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Glucocorticoids Suppress Tumor Necrosis Factor-α Expression by Human Monocytic THP-1 Cells by Suppressing Transactivation through Adjacent NF-κB and c-Jun-Activating Transcription Factor-2 Binding Sites in the Promoter*

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Glucocorticoid drugs suppress tumor necrosis factor-α (TNF-α) synthesis by activated monocyte/macrophages, contributing to an anti-inflammatory action *in vivo*. In lipopolysaccharide (LPS)-activated human monocytic THP-1 cells, glucocorticoids acted primarily on the TNF-α promoter to suppress a burst of transcriptional activity that occurred between 90 min and 3 h after LPS exposure. LPS increased nuclear c-Jun/ATF-2, NF-κB$_1$/Rel-A, and Rel-A/C-Rel transcription factor complexes, which bound specifically to oligonucleotide sequences from the −106 to −88 base pair (bp) region of the promoter. The glucocorticoid, dexamethasone, suppressed nuclear binding activity of these complexes prior to and during the critical phase of TNF-α transcription. Site-directed mutagenesis in TNF-α promoter-luciferase reporter constructs showed that the adjacent c-Jun/ATF-2 (−106 to −99 bp) and NF-κB (−97 to −88 bp) binding sites each contributed to the LPS-stimulated expression. Mutating both sites largely prevented dexamethasone from suppressing TNF-α promoter-luciferase reporters. LPS exposure also increased nuclear Egr-1 and PU.1 abundance. The Egr-1/Sp1 (−172 to −161 bp) binding sites and the PU.1-binding Ets site (−116 to −110 bp) each contributed to the LPS-stimulated expression but not to glucocorticoid response. Dexamethasone suppressed the abundance of the c-Fos/c-Jun complex in THP-1 cell nuclei, but there was no direct evidence for c-Fos/c-Jun transactivation through sites in the −172 to −52 bp region. Small contributions to glucocorticoid response were attributable to promoter sequences outside the −172 to −88 bp region and to sequences in the TNF-α 3′-untranslated region. We conclude that glucocorticoids suppress LPS-stimulated secretion of TNF-α from human monocytic cells largely through antagonizing transactivation by c-Jun/ATF-2 and NF-κB complexes at binding sites in the −106 to −88 bp region of the TNF-α promoter.

The glucocorticoids are prototypic anti-inflammatory drugs, with wide ranging effects on inflammatory cells and tissues. Cells of the monocyte/macrophage lineage release numerous proinflammatory cytokines, including tumor necrosis factor (TNF-α)[1] (1), and are important therapeutic targets for glucocorticoids. Glucocorticoids suppress the release of secreted TNF-α from human monocyte/macrophages *in vivo* (2) and reduce expression of cell surface TNF-α (3). TNF-α is essential for normal inflammatory and immune function but also contributes to the pathogenesis of autoimmune and inflammatory diseases, such as endotoxic shock, rheumatoid arthritis, ulcerative colitis, Crohn's disease, and multiple sclerosis (4–8). Clinically, suppressing TNF-α activity ameliorates rheumatoid arthritis and Crohn's disease (4, 7). Glucocorticoid drugs suppress inflammatory activity in rheumatoid arthritis, ulcerative colitis, and multiple sclerosis.

Monocytic cells release TNF-α in response to many stimuli, including the Gram-negative bacterial endotoxin, lipopolysaccharide (LPS) (9, 10), TNF-α itself (11), phorbol esters (12), superantigens (13, 14) and viral agents (15). Regulation is both transcriptional and post-transcriptional, depending on the stimulus, cell type, and possibly differentiation (16–19). Sequences in the proximal 172 base pairs (bp) and in the −627 to −487 bp region of the TNF-α promoter (Fig. 1), at least, contribute to transcriptional control in monocytic cells (9, 10, 12, 14, 15, 19, 20). Each of these stimuli modifies transcription factors interaction with the −116 to −88 bp region. This region includes putative cAMP-response element/activating transcription factor (CRE/ATF), NF-κB, CCAAT/enhancer-binding protein (C/EBP), and Ets binding sites (9, 10, 12–15, 21). There is an activator protein-1 (AP-1)-like site at −65 to −59 bp (20), an AP-2-like site at −36 to −28 bp (22), and an Egr-1 binding site at −172 to −161 bp, which is functional in LPS-stimulated THP-1 cells (23). Factors binding to the CRE/ATF site (−106 to −99 bp) and the NF-κB site (−97 to −88 bp) site co-operate functionally in both monocytic and lymphocytic cells (9, 24). There is also evidence for cooperativity between the CRE/ATF site and the immediately upstream Ets-like site at −116 to −110 bp (25). LPS, the stimulus used in these studies, activates monocyte/macrophage cells through Toll-like receptors 2 and 4, binding in association with the glycosyl phosphatidylinositol-anchored surface glycoprotein, CD14 (26–28).

Glucocorticoids have pleiotropic actions on cytokine expression by monocyte/macrophages, suppressing gene transcription

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[1] The abbreviations used are: TNF, tumor necrosis factor; AcD, actinomycin D; ATF, activating transcription factor; AP-1, activator protein-1; C/EBP, CCAAT/enhancer-binding protein; CRE, cAMP-response element; CREB, CRE-binding protein; EMSA, electrophoretic mobility shift assay; LPS, lipopolysaccharide; UTR, untranslated region; bp, base pair(s); Dex, dexamethasone.

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(29), reducing mRNA stability (30), or reducing mRNA translational efficiency (31). In LPS-stimulated human THP-1 monocytic cells, glucocorticoids suppress TNF-α promoter activity (18). In murine RAW macrophages, glucocorticoids also suppress TNF-α transcription and, more importantly, inhibit translation of TNF-α mRNA (31). The effect on translation is at least partially mediated through suppression of Jun N-terminal kinase/stress-activated protein kinase activity (16). Like the 3′-UTR of murine TNF-α, the 3′-UTR of human TNF-α includes an AU-rich region, which indicates potential for regulation through mRNA stability or translational efficiency (32).

In this study, we identify transcriptional suppression as the main mechanism for glucocorticoid suppression of TNF-α expression in THP-1 monocytic cells. Transcriptional suppression is largely due to diminished transactivation through sites in the −106 to −88 bp region of the promoter and is associated with reduced binding activity of transcription factor complexes containing NF-κB factors and c-Jun/ATF-2.

EXPERIMENTAL PROCEDURES

Cell Culture—The human promonocytic cell line, THP-1, and the murine macrophage cell line, RAW264.7, were obtained from ATCC. Cells were maintained in RPMI 1640 medium (THP-1) or Dulbecco's modified Eagle's medium (RAW264.7) with 10% low endotoxin fetal bovine serum (CSL, Melbourne, Australia), penicillin, and gentamicin. Fetal bovine serum was charcoal-stripped to remove endogenous glucocorticoids (33). All media and additives were checked for endotoxin contamination using the Limulus amebocyte lysate test (BioWhittaker Inc., Walkersville, MD) and rejected if endotoxin concentration exceeded 0.1 unit/ml. All glassware was baked prior to use, and all plasticware was new.

Construction of Luciferase Fusion Plasmids—The TNF-α promoter fragment −993 to +110 (which includes the 5′-UTR) and the TNF-α 3′-UTR +1957 to +2792 fragment were amplified from genomic DNA, as described previously (34). The promoter fragment was cloned into the *Sac*I and *Hin*dIII restriction sites located upstream of the luciferase gene in the pGL2-Basic plasmid (Promega) to create the *TNF-Luc-SV40* reporter gene construct. The 3′-UTR in this construct is SV40-derived and includes the SV40 polyadenylation signal. The 3′-UTR of TNF-α was cloned into this construct to replace the SV40 3′-UTR after digestion at the *Pfl*MI and *Bam*HI sites, creating *TNF-Luc-TNF*. The same procedure was used to clone the TNF-α 3′-UTR into pGL2-Promoter. The resulting construct includes the SV40 early promoter and is designated *SV40-Luc-TNF*. There is a consensus NF-κB site downstream of the 3′-UTR in the mouse and human genomes (35), which was not included in these reporters. The pGL2-Promoter vector (*SV40-Luc-SV40*) itself also served as a control.

Site-directed mutagenesis was used to alter putative binding sites in the promoter region of *TNF-Luc-SV40* and *TNF-Luc-TNF*, using the QuikChange™ site-directed mutagenesis kit (Stratagene) and appropriate oligonucleotide sequences (Fig. 1). Mutated sequences were confirmed by restriction digestion of the mutated site. DNA was prepared for transfection using the EndoFree™ Plasmid Maxi Kit (Qiagen).

