Physical association and functional antagonism between the p65 subunit of transcription factor NF- κ B and the glucocorticoid receptor

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ABSTRACT Glucocorticoids, which are widely used as antiinflammatory agents, downregulate the expression of the interleukin 6 gene and of additional cytokine genes involved in inflammatory responses. Conversely, the transcription factor NF- κ B, a member of the Rel family of transcription factors, has been implicated in the induction of multiple genes involved in the early processes of immune and inflammatory responses. This prompted us to investigate whether one of the mechanisms by which glucocorticoids exert their antiinflammatory activities is through inhibition of gene activation mediated by NF- κ B. We report that, in intact cells, activation of the interleukin 6 promoter by a combination of the factor NF-IL6 and the p65 subunit of NF- κ B is inhibited by dexamethasone (ligand)-activated glucocorticoid receptor. Conversely, activation of the mouse mammary tumor virus promoter by a combination of dexamethasone and glucocorticoid receptor is inhibited by overexpression of p65. Furthermore, we provide evidence for physical association between glucocorticoid receptor and p65 in protein crosslinking and coimmunoprecipitation experiments, using either *in vitro* translated proteins or those present in cell extracts. These studies suggest that direct interactions between NF- κ B and glucocorticoid receptor may partly account for the antiinflammatory properties of glucocorticoids *in vivo*.

Glucocorticoids strongly repress interleukin 6 (IL-6) gene expression induced by cytokines and by activators of protein kinases A and C (1–4). Glucocorticoids synergize with IL-6 in increased hepatic synthesis of acute-phase plasma proteins (5–7) while inhibiting, via a negative feedback loop, uncontrolled production of IL-6 and other cytokines.

Previous studies in this laboratory established a requirement for wild-type glucocorticoid receptor (GR) for efficient repression of IL-6 gene expression induced by IL-1 (1, 8). Although footprinting studies with the DNA-binding domain of GR revealed binding across the enhancer and basal promoter elements in the IL-6 gene, only weak binding by the intact wild-type GR to the IL-6 promoter was found, suggesting that a combination of DNA-binding and protein-protein interactions may be responsible for efficient downregulation of IL-6 gene expression (8). Repression of the collagenase promoter, the proliferin promoter, and synthetic AP-1 site-chloramphenicol acetyltransferase (CAT) gene constructs by glucocorticoids was shown to be due to interactions between GR and the AP-1 site-binding protein Jun (9–12). It seems unlikely that Fos/Jun-GR interactions play a significant role in the repression of the IL-6 gene by glucocorticoids, since MRE I (multiple cytokine- and second messenger-responsive enhancer I, nucleotides –173 to –151) in the IL-6 gene, which contains an AP-1-like sequence, is not a major contributor to IL-1 induction of the IL-6 gene (1). Two overlapping enhancer sequences, MRE I and MRE II

(–158 to –145) in the IL-6 promoter, when independently linked to heterologous promoters, confer responsiveness to different stimuli (1, 13). MRE II was described as the binding site for nuclear factor NF-IL6 and hypothesized to be responsible for induction of the IL-6 gene by IL-1 (14). Although the NF- κ B site in the IL-6 gene (–64 to –73) was previously implicated in IL-6 gene expression (15, 16), the presence of this site alone in IL-6 promoter constructs fails to activate the reporter gene (17, 18).

The transcriptionally active NF- κ B contains a 65-kDa subunit (p65) that forms a heterodimer with a 50-kDa subunit (p50) (19, 20). Contrary to earlier reports, it has been shown that p65 directly contacts DNA in a heterodimer with p50 (21–23) and that even p65 homodimers can bind DNA (21, 22). The existence of p65 homodimers *in vivo* is yet to be demonstrated. Both DNA-binding and dimerization properties of the NF- κ B subunits reside in a domain of $\approx$ 300 amino acids which is also found in proteins of the Rel family (24). p49/p100, which can be derived by processing of the precursor protein p100 or by alternative splicing, shares a 60% identity in sequence with p105, the precursor of p50 (25, 26). There is increasing evidence that the subunits singly may have independent modes of transactivation (22, 23, 27).

In the present study, we have examined the role of NF- κ B in IL-1-induced IL-6 gene expression. Due to the strikingly opposite roles of NF- κ B and glucocorticoids in inflammation, we have investigated the possibility of functional antagonism between NF- κ B and GR in repression of IL-1-activated expression of the IL-6 gene by glucocorticoids (1, 8).

