day period of the experiment. In contrast to IL-1β, TNFα did not significantly induce ESE-1 expression in synovial fibroblasts (Figure 2b). These results demonstrate that IL-1β induces transient expression of ESE-1 in synovial fibroblasts and implicate ESE-1 in cytokine-mediated responses in this cell type. They also suggest that cytokine-induced prostaglandins may modulate steady-state ESE-1 mRNA levels.

Chondrocytes, the cellular component of cartilage, and osteoblasts, the cells responsible for bone formation, represent additional cell targets of inflammatory cytokines in joint disorders (2,25,26). The immortalized human chondrocyte cell lines T/C28a2, C28/I2, and C20/A4, which express cartilage-specific matrix proteins and other markers of the differentiated phenotype (27,28), were developed as models to study cytokine regulation of gene expression (29). Treatment with IL-1β for 24 hours induced ESE-1 mRNA expression in all chondrocyte cell lines (Figure 2c). In T/C28a2 cells, TNFα stimulated ESE-1 expression to levels similar to those observed with IL-1β treatment, but TNFα stimulation of ESE-1 expression in C28/I2 and C2/A4 cells was significantly less at this time point. Interferon-γ failed to induce ESE-1 expression (Figure 2c), indicating that some, but not all, proinflammatory cytokines are capable of inducing ESE-1 expression. The kinetics of ESE-1 induction was explored in a time course experiment with T/C28a2 chondrocytes. RT-PCR and real-time PCR analysis revealed rapid induction of ESE-1 mRNA by both IL-1β and TNFα, reaching a peak within 2 hours and declining gradually thereafter (Figures 2d and e). Even in T/C28a2 cells, ESE-1 induction by TNFα was much weaker than by IL-1β. IL-1β also induced ESE-1 mRNA in the human articular chondrocyte cell line tsT/AC62 (30) (data not shown).

To examine cellular responses in bone, we used the immortalized osteoblast-like bone marrow stromal cell line LB-12 (31). Northern blot analysis showed that

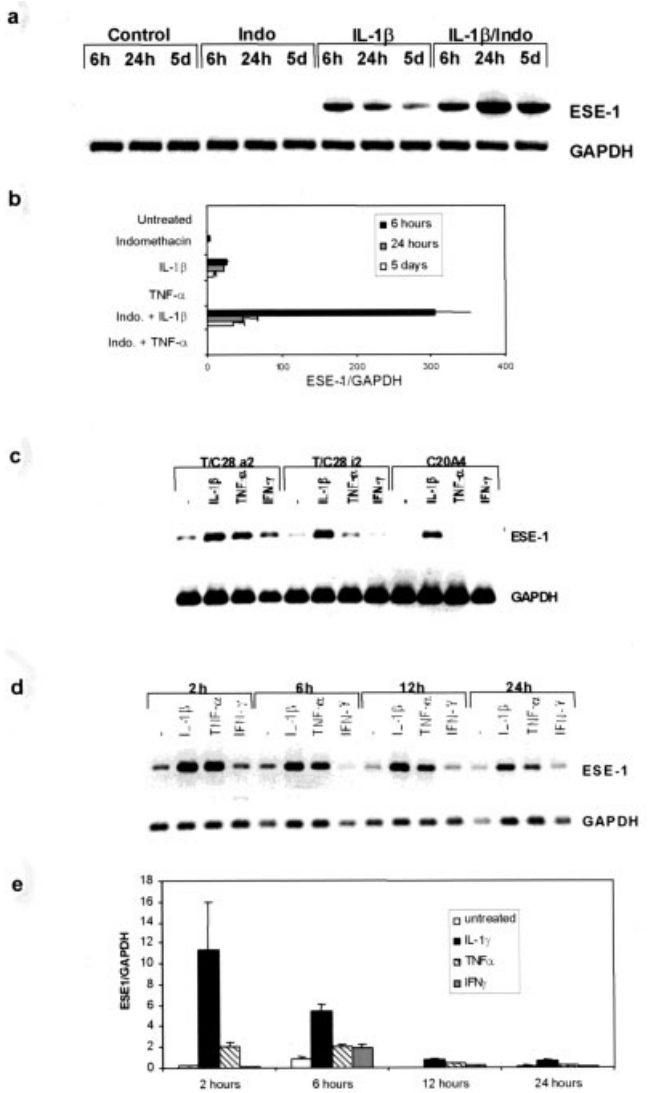

Figure 2. Induction of ESE-1 mRNA in various cultured joint-related cells. **a** and **b,** Primary human synovial fibroblasts at passage 4 were incubated in the absence or presence of interleukin-1β (IL-1β), tumor necrosis factor α (TNFα), indomethacin (Indo.), or a combination of cytokine and indomethacin for 6 hours, 24 hours, or 5 days. Total RNA was extracted and analyzed using ESE-1 and GAPDH specific primers by conventional reverse transcriptase–polymerase chain reaction (RT-PCR) (**a**) and by real-time PCR (**b**). **c,** Induction of ESE-1 mRNA in human chondrocytes by different proinflammatory cytokines. Subconfluent cultures of the human costal chondrocyte cell lines T/C28a2, C28/I2, and C20A4 were incubated in the absence or presence of IL-1β, TNFα, or interferon-γ (IFNγ) for 24 hours. Total RNA was analyzed by RT-PCR using ESE-1 and GAPDH specific primers. **d** and **e,** Kinetics of ESE-1 mRNA induction by proinflammatory cytokines in human chondrocytes. T/C28a2 cells were grown in the absence or presence of IL-1β, TNFα, or IFNγ for 2, 6, 12, or 24 hours. Conventional (**d**) or real-time (**e**) RT-PCR analysis was performed using ESE-1 and GAPDH specific primers. Values are the mean and SD of measurements from 2 experiments.