The *3κB-Luc-SV40* reporter, which contains three Ig NF-κB sites from the interferon-β gene (36) upstream of the luciferase coding region of the pGL2-basic plasmid, was kindly provided by Dr. D. Baltimore.

Transient Transfection of THP-1 Cells—Plasmids were diluted to 100 ng·μl^{-1} in endotoxin-free water, and 5 μl was mixed with 4 μl of 50 μg/μl DEAE-dextran (Amersham Pharmacia Biotech). This was mixed with 250 μl of HEPES-buffered RPMI medium (20 mM HEPES, pH 7.4, without antibiotics) and left at room temperature for 30 min. Prior to transfection, 1.2 × 10^7 THP-1 cells were washed in RPMI/HEPES, pelleted, and then resuspended in 750 μl of RPMI/HEPES. The cells and DEAE-dextran-plasmid mixture were mixed in a well of a 24-well plate (Costar). After a 30-min incubation at 37 °C in a 5% CO_2 atmosphere, cells were washed twice in RPMI/HEPES and resuspended in RPMI with 10% fetal bovine serum, penicillin, and streptomycin. Transfected cells were distributed into remaining wells of the 24-well plate at 2 × 10^5 cells/well and rested overnight before experimentation. For each comparison between constructs, equivalent transfectional efficiency was confirmed by co-transfecting the *Renilla* luciferase vector, pRL-TK (Promega), with each in parallel experiments.

Transfected cell cultures were then stimulated in triplicate with 1

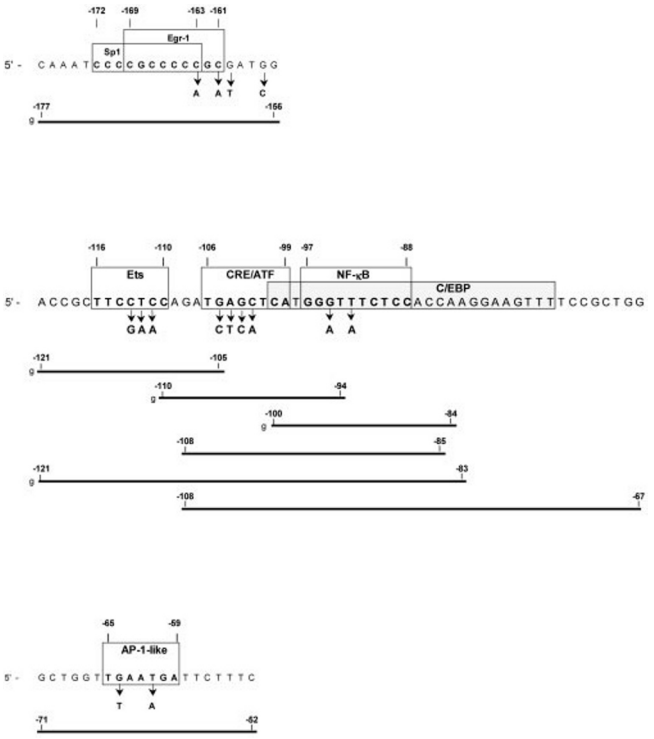

FIG. 1. **TNF-α promoter regions −177 to −156 bp, −121 to −67 bp, and −71 to −52 bp.** Putative binding sites for Egr-1, Sp1, CRE/ATF, NF-κB, and Ets factors are shown. C/EBP factor binding has been localized to sequences within the −100 to −74 region (*shaded*). The −65 to −59 sequence resembles an AP-1 binding site. The *arrows* indicate the mutations created in reporter constructs. Oligonucleotide probes used for EMSA are shown *below* the sequences. Some probes included an additional 5′ guanosine nucleotide (*g*) to allow infill labeling.

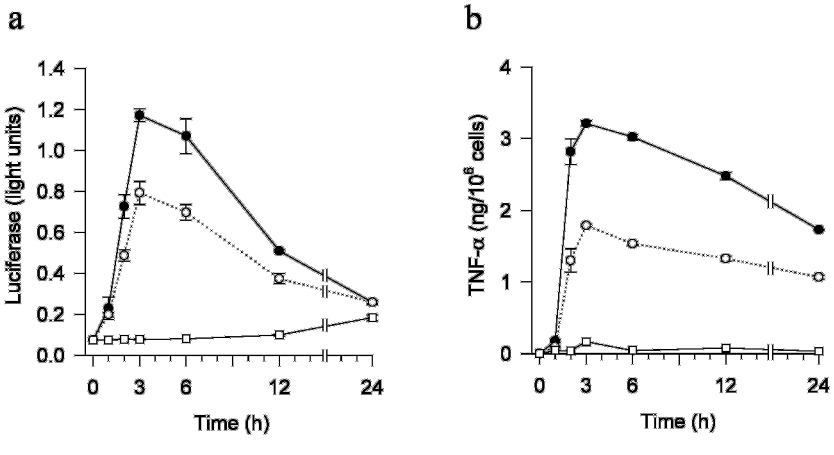

FIG. 2. *a*, luciferase activity (mean ± S.E.) in THP-1 cells transfected with the *TNF-Luc-TNF* reporter construct over 24 h following exposure to LPS (●), LPS plus Dex (○), or neither (□). *b*, TNF-α secretion (mean ± S.E.) by the same transfected THP-1 cells, exposed to LPS, LPS plus Dex, or neither. Results are from single experiments, performed in triplicate.

$\mu g \cdot ml^{-1}$ lipopolysaccharide (*Escherichia coli*, serotype 026-B6; Sigma). Glucocorticoid-treated cultures were exposed to 1 μM dexamethasone (Dex; Sigma) from 30 min before LPS addition. Similar triplicate cultures without LPS or Dex served as controls. Firefly luciferase expression was measured at times up to 24 h after LPS exposure, using the Promega Luciferase Assay System. When *Renilla* luciferase expression was also to be measured, the Promega Dual Luciferase Reporter Assay System was used.

Electrophoretic Mobility Shift Assay (EMSA) and Supershift EMSA—THP-1 cells were exposed to LPS, with or without Dex, as above for up to 6 h. Unexposed cells served as controls. Nuclear extracts were prepared from 2.5×10^7 cells using the method of Li *et al.* (37, 38). For EMSA, nuclear proteins (4 μg) were preincubated for 10 min at room temperature with 0.5 μg of poly(dI-dC) (Amersham Pharmacia Biotech) in a binding buffer (4% Ficoll, 20 mM HEPES (pH 7.9), 1 mM EDTA, 1 mM dithiothreitol, 50 mM KCl, 0.05% IGEPALCA-630) to give a final reaction volume of 10 μl. Appropriate specific antibodies (1 μg; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) were included in the mixture for supershift EMSA. The oligonucleotide probe, which had been labeled with [α-^{32}P]dCTP (Amersham Pharmacia Biotech) using Klenow fragment of *E. coli* DNA polymerase I (Promega) was then added. Probes derived from sequences of the TNF-α promoter are shown in Fig. 1. Consensus binding sequences for CRE, AP-1, NF-κB, Ets/PEA3, and C/EBP were derived from somatostatin promoter (39), collagenase promoter (40), TNF-α 3′-enhancer (35), polyoma enhancer (41), and interleukin-6 promoter (42), respectively. The PU.1 consensus probe was designed to incorporate optimal binding sequences for this protein (43). The sequences were as follows: CRE, 5′-gcaga**tgacgtca**t-gggt-3′; AP-1, 5′-gaagca**tgagtca**gacacg-3′; NF-κB, 5′-gggcat**gggaatttcc**aactc-3′; Ets/PEA3, 5′-gcgagc**aggaag**ttcgacg-3′; C/EBP, 5′-tcgagac**attgcacaa**tctg-3′; PU-1, 5′-gcataa**aggggaa**gttgtag-3′. Boldface lettering indicates the core sequences. When present, unlabeled oligonucleotide probes were at 100-fold molar excess, unless otherwise indicated. Thirty minutes after the addition of probe, samples were loaded onto a 4% polyacrylamide gel, containing 0.25 × Tris-Borate-EDTA buffer, which had been pre-run for 2 h in the same buffer. They were then separated at 150 V for 90 min. Gels were then exposed to Cronex x-ray film, using a single intensifying screen. Films were not preflashed. Retarded bands were quantitated using a Molecular Dynamics ImageQuant densitometer in "wide line" integration mode with film background subtraction.