MATERIALS AND METHODS

Cell Culture and Transfections. HeLa human cervical carcinoma cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (17). F9 mouse embryonal carcinoma cells were plated on gelatin-coated plates and were maintained in DMEM with 15% fetal bovine serum. Transfections and CAT assays were essentially as described (1, 8).

Plasmids. The construct pIC225 was described previously (17). The NF-IL-6 site (MRE II; refs. 1 and 8) and the NF- κ B site in pIC225 were mutated by site-directed mutagenesis (Muta-Gene, Bio-Rad). pmMRE IIB225, containing mutations in the NF-IL6 site, was constructed in two steps using two different oligonucleotides sequentially: (i) 5'-CGTCACATcaCACAAATCT-3' (mMRE IIa) and (ii) 5'-CGTCACATcaCAaAcaCTTAATAAGG-3' (mMRE IIB). The oligonucleotide 5'-TTTATCAAATGTaatATTTTCCCATGA-3' was used to construct pmNF- κ B225. The cDNAs for p49 or p65 in Bluescript plasmids or under the control of the Rous sarcoma virus (RSV) promoter (pRSV49 or pRSV65) were

FIG. 1. Activation of the IL-6 promoter requires both the NF-IL-6 and NF-κB sites. Monolayer cultures of HeLa cells and F9 cells were transfected by the calcium phosphate coprecipitation procedure, essentially as described (1, 13). (A) Subconfluent cultures of HeLa cells were transfected separately with the reporter plasmid (5 μg) pIC225 or pmMRE IIB225 or pmNF-κB225 containing wild-type or mutated NF-IL-6 or NF-κB sites, respectively (see Materials and Methods), various expression vectors (1 μg), and pCH110 (2.5 μg; a constitutive expression vector for β-galactosidase, for monitoring of transfection efficiency). Extracts normalized to equal β-galactosidase activity were assayed for CAT activity. Numbers below the dots denote fold activity relative to that in uninduced lanes. The 8-fold activation of pIC225 by IL-1α represents a 7% absolute conversion of [¹⁴C]chloramphenicol. F9 cells were transfected with the reporter plasmid pIC225. The 10-fold activation by a combination of pRSV65 and CMVNF-IL6 denotes a 6% conversion of [¹⁴C]chloramphenicol. A representative experiment is shown. Each set of experiments was repeated at least two or three times, with deviations between experiments of ≤20%. (B) Nucleotide sequence of the wild-type and mutant NF-IL6 and NF-κB sites present in the reporter constructs. mMRE IIB denotes the mutated NF-IL6 binding site, and mNF-κB denotes the mutated NF-κB site.

gifts of Gary Nabel (University of Michigan). pRSVhGR (with the human GR cDNA under control of the RSV promoter) and pMTV-CAT [CAT gene under control of the mouse mammary tumor virus (MTV) promoter] were provided previously by R. Evans (Salk Institute). CMVNF-IL6 [NF-IL6 cDNA under control of the cytomegalovirus (CMV) promoter] was provided by T. Kishimoto (Osaka University). An ≈1.0-kb fragment containing the basic-leucine zipper domain of mouse NF-IL6 (C/EBPβ) (28), was engineered for *in vitro* expression by fusion of an ATG codon in frame to amino acid 141, and the resulting fragment containing amino acids 141–296 of the protein was subcloned into pGEM-7Zf(+) (Promega) for expression under the control of the T7 promoter. A cDNA fragment encoding GRAS, comprising amino acids 1–596 of human GR, was subcloned into pGEM-4Z (Promega).

Electrophoretic Mobility-Shift Assays. *In vitro* translated p49 or p65 was incubated with the NF-κB probe in 20 μl of reaction mixture containing 20 mM Hepes (pH 7.9), 50 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 0.5% (vol/vol) Nonidet P-40, 5% (vol/vol) glycerol, 1 mM phenylmethanesulfonyl fluoride, 0.5 μg of poly(dI-dC), and 1 μg of bovine serum albumin. The following is the sequence of the double-stranded oligonucleotide probe used in the assay (the NF-κB site of the IL-6 gene is in bold type).

5'-TCGAAATGTGGGATTTTCCCATGAGT-3'
3'-TTACACCCTAAAAGGGTACTCACTAG-5'

For competition experiments, the unlabeled oligonucleotides were used at a 50-fold molar excess.

