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NF-IL6 and AP-1 Cooperatively Modulate the Activation of the *TSG-6* Gene by Tumor Necrosis Factor Alpha and Interleukin-1

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Tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) activate transcription of the *TSG-6* gene in normal human fibroblasts through a promoter region (−165 to −58) that encompasses an AP-1 and a NF-IL6 site. We show by deletion analysis and substitution mutagenesis that both sites are necessary for activation by TNF-α. Activation by IL-1 requires the NF-IL6 site and is enhanced by the AP-1 site. These results suggest that the NF-IL6 and AP-1 family transcription factors functionally cooperate to mediate TNF-α and IL-1 signals. Consistent with this possibility, IL-1 and TNF-α markedly increase the binding of Fos and Jun to the AP-1 site, and NF-IL6 activates the native *TSG-6* promoter. Activation by NF-IL6 requires an intact NF-IL6 site and is modulated by the ratio of activator to inhibitor NF-IL6 isoforms that are translated from different in-frame AUGs. However, the inhibitor isoform can also bind to the AP-1 site and repress AP-1 site-mediated transcription. The finding that the inhibitor isoform antagonizes activation of the native *TSG-6* promoter by IL-1 and TNF-α suggests that NF-IL6 has a physiologic role in these cytokine responses. Thus, the functionally distinct NF-IL6 isoforms cooperate with Fos and Jun to positively and negatively regulate the native *TSG-6* promoter by TNF-α and IL-1.

Tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) are cytokines that exhibit overlapping biological activities and regulate a common set of genes (29, 53). One such gene is *TSG-6*, a new member of the hyaluronan-binding protein family that includes cartilage link protein, proteoglycan core protein, and the adhesion receptor CD44 (16, 47). *TSG-6* is highly homologous to CD44, in particular in the hyaluronic acid-binding domain. It binds hyaluronic acid in vitro (33) and may function as a component of the extracellular matrix. The expression of the *TSG-6* gene is rapidly activated by TNF-α, IL-1, and lipopolysaccharide in normal human fibroblasts, peripheral blood mononuclear cells, synovial cells, and chondrocytes (33, 57). The presence of high levels of *TSG-6* protein in synovial fluids of patients with rheumatoid arthritis (57) suggests that *TSG-6* has a role in inflammation.

Previously we have shown that IL-1 and TNF-α enhance transcription of the *TSG-6* promoter through a region spanning from −165 to −58 (31). This region encompasses a CCAAT box as well as potential binding sites for transcription factors of the AP-1 and NF-IL6 families. The AP-1 site (5′-TGAGTCAG-3′) is identical to that present in the collagenase promoter, which binds Fos/Jun (AP-1) family proteins (3, 7, 9, 10, 54). Fos and Jun contain a bipartite DNA-binding domain consisting of a basic leucine zipper region (b-ZIP), and they regulate target genes by binding as homo- or heterodimers to sequences related to the AP-1 site (15, 24, 50). They are rapidly activated by various extracellular stimuli such as phorbol esters, growth factors, and cytokines, including TNF-α and IL-1 (4). The AP-1 site of the *TSG-6* promoter (−119 to −126) overlaps with a putative interferon responsive element, IRF-E (31), and is separated by only 3 bp from a potential binding site for NF-IL6 (−106 to −115).

NF-IL6 (LAP, IL-6DBP, AGP/EBP, C/EBP-β, or CRP-2) is a transcription factor of the CCAAT/enhancer-binding protein (C/EBP) family (2, 8), whose synthesis is rapidly activated by stimulation with cytokines such as IL-1, IL-6, and TNF-α and by LPS (1, 12, 19, 22). It has also been observed that stimulation with phorbol esters causes NF-IL6 to translocate from the cytoplasm to the nucleus (38). In common with Fos and Jun, NF-IL6 regulates the transcription of target genes by binding as dimers through the b-ZIP region to specific sequences in the promoter (26, 28, 42). Activation by NF-IL6 can be enhanced by phosphorylation near or within the b-ZIP region (39, 55). However, transcriptional activation by NF-IL6, as by its rodent counterpart LAP, is modulated by the ratios of activator to inhibitor isoforms that are translated from in-frame AUGs of the same mRNA species (13, 21). The abundance and ratio of the activator to inhibitor NF-IL6 isoforms vary according to cell type and can be altered by cytokine stimulation (19). NF-IL6 is therefore regulated at multiple levels and may be a component of many cytokine nuclear signaling pathways that lead to regulation of genes encoding immunoglobulins, viral proteins, and cytokines (1, 41, 45, 46). The functions of NF-IL6 may be further modulated by protein-protein interaction, since NF-IL6 associates in vitro with NF-κB (30, 37, 49), the glucocorticoid receptor (40), C/ATF (51), and Fos/Jun (21). The demonstration that NF-IL6 forms stable protein-protein complexes with Jun in vivo in IL-6-induced cells (20) supports a physiologic role for this protein-protein interaction in IL-6 signaling.

The presence of NF-IL6 and AP-1 sites in the cytokine-responsive region of the *TSG-6* promoter suggests that NF-IL6 and Fos/Jun transcription factors also play important roles in mediating the IL-1 and TNF-α signals. In this study we show that the NF-IL6 site is essential for activation of the *TSG-6* promoter by either cytokine. The AP-1 site cooperates with the NF-IL6 site in the activation of the *TSG-6* promoter by both TNF-α and IL-1. Functional analysis suggests that the ratio of activator to inhibitor isoforms of NF-IL6 and the interaction of NF-IL6 with the AP-1 binding site are important in the regulation of *TSG-6* gene expression.

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MATERIALS AND METHODS

Cell cultures and RNA analysis. Normal human FS-4 fibroblasts (32, 34) were cultured in Eagle's minimum essential medium supplemented with 5% fetal bovine serum. For cytokine induction, confluent cultures were incubated in minimum essential medium containing 0.25% serum for 48 h, after which recombinant human TNF-α (20 ng/ml), IL-1α (1 ng/ml), and dexamethasone (8×10^{-6} M) were added singly or in combinations. TNF-α (6×10^6 U/mg) was provided by M. Tsujimoto of the Suntory Institute for Biochemical Research, Osaka, Japan, and IL-1α (1×10^9 U/mg) was a gift of A. Stern and P. Lomedico, Hoffmann-La Roche, Nutley, N.J. The cells were then cultured for an additional 4 h in the same medium before total RNA was isolated as previously described (34). Northern (RNA) blot analysis was performed as previously described (36), using a ^{32}P-labeled *TSG-6* cDNA (32) for detection of *TSG-6* mRNA and a ^{32}P-labeled pHe7 cDNA (5) as a control for sample loading.

Plasmids. The expression vector pCMV-NF-IL6(+) contains a cDNA encoding NF-IL6 directed by the cytomegalovirus promoter, and pCMV-NF-IL6(−) is its antisense derivative (2). pCMV-NF-IL6-2 encodes the activator isoform of NF-IL6 that is translated from the second AUG, and pCMV-NF-IL6-3 encodes the inhibitor form that is translated from the third AUG (21). The reporter plasmid pBD3CAT contains the −163 to +78 region of the *TSG-6* promoter linked to the chloramphenicol acetyltransferase (CAT) gene. 5′ deletion mutants of the *TSG-6* promoter were generated by the exonuclease III-S1 nuclease method (18) from pBBCAT, which contains the −165 to +78 region of this promoter cloned into the *Bam*HI site of pBLCAT3 (35). Briefly, pBBCAT (40 μg) was digested with *Pst*I and *Xba*I. DNA samples were treated with exonuclease III (70 U; Boehringer Mannheim) for 5, 15, 30, or 60 s at 30°C before the reaction was terminated by heating the mixture at 65°C for 5 min. The DNA was then treated with S1 nuclease (40 U; Boehringer Mannheim). The ends were filled in by the Klenow reaction and ligated by using T4 ligase. The mutant constructs (shown in Fig. 2) were verified by sequencing by the dideoxy method (43). pAP-1-TKCAT was generated by inserting in tandem two copies of the AP-1 site of the *TSG-6* promoter (GATCTGAGTCAGT GAGTCAGTCAG) into the *Bam*HI site of pBLCAT2 (TK-CAT) (35). pNF-IL6-TKCAT was constructed by inserting a promoter region of *TSG-6* that encompasses the NF-IL6 site (GATCGATTTGCTTCAGACT) into the same site. The oligonucleotides were prepared by using an automated DNA synthesizer and purified by electrophoresis.