TNF-α Assay—TNF-α in supernatant was assayed by sandwich enzyme-linked immunosorbent assay, as described previously (44, 45).

Statistical Analysis—Data are expressed as mean ± S.E. Suppression of luciferase activity and TNF-α by Dex is expressed as a percentage of the LPS-induced increment. Statistical analyses were made using two-way analysis of variance.

RESULTS

Luciferase Expression Parallels TNF-α Release in THP-1 Cells Transfected with TNF-Luc-TNF—In order to confirm that the reporter constructs responded comparably to the endogenous TNF-α gene, TNF-α release and luciferase activity were measured simultaneously in *TNF-Luc-TNF*-transfected cells during exposure to LPS and Dex. THP-1 cells were transfected, rested overnight, and exposed to LPS with or without Dex. In the absence of LPS stimulation, luciferase activity was initially low and increased only marginally between 12 and 24 h (Fig. 2*a*). Secreted TNF-α concentrations were also close to the limit of assay detection (32 $pg \cdot ml^{-1}$) throughout this time (Fig. 2*b*). Peak expression of luciferase and peak concentration of secreted TNF-α coincided at 3 h after LPS stimulation (Fig. 2, *a* and *b*). Dex suppressed both from 2 to 12 h. At 3 h, luciferase activity was suppressed 35% and TNF-α was suppressed 47% by Dex. Luciferase activity had returned almost to the level of unstimulated cells at 24 h, but the Dex-induced suppression of supernatant TNF-α activity remained. Dex did not influence either TNF-α secretion or luciferase activity in the absence of LPS stimulation (results not shown). The *TNF-Luc-TNF* construct, transfected into THP-1 cells, therefore responds to LPS and Dex like the endogenous TNF-α gene. Neither LPS nor Dex affected cell numbers or viability in this or subsequent experiments.

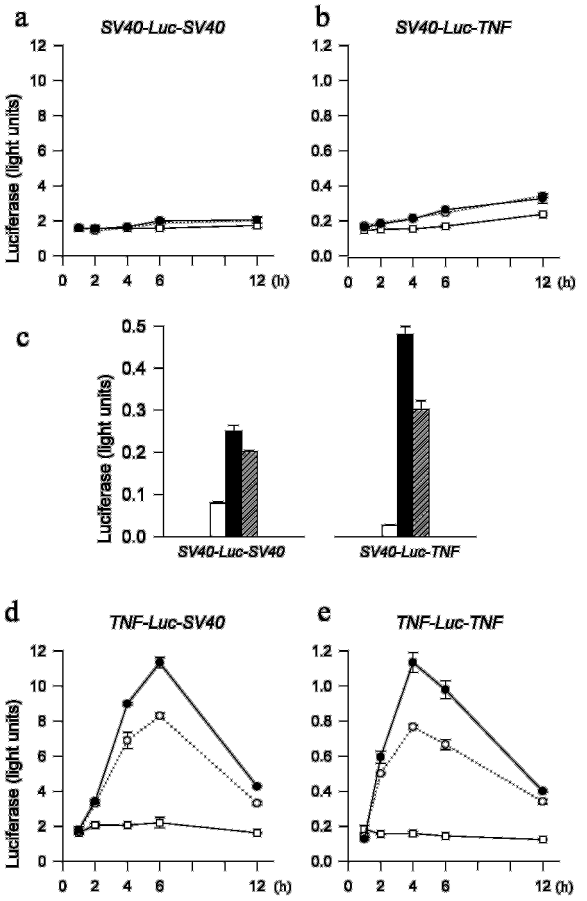

FIG. 3. **THP-1 cells and murine RAW macrophage cells were transfected with luciferase reporter constructs, rested overnight, and then incubated for up to 12 h with LPS (●), LPS plus Dex (○), or neither (□).** Results in *panels a*, *b*, *d*, and *e* are means ± S.E. of representative experiments performed in triplicate. *a* and *b*, reporter constructs that incorporate the SV40 early promoter and either SV40 3′-UTR sequences (*SV40-Luc-SV40*) or the TNF-α 3′-UTR (*SV40-Luc-TNF*) did not respond to Dex after transient transfection into THP-1 cells. *c*, the SV40 promoter construct that incorporates the TNF-α 3′-UTR (*SV40-Luc-TNF*) is more responsive to Dex than the control vector (*SV40-Luc-SV40*) after transfection into murine RAW264.7 macrophage cells. Cells were exposed to LPS (*filled bars*), LPS and Dex (*hatched bars*), or neither (*open bars*) for 6 h. Results are means ± S.E. from one of two experiments, each performed in triplicate. *d* and *e*, Dex suppressed the LPS-induced activity of reporter constructs that incorporate the TNF-α promoter and adjacent 5′-UTR (−993 to +110 bp) in THP-1 cells. Suppression was seen for both *TNF-Luc-SV40* and *TNF-Luc-TNF*.

Glucocorticoids Suppress Activity of TNF-α Promoter Reporter Constructs—Previous work has shown that glucocorticoids suppress both TNF-α gene transcription and TNF-α mRNA translation in the murine RAW264.7 macrophage line (16, 31). Sequences in the 3′-UTR mediate the effect on translational efficiency (32). We therefore expressed reporters that incorporated the TNF-α promoter, the TNF-α 3′-UTR, or both in THP-1 cells and measured suppression of LPS-induced luciferase activity by Dex. Initially, *SV40-Luc-TNF* (which incorporates the 3′-UTR of the TNF-α gene) was compared with *SV40-Luc-SV40* (which incorporates the SV40-derived 3′-UTR). *SV40-Luc-SV40* does not respond to LPS or Dex in THP-1 cells (Fig. 3*a*). *SV40-Luc-TNF*, which expresses at approximately one-tenth of the level of *SV40-Luc-SV40*, was induced by LPS (to a maximum of 1.56-fold at 6 h), but the response was unaffected by Dex (Fig. 3*b*). Because the murine TNF-α 3′-UTR confers Dex responsiveness in the murine RAW264.7 cell line, we performed the same experiments with RAW264.7 cells. In these cells, Dex suppressed LPS-stimulated

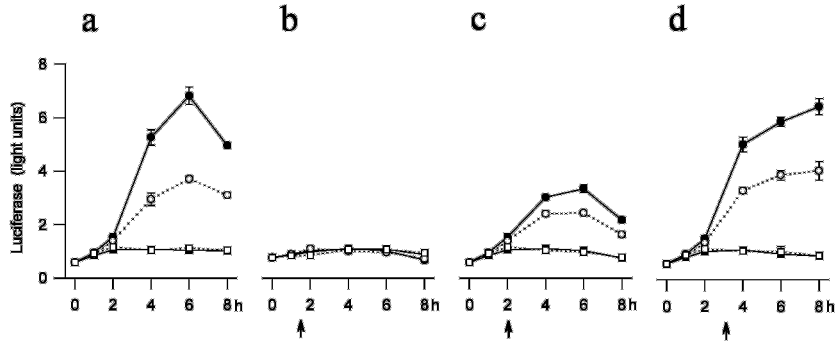

FIG. 4. *a*, luciferase activity (mean ± S.E.) in THP-1 cells transfected with the *TNF-Luc-SV40* reporter construct over 8 h following exposure to LPS (●), LPS plus Dex (○), Dex alone (■), or neither (□). *b*, *c*, and *d*, AcD (2 $\mu g \cdot ml^{-1}$) was added to cultures at the times indicated by the *arrows*. Added at 90 min after LPS/Dex exposure, AcD prevents LPS-induced luciferase activity (*b*). When the addition of AcD is delayed for 3 h after the addition of LPS (with or without Dex), however, both LPS and Dex effects on luciferase activity are observed in their entirety (*d*). Adding AcD at 2 h has an intermediate effect (*c*).

activity of *SV40-Luc-TNF* by 39% at 6 h after exposure (Fig. 3*c*). This confirms the relatively greater importance of the TNF-α 3′-UTR in Dex response of RAW264.7 cells. LPS itself was a more potent inducer in RAW264.7 cells, causing an 18-fold increase in *SV40-Luc-TNF* expression and a 3-fold increase in expression of the control vector, *SV40-Luc-SV40*.