ESE-1 was not expressed in unstimulated LB-12 cells, but was rapidly induced by IL-1β, with a peak between 2 and 6 hours, and a decline within 24 hours (Figure 3). Since the rapid, but transient, induction of ESE-1 resembles that of immediate early genes, we tested the effect of the protein synthesis inhibitor cycloheximide, which superinduces expression of certain genes due to mRNA stabilization (32). Cycloheximide alone had no effect, but it superinduced IL-1β–mediated ESE-1 mRNA expression (Figure 3). We further tested the glucocorticoid hydrocortisone, which is a strong repressor of IL-1β–induced IL-6 gene expression and enhances the osteoblast phenotype in these cells (33). Hydrocortisone by itself did not induce ESE-1 mRNA, but it strongly enhanced IL-1β-induced expression (Figure 3).

Macrophages represent an additional cell type present within inflammatory tissues such as RA synovium and are a major target of bacterial endotoxin. Therefore, we examined the effects of LPS on ESE-1 expression in the human monocytic cell line, THP-1 (Figure 4). Within 1 hour, LPS induced ESE-1 mRNA, thus indicating that endotoxins are inducers of ESE-1 and monocytes are able to express ESE-1 upon activation by this proinflammatory stimulus (Figure 4).

In addition to their roles in joint diseases, TNFα

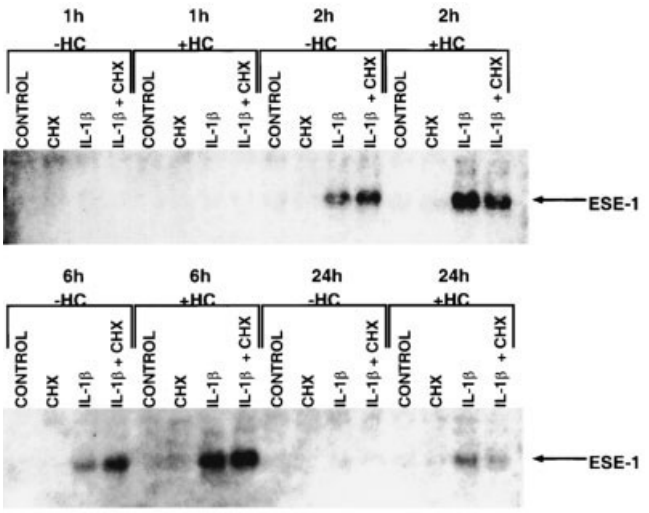

Figure 3. Transient ESE-1 mRNA expression in human osteoblasts upon stimulation with interleukin-1β (IL-1β). Cultures of the human LB-12 osteoblast cell line were incubated in the absence or presence of cycloheximide (CHX), IL-1β, hydrocortisone (HC), or combinations thereof for 1, 2, 6, or 24 hours. Total RNA was extracted and 28S RNA normalized amounts were analyzed on Northern blots using the ESE-1 cDNA probe under stringent conditions as described in Materials and Methods.

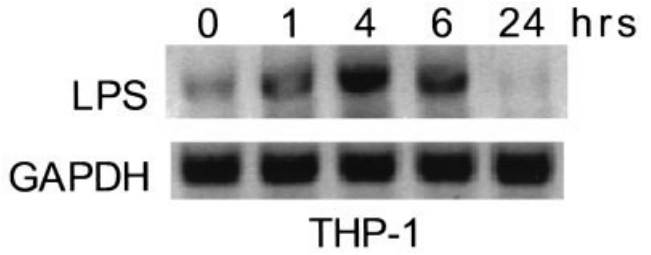

Figure 4. Induction of ESE-1 mRNA in human monocytes by bacterial endotoxin. THP-1 cells were grown in the absence or presence of lipopolysaccharide (LPS) for 1, 2, 4, or 24 hours, and ESE-1 mRNA was analyzed by reverse transcriptase–polymerase chain reaction.

and IL-1β have been shown to be important regulators of inflammatory processes that affect the central nervous system (34). Human glioma cell lines, derived from glioblastomas, are microglial/macrophage-lineage cells that express macrophage-specific antigens and a variety of cytokines and cytokine receptors and serve as surrogate models for studying responses to proinflammatory mediators (35). Therefore, we examined the effects of IL-1β on ESE-1 expression in the U-138 MG and U-373 MG glioma cells. IL-1β rapidly induced transient expression of ESE-1 in these cell lines, reaching a peak between 2 and 6 hours after the addition of IL-1β (Figure 5).

IL-1β–mediated ESE-1 induction was highly specific for this member of the Ets family, since screening with a panel of Ets factors in IL-1β–stimulated chondrocytes and U-138 MG cells revealed that ESE-2, PDEF, GABPα, Tel, ERP, ELK-1, SAP-1, ELF-1, NERF, MEF, ERF, E4TF160, E1-AF, Fli-1, Erg, and Erm mRNA were not induced by IL-1β at any time point

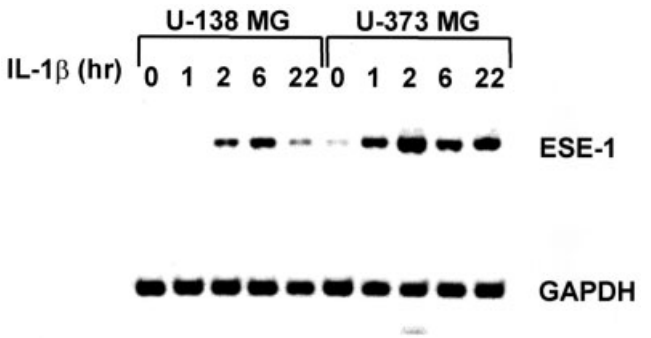

Figure 5. Induction of ESE-1 mRNA by interleukin-1β (IL-1β) in human glioma cell lines. U-138 MG and U-373 MG glioma cells were incubated in the absence or presence of IL-1β for 1, 2, 6, or 22 hours, and ESE-1 and GAPDH mRNA were analyzed by reverse transcriptase–polymerase chain reaction.