Site-directed mutagenesis. In vitro site-directed mutagenesis was performed by the method of Deng and Nickoloff (11). The pBD3CAT plasmid was denatured by being heated at 100°C and annealed with two primers. A selection primer (CCAGT GCCAcGCgTGCATGCCTG) was used to convert the unique *Hin*dIII site into an *Mlu*I site. Mutagenic primers were used to create substitutions of specific nucleotides. The oligonucleotides used are shown in Table 1.

After elongation and ligation, the DNA pool which contains the circularized wild-type and mutated plasmids was used to transform the repair-deficient BMH 71-18 *mutS* strain of *Escherichia coli*. The plasmid pool isolated from transformed cells was linearized with the *Hin*dIII restriction enzyme and used for another round of transformation. The mutated plasmids were amplified and selected for in this process because of their resistance to digestion by the *Hin*dIII restriction enzyme. In all cases, the mutations were verified by sequencing.

Transfections. FS-4 cells were transfected when they reached 70% confluence, by the calcium phosphate precipitation method, as described previously (31). In some experiments, cells (10^6/100-mm^2 dish) were transfected with 20 μg of the reporter plasmids and induced with TNF-α or IL-1 for 24 h. In other experiments, cells were cotransfected with 20 μg of the reporter plasmids and 0.1 to 5 μg of NF-IL6 expression vectors (21) as indicated. At 24 h after transfection, cell extracts were prepared and assayed for CAT activity as previously described (17). The acetylated forms of chloramphenicol were separated by thin-layer chromatography and quantified with a radioactive imaging system (Ambis, Inc., San Diego, Calif.). CAT activity is expressed as the percentage of chloramphenicol that is converted into its acetylated forms.

TABLE 1. Oligonucleotides used in this study

Site	Primer[a]
IRF-E	GTTGCCAATGCtAtTGAGTCAG
AP-1	AATGCAAATGtGaCtGAGGATTTGC
NF-IL6	TCAGAGGATTcGaTTCgGACACTG
Insertion (AP-1/NF-IL6)	TGAGTCAGAGGatgcgATTTGCTTCAG

[a] Substituted and inserted nucleotides are indicated in lowercase letters.

Electrophoretic mobility shift assay. Nuclear extracts from FS-4 cells (4 μg) or purified recombinant glutathione *S*-transferase (GST)–NF-IL6-3 fusion protein (21) at the concentrations indicated were incubated in a buffer containing 5 mM Tris-HCl (pH 7.5), 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES; pH 7.9), 50 mM NaCl, 15 mM EDTA, 5 mM dithiothreitol, and 10% glycerol at 37°C for 15 min. Poly(dI-dC) (1 μg) was added, and incubation was continued for 5 min before the addition of radiolabeled DNA probe. The protein-DNA complexes formed after incubation at room temperature for 10 min were separated from the free probe by electrophoresis in a 6% native polyacrylamide gel in 0.25% Tris-borate-EDTA buffer. Antisera specific for NF-IL6 (19), Fos, and Jun (kindly provided by E. Ziff, New York University Medical Center) were used to confirm the participation of these proteins in the formation of protein-DNA complexes. The oligonucleotide probes used in the electrophoretic mobility shift assays were as follows, with the core binding site underlined and mutations indicated in lowercase letters.

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AP-1:    5′ GATCTGAGTCAGTGAGTCAG 3′
         3′      ACTCAGTCACTCAGTCCTAG 5′

NF-IL6:  5′ AGGATTTGCTTCAGACACTGA 3′
         3′ TCCTAAACGAAGTCTGTGACT 5′

AP-1M:   5′ TGAGgCAcTGAGgCAc 3′
         3′ ACTCcGTgACTCcGTg 5′
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RESULTS

TNF-α and IL-1 activate the *TSG-6* gene through distinct pathways. *TSG-6* mRNA levels increase rapidly in normal human FS-4 fibroblasts stimulated with either TNF-α or IL-1 (32). Simultaneous treatment with TNF-α and IL-1 results in an additive if not synergistic induction of *TSG-6* mRNA (31). Since inflammatory and acute-phase responses are regulated mainly by cytokines and hormones, we examined the effect of dexamethasone on the synthesis of *TSG-6* mRNA. Treatment with dexamethasone alone did not increase *TSG-6* mRNA or influence the levels of *TSG-6* mRNA induced by TNF-α (Fig. 1). However, dexamethasone markedly inhibited the increase of *TSG-6* mRNA that was induced by IL-1. The pathways

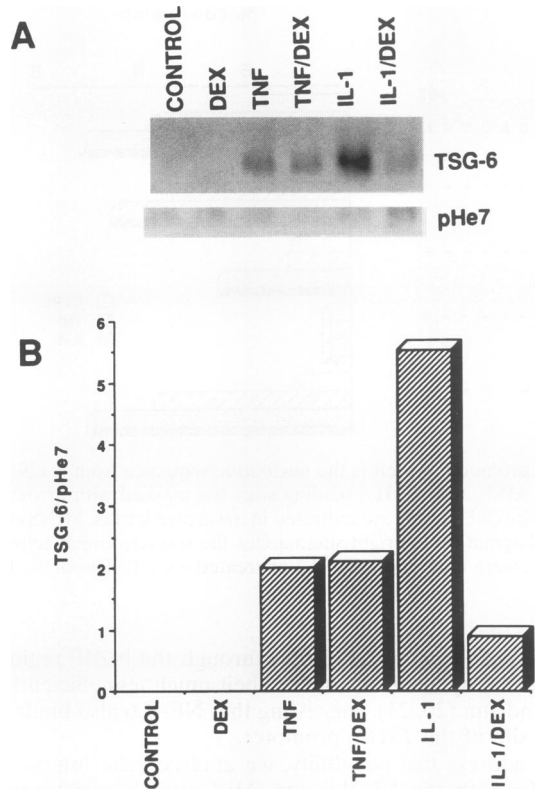

FIG. 1. Regulation of *TSG-6* mRNA synthesis by TNF-α, IL-1, and dexamethasone. (A) FS-4 cells were incubated for 4 h with medium alone (CONTROL), dexamethasone (DEX), TNF-α (TNF), IL-1, or with a combination of cytokine and dexamethasone as indicated. The levels of *TSG-6* mRNA were analyzed by Northern blotting, using the invariant pHe7 mRNA as a control for RNA loading. (B) Densitometric analysis of the data shown in panel A, presented as the ratios of *TSG-6* to pHe7.

through which IL-1 and TNF-α activate the *TSG-6* gene are therefore distinguishable.

The AP-1 and NF-IL6 sites are required for cytokine activation. Previously we showed that the −165 to −58 region of the *TSG-6* promoter was required for the basal promoter activity as well as for its activation by TNF-α and IL-1 in normal human FS-4 fibroblasts (31). To identify the regulatory elements in this region, we created serial 5′ deletions linked to the CAT reporter gene and analyzed their responsiveness to IL-1 and TNF-α in transiently transfected FS-4 cells (Fig. 2). Deletion of the CCAAT box (pBD4CAT) led to significant reduction of the IL-1 response. Removal of the overlapping IRF-E/AP-1 sites (pBD56CAT) diminished the basal promoter activity and markedly reduced the TNF-α response. However, activation by IL-1 was significantly retained (~50% of the pBD3CAT activity). Deletion of the NF-IL6 site (pBD6CAT) rendered the promoter unresponsive to TNF-α and marginally responsive to IL-1, coincidental with a further reduction of the basal promoter activity. These results suggest that although the NF-IL6 and AP-1 sites enhance the basal promoter activity incrementally, they are critical for activation of the *TSG-6* promoter by TNF-α and IL-1.