We then examined the role of the TNF-α promoter in glucocorticoid response of LPS-stimulated THP-1 cells, using the *TNF-Luc-SV40* and *TNF-Luc-TNF* reporters. These included the TNF-α promoter in the place of the SV40 promoter. Dex suppressed activity from 4 h (*TNF-Luc-SV40*) or 2 h (*TNF-Luc-TNF*). Dex suppressed the peak LPS-induced luciferase activity by 31% in *TNF-Luc-SV40*-transfected cells and 38% in *TNF-Luc-TNF*-transfected cells (Fig. 3, *d* and *e*). In separate experiments, Dex consistently produced slightly greater suppression of *TNF-Luc-TNF* than *TNF-Luc-SV40* (52% maximum suppression compared with 41%, $p = 0.003$, data from eight independent experiments), implying that the TNF-α 3′-UTR also contributed to Dex response in these TNF-α promoter reporters, although it had no measurable effect in the SV40 promoter vectors in THP-1 cells (Fig. 3*b*). Dex did not alter the activity in either construct in the absence of LPS stimulation (results not shown). *TNF-Luc-TNF* exhibited lower absolute expression than *TNF-Luc-SV40* but greater inducibility with LPS (Fig. 3, *d* and *e*), similar to the distinction that had been observed between *SV40-Luc-SV40* and *SV40-Luc-TNF* (Fig. 3, *a* and *b*).

From these experiments, we concluded that promoter sequences were essential for glucocorticoid suppression of TNF-α in LPS-stimulated THP-1 cells and that there was also a small effect mediated through sequences in the TNF-α 3′-UTR.

We then timed the onset and duration of these promoter-mediated effects of LPS and Dex. THP-1 cells were transiently transfected with *TNF-Luc-SV40* and exposed to LPS, with or without Dex, as described under "Experimental Procedures." Actinomycin D (AcD; 2 $\mu g \cdot ml^{-1}$) was added at 90 min, 2 or 3 h after LPS. In the absence of AcD, the effects of LPS and Dex on luciferase activity were apparent at 2 h and were maximal at 6 h (Fig. 4*a*). Added at 90 min, AcD completely prevented both the LPS and Dex effects (Fig. 4*b*). Added at 3 h, it failed to prevent either (compare Fig. 4, *a* and *d*). Adding AcD at 2 h partially prevented the actions of both LPS and Dex (Fig. 4*c*). We concluded that LPS induced a burst of transcription from the TNF-α promoter-luciferase reporter construct between 90 min and 3 h after exposure and that Dex suppressed this. This is consistent with the timing of maximal effects of LPS and Dex on TNF-α protein secretion at 3 h (Fig. 2). Later experiments examined control of the TNF-α promoter before and during this brief phase of transcription.

Glucocorticoids Suppress LPS-induced TNF-α Promoter Binding Activity and Transactivation by NF-κB—A series of functional transcription factor binding sites exists in the −177 to −59 bp region of the TNF-α promoter, with binding sequences for NF-κB factors, Ets, AP-1, CRE/ATF, NF-AT, C/EBP, Egr-1, and Sp.1 (Fig. 1). We went on to investigate whether glucocorticoids altered transcription factor binding at these sites in LPS-stimulated THP-1 cells and whether these sequences also mediated the suppression of TNF-α promoter activity by glucocorticoids.

A putative NF-κB-binding site exists at −97 to −88 bp in the TNF-α promoter (Fig. 1). Nuclear extracts from LPS-stimulated THP-1 cells contained four protein complexes that retarded a NF-κB consensus probe. Supershift analysis identified NF-κB_1/Rel-A complex, Rel-A/C-Rel complex, NF-κB_1/NF-κB_1 homodimer, and a further, slowly migrating Rel-A-containing complex (Fig. 5*a*). Only the slowly migrating complex was detected in unstimulated cells. Each of the Rel-A-containing complexes increased within 1 h of LPS stimulation and peaked at 2 or 4 h (Fig. 5*b*). The NF-κB_1 homodimer peaked later than the Rel-A complexes, at 4–6 h. Rel-A/C-Rel had largely disappeared by 6 h, while the other complexes remained readily detectable. Nuclei of Dex-treated cells contained less binding activity for each of the complexes from 1 to 4 h after LPS stimulation. (Fig. 5*b*). Dex therefore suppressed the nuclear abundance of NF-κB-containing complexes before and during the critical period for TNF-α promoter-driven transcription, between 90 min and 3 h after LPS exposure (Fig. 4).

An oligonucleotide probe corresponding to bases −100 to −84 of the TNF-α promoter, which includes the putative NF-κB-binding site (Fig. 1), also bound NF-κB_1/Rel-A, Rel-A/C-Rel, NF-κB_1 homodimer, and the slower migrating, Rel-A-containing complex (Fig. 5*c*).

NF-κB transactivating activity, as measured by the NF-κB reporter construct, *3×κB-Luc-SV40*, corresponded with changes in nuclear NF-κB DNA binding activity. This vector was transfected into THP-1 cells, as for the TNF-α reporter constructs. LPS exposure increased expression to a maximum of 94-fold at 6 h (Fig. 5*d*). Dex suppressed the maximum response by 36%. The timing of the *3×κB-Luc-SV40* response closely reflected the response of *TNF-Luc-SV40* (compare Figs. 4*a* and 5*d*).

The functional importance of suppressed NF-κB factor binding in Dex response of TNF-α was shown in gene transfer experiments using TNF-α promoter reporter constructs (*TNF-Luc-SV40* and *TNF-Luc-TNF*) that incorporated nonbinding mutations of the NF-κB site (Fig. 1). The mutants were shown to be binding-defective by competition EMSA against wild-type oligonucleotides (Fig. 6*a*). Constructs were transiently transfected into THP-1 cells, and the cells were then exposed to LPS, with or without Dex. The mutants still responded to LPS, although significantly less than the wild-type promoter constructs ($p < 0.05$ in each case; Fig. 6*b*). Both mutant constructs exhibited impaired responsiveness to Dex in LPS-stimulated THP-1 cells. Whereas Dex maximally suppressed LPS-induced activity of *TNF-Luc-SV40* by 40%, the mutation in the NF-κB site limited suppression to 20.3% ($p < 0.01$; $n = 6$; Fig. 6*b*). Likewise, mutating the NF-κB sites in *TNF-Luc-TNF* reduced

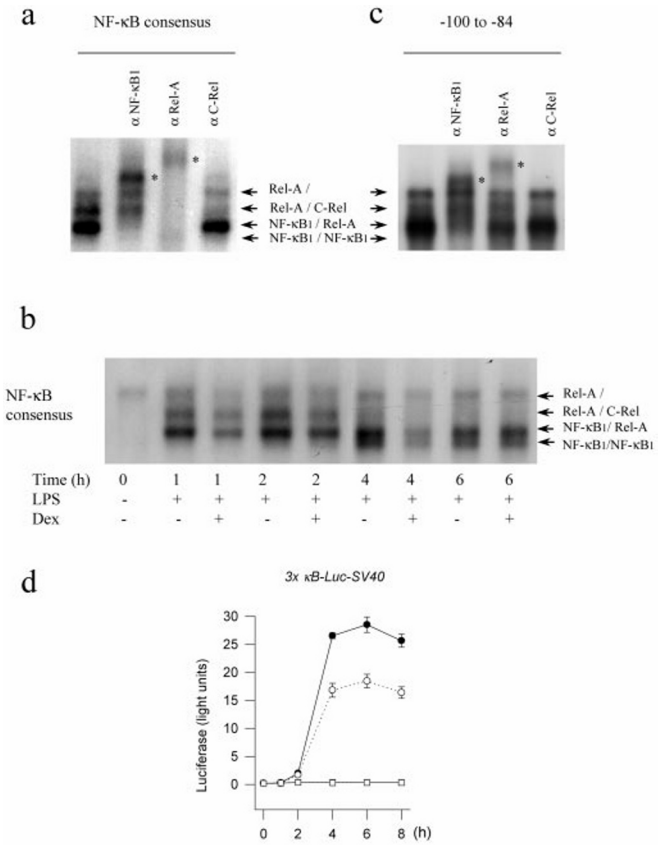

FIG. 5. *a*, at 2 h after LPS exposure, THP-1 cell nuclei contained four protein complexes that retarded a NF-κB consensus probe. Supershift analysis with specific antibodies to NF-κB_1, Rel-A, and C-Rel identified the NF-κB_1/Rel-A complex, Rel-A/C-Rel complex, NF-κB_1/NF-κB_1 complex, and a further Rel-A-containing complex (*arrow*). The *asterisks* designate supershifted bands. The anti-C-Rel antibody antagonized C-Rel complex binding but did not generate a supershifted band. *b*, THP-1 cells were exposed to LPS, with or without Dex for 6 h. Nuclear extracts were made at the indicated times and analyzed by EMSA with the NF-κB consensus probe. NF-κB_1/Rel-A, Rel-A/C-Rel, NF-κB_1 homodimer, and the further Rel-A-containing complex (*arrow*) each increased after LPS stimulation but with differing time courses. Dex diminished the binding of each complex between 1 and 4 h after LPS stimulation. *c*, NF-κB factors in nuclear extracts from LPS-stimulated THP-1 cells bound to an oligonucleotide probe corresponding to bases −100 to −84 of the TNF-α promoter. The cells were exposed to LPS for 2 h. NF-κB factors were identified by supershift EMSA with specific antibodies to NF-κB_1, Rel-A, and C-Rel. Complexes are designated by *arrows*, and supershifted bands are indicated by *asterisks*. *d*, luciferase activity (mean ± S.E.) in THP-1 cells transfected with the NF-κB reporter construct, 3×κB-*Luc-SV40*, over 8 h following exposure to LPS (●), LPS plus Dex (○), or neither (□). Dex alone did not alter expression.