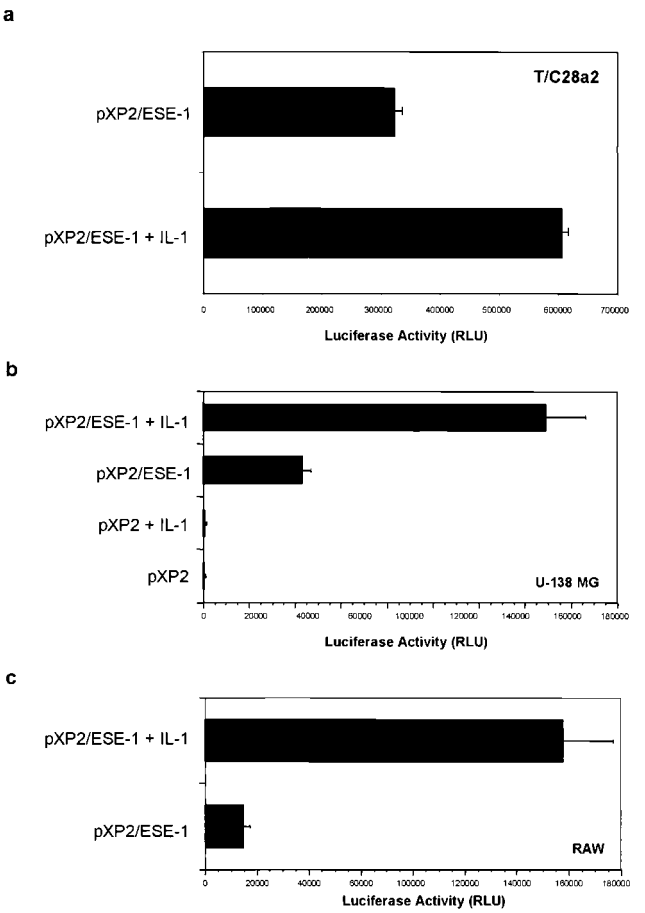

Figure 6. Transcriptional activation of ESE-1 promoter by interleukin-1β (IL-1β) and lipopolysaccharide (LPS). **a,** T/C28a2 and **b,** U-138 MG cells were transfected with either the parental pXP2 luciferase plasmid or the pXP2 luciferase construct containing the ESE-1 promoter and incubated in the absence or presence of IL-1β. Luciferase activity in the lysates was determined 16 hours later, as described in Materials and Methods. The experiment was repeated 4 times with different plasmid preparations with comparable results. **c,** RAW cells were transfected with the pXP2 luciferase construct containing the ESE-1 promoter and incubated in the absence or presence of LPS. Luciferase activity in the lysates was determined 16 hours later, as described. The experiment was repeated 3 times with different plasmid preparations with comparable results. Values shown are the mean and SD of duplicate measurements from 1 representative transfection.

(data not shown). Only Ets-1 was weakly up-regulated by IL-1β (data not shown). These findings most vividly demonstrate the highly specific inducibility of ESE-1, compared with other members of the Ets family, by proinflammatory cytokines in cells that mediate tissue-specific responses.