The regulatory role of the putative IRF-E, AP-1, and NF-IL6 binding sites was studied further by substitution mutagenesis (Fig. 3). The IRF-E is apparently dispensable for TNF-α and IL-1 activation, because mutations of this site (IRF-EM) did not appreciably alter either the basal promoter activity or the responsiveness to either cytokine (Fig. 3). In agreement with the deletion analyses, disruption of the AP-1 site (AP-1M) abolished TNF-α activation but retained the IL-1 responsiveness substantially (60%). Mutations in the NF-IL6 site (NF-IL6M) completely abolished activation by either TNF-α or IL-1 and reduced the basal promoter activity markedly. A mutant disrupted in both AP-1 and NF-IL6 sites was not activated by either cytokine, thus confirming the critical role of these two sites (27). The functional cooperation between the NF-IL6 and AP-1 sites, however, does not appear to have a spatial constraint, since increasing the spacing between the two sites by 5 bp (MINS) did not perturb the IL-1

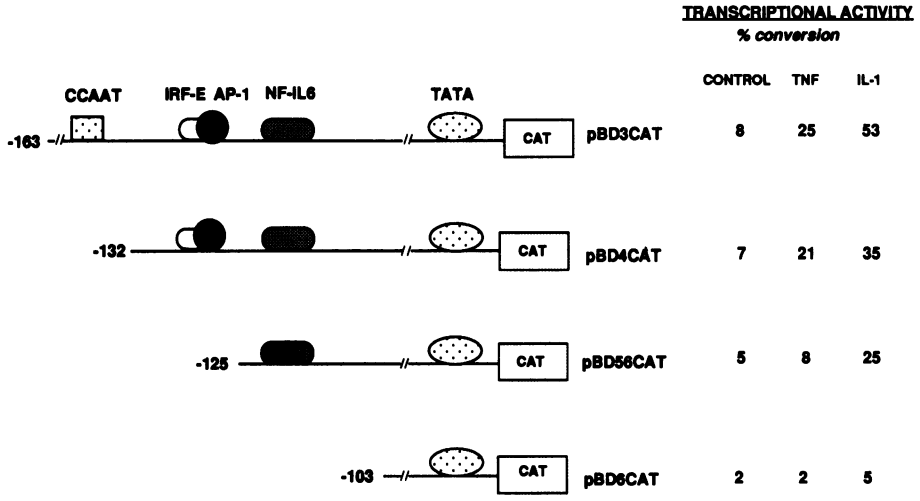

FIG. 2. Deletion analysis of the cytokine-responsive region in the *TSG-6* promoter. Serial 5′ deletions of the −163 to +78 region of a *TSG-6* promoter-CAT construct were generated as described in Materials and Methods. After transfection with the mutant constructs, FS-4 cells were cultured in the absence (CONTROL) or presence of either TNF-α or IL-1 for 24 h. Cell extracts were prepared and assayed for CAT activity. The results shown are representative of five independent experiments, which are within 20% variation and in which different preparations of each plasmid construct were used.

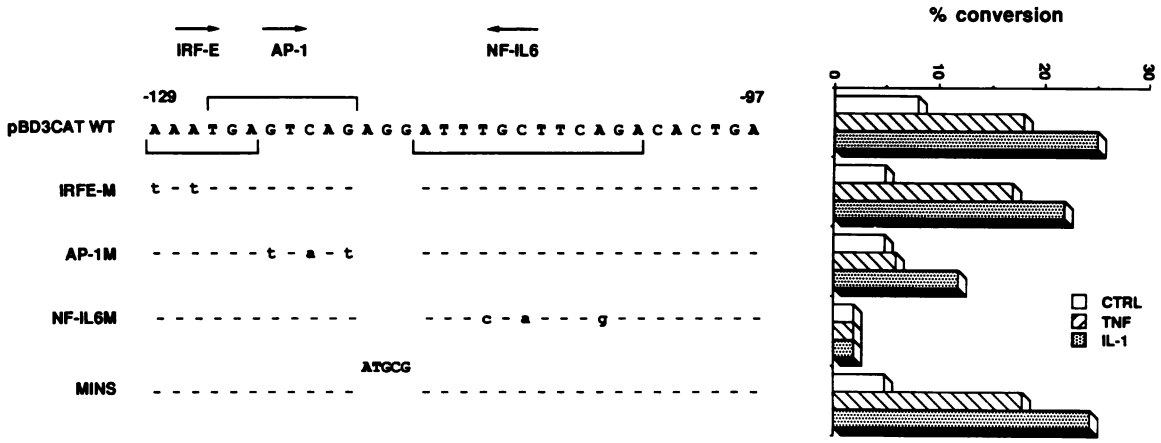

FIG. 3. Mutational analysis of the cytokine responsive elements in the *TSG-6* promoter. At left is the nucleotide sequence from −129 to −97 of the wild-type *TSG-6* promoter (pBD3CAT). The orientations of the IRF-E, AP-1, and NF-IL6 binding sites are marked with arrows. Base substitutions in the IRF-E site (IRF-EM), the AP-1 site (AP-1M) and the NF-IL6 site (NF-IL6M) are indicated in lowercase letters. MINS contains a five-nucleotide (ATGCG) insertion between the AP-1 and NF-IL6 sites. The diagram on the right summarizes the transcriptional activities of the wild-type and mutant promoter CAT constructs in transfected FS-4 cells that were untreated (CTRL) or treated with TNF-α or IL-1.

and TNF-α responsiveness (Fig. 3). These results confirm that the NF-IL6 site is indispensable for activation by either TNF-α or IL-1 and that the AP-1 site is also essential for TNF-α stimulation and enhances activation by IL-1.

NF-IL6 binds to NF-IL6 and AP-1 sites with different efficiencies. The requirement of an intact NF-IL6 site for activation of the *TSG-6* promoter by IL-1 and TNF-α suggests that the transcription factor NF-IL6 plays an essential role in mediating these cytokine signals. Fos/Jun and NF-IL6 differ significantly in their DNA-binding specificities. NF-IL6 binds to DNA sequences related to the consensus NF-IL6 binding site 5′ TT/GNNGNAAT/G 3′ (1) with different efficiencies (21). Fos and Jun do not bind to NF-IL6 sites. However, NF-IL6 can bind to AP-1 sites through the b-ZIP region in a sequence-dependent manner, albeit much less efficiently than Fos and Jun (20, 21), suggesting that NF-IL6 also binds to the AP-1 site of the *TSG-6* promoter.