Crosslinking and Immunoprecipitations. For protein crosslinking and immunoprecipitations, proteins were synthesized *in vitro* with the TNT system (Promega) using [³⁵S]methionine or unlabeled amino acids. Individual lysates

were appropriately mixed and incubated in 20 mM Hepes, pH 7.9/50 mM KCl/2.5 mM MgCl₂/1 mM dithiothreitol/1 mM phenylmethanesulfonyl fluoride/10% glycerol with aprotinin at 10 μg/ml for 30 min at room temperature. The proteins were crosslinked with dithiobis(succinimidyl propionate) (5 mM) for 15 min at room temperature and the reaction was terminated by addition of ethanolamine to 0.1 M. The crosslinked proteins were diluted to 200 μl with a modified radioimmunoprecipitation assay (RIPA) buffer (10 mM Tris-HCl, pH 7.5/150 mM NaCl/2 mM EDTA/0.5% sodium deoxycholate/0.5% Nonidet P-40/0.1 M ethanolamine/1 mM phenylmethanesulfonyl fluoride with aprotinin at 10 μg/ml), precleared, and subjected to immunoprecipitation with anti-GR antibody (gift of W. Hoeck, Syntex Research) or anti-p65 antibody (Santa Cruz Biotechnology). Coimmunoprecipitation of GR/p65 complexes from metabolically labeled cells was carried out by procedures described in ref. 29.

RESULTS

Activation of the IL-6 Promoter Requires both NF-IL6 and NF-κB Binding Sites. Earlier studies have established that 225 bp of 5' flanking sequences of the IL-6 gene linked to the CAT gene (pIC225) is sufficient for conferring IL-1 response on the IL-6 promoter (1, 13). The construct pIC110, which contains 110 bp of IL-6 gene 5' flanking sequence including the NF-κB site between -64 and -73, is, however, not induced by IL-1 or by additional stimuli (1, 13, 17, 18). In view of the implication of this NF-κB site in the induction of the IL-6 gene (15, 16), we explored the IL-1-inducibility of constructs pmMRE IIB225 and pmNF-κB225, mutated at the NF-IL6-binding site MRE II and the NF-κB site, respectively (Fig. 1). The IL-1 responsiveness of both these mutants was greatly

FIG. 2. Functional antagonism between p65 and GR. (A) HeLa cells or F9 cells were transfected with pIC225 (5 μ g), RSVhGR (expression vector for GR, 5 μ g), combinations of additional expression vectors (2.5 μ g), and pCH110 (2.5 μ g), with or without IL-1 α and dexamethasone (Dex, 1 μ M). Cells were grown, harvested, and assayed for β -galactosidase and CAT activity as in Fig. 1. The 4-fold activation by NF-IL6 or p65 in HeLa cells represents a 5% conversion of [14 C]chloramphenicol. The 50-fold activation of pIC225 by the same combination in F9 cells represents a 7.5% conversion of [14 C]chloramphenicol. A representative experiment is shown. The experiment was repeated three times with deviations of $\leq 20\%$ between experiments. (B) GR decreases the binding of p65 to the NF- κ B site of the IL-6 gene. Reticulocyte lysates containing *in vitro* translated p49 or p65 (2 μ l/50 μ l) were incubated with the labeled probe. Each binding reaction mixture was adjusted to equal amounts of reticulocyte lysate by adding appropriate amounts of unprogrammed lysate. The result of incubation of 2, 4, and 6 μ l of *in vitro* translated GRAS alone with the probe (lanes 1, 3, and 5 respectively) or with 2 μ l of p65 (15 min at room temperature) before addition of the probe (lanes 2, 4, and 6 respectively) and subsequent analysis by 6% PAGE is shown. This figure is representative of two experiments.

diminished in HeLa cells (Fig. 1A). To further demonstrate that the activation of pIC225 depends on the presence of both the MRE II and NF- κ B sites, we performed a similar experiment in undifferentiated F9 embryonal carcinoma cells. F9 cells lack endogenous NF-IL6 (7), AP-1 (9), and possibly also Rel-like activities (30) and are therefore the cells of choice when investigating the role of the particular transcription factors in gene expression. No pIC225 expression was detected with any of the singly cotransfected expression vectors in F9 cells (Fig. 1A). Only a combination of the expression vectors CMVNF-IL6 and pRSV65 yielded a 10-fold increase in CAT expression over basal expression (Fig. 1A). Interestingly, coexpression of p49, the processed product of p100,

