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Glucocorticoids Inhibit Calcium- and Calcineurin-Dependent Activation of the Human IL-4 Promoter

Rongbing Chen,* Thomas F. Burke,* John E. Cumberland,* Mary Brummet, † Lisa A. Beck, † Vincenzo Casolaro, † and Steve N. Georas2*†

The mechanism by which glucocorticoids (GC) inhibit IL-4 gene expression is currently unknown. In T lymphocytes, IL-4 gene expression is regulated at the level of transcription by increases in intracellular calcium concentration and by the calcium-activated phosphatase calcineurin. In this paper we report that dexamethasone (Dex) inhibits calcium ionophore-induced activation of the human IL-4 promoter in transiently transfected Jurkat T cells. Inhibition of the promoter by Dex is dependent on expression of the GC receptor (GR), because it does not occur in GR-deficient cells. Dex also represses activation of the promoter induced by cotransfecting cells with a constitutively active mutant of calcineurin. Using a series of deletion constructs, we show that the proximal 95 bp of the IL-4 promoter contain a Dex-sensitive regulatory element. This region contains the P1 sequence, a proximal binding site for NF-AT. A calcium-induced but Dex-inhibited nuclear complex containing NF-AT binds to the P1 element in EMSA. Using immunoprecipitation under nondenaturing conditions, we found that the GRα isoform coprecipitates with NF-ATc in nuclear extracts of calcium ionophore- and Dex-treated cells. Taken together, our results show that GC inhibit IL-4 gene expression by interfering with NF-AT-dependent transactivation of the proximal human IL-4 promoter. 

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Glucocorticoids (GC) are the most potent immunosuppressive agents available for inflammatory diseases and work largely by inhibiting the production of proinflammatory molecules such as cytokines (1). Recently, considerable progress has been made in identifying the molecular mechanisms by which GC exert their effects. GC bind the cytosolic GC receptor (GR), which then translocates to the nucleus and inhibits the transcriptional activation of target genes (2). In general, the GR mediates transcriptional repression through: 1) interfering with the function of transacting factors (e.g., AP-1 and NF-κB) via protein-protein interactions; 2) direct DNA binding to poorly conserved negative GC response elements (GRE); or 3) inducing the expression of inhibitory factors such as IκBα (3–8).

IL-4, a pleiotropic cytokine produced by activated T cells, basophils, and mast cells, regulates many cellular and humoral immune responses (9, 10). Dysregulated IL-4 gene expression has been linked with several inflammatory, allergic, and autoimmune diseases (11–15). GC readily inhibit IL-4 expression in vivo (16–18) and also suppress IL-4 production in isolated cell types including T cells, basophils, and mast cells (19–22). The molecular mechanisms by which GC inhibit IL-4 gene expression have not previously been defined.

In T cells, IL-4 expression is tightly controlled at the level of transcription by multiple regulatory elements located within a proximal promoter region (23–30). Regulatory elements outside of the promoter have also been detected (31, 32), NF-AT can enhance IL-4 transcription by binding up to five related sequences in the IL-4 promoter (termed the P elements P0–P4) (24). NF-AT is now known to comprise a group of related factors that recognize a common DNA motif (5′-GGAAAAA-3′) (33). The precise roles of individual NF-AT proteins in regulating IL-4 gene expression are currently not clear. Experiments using NF-AT-deficient mice have demonstrated a critical role for NF-ATc in enhancing the differentiation of IL-4-secreting Th2 cells, whereas NF-ATp and NF-AT4 down-regulate this process (34–37). In contrast, IL-4 expression in activated T cells appears to be due in part to NF-ATp-dependent promoter transactivation (38).

Unlike the IL-2 promoter, which requires costimulation of calcium- and protein kinase C (PKC)-mediated signaling pathways for full activation, the IL-4 promoter can be maximally induced by a calcium signal alone (26, 39, 40). A constitutively active mutant of the calcium-sensitive phosphatase calcineurin (CN) can efficiently substitute for this signal (40–42). Of the potential factors that are activated by CN in T cells (43–46), NF-ATp appears to be the most likely target in the IL-4 promoter. Currently, whether GC inhibit NF-AT-dependent transactivation is controversial. In one study, transcription driven by the IL-2 promoter-distal NF-AT site was partially inhibited by dexamethasone (Dex) in transfected Jurkat T cells (47). Because the IL-2 promoter requires cooperative interactions between NF-AT and AP-1 proteins for maximal induction, it was not possible to separate the repressive effects of GC on NF-AT from their known inhibition of AP-1 activity (3). Additionally, NF-AT activity was not inhibited by GC in other studies of the IL-2 promoter (48, 49).