To address this possibility, we analyzed the interaction of NF-IL6 with the NF-IL6 and AP-1 sites by electrophoretic mobility shift assay, using purified recombinant GST–NF-IL6-3 fusion protein, which contains the smallest of the three NF-IL6 isoforms (21). Oligonucleotides that contain either the NF-IL6 site (NF-IL6 probe) or two copies of the AP-1 site (AP-1 probe) of the *TSG-6* promoter were used as probes. NF-IL6-3 bound to the NF-IL6 probe in a concentration-dependent manner (Fig. 4A, lanes 2 to 4). Competitions with

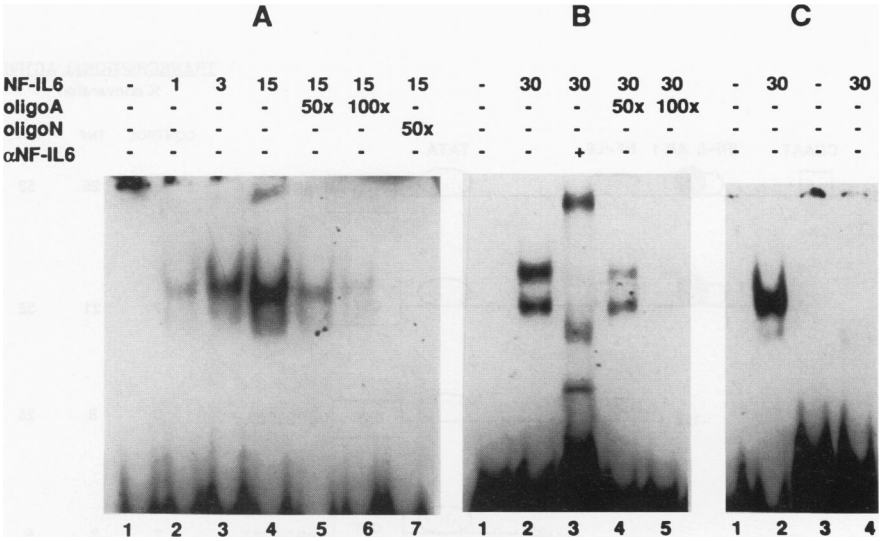

FIG. 4. NF-IL6 binds to the NF-IL6 and AP-1 sites. The binding of purified recombinant GST–NF-IL6-3 (NF-IL6) to an oligonucleotide probe containing either the NF-IL6 site (A) or two copies of the tandemly repeated AP-1 site (B) of the *TSG-6* promoter was analyzed by electrophoretic mobility assay. The amounts of NF-IL6 protein (in micrograms) used for each binding reaction were as indicated. Competition with unlabeled NF-IL6 oligonucleotide (oligoN) or unlabeled AP-1 oligonucleotide (oligoA) at a 50-fold (50×) or 100-fold (100×) molar excess or preincubation with an anti-NF-IL6 antibody (αNF-IL6) in the binding reaction mixture was as indicated. (C) The AP-1 probe was used in lanes 1 and 2, and the AP-1M probe, an oligonucleotide containing a mutated AP-1 site (see Materials and Methods), was used in lanes 3 and 4.

unlabeled NF-IL6 and AP-1 oligonucleotides suggested that NF-IL6-3 also bound to the AP-1 probe (lanes 5 and 6), although less efficiently than to the NF-IL6 probe (lane 7). These results are consistent with those of previous studies (21). Binding of NF-IL6-3 to the AP-1 probe led to the formation of two protein-DNA complexes, most likely due to protein occupancy at either or both of the two tandemly repeated AP-1 sites present in the probe (Fig. 4B, lane 2). Both complexes contained NF-IL6 and were specific to the AP-1 site, since their formation was inhibited by antibodies specific for NF-IL6 (lane 3) and by a molar excess of unlabeled AP-1 oligonucleotide (lanes 4 and 5). Confirming these results, the protein-DNA complex formation could not be inhibited by oligonucleotides containing mutated NF-IL6 or AP-1 sites (20, 27) and NF-IL6-3 did not bind to an oligonucleotide containing a mutated AP-1 site (Fig. 4C, lanes 3 and 4). Thus, NF-IL6-3 binds to both the NF-IL6 and the AP-1 sites present in the *TSG-6* promoter, but with different efficiencies.

The NF-IL6 site mediates activation by NF-IL6. The NF-IL6 binding consensus sequence is present in the promoters of many genes, including those encoding cytokines (Fig. 5A). The finding that recombinant NF-IL6-3 binds to both NF-IL6 and AP-1 sites (Fig. 4) suggests that NF-IL6 regulates the *TSG-6* promoter via either, or both, of these sites. To address this possibility, we analyzed the sequence requirement for activation of the *TSG-6* promoter by NF-IL6 in FS-4 cells transfected with reporter plasmids driven by the wild-type *TSG-6* promoter and its substitution mutants. Expression of NF-IL6, but not its antisense derivative, enhanced the *TSG-6* promoter activity three- to fivefold (Fig. 5B). Mutations of the IRF-E (IRF-EM) or the AP-1 site (AP-1M) or alteration of the spacing between the NF-IL6 and AP-1 sites (MINS) did not interfere with activation by NF-IL6 (Fig. 5C). However, activation by NF-IL6 required the presence of an intact NF-IL6 binding site, because disruption of this site (NF-IL6M) completely abolished transactivation by NF-IL6. Thus, NF-IL6 activates the native *TSG-6* promoter in a NF-IL6 site-dependent manner.

IL-1 and TNF-α increase the binding of Fos and Jun to the AP-1 site. The AP-1 site is indispensable for activation of the *TSG-6* promoter by TNF-α, and it enhances activation by IL-1 (Fig. 2 and 3). The AP-1 site, however, was insufficient to mediate activation by NF-IL6 (Fig. 5C), despite its ability to bind NF-IL6-3 in vitro (Fig. 4B). Since IL-1 and TNF-α are known to increase the synthesis of *fos* and *jun* mRNAs (34, 44), it appeared likely that IL-1 and TNF-α mediate their responses through enhanced interaction of Fos and Jun with the AP-1 site present in the *TSG-6* promoter. This possibility was examined by analyzing the Fos/Jun DNA-binding activity present in nuclear extracts prepared from IL-1- and TNF-α-treated FS-4 cells, using an oligonucleotide containing the AP-1 site as a probe in the electrophoretic mobility assay. The AP-1 DNA-binding activity was low in unstimulated FS-4 cells (Fig. 6, lane 1) but increased rapidly (within 30 min) and markedly after stimulation with IL-1 (lanes 3 and 5). TNF-α also rapidly enhanced AP-1 DNA-binding activity, although less effectively than IL-1 (lanes 2 and 4). The protein-DNA complex was specific for the AP-1 site, since its formation was inhibited by competition with a molar excess of the unlabeled AP-1 probe (lane 6), but not with an NF-IL6 probe (lane 7). Preincubation with anti-Fos and anti-Jun antibodies (lane 9), but not with anti-NF-IL6 antibodies (lane 8), led to inhibition of the protein-DNA complex and generation of a more slowly migrating complex. These results suggest that activation of the *TSG-6* promoter by IL-1 and TNF-α is therefore in part due to enhanced binding of Fos and Jun to the AP-1 site.