similar to p50 of NF- κ B, completely inhibited the increased CAT expression obtained by a combination of pRSV65 and CMVNF-IL6 (Fig. 1A). The p50 subunit of NF- κ B was recently shown to interact with NF-IL6, although the functional significance of this association was not addressed (31). The inhibition of NF-IL6/p65-mediated activation of the IL-6 promoter by overexpression of p49 may be due to sequestration of p65 and NF-IL6 by p49. We have not determined the effect of coexpression of p50 on IL-6 promoter activity, either alone or in combination with other transcription factors. Expression vectors for Fos/Jun and NF-IL6 or Fos/Jun and p49/p65, in all possible combinations, had no effect on CAT expression from pIC225 (data not shown).

Functional Antagonism Between GR and p65 and Inhibition of DNA-Binding Activity of p65 by GR. Having established that NF-IL6 and p65 are required for activation of the IL-6 promoter, we examined the possibility that repression by glucocorticoids might be achieved by association of one of these proteins with GR. We cotransfected combinations of expression vectors for various transcription factors and GR in HeLa cells and asked whether GR was able to inhibit activation of pIC225 by NF-IL6 and p65. In contrast to what was observed in F9 cells, in HeLa cells, expression of either one of these factors could elicit CAT expression (Fig. 2A). This may be due to the interaction of NF-IL6 or p65 with endogenous transcription factors interacting with other motifs in the IL-6 promoter such as MRE I (1, 13). GR and dexamethasone only partially repressed activation of pIC225 by NF-IL6 alone in HeLa cells (Fig. 2A), in contrast to their total repression of IL-1-induced IL-6 gene expression, observed in these and earlier studies (1, 8). However, the fact that activation by p65 of pIC225 was reduced to basal levels by GR and dexamethasone (Fig. 2A) suggested that p65 might be a target of GR. Similar transfection experiments in F9 cells with 2.5 μ g each of coexpressed NF-IL6 and p65 vectors gave about 70% inhibition with a combination of GR (5 μ g) and dexamethasone (Fig. 2). This inhibition could be titrated down to almost basal levels by increasing the ratio of GR to CMVNF-IL6 and pRSV65 (data not shown).

To investigate whether GR affected the binding of p65 to the IL-6 κ B oligonucleotide probe, proteins synthesized in reticulocyte lysates in the presence of [35 S]methionine were tested in electrophoretic mobility shift assays. GR expressed *in vitro* which lacked the steroid-binding domain is referred to as GRAS. This receptor construct retained (i) the two zinc fingers, which are required for its repressor function on the IL-6 promoter (1, 8) and the AP-1 site (9–12), and (ii) the adjacent putative amphipathic helix that has been proposed to be involved in protein–protein interactions (32).

In contrast to p49, p65 bound very strongly to the probe (Fig. 2B). p49 also binds weakly to the κ B site in the human immunodeficiency virus enhancer (26). Holding the level of p65 constant in lanes 2, 4, and 6, and increasing the amount of GRAS approximately 1-, 2-, and 3-fold over p65, incrementally inhibited the DNA-binding ability of p65 (Fig. 2B). Densitometric scanning of the autoradiograph revealed a 65% reduction in the ability of p65 to bind DNA in the presence of excess (3-fold) GRAS. GRAS alone did not bind to the probe (Fig. 2B, lanes 1, 3, and 5). Preincubation of excess GRAS with p65 resulted in significant but not complete inhibition of the DNA-binding ability of p65; the reason for partial inhibition is probably due to competition from endogenous NF- κ B-like activity present in rabbit reticulocyte extracts (33). In a recent report, Nishio *et al.* (7) demonstrated interaction between GR and the basic-leucine zipper domain of NF-IL6 in coimmunoprecipitation experiments (7). However, their attempts to detect such an interaction by means of gel shift assays were not successful.