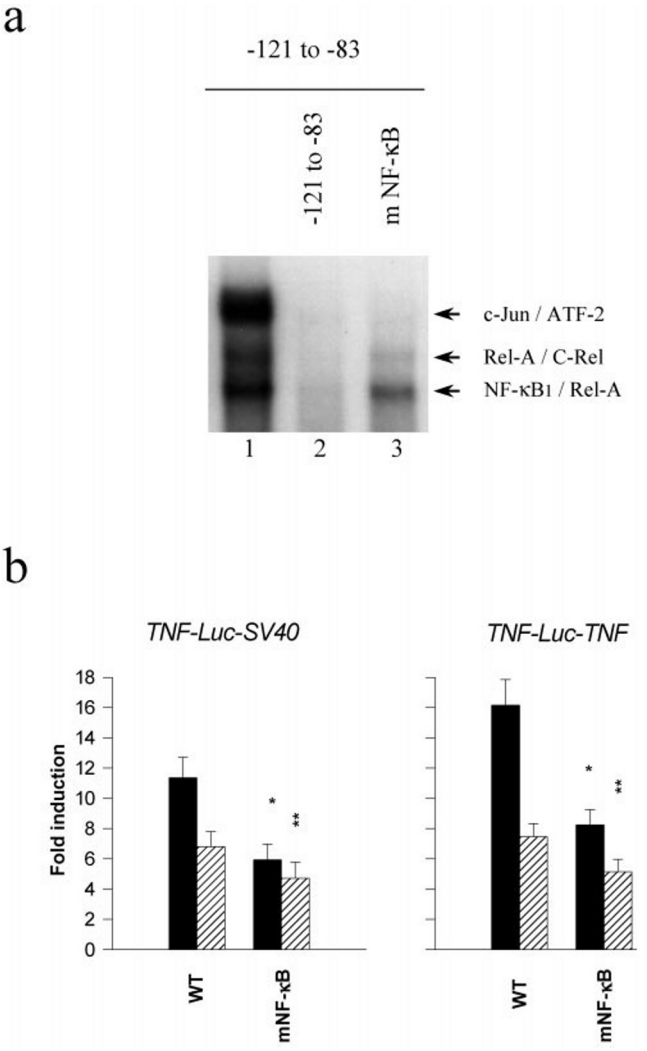

FIG. 6. *a*, an oligonucleotide probe corresponding to the −121 to −83 bp region of the TNF-α promoter bound NF-κB factors. A 50-fold excess of an oligonucleotide corresponding to the same region but incorporating mutations in the NF-κB site (*mNF-κB*), failed to compete with the NF-κB binding (*lane 3*). The wild-type probe itself competed efficiently under the same conditions (*lane 2*). The slowly migrating band is c-Jun/ATF-2 (see "Results" and Fig. 8*b*). The wild-type and NF-κB mutant probes compete for it with comparable affinity. *b*, mutants of *TNF-Luc-SV40* and *TNF-Luc-TNF* that incorporate the nonbinding NF-κB mutation (*mNF-κB*) are less induced by LPS (*, $p < 0.05$ in each case) and less suppressed by Dex (**, $p < 0.01$ in each case) than the respective parent vectors (*WT*). Luciferase activity was measured at the time of peak induction, 6 h after LPS exposure in the case of *TNF-Luc-SV40* and 4 h after exposure in the case of *TNF-Luc-TNF*. Results are means ± S.E. of six (*TNF-Luc-SV40*) and five (*TNF-Luc-TNF*) experiments, each performed in triplicate, and are expressed as -fold induction over unstimulated cells. *Filled bars*, LPS-stimulated; *hatched bars*, LPS-stimulated and Dex-exposed.

maximal Dex suppression from 53.8 to 37.7% ($p < 0.01$; $n = 5$, Fig. 6*b*).

From these experiments, we concluded that the NF-κB-binding site at −97 to −88 bp of the TNF-α promoter contributed to glucocorticoid response and that glucocorticoids diminished NF-κB factor binding to the site.

Glucocorticoids Suppress LPS-induced TNF-α Promoter Binding Activity and Transactivation by c-Jun/ATF-2—A putative CRE/ATF-binding site exists at −106 to −99 bp in the TNF-α promoter (Fig. 1). Nuclear extracts from both unstimulated and LPS-stimulated THP-1 cells contained three protein complexes that retarded a consensus CRE/ATF probe. Specific antibodies to c-Jun and ATF-2 individually produced supershifted bands in gel shift analysis of THP-1 cell extracts made at 2 h after LPS exposure (Fig. 7*a*, *lanes 2* and *3*; supershifted bands are designated with *asterisks*). Together, the two antibodies also reduced binding of the slowest migrating band (*lane 5*). A specific antibody to $CREB_1$ supershifted the bands of rapid and intermediate mobility (*lane 6*).

LPS increased nuclear abundance of c-Jun/ATF-2 and the $CREB_1$ complexes from 1 h after exposure (Fig. 7*b*). Dex reduced the abundance of c-Jun/ATF-2 from 1 to 6 h and reduced the $CREB_1$ complexes from 1 to at least 4 h (Fig. 7*b*).

An oligonucleotide probe corresponding to bases −110 to −94 bp of the TNF-α promoter, including the putative CRE/ATF site, also bound the c-Jun/ATF-2 complex (Fig. 7*c*, *lanes 2*, *3*, and *5*) but did not bind the $CREB_1$ complexes (*lane 6*) or displace $CREB_1$ complexes from the CRE/ATF consensus probe (Fig. 7*d*). It bound a distinct rapidly migrating complex that