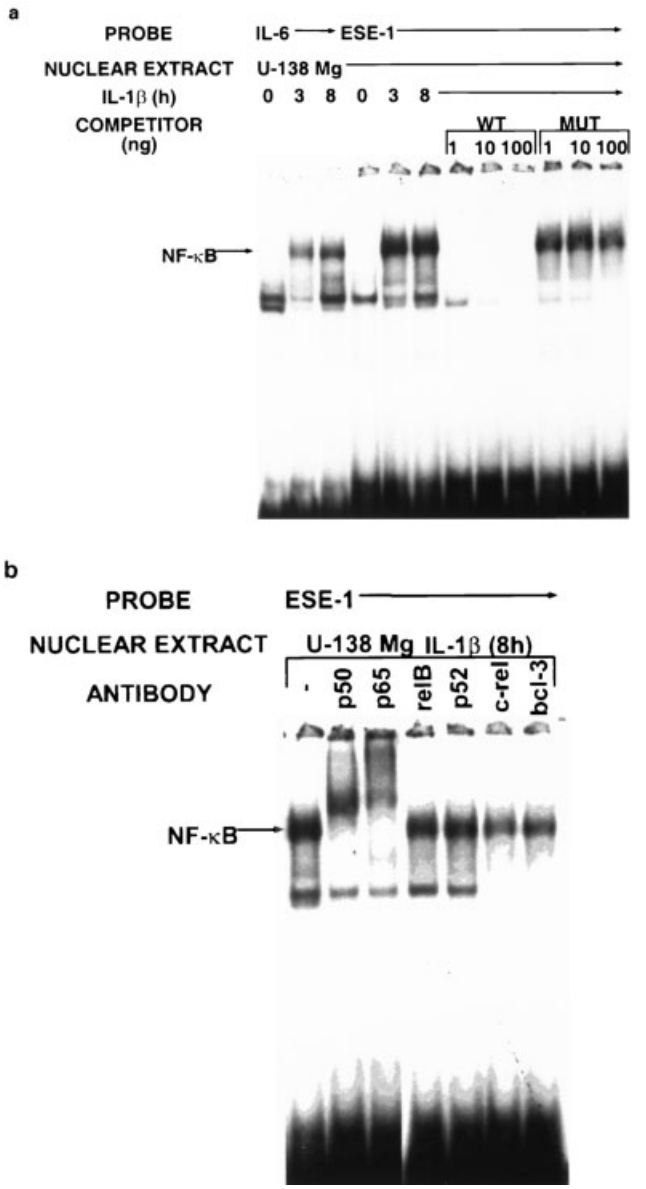

Figure 7. Interaction of the nuclear factor κB/Rel (NF-κB/Rel) family members p50 and p65 with the NF-κB binding site in the ESE-1 promoter. **a,** Interaction of NF-κB with the NF-κB binding site in the ESE-1 promoter. Whole cell extracts isolated from U-138 MG cells stimulated with interleukin-1β (IL-1β) for 0, 3, or 8 hours were analyzed by electrophoretic mobility shift assay (EMSA) using the labeled human ESE-1/NF-κB site oligonucleotide or the human IL-6/NF-κB site oligonucleotide as probes. Competitions were carried out with either no competitor or 1, 10, or 100 ng of unlabeled wild-type (WT) or mutant (MUT) ESE-1/NF-κB oligonucleotides. **Arrow** indicates the specific IL-1β–inducible DNA–protein complex. **b,** Interaction of NF-κB/Rel family members p50 and p65 with the NF-κB binding site in the ESE-1 promoter. Supershift EMSAs using whole cell extracts isolated from U-138 MG cells stimulated with IL-1β for 8 hours and the labeled human ESE-1/NF-κB oligonucleotide probe were carried out with either no antibody or antibodies against p50, p65, RelB, p52, c-Rel, or Bcl-3. **Arrow** indicates the NF-κB DNA–protein complex.

Induction of ESE-1 promoter by IL-1β and LPS. To investigate the molecular mechanism by which IL-1β regulates ESE-1 expression, we examined the response of the pXP2/ESE-1 luciferase reporter construct in transfections using the T/C28a2, RAW 264-7, and U-138 MG cell lines. Whereas the parental pXP2 vector was not stimulated by IL-1β or by LPS, IL-1β increased ESE-1 promoter activity 2-fold in T/C28a2 chondrocytes (Figure 6a) and up to 5-fold in U-138 MG cells (Figure 6b). Similarly, LPS enhanced ESE-1 promoter activity approximately 9-fold in RAW 264-7 cells (Figure 6c). TNFα also induced ESE-1 promoter activity in these cells.

IL-1β induces binding of NF-κB family members p50 and p65 to a high-affinity site within the ESE-1 promoter. NF-κB has been shown to be a critical regulatory molecule involved in transducing cellular responses to IL-1β and endotoxin (36). Using EMSAs, we compared the ability of the highly conserved human ESE-1 promoter NF-κB site (ESE-1/NF-κB), previously identified between nucleotides −88 and −79 bp upstream of the transcription start site (13), and the well-characterized IL-6 promoter NF-κB site (IL-6/NF-κB) (37) to form complexes with proteins present in whole cell extracts from unstimulated and IL-1β–stimulated U-138 MG cells (Figure 7a). Using the ESE-1/NF-κB probe, we observed an inducible, high-affinity protein–DNA complex in IL-1β–treated, but not unstimulated, U-138 MG extracts, which comigrated with a complex formed by the IL-6/NF-κB site (Figure 7a). The complex formed with the ESE-1/NF-κB site was significantly stronger than that formed with the IL-6/NF-κB site, indicating that the ESE-1/NF-κB site is a high-affinity binding site for NF-κB. Competition with either wild-type or mutant ESE-1/NF-κB oligonucleotides confirmed the specificity of the inducible complex (Figure 7a).

Using a supershift EMSA, we determined that antibodies against the NF-κB/Rel family members p50 and p65, but not other family members, completely shifted the inducible complex formed on the ESE-1/NF-κB site (Figure 7b). NF-κB was induced as early as 30 minutes after IL-1β stimulation, correlating well with the rapid induction of ESE-1 mRNA expression (results not shown).

NF-κB site mediates inducibility of the ESE-1 promoter by IL-1β. To examine whether the NF-κB site was responsible for IL-1β–mediated activation of ESE-1 gene transcription, we introduced a mutation into the ESE-1/NF-κB site (Figure 8a). T/C28a2 chondrocytes and U-138 MG cells transfected with wild-type or mutant ESE-1 promoter/luciferase plasmids were incubated

in the absence or presence of IL-1β. In contrast to the wild-type ESE-1 promoter, which was induced by IL-1β, inducibility of the mutant promoter was completely abolished (Figures 8b and c). These results demonstrate that an intact NF-κB binding site is essential for induction of the ESE-1 gene by IL-1β and that the inducibility of the ESE-1 gene by proinflammatory stimuli such as IL-1β may be explained in large part by activation of NF-κB.

Inhibition of NF-κB activation by an adenovirus expressing IκB blocks IL-1β–mediated induction of endogenous ESE-1 mRNA expression. To further investigate the hypothesis that NF-κB is required for IL-1β–mediated induction of endogenous ESE-1 gene expression, we tested the effect of blocking NF-κB activation by overexpressing IκB (23). U-138 MG cells were infected with either an adenovirus expressing the IκB inhibitor or, as a control, an adenovirus expressing β-galactosidase, and subsequently stimulated with IL-1β. ESE-1 expression was then analyzed by RT-PCR. Ad/βGAL infection did not prevent the induction of ESE-1 by IL-1β, nor did the use of an adenovirus seem to have an effect on ESE-1 expression by itself. In contrast, prior infection of the cells with the IκB adenovirus drastically reduced the ability of IL-1β to induce ESE-1, although some residual ESE-1 transcript was detectable (Figure 9). These data most vividly demonstrate that NF-κB activation is a critical step involved in ESE-1 induction by IL-1β, although additional factors may contribute to ESE-1 induction.