Direct Physical Interaction Between p65 and GR. To investigate the possibility that the antagonism between GR and p65

FIG. 3. Crosslinking of *in vitro* synthesized p65 and GRAS. (A and B) Experiments were performed with 15- μ l samples of [35 S]methionine-labeled translation mixtures. For A, [35 S]-labeled p65 was crosslinked to unlabeled GRAS and anti-GR antibody raised against rat GR was used for immunoprecipitation; B illustrates the result of the converse combination, with anti-p65 antibody for immunoprecipitation. In each case, after crosslinking, the reaction mixture was divided into two parts; to one part was added either anti-GR (A) or anti-p65 antibody (B) and to the other part was added control rabbit nonimmune serum (NI Ser). *In vitro* translation of p65 gave multiple bands probably resulting from proteolytic digestion of the larger fragment. This was minimized on inclusion of protease inhibitors such as aprotinin (10 μ g/ml) and phenylmethanesulfonyl fluoride (1 mM) to the *in vitro* translation reactions (see Fig. 4).

The immunoprecipitates were washed extensively before crosslinks were reduced by boiling in 2 $\times$ SDS/PAGE sample buffer containing 10% (vol/vol) 2-mercaptoethanol. The products were resolved in an SDS/15% polyacrylamide and the gel was subjected to fluorography and autoradiography. The lanes showing the immunoprecipitated products represent a 2-day exposure. The arrow indicates the p65 band immunoprecipitated specifically with the anti-p65 antibody. Molecular size (kDa) markers are indicated. (C) A 10- μ l sample of [35 S]methionine-labeled p49 or truncated murine NF-IL6 (Δ NF-IL-6, containing amino acids 141–296) was incubated with 10 μ l of unlabeled GRAS, crosslinked with 5 mM dithiobis(succinimidyl propionate), and immunoprecipitated with anti-GR antibody. The reaction mixtures were further processed as above.

might be due to direct association, we tested whether the proteins in solution could be chemically crosslinked. Due to the similar molecular sizes of p65 and GRAS, in experiments utilizing both (Fig. 3 A and B), only one was labeled. In one set of experiments, cell-free translated p65 was labeled with [35 S]methionine while similarly synthesized GRAS was left unlabeled. After the two proteins were incubated together and crosslinked with dithiobis(succinimidyl propionate), the mixture was divided into two parts. One part was treated with control rabbit nonimmune serum and the other part was immunoprecipitated with anti-GR antiserum. After reversal of crosslinks by reduction, the proteins were analyzed by SDS/PAGE. Fig. 3A shows specific immunoprecipitation of p65 with the receptor-specific antiserum and not the control serum. In the reciprocal experiment, the ability of anti-p65 antiserum to precipitate [35 S]methionine-labeled GRAS when coincubated with unlabeled p65 was studied. Fig. 3B illustrates that the p65 specific band can be detected only with the specific antiserum. The anti-p65 antiserum was not very efficient for quantitative immunoprecipitations, most probably owing to its anti-peptide nature. In similar experiments, we failed to detect any association between GRAS and either p49 or Δ NF-IL6 (Fig. 3C).

We also asked whether a complex between GR and p65 can form in intact cells. To enhance our chances of detecting such an interaction in cell lysates, HeLa cells were transfected with expression vectors for full-length GR and p65 and cells were cultured in methionine-free medium supplemented with [35 S]methionine. A nuclear extract was prepared from these cells for a double immunoprecipitation. A part of the extract was split into two equal parts and immunoprecipitation was performed in two steps with each part. Anti-GR antiserum, and not nonimmune serum, specifically coimmunoprecipitated a protein that when released from the complex was again specifically recognized by anti-p65 antibody (Fig. 4). The $\approx$ 100-kDa nonspecific band seen in both lanes when anti-GR antiserum was used as the first antibody was reproducibly detected and was not further characterized.

p65 Represses GR-Mediated Activation. The experiments illustrated in Figs. 2–4 suggested that GR could interact with p65 to inhibit its activity. In the event of such an interaction, it was also possible that p65 would inhibit the ability of GR to activate transcription. The experiment illustrated in Fig. 5 confirms this hypothesis. In HeLa cells, under our transfection conditions, wild-type GR is a potent hormone-dependent activator of the mouse MTV long terminal repeat. Cotransfection of pRSV65 inhibited the GR-dependent activation of

pMTV-CAT. This inhibition was p65-specific and not due to cross-competition for common transcription factors between the RSV and MTV promoters, since cotransfection with pRSV49 had no effect on transactivation of the MTV promoter by GR and dexamethasone (Fig. 5).