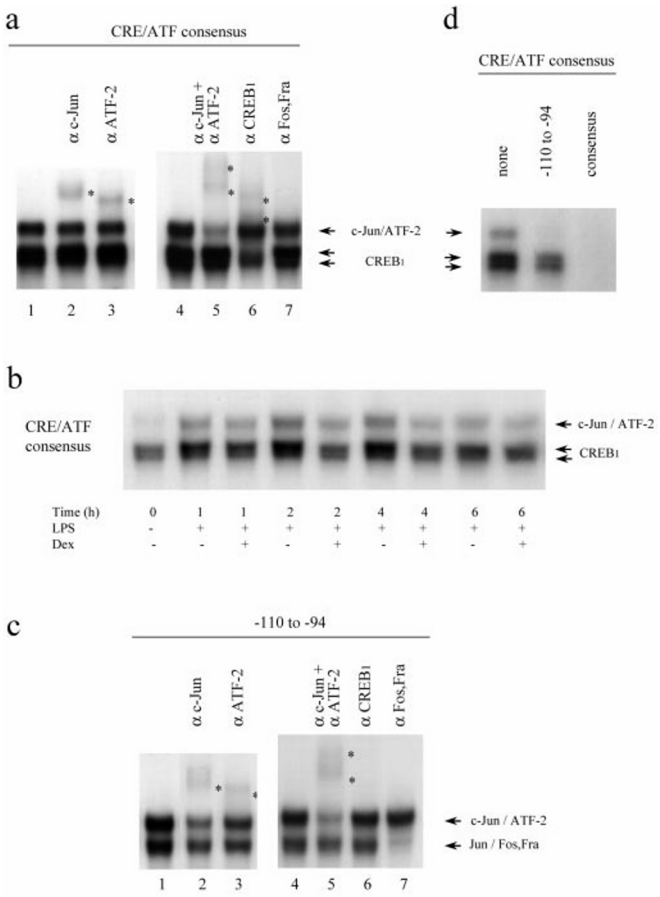

FIG. 7. *a*, at 2 h after LPS exposure, THP-1 cell nuclei contained three protein complexes that retarded a CRE/ATF consensus probe (*lanes 1* and *4*). The intermediate and rapidly migrating bands appear confluent at the exposures required for supershift analysis (compare with *b* and *d*). Specific antibodies to c-Jun and ATF-2 each produced supershifted bands in supershift analysis (*lanes 2* and *3*). Together, the two antibodies also diminished binding to the slowly migrating band (*arrow*, *lane 5*). A specific antibody to $CREB_1$ produced two supershifted bands, accompanied by the partial disappearance of the bands of intermediate and rapid mobility (*arrow*, *lane 6*). The *asterisks* designate supershifted bands. No supershifts were seen with an antibody that had broad specificity against c-Fos, Fos-B, Fra-1, and Fra-2 (*lane 7*). *b*, THP-1 cells were exposed to LPS, with or without Dex, for 6 h. Nuclear extracts were made at the indicated times and analyzed by EMSA with the CRE/ATF consensus probe. The c-Jun/ATF-2 and $CREB_1$-containing complexes (*arrow*) both increased after LPS stimulation. Dex diminished the binding of each. *c*, nuclear extracts from LPS-stimulated THP-1 cells contained two complexes that bound to an oligonucleotide probe corresponding to bases −110 to −94 of the TNF-α promoter. The cells were exposed to LPS for 2 h. Supershift analysis was performed, as for *a*. Specific antibodies to c-Jun (*lane 2*), ATF-2 (*lane 3*), and c-Jun and ATF-2 together (*lane 5*) yielded a similar result as when used with the consensus CRE/ATF probe. However, the rapidly migrating complex (which appeared as a single band, *arrow*) was not supershifted by a specific antibody against $CREB_1$ (*lane 6*) but was displaced by an antibody with specificity toward Fos/Fra factors (*lane 7*). Complexes are designated by *arrows*, and supershifted bands are indicated by *asterisks*. *d*, a 100-fold excess of an oligonucleotide corresponding to the −110 to −94 bp region of the TNF-α promoter competed for the c-Jun/ATF-2 complex binding to the consensus CRE/ATF probe but failed to compete for the $CREB_1$-containing complexes. Under the same conditions, the consensus CRE/ATF probe competed efficiently for all complexes.

was subject to binding competition from an antibody of broad specificity to c-Fos, Fos-B, Fra-1, and Fra-2 (Fig. 7*c*, *lane 7*). The same antibody failed to displace the $CREB_1$ complexes from the CRE/ATF consensus probe (Fig. 7*b*, *lane 7*).

TNF-α promoter oligonucleotide probes that included the NF-κB site as well as the c-Jun/ATF-2-binding site (*e.g.* the −108 to −85 bp probe) did not bind either the $CREB_1$-containing complexes or the Fos/Fra-containing complexes (Fig. 8*a* and results not shown).

The functional importance of suppressed c-Jun/ATF-2 binding in Dex response was shown in gene transfer experiments using TNF-α promoter reporter constructs (*TNF-Luc-SV40* and *TNF-Luc-TNF*) that incorporated nonbinding mutations of the c-Jun/ATF-2 site (Fig. 1; Fig. 8*b*). Transiently transfected mutant reporters still responded to LPS although significantly less than the wild-type promoter constructs ($p < 0.05$ in each case; Fig. 8*c*). Both mutant constructs were less responsive to Dex than the wild-type controls. Dex suppressed the c-Jun/ATF-2 site mutant of *TNF-Luc-SV40* by 22.8%, compared with 40% for the wild-type control at 6 h ($p < 0.01$; $n = 6$; Fig. 8*c*). Similarly, mutating the c-Jun/ATF-2 site in *TNF-Luc-TNF* reduced maximal Dex suppression from 53.8 to 32.2% at 4 h ($p < 0.01$; $n = 5$, Fig. 8*c*).

We concluded that the c-Jun/ATF-2-binding site at −106 to −99 bp of the TNF-α promoter contributed to glucocorticoid response and that glucocorticoids diminished c-Jun/ATF-2 factor binding to the site.

The NF-κB and CRE/ATF Sites Cooperatively Mediate Glucocorticoid Response—The NF-κB and c-Jun/ATF-2 sites are adjacent in the TNF-α promoter, raising the possibility that they interact in respect to factor binding and transactivation. To investigate interactions in Dex response, we examined transcription factor binding to the −108 to −85 bp oligonucleotide probe that incorporated both sites and performed gene transfer studies with TNF-α promoter reporters that had mutations in both sites. The probe bound three prominent nuclear protein complexes from LPS-stimulated THP-1 cells. Competition studies with consensus sequences and the shorter probes from the TNF-α promoter confirmed that these were c-Jun/ATF-2, Rel-A/C-Rel, and NF-$κB_1$/Rel-A (Fig. 8*a*). Binding activity for each of these increased between 1 and 4 h after LPS exposure (Fig. 9, *a* and *b*). Dex suppressed binding of each complex at each time. At 6 h, the effect of LPS had diminished, and the effect of Dex had disappeared. The LPS and Dex-induced changes in nuclear transcription factor abundance therefore occurred before and during the critical period of TNF-α promoter-driven transcription (compare Fig. 9*b* with Fig. 4)

We investigated functional interactions between the NF-κB and c-Jun/ATF-2 sites by transiently transfecting *TNF-Luc-SV40* and *TNF-Luc-TNF* constructs that incorporated nonbinding mutations in both the NF-κB and c-Jun/ATF-2 sites (Fig. 8*b*). As with the single mutants, they were less responsive to LPS than wild-type promoter constructs (Fig. 9*c*). Dex response was more impaired in the double mutants than in single mutants of either *TNF-Luc-SV40* or *TNF-Luc-TNF*. When both sites were mutated in *TNF-Luc-SV40*, Dex suppressed LPS response by no more than 17.7% ($p < 0.01$; $n = 12$; Fig. 9*c*). Dex suppressed the LPS response of *TNF-Luc-TNF* by no more than 27.6% ($p < 0.01$; $n = 7$, Fig. 9*c*) when both sites were mutated.

From these experiments, we concluded that juxtaposition of the NF-κB and c-Jun/ATF-2 binding sites conferred binding selectivity on the region and that the two sites behaved cooperatively in respect to glucocorticoid response.

Glucocorticoids Do Not Affect C/EBP Binding to the −121 to −67 bp Region of the TNF-α Promoter in THP-1 Cell Nuclear Extracts—A consensus C/EBP probe bound three protein complexes specifically in EMSA of nuclear extracts from LPS-stimulated cells. A broad specificity antibody against C/EBPβ, C/EBPα, C/EBPδ, and C/EBPε supershifted one of these bands, confirming the presence of at least one C/EBP factor in THP-1 cell nuclei (results not shown). Dex did not consistently alter the abundance of any of the three complexes that bound the