The IL-4 promoter provides a unique opportunity to study the regulation of NF-AT-dependent transactivation by GC in T cells.
In this paper we report that transcription driven by the intact human IL-4 promoter is strongly inhibited by GC in Jurkat T cells in a GR-dependent manner. We show that the repressive effects of Dex map downstream of CN activation and by EMSA identify the P1 NF-AT element as the site of an activation-induced and GC-inhibited nuclear protein complex that contains NF-ATc.

Materials and Methods
Plasmid construction
Human IL-4 promoter constructs were amplified from genomic DNA using the PCR. Twenty-five base pair primers annealing 372, 265, 225, 95, 65, and 35 bp upstream from the transcription start site (according to Otsuka et al. (50)) were used with a 25-bp primer ending at position +65. PCR products were ligated into the SrfI site of pCR-Script (Stratagene, La Jolla, CA) and sequenced to confirm accurate replication. pLuc 372 was synthesized by ligating the KpnI and SacI restriction fragment from pCR-Script 372 into compatible sites in pGL3 (Promega, Madison, WI). pCAT plasmids were synthesized by ligating the HindIII and XhoI restriction fragments from the corresponding pCR-Script plasmids (generated using PCR primers with those restriction sites included) into compatible sites in pCAT Basic (Promega). The IL-2 promoter reporter construct IL-2.125ACAT has been described (51) and was a gift of Dr. Gerald Crabtree (Stanford University, Stanford, CA). The respiratory syncytial virus long terminal repeat-driven full-length human GRs expression vector (pRS hGRs, see Ref. 52) was a kind gift of Dr. Ron Evans (Salk Institute, La Jolla, CA). The plasmid ΔCaMAI contains a constitutively active fragment of CN, lacking the calmodulin-binding and autoinhibitory domains (53), and was kindly donated by Dr. Randall Kincaid (Veritas, Rockville, MD). Construction of the pREP4-based NF-ATc expression vector (provided by Dr. Timothy Hoey, Tularik, South San Francisco, CA) has been described (54).

Cell lines and transfections
Three different lines of human Jurkat T cells were used in these experiments. One line deficient in GR expression was identified on the basis of minimal immunoreactive GR using nuclear extracts from Dex-stimulated cells in EMSA with a radiolaabeled GRE (Promega; not shown). The generation of a stably transfected GR-expressing subline of Jurkat T cells, derived from a parental GR-negative line, has been described (55). These cells (A11 cells), expressing roughly 70,000 copies/cell of the GR under control of the β-actin promoter (55), and a subline transfected with empty vector (β-actin cells), were both kindly donated by Dr. Michael Karin (University of California at San Diego, La Jolla, CA). Cells were maintained in RPMI 1640 supplemented with 10% FCS (Life Technologies, Grand Island, NY) and 100 U/ml gentamicin (Life Technologies), and, in the case of A11 and β-actin cells, 1.5 mg/ml G418 (60% active compound). Cells (5 × 10⁶) were washed and resuspended in RPMI 1640 containing 5% charcoal-filtered FCS (Gemini Bioproducts, Calabasas, CA) for 3 h and then transfected in duplicate using the Superfect method (Qiagen; not shown) with either 25 ng of the intact IL-4 promoter linked to the firefly luciferase gene (pLuc 372). As we and others have previously observed with different NF-ATc, lysates were precleared with 1 µg of mouse IgG (Sigma) and 20 µl protein G-coated Sepharose beads (protein G PLUS, Sigma) and a full-length ex- pressed NF-ATc (7A6, Affinity BioReagents) and 20 µl protein G PLUS for 18 h under condition to constant reaction at 4°C. Equal amounts of species-matched control anti-sera were used for each condition. After centrifugation, beads were extensively washed, and immunoprecipitated proteins were collected by boiling in sample buffer (50 mM Tris (pH 6.8), 10 mM DTT, 0.1% bromophenol blue, and 10% glycerol). Forty-microliter aliquots were separated by 6% SDS-PAGE and then transferred to Trans-Blot transfer medium polyvinylidene difluoride membrane (Bio-Rad). After blocking in PBS-5% BSA/0.1% Tween 20 for 1 h, membranes were incubated with anti-GR (E20, 1:200) for 1 h. After two washes (5 min each) with PBS/0.1% Tween, membranes were incubated for 1 h with the second antibody (Ab) and then with biotinylated goat anti-rabbit IgG (Pierce) followed by 0.8 µg nuclear protein, 0.8 µg matched control Ab, and 0.8 µg mouse IgG (Sigma) or isotype- and species-matched control IgG (Sigma). After blocking in PBS-5% BSA/0.1% Tween, membranes were incubated with anti-NF-ATp (Upstate Biotechnology, Lake Placid, NY) and anti-NF-ATc (7A6, Affinity BioReagents, Golden, CO), each non-cross-reactive with other NF-AT family members; anti-c-Fos and anti-c-Jun, both broadly reactive with other Fos and Jun family members, respectively (Santa Cruz Biotechnology, Santa Cruz, CA); and isotype- and species-matched control IgG (Sigma).