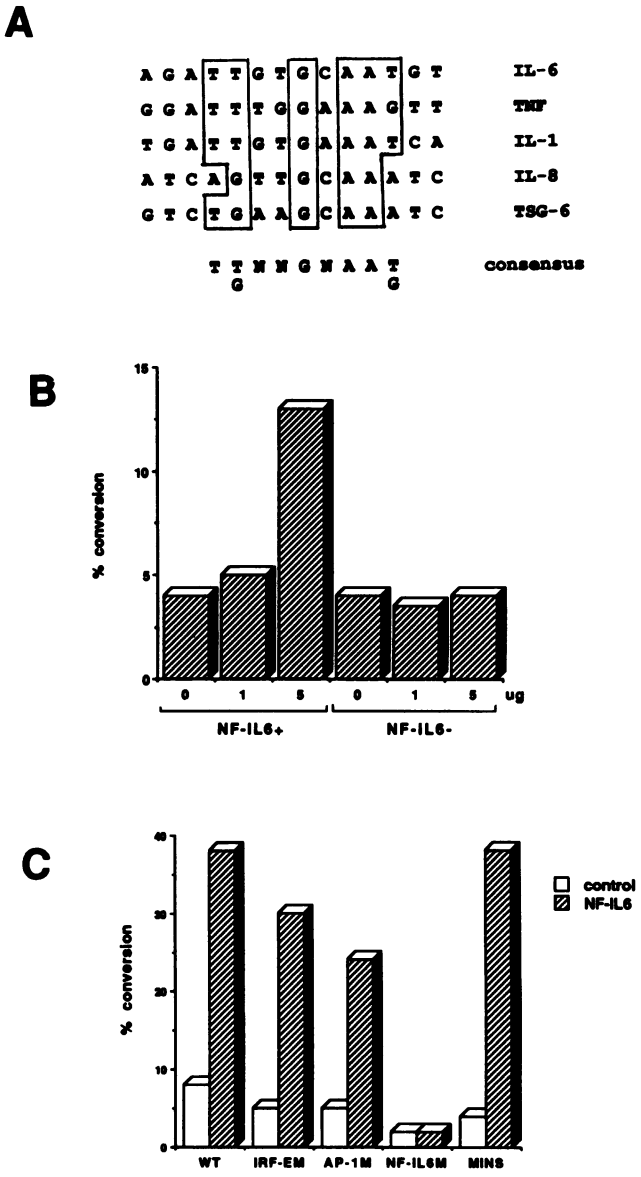

FIG. 5. NF-IL6 activates the *TSG-6* promoter through the NF-IL6 site. (A) Nucleotide sequences of the NF-IL6 binding sites present in the promoters of genes encoding cytokines and *TSG-6* protein. (B) Analysis of the activity of the wild-type *TSG-6* promoter (pBD3CAT) in FS-4 cells cotransfected with increasing amounts of the NF-IL6 expression vector [pCMV-NF-IL6(+)] or its antisense derivative [pCMV-NF-IL6(−)] or without NF-IL6 (0 μg). (C) Analysis of the pBD3CAT (WT) or mutant *TSG-6* promoter activity in FS-4 cells transfected without (open bars) or with (hatched bars) pCMV-NF-IL6(+). CAT activity was determined as described in the legend to Fig. 2.

NF-IL6-3 represses AP-1 site-mediated transcription. NF-IL6 encodes three isoforms translated from different in-frame AUGs, two activators (NF-IL6-1 and NF-IL6-2) and an inhibitor (NF-IL6-3), analogous to its rodent counterpart LAP (13, 19). Positive regulation and negative regulation by NF-IL6 are modulated by the ratio of activator to inhibitor isoforms (13, 21). However, the NF-IL6 function may also be influenced by the DNA-binding affinity of its isoforms, because the inhibitor form of LAP (NF-IL6-3) has a higher affinity for the LAP-

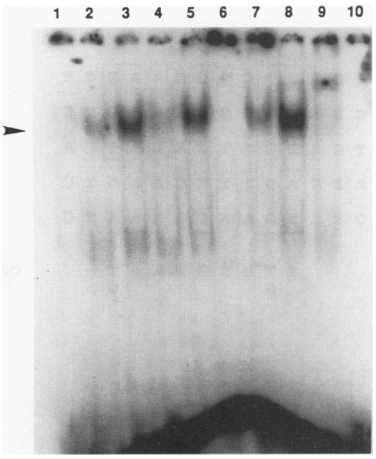

FIG. 6. TNF-α and IL-1 enhance the binding of Fos/Jun to the AP-1 site. The binding of proteins present in nuclear extracts of FS-4 cells to an AP-1 probe was analyzed by electrophoretic mobility assay. Nuclear extracts were prepared from cells that were untreated (lane 1), treated for 30 min with TNF-α (lane 2) or IL-1 (lane 3), or treated for 120 min with TNF-α (lane 4) or IL-1 (lane 5). Nuclear extract prepared from cells treated with IL-1 for 30 min was preincubed with a 50× molar excess of unlabeled AP-1 probe (lane 6), unlabeled NF-IL6 probe (lane 7), anti-NF-IL6 antibody (lane 8), or anti-Fos and anti-Jun antibodies (lane 9). Lane 10, labeled probe only. The AP-1 complex, separated from the free probe, is marked with an arrowhead.

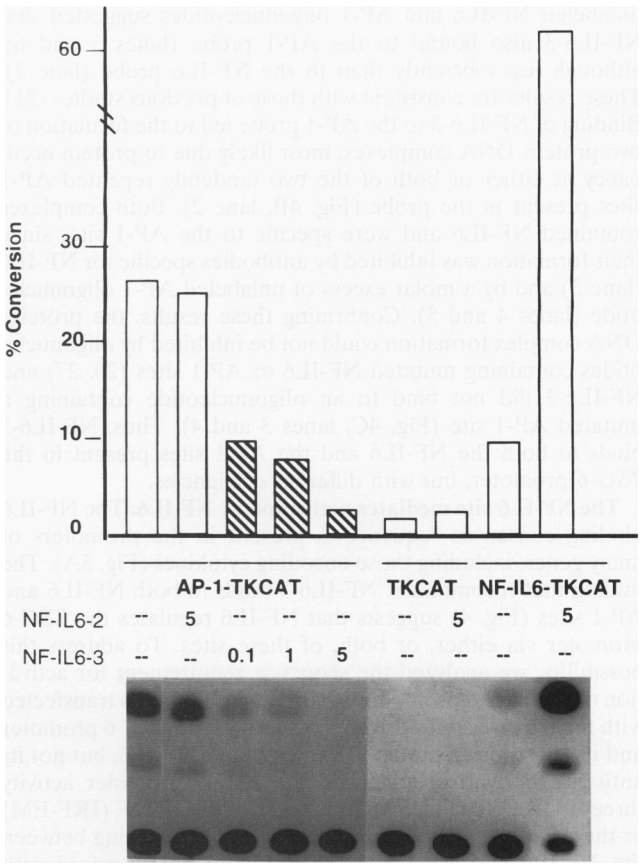

FIG. 7. Differential regulation of AP-1 site- and NF-IL6 site-mediated transcription by NF-IL6. Regulation of the TK promoter (TKCAT) or the TK promoter linked to two copies of the AP-1 site (AP-1-TKCAT) or the NF-IL6 site (NF-IL6-TKCAT) of the *TSG-6* promoter by NF-IL6-2 and NF-IL6-3 in transiently transfected FS-4 cells is shown. The top panel represents the densitometric analysis of the data shown in the bottom panel. The amount of reporter plasmid used in each transfection mixture was 20 μg. The amount (in micrograms) of pCMV-NF-IL6-2 (NF-IL6-2) or pCMV-NF-IL6-3 (NF-IL6-3) used in each cotransfection was as indicated.

responsive element than the activator form (13). The ability of NF-IL6-3 to bind to the AP-1 site of the *TSG-6* promoter (Fig. 4) suggested that NF-IL6-3 inhibits transcription mediated by this AP-1 site. Indeed, in a dose-dependent manner, NF-IL6-3 effectively inhibited the transcription of a thymidine kinase (TK) promoter linked to two copies of the AP-1 site (AP-1TKCAT; 9-fold) (Fig. 7). In contrast, the activator isoform NF-IL6-2 had no appreciable effect, although the transactivating activity of NF-IL6-2 was confirmed by a sevenfold enhancement of transcription mediated by the NF-IL6 site of the *TSG-6* promoter (NF-IL6TKCAT) (Fig. 7). Activation by NF-IL6-2 was modulated by the ratio of NF-IL6-2 to NF-IL6-3 (27) and was NF-IL6 site specific, as shown by the lack of activation of the control TK promoter in TKCAT (Fig. 7). (Repression of the TK promoters cannot be studied because of its extremely low basal activity in the absence of an AP-1 site or an NF-IL6 site in FS-4 cells.) Since the expression of NF-IL6 isoforms in transfected cells reflects the concentration of input plasmids (20) and NF-IL6-3 differs from NF-IL-2 only in the absence of the transactivating region of NF-IL6, selective inhibition of the AP-1 site by NF-IL-3 must be a function of protein-DNA interaction. Thus, although activation of the *TSG-6* promoter by NF-IL6 is mediated by the NF-IL6 site, repression by NF-IL6 can also be mediated by the AP-1 site.