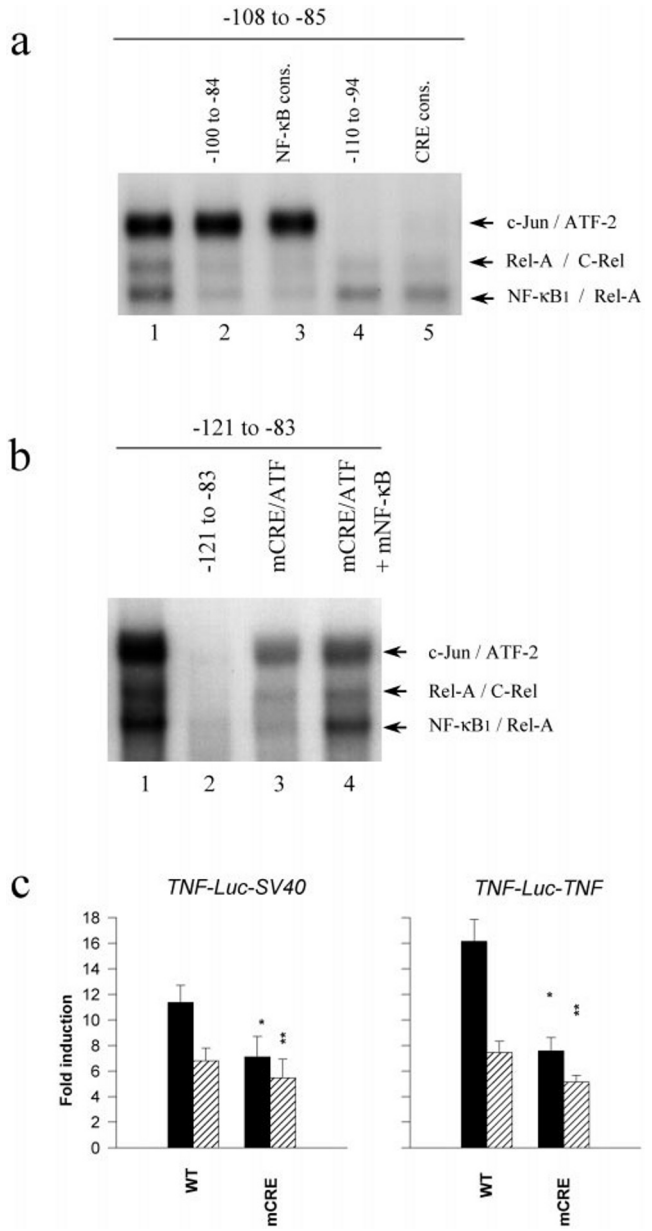

FIG. 8. *a*, an oligonucleotide probe corresponding to the −108 to −85 bp region of the TNF-α promoter bound three protein complexes in nuclear extracts prepared from THP-1 cells at 2 h after LPS stimulation. A consensus NF-κB probe (*lane 3*) and an oligonucleotide comprising the −100 to −84 bp region (which includes the NF-κB site; *lane 2*) efficiently compete for the intermediate and rapidly migrating complexes (which contain NF-κB factors), while a consensus CRE/ATF probe (*lane 5*) and an oligonucleotide comprising the −110 to −94 bp region (*lane 4*) compete efficiently for c-Jun/ATF-2 binding. The probe did not bind $CREB_1$ or Fos/Fra-containing complexes. *b*, in the absence of an oligonucleotide competitor, an oligonucleotide probe corresponding to bases −121 to −83 of the TNF-α promoter bound c-Jun/ATF-2, Rel-A/C-Rel, and NF-$κB_1$/Rel-A complexes (*lane 1*). At a 50-fold molar excess, the same probe, unlabeled (−121 to −83), competed for binding to all factors (*lane 2*). When this probe included mutations in the CRE/ATF site (*mCRE/ATF*), it failed to compete for c-Jun/ATF-2 (*lane 3*). Mutating both the NF-κB site and CRE/ATF site (*mNF-κB + mCRE/ATF*) impaired competition for both NF-κB factors and c-Jun/ATF-2 (*lane 4*). *c*, mutants of *TNF-Luc-SV40* and *TNF-Luc-TNF* that incorporate the nonbinding CRE/ATF (*mCRE*) mutation were less induced by LPS (*, $p < 0.05$ in each case) and less suppressed by Dex (**, $p < 0.01$ in each case) than the respective parent vectors (*WT*). Luciferase activity was measured at the time of peak induction, 6 h after LPS exposure in the case of *TNF-Luc-SV40* and 4 h after exposure in the case of *TNF-Luc-TNF*. Results are means ± S.E. of six (*TNF-Luc-SV40*) and five (*TNF-Luc-TNF*) experiments, each performed in triplicate. *Filled bars*, LPS-stimulated; *hatched bars*, LPS-stimulated and Dex-exposed.

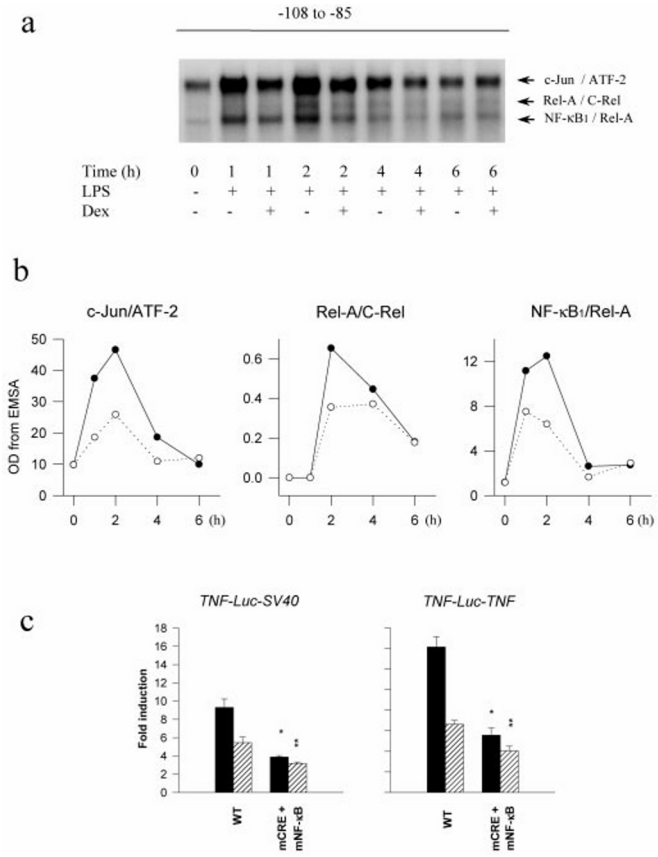

FIG. 9. *a*, nuclear extracts of THP-1 cells contained increased amounts of c-Jun/ATF-2, Rel-A/C-Rel, and NF-$κB_1$/Rel-A after LPS exposure, shown by increased binding to the −108 to −85 bp oligonucleotide probe. The time courses are similar to those for binding to the respective consensus probes (Figs. 5*b* and 7*b*). Dex antagonized the LPS-induced increases in all three complexes. *b*, densitometric measurement of c-Jun/ATF-2, Rel-A/C-Rel, and NF-$κB_1$/Rel-A binding activities in *a*. The changes occur before and during the critical period for TNF-α promoter-driven transcription (compare with Fig. 4). *c*, mutants of *TNF-Luc-SV40* and *TNF-Luc-TNF* that incorporate the nonbinding mutations of both the NF-κB and CRE/ATF site were less induced by LPS (*, $p < 0.05$ in each case) and less suppressed by Dex (**, $p < 0.01$ in each case) than the respective parent vectors. Luciferase activity was measured in THP-1 cells at the time of peak induction. Results are means ± S.E. of 12 (*TNF-Luc-SV40*) and seven (*TNF-Luc-TNF*) experiments, each performed in triplicate. *Filled bars*, LPS-stimulated; *hatched bars*, LPS-stimulated and Dex-exposed.

consensus probe.

The −100 to −74 bp region of the TNF-α promoter can bind overexpressed C/EBPβ (12) and C/EBP factors in nuclear extracts of other cell types (46). However, neither supershift EMSA with the anti-C/EBP antibody nor competition EMSA with the consensus probe revealed evidence that C/EBP factors in nuclei of LPS-stimulated THP-1 cells bound this region (result not shown).

The sequences that determine C/EBP binding to the −100 to −74 region remain incompletely defined, but they overlap with the NF-κB binding region (12, 46, 47). We were therefore unable to prepare mutants that we were confident had specific deficits in C/EBP factor binding. Consequently, we cannot exclude the possibility that Dex modifies C/EBP transactivation at this region of the TNF-α promoter.

Glucocorticoids Do Not Affect Transactivation through PU.1-binding and Egr-1/Sp1 Sites in the −172 to −110 Region of the TNF-α Promoter or through an AP-1-like Site at −65 to −59 bp—The −116 to −110 bp sequence of the TNF-α promoter has been shown to bind Ets factors (25) and the −172 to −161 bp sequence includes overlapping Egr-1 and Sp1 sites (9). Both

regions are functional (9, 25). EMSA and supershift analysis with oligonucleotide probes based on these regions showed that LPS increased nuclear levels of Egr-1 and the Ets factor PU.1 (results not shown). Dex slightly and transiently suppressed PU.1 but did not alter the abundance of Egr-1.

Mutations that impair PU.1 binding at the Ets site (25) or binding to the Egr-1 site reduced the LPS responsiveness of *TNF-Luc-SV40* and *TNF-Luc-TNF* in THP-1 cells ($p < 0.05$, $n = 6$ for the Ets site mutant and $n = 5$ for the Egr-1 site mutant). None of the mutants exhibited impaired Dex response ($p > 0.05$ in each case; results not shown). We concluded that interactions of PU.1 with the Ets site and Egr-1 with the Egr-1 site contribute to LPS response in THP-1 cells but that glucocorticoids did not impair transcriptional activation through either site.