Immunoprecipitation and Western blot analysis
Fifty micrograms of nuclear protein per condition (extracted from A11 cells using the method of Schreiber et al. (56)) were adjusted to a final concentration of 430 mM KCl and 15 mM Tris-HCl. To immunoprecipitate NF-ATc, lysates were precleared with 1 µg of mouse IgG (Sigma) and 0.5 µg protein G-coated Sepharose beads (protein G PLUS, Sigma) and a full-length expression vector (pRS hGRs, see Ref. 52) were added. Nuclear extracts were used for each condition. After centrifugation, beads were extensively washed, and immunoprecipitated proteins were collected by boiling in sample buffer (50 mM Tris (pH 6.8), 10 mM DTT, 0.1% bromophenol blue, and 10% glycerol). Forty-microliter aliquots were separated by 6% SDS-PAGE and then transferred to Trans-Blot transfer medium polyvinylidene difluoride membrane (Bio-Rad). After blocking in PBS-5% BSA/0.1% Tween 20 for 1 h, membranes were incubated with anti-GR (E20, 1:200) for 1 h. After two washes (5 min each) with PBS/0.1% Tween, membranes were incubated for 1 h with the second antibody (Ab) and then with biotinylated goat anti-rabbit IgG (Pierce) followed by 0.8 µg nuclear protein, 0.8 µg matched control Ab, and 0.8 µg mouse IgG (Sigma) or isotype- and species-matched control IgG (Sigma).

Results
Calcium- and PKC-mediated IL-4 promoter activity is inhibited by Dex
We studied the effects of the synthetic hormone Dex on transcription driven by a comprehensive panel of human IL-4 promoter deletion constructs transiently transfected into GR-deficient Jurkat T cells (see Materials and Methods). Fig. 1 shows the results of experiments using a full-length promoter construct that contains 372 bp of the IL-4 promoter linked to the firefly luciferase gene (pLuc 372). As we and others have previously observed with different IL-4 promoter constructs (26, 39), pLuc 372 was maximally inducible with a calcium signal alone. Promoter activity was not affected by Dex alone, consistent with the negligible expression of nuclear GR protein in these cells. We next used a full-length expression vector encoding the wild-type human GR (pRShGRα) in transient cotransfection experiments. As shown in Fig. 1, Dex strongly inhibited calcium-induced pLuc 372 activity in a GR-dependent manner.
NF-κB is a well-established molecular target of GC (7, 8). We have previously shown that PMA-induced NF-κB proteins down-regulate the human IL-4 promoter by competing with NF-AT for binding to the P1 element (39). Therefore, GC might be expected to relieve NF-κB-mediated repression of the IL-4 promoter induced by PMA. However, as shown in Fig. 1, pLuc 372 activity in Jurkat cells costimulated with calcium ionophore and PMA was inhibited by Dex in GR-cotransfected cells. This observation suggests that GC inhibit another factor that activates the proximal IL-4 promoter in our system.