NF-IL6-3 represses activation of the native *TSG-6* promoter by NF-IL6-2, TNF-α, or IL-1. NF-IL6-3 dimerizes with the NF-IL6 activator isoforms as well as Fos/Jun through the b-ZIP region, thereby modulating the activity of both C/EBP and AP-1 transcription factors (20, 21). While the NF-IL6 activator isoform and Fos/Jun mediate the IL-1 and TNF-α signals to activate the *TSG-6* promoter (Fig. 5 and 6), NF-IL6-3 may function as a powerful negative regulator, by modulating the activator isoforms of NF-IL6 and AP-1 proteins and by interacting with both NF-IL6 and AP-1 sites. In support of this possibility, the *TSG-6* promoter was markedly activated by NF-IL6-2, and the activation was suppressed by NF-IL6-3 in a concentration-dependent manner (Fig. 8A). More importantly, NF-IL6-3 very effectively antagonized activation of the *TSG-6* promoter by either IL-1 or TNF-α (Fig. 8B and C). These results demonstrate that NF-IL6 activates the native *TSG-6* promoter according to the ratio of its isoforms rather than the absolute concentration of the activator form. Inhibition of IL-1 and TNF-α responses by NF-IL6-3 suggests a physiologic role for this naturally occurring negative regulator in the modulation of IL-1 and TNF-α actions.

DISCUSSION

The IL-1 and TNF-α nuclear signaling pathways are overlapping but distinct. We have used the native TSG-6 promoter as a model to study IL-1- and TNF-induced nuclear signaling that leads to activation of common target genes (29, 31, 32, 53). The two pathways are distinguishable by their differential sensitivity to suppression by dexamethasone (Fig. 1). They are also distinct in the sequence requirement for activation of the *TSG-6* promoter; the AP-1 site augments the IL-1 response, whereas it is essential for activation of the *TSG-6* promoter by TNF-α. In contrast, the NF-IL6 site is indispensable for

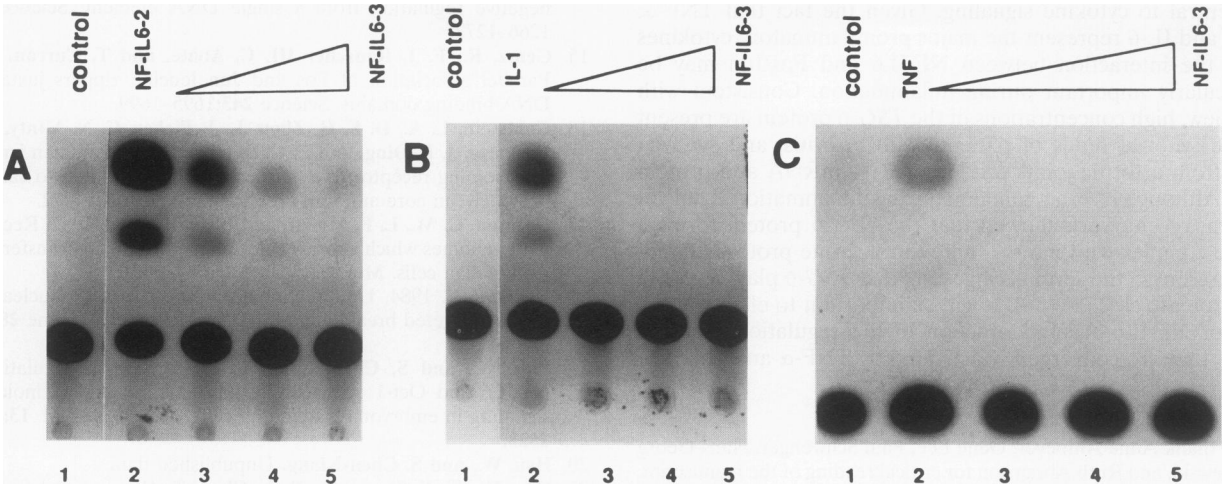

FIG. 8. NF-IL6-3 represses activation of transcription by NF-IL6-2, IL-1, and TNF-α. In all panels, FS-4 cells were transfected with the reporter *TSG-6* promoter construct pBD3CAT (20 μg) (see Fig. 2). (A) Cells were cotransfected with pCMV-NF-IL6-2 (5 μg) (lanes 2 to 5) and 0.1 μg (lane 3), 1 μg (lane 4), or 5 μg (lane 5) of pCMV-NF-IL6-3. (B) Cells were not treated (lane 1) or were treated with IL-1 for 24 h (lanes 2 to 5) after cotransfection with 0.1 μg (lane 3), 1 μg (lane 4), or 5 μg (lane 5) of pCMV-NF-IL6-3. (C) Cells were not treated (lane 1) or were treated with TNF-α for 24 h (lanes 2 to 5) after cotransfection with 0.1 μg (lane 3), 1 μg (lane 4), or 5 μg (lane 5) of pCMV-NF-IL6-3.

activation of this promoter by either cytokine. Integration of IL-1 and TNF-α signals by NF-IL6 and AP-1 transcription factors appears to be one of the mechanisms by which these two cytokines convergently activate target genes and exert overlapping biological activities.

Role of NF-IL6 in mediating IL-1 and TNF-α signals. NF-IL6 is the major member of the C/EBP family in human cells (8). We showed that NF-IL6 can positively or negatively regulate the *TSG-6* promoter depending on the ratio of functionally distinct NF-IL6 isoforms (Fig. 8). This finding provides experimental evidence that a native promoter can be regulated by the interplay of naturally occurring NF-IL6 isoforms, a model previously based only on studies of synthetic promoters (13, 21).

Although activation by NF-IL6 is mediated by the NF-IL6 site, inhibition by NF-IL6 can be mediated by both NF-IL6 and AP-1 sites (Fig. 7 and 8). Inhibition of AP-1 site-mediated transcription by the inhibitor form of NF-IL6 was unexpected. It may be explained by competition of NF-IL6-3 at molar excess with Fos/Jun for binding to the AP-1 site, as suggested by the interaction of NF-IL6-3 with the AP-1 site (Fig. 4). Alternatively, NF-IL6-3 may form heterodimers with Fos and Jun, thereby dominantly inhibiting activation by Fos and Jun. Whether the heterodimers do not bind to the AP-1 site at all or they bind to the AP-1 site but are inactive is not known. The demonstration that NF-IL6-3 dimerizes with Fos/Jun in vitro, leading to heterodimers with reduced affinity for NF-IL6 and AP-1 sites (20, 21), is consistent with the latter possibility. NF-IL6-2 has a lower affinity than NF-IL6-3 for both AP-1 and NF-IL6 sites (20, 58). This reduced DNA-binding affinity may also contribute to the lack of activation of AP-1 site-mediated transcription by NF-IL6-2.

Activation of the *TSG-6* promoter by either IL-1 or TNF-α requires an intact NF-IL6 site (Fig. 2 and 3). The responses to these cytokines are antagonized by the inhibitor isoform of NF-IL6 (Fig. 8), presumably through the NF-IL6 and AP-1 sites. The NF-IL6 and AP-1 activities in intact cells are likely to be modulated by their concentrations in the cells, in addition to the relative affinity of the NF-IL6 isoforms and the various heterodimers for the NF-IL6 and AP-1 sites. Earlier work showed that the endogenous *TSG-6* gene was readily inducible by TNF-α and IL-1 in certain normal cells (fibroblasts, peripheral blood mononuclear cells, chondrocytes, and synovial cells), but not in normal vascular endothelial cells or the majority of malignant cells examined (33, 57). The predominant expression of NF-IL6-3 in transformed cells (19) and of NF-IL6-2 in FS-4 cells (27) suggests that the ratio of NF-IL6 isoforms contributes to the determination of cell-type-dependent activation of the *TSG-6* gene. This ratio is regulated in favor of the activator forms by IL-6 in human embryonal carcinoma and T cells (21) and by IL-1 and TNF-α in FS-4 cells (27), suggesting that it plays a physiologic role in the responses to these cytokines.