An AP-1-like sequence is present at bases −65 to −59 in the TNF-α promoter. Nuclear extracts from THP-1 cells contained a complex of c-Jun in association with c-Fos and other Fos/Fra factors, identified by supershift analysis with specific antibodies and a consensus AP-1 probe. The nuclear abundance of the complex increased with LPS stimulation and was suppressed by Dex (results not shown). However, the complex did not bind to a probe corresponding to the −71 to −52 bp region, which incorporates the putative AP-1 site. At a 100-fold molar excess, this probe also failed to compete with the consensus probe for binding to the c-Jun/Fos/Fra complex (results not shown). When this site in *TNF-Luc-SV40* was mutated to destroy potential for AP-1 binding, neither LPS nor Dex response was impaired ($p > 0.05$ in each case, $n = 5$; results not shown). It was concluded that the −65 to −59 bp region of the TNF-α promoter did not bind AP-1 factors and was not relevant to LPS or Dex response in THP-1 cells.

DISCUSSION

Dex suppressed transcription of TNF-α in promonocytic THP-1 cells by preventing transactivation at NF-κB and CRE/ATF sites between −106 and −88 bp of the promoter between 90 min and 3 h after LPS exposure. This corresponded chronologically with reduced nuclear DNA binding activity of NF-κB_1/Rel-A, Rel-A/C-Rel and c-Jun/ATF-2 complexes. The NF-κB and CRE/ATF sites contributed independently but additively to glucocorticoid response. Sequence in the TNF-α 3′-UTR contributed to glucocorticoid response in THP-1 cells, but to a lesser extent than in murine RAW264.7 macrophage cells.

Glucocorticoids can suppress NF-κB-transactivation through direct physical association of ligand-activated glucocorticoid receptors with Rel-A (48, 49) and, in some cell types, by enhancing expression of I-κBα (50–52). Physical association with Rel-A prevents DNA binding and transactivation by Rel-A-containing complexes (48), while I-κBα maintains NF-κB_1 and Rel-A in an inactive cytoplasmic complex (53). Glucocorticoid treatment also diminishes nuclear DNA binding activity of NF-κB_1 and C-Rel-containing complexes in COS cells that overexpress both glucocorticoid receptor and the NF-κB subunits (49), but these phenomena may not be directly relevant to glucocorticoid suppression of NF-κB transactivation (54).

Unstimulated THP-1 cells express ATF-2 homodimer (10), c-Jun/ATF-2, c-Fos/c-Jun, and at least one other complex of c-Jun with another Fos/Fra family member. LPS exposure increased the nuclear DNA-binding activity of c-Jun/ATF-2 and c-Fos/c-Jun, at least, and Dex partially prevented the increases. An intact c-Jun/ATF-2 binding sequence at −106 to −99 bp was required for full LPS and Dex responses. In context, this site did not bind the c-Jun/Fos/Fra complex(es) from THP-1 cell nuclei, although the isolated short sequence from −110 to −94 bp did bind.

Glucocorticoids potentially suppress transactivation by c-Jun-containing complexes through preventing c-Jun activation by mitogen-activated protein kinase pathways (55–57), by direct physical interaction between ligand-activated glucocorticoid receptor and the c-Jun containing complex (58–60), or through reducing *de novo* synthesis of c-Jun (61, 62). Glucocorticoids suppress activity of the c-Jun N-terminal kinase/stress-activated protein kinase in LPS-stimulated RAW264.7 macrophage cells (56). Jun N-terminal kinase/stress-activated protein kinase activates both c-Jun and ATF-2 through amino-terminal phosphorylation (55–57). Direct physical interaction of glucocorticoid receptor with c-Jun underlies glucocorticoid inhibition of c-Fos/c-Jun (AP-1) transactivating function and may contribute to glucocorticoid inhibition of c-Jun/ATF-2 transactivation (58–60). *De novo* synthesis of c-Jun itself is subject to transcriptional regulation by c-Jun/ATF-2 (62), providing an opportunity for glucocorticoids to modulate c-Jun levels by interfering with this feedback. LPS stimulates the *de novo* synthesis of c-Jun (61).

Simultaneous mutations in the c-Jun/ATF-2-binding and NF-κB-binding regions maximally suppressed glucocorticoid response. The c-Jun/ATF-2 and NF-κB sites in the TNF-α promoter act synergistically in response to LPS stimulation. Synergism depends on the proximity of the sites and is most apparent when they are linked to a minimal heterologous promoter (9). Proximate, cooperating c-Jun/ATF-2 and NF-κB sites are also present in the promoters of the E-selectin and interferon-β genes. The sites are separated by a greater distance than in the TNF-α promoter. Binding of high mobility group I(Y) family proteins to the regions in the E-selectin and interferon-β promoters alters the DNA conformation and facilitates factor binding and transcriptional activation (63, 64). We have not investigated the role of the high mobility group proteins in LPS or glucocorticoid response of TNF-α.

TNF-α promoter mutants that did not bind NF-κB factors or c-Jun/ATF-2 still showed some response to glucocorticoid, indicating additional effects that were independent of these two sites. There are six other NF-κB-like sites in the 993 bases of the TNF-α promoter upstream of the transcription start site. The sites between −627 and −589 bp are functional in Mono Mac 6 human monocytic cells (19), while others appear nonfunctional (9, 65, 66) or have not been tested. The functional sites may contribute to the residual glucocorticoid action.

C/EBP factors were present in nuclear extracts of unstimulated THP-1 cells. The −100 to −74 bp region of the TNF-α promoter region has been shown previously to bind C/EBPα and C/EBPβ in nuclear extracts of Mono Mac 6 monocytic cells and to bind recombinant C/EBP factors (12, 46). Functional studies with overexpressed full-length C/EBPβ (47) and a dominant negative C/EBPβ mutant (12) indicate that the interaction is important for full transactivation of TNF-α. However, we were not able to demonstrate a role for C/EBP factors in glucocorticoid response of LPS-stimulated THP-1 cells. C/EBPβ synergizes with other LPS-regulated transcription factors, including NF-κB, c-Jun and glucocorticoid receptor (47, 67, 68). It therefore may have a permissive role in glucocorticoid response of TNF-α in monocytic cells, which our experiments did not reveal.

The TNF-α 3′-UTR strongly influenced basal expression and contributed to LPS response of luciferase reporter constructs in THP-1 cells. Although the TNF-α 3′-UTR did not mediate glucocorticoid response in SV40 promoter reporter constructs, TNF-α promoter reporter constructs that incorporated the TNF-α 3′-UTR were more responsive to glucocorticoid than constructs that incorporated the SV40 3′-UTR. The effect was small but was consistent across all of the TNF-α promoter constructs and mutants that were used in this study. Se-

quences in the 3′-UTR were substantially more important to TNF-α glucocorticoid response in murine macrophage RAW264.7 cells than in THP-1 cells. In RAW264.7 cells, glucocorticoids also inhibit translation by inhibiting activation of Jun N-terminal kinase/stress-activated protein kinase, which is necessary for efficient translation of TNF-α mRNA (29, 56).

In summary, therefore, glucocorticoids act to reduce TNF-α secretion by THP-1 cells largely through suppressing TNF-α gene transcription. Suppression is mediated through reduced binding of Rel-A/NF-κB_1, Rel-A/C-Rel, c-Jun/ATF-2, and possibly other complexes to binding sites between −106 and −88 bp of the promoter. Smaller, additional effects on transcription are unrelated to these sites. There is evidence for a small effect mediated through the 3′-UTR, but it is much less important than in RAW264.7 cells.

Glucocorticoids suppress the stimulus-dependent expression of many proinflammatory proteins by macrophages, including TNF-α. Most macrophage stimuli are subject to glucocorticoid modulation. Glucocorticoids exhibit such breadth of action because they interact with a range of regulatory pathways and because the pathways employed by different macrophage stimuli overlap. This diversity seems to offer many opportunities for development of drugs with glucocorticoid-like anti-inflammatory action, but also means that drugs that emulate only one aspect of glucocorticoid action are unlikely to exhibit full clinical anti-inflammatory efficacy. Drugs that act against discrete glucocorticoid-sensitive processes may find application in inflammatory or immune diseases that are particularly dependent on that pathway.

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