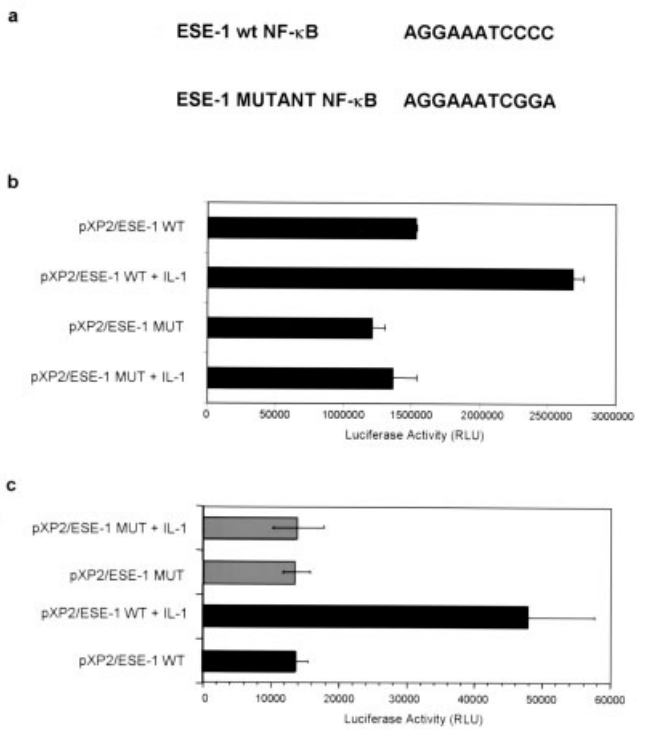

Figure 8. Mutation of the nuclear factor κB (NF-κB) site within the ESE-1 promoter abolishes induction by interleukin-1β (IL-1β). **a,** Sequences of the wild-type (WT) ESE-1/NF-κB site and the mutation introduced within the ESE-1 promoter. **b** and **c,** T/C28a2 and U-138 MG cells were transfected with the ESE-1 promoter/pXP2 luciferase construct containing either the WT or a mutant (MUT) NF-κB site and incubated in the absence or presence of IL-1β. Luciferase activity in the lysates was determined 16 hours later, as described in Materials and Methods. The experiment was repeated 4 times with different plasmid preparations, with comparable results. Values shown are the mean and SD of duplicate measurements from 1 representative transfection.

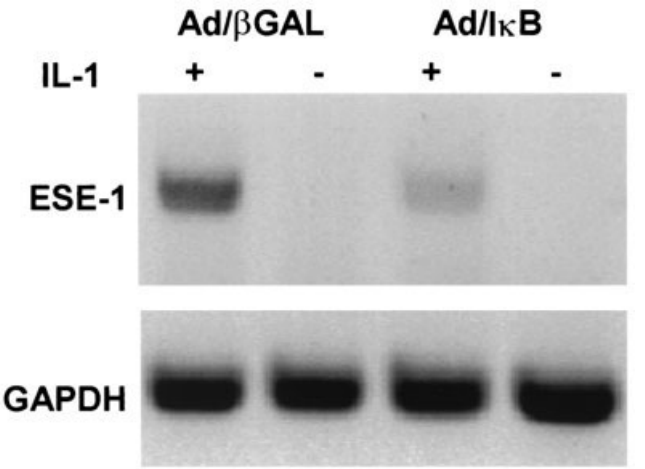

Figure 9. Adenovirus (Ad)–mediated overexpression of inhibitor of nuclear factor κB (IκB) inhibits ESE-1 induction by interleukin-1β (IL-1β). U-138 MG cells were infected with an adenovirus expressing IκB or, as a control, an adenovirus expressing β-galactosidase prior to stimulation with IL-1β for 8 hours. Total RNA was extracted and analyzed by reverse transcriptase–polymerase chain reaction using ESE-1 and GAPDH specific primers.

Binding of NF-κB to the endogenous ESE-1 promoter. To confirm that NF-κB p65 binds to the endogenous ESE-1 promoter in vivo, we performed a ChIP assay. THP-1 cells were treated with LPS for 1 hour to induce NF-κB translocation to the nucleus, and then with formaldehyde to crosslink the transcription factors to the DNA. Immunoprecipitation of the protein–DNA complexes with an antibody against NF-κB p65 was followed by analysis of the immunoprecipitated DNA by PCR using promoter-specific primers spanning the NF-κB site of the ESE-1 promoter. As shown in Figure 10A, fractionation of chromatin with anti–NF-κB p65 antibody, but not with the control rabbit IgG, caused specific enrichment for endogenous ESE-1 promoter DNA, thus demonstrating the ability of the

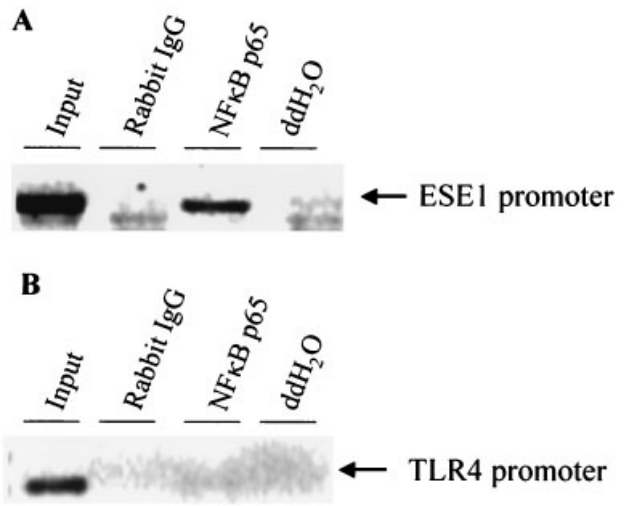

Figure 10. Binding of nuclear factor κB (NF-κB) family member p65 to endogenous ESE-1 promoter around its NF-κB site. The anti–NF-κB p65 antibody was used to specifically enrich ESE-1 promoter DNA sequences in a chromatin immunoprecipitation assay. Chromatin proteins were crosslinked to DNA in THP-1 cells with formaldehyde, and purified nucleoprotein complexes were immunoprecipitated using either anti–NF-κB p65 antibody or nonspecific rabbit IgG. The precipitated DNA fractions were analyzed by polymerase chain reaction (PCR) for the presence of the ESE-1 promoter region (**A**) or a region of the hTLR4 locus (**B**). In each case, the input DNA was used as a positive control, and no template (deionized and distilled H_2O) was used as a negative control for PCR. Amplification products were analyzed on a 2% agarose gel and visualized by ethidium bromide staining.