Role of AP-1 family transcription factors in TNF-α and IL-1 responses. The AP-1 site of the *TSG-6* promoter is required for activation by TNF-α and it enhances the response to IL-1. Although the precise mechanism by which NF-IL6 and AP-1 sites cooperate to activate this promoter remains to be investigated further, activation of *fos* and *jun* mRNA synthesis (34, 44) and AP-1 DNA-binding activity by TNF-α and IL-1 (Fig. 6) indicates that Fos and Jun mediate the cytokine signals to activate the AP-1 site. However, there is a cooperation between NF-IL6 and AP-1 sites since activation of the *TSG-6* promoter by IL-1 and TNF-α does not occur in the absence of the NF-IL6 site (Fig. 2). Binding of NF-IL6-3 to the AP-1 site (Fig. 4) and repression of AP-1 site-mediated transcription by NF-IL6-3 (Fig. 7) suggested that NF-IL6 is a negative regulator for AP-1 site-mediated transcription. Since the AP-1 site of the *TSG-6* promoter is identical to those present in the collagenase and stromelysine promoters (4), NF-IL6-3 may have a role in the negative regulation of these promoters as well.

Fos and Jun have been thought to regulate diverse biological activities through protein-protein interactions, as suggested by their abilities to associate with many transcription factors in the presence of DNA in vitro, for example, the glucocorticoid receptor (14, 25), MyoD (6), NFAT (23), and NF-κB (48). Our demonstration that NF-IL6 associates with Fos/Jun in vivo when both NF-IL6 and Fos/Jun are regulated by IL-6 (20) suggests that the NF-IL6–AP-1 interaction is dynamic and may

be central to cytokine signaling. Given the fact that TNF-α, IL-1, and IL-6 represent the major proinflammatory cytokines (29), the interaction between NF-IL6 and Fos/Jun may be particularly important during inflammation. Consistent with this view, high concentrations of the *TSG-6* protein are present in the synovial fluids of patients with arthritis, and synovial cells from arthritic joints produce *TSG-6* mRNA and protein (57). Although its exact function during inflammation is not yet known, recent work showed that the *TSG-6* protein forms a stable complex with inter-α-inhibitor, a serine protease inhibitor present in the serum, suggesting that *TSG-6* plays a role in the protease cascade (56). It will be important to elucidate the role of NF-IL6–AP-1 interaction in the regulation of other genes that are convergently activated by TNF-α and IL-1.

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REFERENCES

1. **Akira, S., H. Isshiki, T. Nakajima, S. Kinoshita, Y. Nishio, S. Hashimoto, S. Natsuka, and T. Kishimoto.** 1992. A nuclear factor for the IL-6 gene (NF-IL6). Chem. Immunol. **51**:299–322.
2. **Akira, S., H. Isshiki, T. Sugita, O. Tanabe, S. Kinoshita, Y. Nishio, T. Nakajima, T. Hirano, and T. Kishimoto.** 1990. A nuclear factor for IL-6 expression (NF-IL6) is a member of C/EBP family. EMBO J. **9**:1897–1906.
3. **Angel, P., E. A. Allegretto, S. T. Okino, K. Hattori, W. J. Boyle, T. Hunter, and M. Karin.** 1988. Oncogene *jun* encodes a sequence-specific *trans*-activator similar to AP-1. Nature (London) **332**:166–171.
4. **Angel, P., and M. Karin.** 1991. The role of jun, fos and the AP-1 complex in cell-proliferation and transformation. Biochim. Biophys. Acta **1072**:129–157.
5. **Babich, A., T. L. Feldman, J. R. Nevins, J. E. Darnell, and C. Weinberg.** 1983. Effect of adenovirus on metabolism of specific host mRNAs: transport control and specific translational discrimination. Mol. Cell. Biol. **3**:1212–1221.
6. **Bengal, E., L. Ransone, R. Schaarfmann, V. J. Dwarki, S. J. Tapscott, H. Weintraub, and I. M. Verma.** 1992. Functional antagonism between c-Jun and MyoD proteins: a direct physical association. Cell **68**:507–519.
7. **Bohmann, D., T. J. Bos, A. Admon, T. Nishimura, P. K. Vogt, and R. Tjian.** 1987. Human proto-oncogene c-*jun* encodes a DNA binding protein with structural and functional properties of transcription factor AP-1. Science **238**:1386–1392.
8. **Chen-Kiang, S., W. Hsu, Y. Natkunam, and X. Zhang.** 1993. Nuclear signaling by interleukin-6. Curr. Opin. Immunol. **5**:124–128.
9. **Chiu, R., W. J. Boyle, J. Meek, T. Smeal, T. Hunter, and M. Karin.** 1988. The c-Fos protein interacts with c-Jun/AP-1 to stimulate transcription of AP-1 responsive genes. Cell **54**:541–552.
10. **Curran, T., and B. R. Franza, Jr.** 1988. Fos and Jun: the AP-1 connection. Cell **55**:395–397.
11. **Deng, W. P., and J. A. Nickoloff.** 1992. Site-directed mutagenesis of virtually any plasmid by eliminating a unique site. Anal. Biochem. **200**:81–87.
12. **Descombes, P., M. Chojkier, S. Lichtsteiner, E. Falvey, and U. Schibler.** 1990. LAP, a novel member of the C/EBP gene family, encodes a liver-enriched transcriptional activator protein. Genes Dev. **4**:1541–1551.
13. **Descombes, P., and U. Schibler.** 1991. A liver-enriched transcriptional activator protein, LAP, and a transcriptional inhibitory protein, LIP, are translated from the same mRNA. Cell **67**:569–579.
14. **Diamond, M. I., J. I. Miner, S. K. Yoshinaga, and K. Yamamoto.** 1990. Transcription factor interactions: selectors of positive or negative regulation from a single DNA element. Science **249**:1266–1272.
15. **Gentz, R., F. J. Rauscher III, C. Abate, and T. Curran.** 1989. Parallel association of Fos and Jun leucine zippers juxtaposes DNA binding domains. Science **243**:1695–1699.
16. **Goldstein, L. A., D. F. H. Zhou, L. J. Picker, C. N. Minty, R. F. Bargatze, J. F. Ding, and E. C. Butcher.** 1989. A human lymphocyte homing receptor, the hermes antigen, is related to cartilage proteoglycan core and link proteins. Cell **56**:1063–1072.
17. **Gorman, C. M., L. F. Moffat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2**:1044–1051.
18. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene **28**:351–359.
19. **Hsu, W., and S. Chen-Kiang.** 1993. Convergent regulation of NF-IL6 and Oct-1 synthesis by interleukin-6 and retinoic acid signaling in embryonal carcinoma cells. Mol. Cell. Biol. **13**:2515–2523.
20. **Hsu, W., and S. Chen-Kiang.** Unpublished data.
21. **Hsu, W., T. K. Kerppola, P. L. Chen, T. Curran, and S. Chen-Kiang.** 1994. Fos and Jun repress transcription activation by NF-IL6 through association at the basic zipper region. Mol. Cell. Biol. **14**:268–276.
22. **Isshiki, H., S. Akira, O. Tanabe, T. Nakajima, T. Shimamoto, T. Hirano, and T. Kishimoto.** 1990. Constitutive and interleukin-1 (IL-1)-inducible factors interact with the IL-1 responsive element in the IL-6 gene. Mol. Cell. Biol. **10**:2757–2764.
23. **Jain, J., P. G. McCaffrey, Z. Miner, T. K. Kerppola, J. N. Lambert, G. L. Verdine, T. Curran, and A. Rao.** 1993. The T-cell transcription factor $NFAT_p$ is a substrate for calcineurin and interacts with Fos and Jun. Nature (London) **365**:352–355.
24. **Kerppola, T. K., and T. Curran.** 1991. Fos-Jun heterodimers and Jun homodimers bend DNA in opposite orientations: implications for transcription factor cooperativity. Cell **66**:317–326.
25. **Kerppola, T. K., D. Luk, and T. Curran.** 1993. Fos is a preferential target of glucocorticoid receptor inhibition of AP-1 activity in vitro. Mol. Cell. Biol. **13**:3782–3791.
26. **Kinoshita, S., S. Akira, and T. Kishimoto.** 1992. A member of the C/EBP family, NF-IL6β, forms heterodimer and transcriptionally synergizes with NF-IL6. Proc. Natl. Acad. Sci. USA **89**:1473–1476.
27. **Klampfer, L., S. Chen-Kiang, and J. Vilcek.** Unpublished data.
28. **Landschulz, W. H., P. F. Johnson, and S. L. McKnight.** 1989. The DNA binding domain of the rat liver nuclear protein C/EBP is bipartite. Science **243**:1681–1688.
29. **Le, J., and J. Vilcek.** 1987. Tumor necrosis factor and interleukin 1: cytokines with multiple overlapping activities. Lab. Invest. **56**:234–248.
30. **LeClair, K., M. A. Blanar, and P. A. Sharp.** 1992. The p50 subunit of NF-κB associates with the NF-IL6 transcription factor. Proc. Natl. Acad. Sci. USA **89**:8145–8149.
31. **Lee, T. H., L. Klampfer, T. B. Shows, and J. Vilcek.** 1993. Transcriptional regulation of TSG6, a tumor necrosis factor- and interleukin-1-inducible primary response gene coding for a secreted hyaluronan-binding protein. J. Biol. Chem. **268**:6154–6160.
32. **Lee, T. H., G. W. Lee, E. B. Ziff, and J. Vilcek.** 1990. Isolation and characterization of eight tumor necrosis factor-inducible gene sequences from human fibroblasts. Mol. Cell. Biol. **10**:1982–1988.
33. **Lee, T. H., H.-G. Wisniewski, and J. Vilcek.** 1992. A novel secretory tumor necrosis-inducible protein (TSG-6) is a member of the family of hyaluronate binding proteins, closely related to the adhesion receptor CD44. J. Cell. Biol. **116**:545–557.
34. **Lin, J.-X., and J. Vilcek.** 1987. Tumor necrosis factor and interleukin-1 cause a rapid and transient stimulation of c-*fos* and c-*myc* mRNA levels in human fibroblasts. J. Biol. Chem. **262**:11908–11911.
35. **Luckow, B., and G. Schütz.** 1987. CAT constructions with multiple unique restriction sites for the functional analysis of eukaryotic promoters and regulatory elements. Nucleic Acids Res. **15**:5490.
36. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
37. **Matsusaka, T., K. Fujikawa, Y. Nishio, N. Mukaida, K. Matsu-**