**IL-4 promoter inhibition by Dex maps downstream of CN activation**

CN is a critical phosphatase that mediates calcium-induced gene expression in T cells (43), and activated CN alone is sufficient to maximally induce the IL-4 promoter in Jurkat cells (42). Unlike other potential targets of CN in T cells that require concomitant stimulation with PMA for full induction (44, 45), NF-AT can be exclusively activated by CN alone (46). To better characterize the inhibition of calcium ionophore-induced IL-4 transcription by GC, we studied the repressive effect of Dex on IL-4 promoter activity induced by cotransfecting cells with a constitutively active form of CN (ΔCaMAI, see Materials and Methods). Fig. 2 shows that cotransfection of ΔCaMAI was sufficient to strongly induce pLuc 372 activity. Interestingly, this induction was inhibited by Dex in a GR-dependent manner. These results map the repressive effect of Dex on calcium-induced IL-4 promoter activity downstream of CN activation.

A Dex-sensitive regulatory element is located within the proximal 95 bp of the IL-4 promoter

To map the Dex-responsive element(s) in the IL-4 promoter, we next used a series of promoter deletion constructs in additional transfection experiments. The calcium-induced activation of each construct examined was inhibited by Dex in the presence of the GR (data not shown). Fig. 3 shows the results of experiments using a minimal promoter construct containing 95 bp upstream from the transcription start site linked to the chloramphenicol acetyltransferase (CAT) gene (pCAT 95). In these experiments we used the A11 subline of Jurkat cells, which was stably transfected with a GR expression vector under the control of the β-actin promoter, to ensure constant GR protein expression (55). Dex completely inhibited calcium-induced pCAT 95 activity in A11 cells and further

**FIGURE 1.** Dex inhibits calcium-induced IL-4 promoter activity in a GR-dependent manner. A schematic drawing of the full-length human IL-4 promoter construct pLuc 372 is shown (top). GR-deficient Jurkat T cells (see Materials and Methods) were cotransfected with 1 µg pLuc 372, 0.5 and 1.0 µg of the GR-expression vector pRShGRα, or empty vector to keep total DNA constant. Cells were then stimulated with calcium ionophore (0.5 µM A23187) without or with 20 ng/ml PMA as indicated for 18 h in the absence (□) or presence of 1 µM Dex (■) before cell lysis and analysis of reporter gene expression by lumimetry. Results are expressed relative to promoter activity in Dex-treated cells cotransfected with empty vector alone and are the mean ± SEM of five independent experiments. *p < 0.05 for the effect of Dex, as determined by the Wilcoxon signed-rank test.

**FIGURE 2.** Dex inhibits CN-driven IL-4 promoter activity in a GR-dependent manner. GR-deficient Jurkat T cells were cotransfected with 1 µg pLuc 372 and 1 µg of ΔCaMAI (containing a constitutively active form of CN, see Materials and Methods) without or with 0.5 µg of the GR-expression vector pRShGRα as indicated or empty vector to keep total DNA constant. Cells were then incubated without (□) or with Dex (1 µM) (■) for 18 h, followed by cell lysis and analysis of reporter gene expression by lumimetry. Results are expressed relative to pLuc 372 activity in cells transfected with empty vector alone and are the mean ± SEM of six independent experiments. *p < 0.05 for the effect of Dex, as determined by the Wilcoxon signed-rank test.