NF-κB p65 protein to bind to the endogenous ESE-1 promoter. As a negative control, PCR amplification with primers from the TLR4 locus, which does not encompass any possible NF-κB binding site, showed no enriched bands with either antibody (Figure 10B). Our results clearly indicated that proinflammatory stimuli induced ESE-1 expression via activation of NF-κB.

DISCUSSION

IL-1 and TNFα play a central role in RA by acting on resident cells in the joint to up-regulate products such as cytokines, chemokines, metalloproteinases, nitric oxide, and prostaglandins (24). Disruption in the normal physiologic activity of these cells by proinflammatory mediators provides the mechanism for tissue destruction in various forms of arthritis. Similarly, during infection, LPS acts on articular tissues and cells in the central nervous system to produce inflammatory and destructive tissue changes. The associated gene products are expressed in response to the activation of their promoters by transcription factors that are regulated by IL-1 and TNFα. Understanding the pathophysiology of the disease and identifying novel therapeutic targets require a detailed study of these transacting factors. Several cytokine-induced transcription factors, such as NF-κB, AP-1, C/EBP, and ETS-1, have been detected in RA synovium (38–43). However, these transcription factors account only partially for the activation of genes involved in inflammatory and destructive processes, and direct binding to responsive promoters has not been demonstrated in many cases.

We now provide evidence that the Ets transcription factor ESE-1 may be one of the factors that regulates or refines the responses of cells to proinflammatory stimuli. Although ESE-1 was described originally as an epithelial-specific factor expressed under normal physiologic conditions, other Ets factors are known to be involved in cytokine-induced responses. In surveying other cell types in which ESE-1 could play a potential regulatory role, we uncovered an unexpected function for ESE-1 in the vascular system (18). Our present study shows that ESE-1 is a potentially important component of the inflammatory response in RA as well as OA, a disease in which inflammatory changes have been described (44). Since ESE-1 expression is induced rapidly and transiently in response to IL-1β or TNFα and endotoxin in cell types resident in joint tissues, this factor may be a key regulator of genes whose expression is associated with inflammation as well as tissue destruction. We also demonstrate that induction of ESE-1 expression by proinflammatory stimuli is in large part dependent on activation of the NF-κB family members p50 and p65, which induce ESE-1 expression via a high-affinity NF-κB binding site within the ESE-1 promoter. These results firmly place ESE-1 as a downstream target of NF-κB.

Our hypothesis that ESE-1 may function as a novel mediator of the inflammatory response is supported by our findings that ESE-1 can activate the expression of several genes in response to proinflammatory mediators. Namely, we have shown that iNOS is activated by ESE-1 in endothelial cells, and COX-2 is another target for ESE-1 in monocyte/macrophages (Grall F, et al: unpublished observations). Matrix metalloproteinases (MMPs) 1 and 13, which are involved in cartilage and bone destruction in RA and OA, are regulated by ESE-1 as well (Gu X, et al: unpublished observations). These data suggest that ESE-1 may play a role in regulating the expression of genes that are involved in the initiation and perpetuation of inflammation and tissue destruction. The implication of the role

of ESE-1 in RA and OA is currently being investigated in more detail. However, our results shown here clearly demonstrate that synovium obtained from patients with RA expresses ESE-1 in synovial lining cells as well as other cell types resident at sites of inflammation. Overall, our findings are consistent with the notion that ESE-1 represents a novel target for the treatment of inflammatory joint diseases.

Several other ESE-1 target genes may be associated with inflammatory processes. For example, IL-1–induced SPRR1 gene expression in differentiating keratinocytes associated with terminal differentiation correlates directly with the up-regulation of ESE-1 and IL-1 receptor type I levels (45). Furthermore, in skin cells from patients with psoriasis, there is marked up-regulation of the expression of SPRR1 and SPRR2A, a related keratinocyte gene (46), and we have preliminary evidence that ESE-1 expression is enhanced in psoriasis (Oettgen P: unpublished observations). The potent and transient ESE-1 induction by inflammatory cytokines appears to be distinct from most other members of the Ets family, since our analysis of 17 additional Ets family members revealed only 1 additional Ets factor, Ets-1, that was inducible by IL-1β in cell types studied here. Indeed, IL-1β and TNFα have been shown to enhance Ets-1 expression in human fibroblasts (47). Furthermore, several genes, including urokinase-type plasminogen activator, (MMP-1), MMP-3, TNFα, scavenger receptor, COX-2, intercellular adhesion molecule 1 (ICAM-1), ICAM-2, and IL-12, have been shown to depend on Ets factors for their inducibility by cytokines such as IL-1 or TNFα (48–51). Many additional cytokine-responsive genes, including iNOS and MMP-13, contain putative Ets binding sites within their regulatory regions (52,53).

In summary, we have shown that in cells resident in the joint, ESE-1 expression is induced by 2 of the critical cytokines involved in inflammation, IL-1 and TNFα. This phenomenon is mediated through NF-κB. We hypothesized that ESE-1 could be up-regulated in conditions such as RA. The central role for ESE-1 in inflammation warrants further studies directed toward the identification of additional target genes for ESE-1 associated with inflammation and tissue destruction in arthritic disorders. If ESE-1 acts as a primary and direct activator of expression of these genes, then specific blockade of its expression and/or activation could leave intact other upstream or parallel pathways that are important for normal homeostasis.