DISCUSSION

Our data provide a mechanism by which steroids can control inflammation by interfering with the activity of NF- κ B. Our transient-transfection experiments have demonstrated synergistic transactivation functions of p65 with a non-Rel transcription factor, NF-IL6. We do not know whether p49 is a

FIG. 4. p65 is immunoprecipitated with GR from HeLa cell nuclear extracts with anti-GR antiserum. Nuclear lysate prepared from HeLa cells transfected with expression vectors for GR and p65 (10 μ g of each) and labeled with [35 S]methionine was immunoprecipitated with nonimmune serum (NI Ser) or anti-GR antiserum for double immunoprecipitation or with anti-p65 antibody. Immunoprecipitated material on the beads was washed with the same buffer. The complexes were disrupted by adding RIPA buffer containing 1% SDS. After the beads were spun down, the supernatants were diluted 1:10 in RIPA buffer, split into two, and transferred to new tubes. Polypeptides recovered in the supernatant were subjected to a second round of immunoprecipitation with either nonimmune serum or anti-p65 antibody. The beads were washed in RIPA buffer and boiled in 2 $\times$ SDS/PAGE sample buffer. The products were resolved in a 10% polyacrylamide gel and subjected to fluorography and autoradiography. The lane showing precipitation by anti-p65 only is a 24-hr exposure; the rest of the lanes were exposed for 4 days. Molecular size (kDa) markers are indicated. Ab, antibody.

FIG. 5. p65 but not p49 inhibits activation of pMTV-CAT by GR and dexamethasone. HeLa cells were cotransfected with pMTV-CAT (1 μ g), GR expression vector (RSVhGR; 1 μ g), and either pRSV65 (2.5 μ g) or pRSV49 (2.5 μ g). After transfection, cells were incubated either in medium alone without serum or in the presence of dexamethasone (Dex, 1 μ M) before harvesting and determination of CAT activity. Almost 95% of the [14 C]chloramphenicol was converted to its acetylated derivatives by a combination of RSVhGR and Dex under these conditions. Inclusion of pRSV65 resulted in a 30% conversion of [14 C]chloramphenicol ($\approx$ 70% inhibition), whereas pRSV49 had no effect. The experiment was repeated three times with deviations of $\leq$ 15% between experiments.

true physiological inhibitor of IL-6 gene expression and, since p49 is a closely related homolog of p50, whether p50 is also inhibitory for IL-6 gene expression. If indeed such is the case, in response to IL-1, p65 will need to selectively interact with NF-IL6 to activate the IL-6 promoter. Possible mechanisms by which this might be achieved are posttranslational modifications or enhanced transcription of p65 by IL-1. An inhibition of p49/p50 activity (if both have inhibitory properties on the IL-6 promoter) is also a possibility. A negative effect of p50 on p65-mediated activation of a reporter construct driven by two NF- κ B sites was reported (27).

Our present results extend previous studies on mechanisms of repression of the IL-6 promoter by GR (1, 8) by demonstrating physical association between GR and p65. A possible explanation for our failure to detect an interaction between GR and NF-IL6, as reported by Nishio *et al.* (7), is that the complex was unstable under our assay conditions. In the present study, while activation of the IL-6 promoter by NF-IL6 alone was only partially repressed by GR and dexamethasone, that by p65 was totally inhibited (Fig. 2A). This suggests that inhibition of p65 activity may be a key mechanism for efficient repression of IL-6 gene expression induced by IL-1 and perhaps by additional stimuli.

In earlier studies, although intact GR did not bind as avidly to IL-6 sequences as to the glucocorticoid response element present in the MTV promoter (8), its DNA-binding domain was important for repressor function in functional studies (1, 8). This observation was not entirely surprising, since the DNA-binding domain of GR is also important for repression of Jun/AP-1 function, although DNA-binding activity itself is apparently not necessary for repression (11, 12). In this role, the DNA-binding domain may be responsible for a dimerization function rather than for a DNA-binding function. These observations suggest that the DNA-binding domain is also involved in protein-protein interactions with p65/NF-IL6.

This mechanism of inhibition by GR by antagonism of p65 activity bypasses the need for a negative glucocorticoid

response element for efficient repression by glucocorticoids. The mechanism does not rule out the possibility that a combination of DNA-binding and protein-protein interactions accounts for the total repression of IL-6 gene expression by glucocorticoids. Thus, regulation of IL-6 gene expression is an excellent example of how cross-coupling of distinct pathways (NF-IL6 and NF- κ B) in certain situations can activate gene transcription and in others (GR and NF- κ B) can repress gene expression. The reciprocal functional antagonism between p65 and GR provides a framework for the opposing actions of inflammatory mediators and steroids.

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