shima, T. Kishimoto, and S. Akira. 1993. Transcription factors NF-IL6 and NFκB synergistically activate transcription of the inflammatory cytokines, interleukin 6 and interleukin 8. Proc. Natl. Acad. Sci. USA **90:**10193–10197.

38. **Metz, R., and E. Ziff.** 1991. cAMP stimulates the C/EBP-related transcription factor NF-IL6 to translocate to the nucleus and induce c-fos transcription. Genes Dev. **5:**1754–1766.
39. **Nakajima, T., S. Kinoshita, T. Sasagawa, K. Sasaki, M. Naruto, T. Kishimoto, and S. Akira.** 1993. Phosphorylation at threonine-235 by a *ras*-dependent mitogen-activated protein kinase cascade is essential for transcription factor NF-IL6. Proc. Natl. Acad. Sci. USA **90:**2207–2211.
40. **Nishio, Y., H. Isshiki, T. Kishimoto, and S. Akira.** 1993. A nuclear factor for interleukin-6 expression (NF-IL6) and the glucocorticoid receptor synergistically activate genes via direct protein-protein interaction. Mol. Cell. Biol. **13:**1854–1862.
41. **Raynal, M. C., Z. Liu, T. Hirano, L. Mayer, T. Kishimoto, and S. Chen-Kiang.** 1989. Interleukin-6 induces secretion of IgG1 by coordinated transcriptional activation and differential mRNA accumulation. Proc. Natl. Acad. Sci. USA **86:**8024–8028.
42. **Roman, C., J. S. Platero, J. Shuman, and K. Calame.** 1990. Ig/EBP-1: a ubiquitously expressed immunoglobulin enhancer binding protein that is similar to C/EBP and heterodimerizes with C/EBP. Genes Dev. **4:**1404–1415.
43. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
44. **Sciavolino, P. J., T. H. Lee, and J. Vilcek.** Interferon-β induces metalloproteinase expression in human fibroblasts. Role of AP-1. J. Biol. Chem., in press.
45. **Spergel, J. M., and S. Chen-Kiang.** 1991. Interleukin-6 enhances a cellular activity that functionally substitutes for E1A protein in transactivation. Proc. Natl. Acad. Sci. USA **88:**6472–6476.
46. **Spergel, J. M., W. Hsu, S. Akira, B. Thimmappaya, T. Kishimoto, and S. Chen-Kiang.** 1992. NF-IL6, a member of the C/EBP family, regulates E1A-responsive promoters in the absence of E1A. J. Virol. **66:**1021–1030.
47. **Stamenkovic, I., M. Amiot, J. M. Pesando, and B. Seed.** 1989. A lymphocyte molecule implicated in lymph node homing is a member of the cartilage link protein family. Cell **56:**1057–1062.
48. **Stein, B., S. A. Baldwin, D. W. Ballard, C. W. Greene, P. Angel, and P. Herrlich.** 1993. Cross-coupling of the NFκB p65 and Fos/Jun transcription factors produces potentiated biological function. EMBO J. **12:**3879–3891.
49. **Stein, B., P. C. Cogswell, and A. S. Baldwin, Jr.** 1993. Functional and physical associations between NF-κB and C/EBP family members: a Rel domain-bZIP interaction. Mol. Cell. Biol. **13:** 3964–3974.
50. **Turner, R., and R. Tjian.** 1989. Leucine repeats and an adjacent DNA binding domain mediate the formation of functional c-fos-c-jun heterodimers. Science **243:**1689–1694.
51. **Vallejo, M., D. Ron, C. P. Miller, and J. F. Habener.** 1993. C/ATF, a member of the activating transcription factor family of DNA-binding proteins, dimerizes with CAAT/enhancer-binding proteins and directs their binding to cAMP response elements. Proc. Natl. Acad. Sci. USA **90:**4679–4683.
52. **Vietor, I., P. Schwenger, W. Li, J. Schlessinger, and J. Vilcek.** 1993. Tumor necrosis factor-induced activation and increased tyrosine phosphorylation of mitogen activated protein (MAP) kinase in human fibroblasts. J. Biol. Chem. **268:**18994–18999.
53. **Vilcek, J., and T. H. Lee.** 1991. Tumor necrosis factor. New insights into the molecular mechanisms of its multiple actions. J. Biol. Chem. **266:**7313–7316.
54. **Vogt, P. K., T. J. Bos, and R. F. Doolittle.** 1987. Homology between the DNA-binding domain of the GCN4 regulatory protein of yeast and the carboxyl-terminal portion of a protein coded for by the oncogene jun. Proc. Natl. Acad. Sci. USA **84:**3316–3319.
55. **Wegner, M., Z. Cao, and M. G. Rosenfeld.** 1992. Calcium-regulated phosphorylation within the leucine zipper of C/EBPβ. Science **256:**370–373.
56. **Wisniewski, H.-G., W. H. Burgess, J. D. Oppenheim, and J. Vilcek.** 1994. TSG-6, an arthritis-associated hyaluronan binding protein, forms a stable complex with the serum protein inter-α-inhibitor. Biochemistry **33:**7423–7429.
57. **Wisniewski, H.-G., R. Maier, M. Lotz, S. Lee, L. Klampfer, T. H. Lee, and J. Vilcek.** 1993. TSG-6: a TNF-, IL-1-, and LPS-inducible secreted glycoprotein associated with arthritis. J. Immunol. **151:** 6593–6601.
58. **Zheng, X., and S. Chen-Kiang.** Unpublished data.