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REFERENCES

1. Arend WP, Dayer JM. Inhibition of the production and effects of interleukin-1 and tumor necrosis factor α in rheumatoid arthritis. Arthritis Rheum 1995;38:151–60.
2. Goldring MB. The role of the chondrocyte in osteoarthritis. Arthritis Rheum 2000;43:1916–26.
3. Karin M, Delhase M. The IκB kinase (IKK) and NF-κB: key elements of proinflammatory signalling. Semin Immunol 2000;12: 85–98.
4. Thomas B, Berenbaum F, Humbert L, Bian H, Bereziat G, Crofford L, et al. Critical role of C/EBPδ and C/EBPβ factors in the stimulation of the cyclooxygenase-2 gene transcription by interleukin-1β in articular chondrocytes. Eur J Biochem 2000;267: 6798–809.
5. Vincenti MP, Brinckerhoff CE. Early response genes induced in chondrocytes stimulated with the inflammatory cytokine interleukin-1β. Arthritis Res 2001;3:381–8.
6. Auron PE. The interleukin 1 receptor: ligand interactions and signal transduction. Cytokine Growth Factor Rev 1998;9:221–37.
7. Andreoli JM, Jang SI, Chung E, Coticchia CM, Steinert PM, Markova NG. The expression of a novel, epithelium-specific ets transcription factor is restricted to the most differentiated layers in the epidermis. Nucleic Acids Res 1997;25:4287–95.
8. Chang CH, Scott GK, Kuo WL, Xiong X, Suzdaltseva Y, Park JW, et al. ESX: a structurally unique Ets overexpressed early during human breast tumorigenesis. Oncogene 1997;14:1617–22.
9. Choi SG, Yi Y, Kim YS, Kato M, Chang J, Chung HW, et al. A novel ets-related transcription factor, ERT/ESX/ESE-1, regulates expression of the transforming growth factor-β type II receptor. J Biol Chem 1998;273:110–7.
10. Graves BJ, Petersen JM. Specificity within the ets family of transcription factors. Adv Cancer Res 1998;75:1–55.
11. Kas K, Finger E, Grall F, Gu X, Akbarali Y, Boltax J, et al. ESE-3, a novel member of an epithelium-specific ets transcription factor subfamily, demonstrates different target gene specificity from ESE-1. J Biol Chem 2000;275:2986–98.
12. Oettgen P, Carter KC, Augustus M, Barcinski M, Boltax J, Kunsch C, et al. The novel epithelial-specific Ets transcription factor gene ESX maps to human chromosome 1q32.1. Genomics 1997;45: 456–7.
13. Oettgen P, Barcinski M, Boltax J, Stolt P, Akbarali Y, Libermann TA. Genomic organization of the human ELF3 (ESE-1/ESX) gene, a member of the Ets transcription factor family, and identification of a functional promoter. Genomics 1999;55:358–62.
14. Oettgen P, Alani RM, Barcinski MA, Brown L, Akbarali Y, Boltax J, et al. Isolation and characterization of a novel epithelium-specific transcription factor, ESE-1, a member of the ets family. Mol Cell Biol 1997;17:4419–33.
15. Oettgen P, Kas K, Dube A, Gu X, Grall F, Thamrongsak U, et al. Characterization of ESE-2, a novel ESE-1-related Ets transcription factor that is restricted to glandular epithelium and differentiated keratinocytes. J Biol Chem 1999;274:29439–52.
16. Oettgen P, Finger E, Sun Z, Akbarali Y, Thamrongsak U, Boltax J, et al. PDEF, a novel prostate epithelium-specific ets transcription factor, interacts with the androgen receptor and activates prostate-specific antigen gene expression. J Biol Chem 2000;275: 1216–25.
17. Zhou J, Ng AY, Tymms MJ, Jermiin LS, Seth AK, Thomas RS, et al. A novel transcription factor, ELF5, belongs to the ELF subfamily of ETS genes and maps to human chromosome 11p13-