**FIGURE 3.** Dex inhibits a minimal IL-4 promoter construct in Jurkat cells stably expressing the GR. A schematic drawing of pCAT 95, which contains only the P1 and P0 NF-AT elements linked to the CAT gene, is indicated (top). The Jurkat cells used in these experiments were derived from a GR-negative parental line and stably express the GR (A11 cells, see Materials and Methods). A11 cells were transfected with 1 µg of pCAT 95 before cell stimulation with calcium ionophore (0.5 µM A23187, A23) alone or with PMA (20 ng/ml, A23/PMA) in the presence (■) or absence (□) of Dex (1 µM) for 18 h. Next, the cells were subjected to cell lysis and analysis of reporter gene expression by ELISA. Results are the mean ± SEM of four independent experiments. *p < 0.05 for the effect of Dex as determined by the Wilcoxon signed-rank test.
complex formation on the P1 element by Dex. Fig. 4 shows that a
broad complex formed using nuclear extracts isolated from cal-
cium ionophore-stimulated, but not resting, A11 cells (Fig. 4A,
complex I, lane 3) which was strikingly prevented by Dex (Fig. 4A,
lane 4). We have previously observed a factor that binds constit-
tutively in this region (Fig. 4A, complex II; Ref. 39), which was not
affected by Dex treatment. To determine the identity of complex I,
we used specific antisera in additional EMSA. Fig. 4B shows that
complex I contains predominantly NF-ATc because its formation
was largely inhibited by a specific anti-NF-ATc antiserum. The
formation of complex I was not affected by anti-NF-ATp Abs (Fig.
4B) or by antisera that were broadly reactive for c-Fos, c-Jun, and
related AP-1 family members (data not shown).

We wondered whether the inhibition of NF-AT binding to the
IL-4 promoter in Dex-treated cells was due to a noncanonical GR
binding site overlapping the P1 element. To test this hypothesis,
we analyzed the ability of the GR DBD to interact with the P1
element in EMSA. Using concentrations of the GR DBD that
formed strong monomeric and dimeric complexes on a canonical
GRE (Fig. 5A, lane 3), we found that the IL-4 P1 element sup-
ported only weak monomeric binding of the GR (Fig. 5A, lane 1).
However, even high concentrations of this factor were unable to
displace the NF-AT DBD from its cognate site in this region (Fig.
5B). In competition experiments, up to 50-fold molar excess of
oligonucleotides containing the P1 element were unable to com-
pete for binding of the GR to a canonical GRE in EMSA (Fig. 6,
lanes 8 and 9). Taken together, these results suggest that the GR
interacts only weakly with the P1 element and that inhibition of
NF-AT binding to the P1 element by Dex is not due to competitive
binding of the GR to an overlapping monomeric binding site.

To further rule out the possibility that the GR interacted directly
with promoter elements located downstream of P1, we first studied
the ability of the GR to bind to an oligonucleotide probe contain-
ing the P0 element (–69 to –36). No binding to this element was
detected in EMSA (Fig. 5C). We next studied the ability of a
100-bp promoter fragment (containing nucleotides –35 to +65, see
Materials and Methods) to compete for binding of the GR to a
canonical GRE. No competition was observed under these con-
ditions (Fig. 6, lanes 6 and 10). Thus, the contiguous 134 bp down-
stream of P1 do not appear to contain a high-affinity GR
binding site.

Dex does not inhibit nuclear accumulation of NF-ATc

We next analyzed nuclear extracts isolated from resting and acti-
vated Jurkat A11 cells for the expression of NF-AT proteins and
the GR using immunoprecipitation and Western blot analysis. To
detect potential protein-protein interactions between these factors,
these experiments were performed under nonnaturating conditions
(see Materials and Methods). As shown in Fig. 7A, multiple iso-
forms of NF-ATc were immunoprecipitated under these condi-
tions. A predominant band of ~86 kDa was observed (Fig. 7A,
binding III) together with two more slowly migrating species corre-
spanding to the ~110-kDa (Fig. 7A, binding II) and ~140-kDa (Fig.
7A, binding I) NF-ATc isoforms recently reported by Lyakh et al.
(62). Interestingly, a quickly migrating, NF-ATc-specific band was
also detected just above the Ig heavy chain (Fig. 7A, band IV),
possibly corresponding to a ~56-kDa NFATc isoform previously
detected in murine mast cells (63). The nuclear expression of all
isoforms was increased by cell stimulation with calcium ionophore
(compare Fig. 7A, lanes 2 and 4), but was not noticeably inhibited
by Dex (Fig. 7A, lane 6). When the membrane was stripped and
reanalyzed for expression of the GR (Fig. 7B), a single band of
~94 kDa was immunoprecipitated with the anti-GR Ab from Dex-
treated nuclear extracts (Fig. 7B, lane 3). This corresponds to the
known stable expression of the Grα isoform in these cells (55).

inhibited the promoter in combination with PMA. Importantly,
Dex did not inhibit pCAT 95 or other promoter constructs in con-
trol cells stably transfected with empty vector alone (data not
shown).