15, a region subject to LOH and rearrangement in human carcinoma cell lines. Oncogene 1998;17:2719–32.
18. Rudders S, Gaspar J, Madore R, Voland C, Grall F, Patel A, et al. ESE-1 is a novel transcriptional mediator of inflammation that interacts with NF-κB to regulate the inducible nitric-oxide synthase gene. J Biol Chem 2001;276:3302–9.
19. Goldring SR, Schiller AL, Roelke M, Rourke CM, O'Neil DA, Harris WH. The synovial-like membrane at the bone-cement interface in loose total hip replacements and its proposed role in bone lysis. J Bone Joint Surg Am 1983;65:575–84.
20. Sokoloff MH, Tso CL, Kaboo R, Taneja S, Pang S, deKernion JB, et al. In vitro modulation of tumor progression-associated properties of hormone refractory prostate carcinoma cell lines by cytokines. Cancer 1996;77:1862–72.
21. Akbarali Y, Oettgen P, Boltax J, Libermann TA. ELF-1 interacts with and transactivates the IgH enhancer π site. J Biol Chem 1996;271:26007–12.
22. Oettgen P, Akbarali Y, Boltax J, Best J, Kunsch C, Libermann TA. Characterization of NERF, a novel transcription factor related to the Ets factor ELF-1. Mol Cell Biol 1996;16:5091–106.
23. Bondeson J, Brennan F, Foxwell B, Feldmann M. Effective adenoviral transfer of IκBα into human fibroblasts and chondrosarcoma cells reveals that the induction of matrix metalloproteinases and proinflammatory cytokines is nuclear factor-κB dependent. J Rheumatol 2000;27:2078–89.
24. Feldmann M, Brennan FM, Maini RN. Role of cytokines in rheumatoid arthritis. Annu Rev Immunol 1996;14:397–440.
25. Goldring MB. Control of collagen synthesis in human chondrocyte cultures by immune interferon and interleukin-1. J Rheumatol 1987;14 Spec No:64–6.
26. Bogoch ER, Moran E. Abnormal bone remodelling in inflammatory arthritis. Can J Surg 1998;41:264–71.
27. Kokenyesi R, Tan L, Robbins JR, Goldring MB. Proteoglycan production by immortalized human chondrocyte cell lines cultured under conditions that promote expression of the differentiated phenotype. Arch Biochem Biophys 2000;383:79–90.
28. Loeser RF, Sadiev S, Tan L, Goldring MB. Integrin expression by primary and immortalized human chondrocytes: evidence of a differential role for $\alpha1\beta1$ and $\alpha2\beta1$ integrins in mediating chondrocyte adhesion to types II and VI collagen. Osteoarthritis Cartilage 2000;8:96–105.
29. Goldring MB, Birkhead JR, Suen LF, Yamin R, Mizuno S, Glowacki J, et al. Interleukin-1β-modulated gene expression in immortalized human chondrocytes. J Clin Invest 1994;94:2307–16.
30. Robbins JR, Thomas B, Tan L, Choy B, Arbiser JL, Berenbaum F, et al. Immortalized human adult articular chondrocytes maintain cartilage-specific phenotype and responses to interleukin-1β. Arthritis Rheum 2000;43:2189–201.
31. Apperley JC, Manning CA, Williams DA, Goldring SR. Immortalization of human osteoblast-like cells with SV-40 large T antigen. J Bone Miner Res 1990;5 Suppl 2:S93.
32. Newton R, Stevens DA, Hart LA, Lindsay M, Adcock IM, Barnes PJ. Superinduction of COX-2 mRNA by cycloheximide and interleukin-1β involves increased transcription and correlates with increased NF-κB and JNK activation. FEBS Lett 1997;418:135–8.
33. Kassem M, Harris SA, Spelsberg TC, Riggs BL. Estrogen inhibits interleukin-6 production and gene expression in a human osteoblastic cell line with high levels of estrogen receptors. J Bone Miner Res 1996;11:193–9.
34. Lee SC, Dickson DW, Brosnan CF. Interleukin-1, nitric oxide and reactive astrocytes. Brain Behav Immun 1995;9:345–54.
35. Lee YB, Nagai A, Kim SU. Cytokines, chemokines, and cytokine receptors in human microglia. J Neurosci Res 2002;69:94–103.
36. Ghosh S, May MJ, Kopp EB. NF-κ B and Rel proteins: evolutionarily conserved mediators of immune responses. Annu Rev Immunol 1998;16:225–60.
37. Libermann TA, Baltimore D. Activation of interleukin-6 gene expression through the NF-κB transcription factor. Mol Cell Biol 1990;10:2327–34.
38. Asahara H, Fujisawa K, Kobata T, Hasunuma T, Maeda T, Asanuma M, et al. Direct evidence of high DNA binding activity of transcription factor AP-1 in rheumatoid arthritis synovium. Arthritis Rheum 1997;40:912–8.
39. Han Z, Boyle DL, Manning AM, Firestein GS. AP-1 and NF-κB regulation in rheumatoid arthritis and murine collagen-induced arthritis. Autoimmunity 1998;28:197–208.
40. Handel ML, McMorrow LB, Gravallese EM. Nuclear factor–κB in rheumatoid synovium: localization of p50 and p65. Arthritis Rheum 1995;38:1762–70.
41. Nishioka K, Ohshima S, Umeshita-Sasai M, Yamaguchi N, Mima T, Nomura S, et al. Enhanced expression and DNA binding activity of two CCAAT/enhancer-binding protein isoforms, C/EBPβ and C/EBPδ, in rheumatoid synovium. Arthritis Rheum 2000;43:1591–6.
42. Pope RM, Lovis R, Mungre S, Perlman H, Koch AE, Haines GK III. C/EBPβ in rheumatoid arthritis: correlation with inflammation, not disease specificity. Clin Immunol 1999;91:271–82.
43. Redlich K, Kiener HP, Schett G, Tohidast-Akrad M, Selzer E, Radda I, et al. Overexpression of transcription factor Ets-1 in rheumatoid arthritis synovial membrane: regulation of expression and activation by interleukin-1 and tumor necrosis factor α. Arthritis Rheum 2001;44:266–74.
44. Fernandes JC, Martel-Pelletier J, Pelletier JP. The role of cytokines in osteoarthritis pathophysiology. Biorheology 2002;39: 237–46.
45. Sark MW, Fischer DF, de Meijer E, van de Putte P, Backendorf C. AP-1 and ets transcription factors regulate the expression of the human SPRR1A keratinocyte terminal differentiation marker. J Biol Chem 1998;273:24683–92.
46. Hohl D, de Viragh PA, Amiguet-Barras F, Gibbs S, Backendorf C, Huber M. The small proline-rich proteins constitute a multigene family of differentially regulated cornified cell envelope precursor proteins. J Invest Dermatol 1995;104:902–9.
47. Gilles F, Raes MB, Stehelin D, Vandenbunder B, Fafeur V. The c-ets-1 proto-oncogene is a new early-response gene differentially regulated by cytokines and growth factors in human fibroblasts. Exp Cell Res 1996;222:370–8.
48. Westermarck J, Seth A, Kahari VM. Differential regulation of interstitial collagenase (MMP-1) gene expression by ETS transcription factors. Oncogene 1997;14:2651–60.
49. White LA, Maute C, Brinckerhoff CE. ETS sites in the promoters of the matrix metalloproteinases collagenase (MMP-1) and stromelysin (MMP-3) are auxiliary elements that regulate basal and phorbol-induced transcription. Connect Tissue Res 1997;36: 321–35.
50. Wasylyk C, Gutman A, Nicholson R, Wasylyk B. The c-Ets oncoprotein activates the stromelysin promoter through the same elements as several non-nuclear oncoproteins. EMBO J 1991;10: 1127–34.
51. McLaughlin F, Ludbrook VJ, Kola I, Campbell CJ, Randi AM. Characterisation of the tumour necrosis factor (TNF)-α response elements in the human ICAM-2 promoter. J Cell Sci 1999;112: 4695–703.
52. Mengshol JA, Vincenti MP, Brinckerhoff CE. IL-1 induces collagenase-3 (MMP-13) promoter activity in stably transfected chondrocytic cells: requirement for Runx-2 and activation by p38 MAPK and JNK pathways. Nucleic Acids Res 2001;29:4361–72.
53. Laumonnier Y, Nadaud S, Agrapart M, Soubrier F. Characterization of an upstream enhancer region in the promoter of the human endothelial nitric-oxide synthase gene. J Biol Chem 2000;275: 40732–41.