These experiments localized the repressive effect of Dex to the
proximal 95 bp of the IL-4 promoter. There are two P elements
within this region. First, the P1 site is a well-characterized NF-AT
response element that is critical for maximal activation of the IL-4
promoter (23–25, 29) and is the site of an activation-induced nu-
clear protein complex in EMSA (29, 60). Second, the P0 element
is located just downstream and has been shown to interact with
NF-AT, C-Maf, and CCAAT/enhancer-binding protein (28, 61). To
better map potential Dex-responsive elements in this region of the
IL-4 promoter, we synthesized a minimal promoter construct con-

Figure 4. Dex inhibits the formation of an NF-ATc-containing nu-
clear complex on the P1 element. A, Nuclear extracts were isolated from
resting and ionophore-activated (A23187, 0.5 μM) A11 cells treated with-
out and with Dex (1 μM) for 4 h as indicated, and were analyzed by EMSA
using a radiolabeled oligonucleotide encompassing the P1 NF-AT element.
A broad complex (complex I) formed using nuclear extracts isolated from
ionophore-activated cells (lane 3), whose formation was almost completely
prevented by Dex (lane 4). Binding of a constitutive nuclear factor (com-
plex II) was not affected by Dex. The relative migration of the free probe
is not indicated in this figure. Similar results were obtained in two addi-
tional experiments. B, Complex I has a nuclear factor that bound to the
P1 element. Nuclear extracts were isolated from ionophore-stimulated, but
not resting, A11 cells (Fig. 4B, lane 3) and were analyzed by EMSA
using a radiolabeled oligonucleotide encompassing the P1 NF-AT element.
It was clear that complex I contains predominantly NF-ATc because
its formation is almost completely inhibited by anti-NF-ATc Abs, but not
anti-NF-ATp or control antisera. No other complex was affected by any of
the antisera tested.
Interestingly, the GRα coprecipitated with NF-ATc using nuclear extracts from calcium ionophore- and Dex-treated cells (Fig. 7B, lane 6). Importantly, this factor was not immunoprecipitated using control antisera and nuclear extracts from cells treated with either calcium ionophore or Dex alone (Fig. 7B, lane 5; and data not shown). These results are suggestive of direct protein-protein interactions between the GRα and NF-ATc within the nuclear environment.

Overexpressed NF-ATc can restore promoter activity in the presence of Dex

If the GR was interfering with the ability of NF-ATc to transactivate the IL-4 promoter, we reasoned that overexpression of this factor would restore promoter inducibility in the presence of Dex. To test this hypothesis, Jurkat cells were cotransfected with an expression vector encoding the GR and full-length NF-ATc, and promoter activity was analyzed in calcium ionophore-activated cells treated with Dex. As shown in Fig. 8, overexpression of NF-ATc modestly but consistently increased the calcium-dependent activation of pLuc 372. However, note that under these conditions transcriptional activity was rendered resistant to inhibition by Dex in cells cotransfected with the GR.

Discussion

GC are known to interfere with the expression and/or function of several transcription factors (2). Based on the experiments described in this report, we conclude that calcium- and CN-dependent IL-4 promoter activity is inhibited by GC. This involves antagonism of NF-ATc-driven promoter activity by the GRα, which may alter the DNA-binding ability of NF-ATc via protein-protein interactions. The observation that NF-ATc itself is a target of the GR in T cells is a novel finding and was facilitated by the unique calcium inducibility of the IL-4 promoter. This allowed us to more precisely analyze transcriptional activation mediated by NF-AT without the concurrent activation of PKC-induced cofactors. Concurrent stimulation of calcium- and PKC-mediated pathways resulted in lower IL-4 promoter activity compared with calcium activation alone (Fig. 1), which we previously found was due to inhibition of NF-AT binding to the P1 element by PMA-induced NF-κB heterodimers (39). This observation suggested that inhibition of NF-κB by the GR is not responsible for the down-regulation of IL-4 promoter activity by Dex.

In conjunction with previous reports (40–42), we found that activated CN can efficiently substitute for the calcium-mediated signal necessary for IL-4 promoter activity. Unlike other potential downstream targets of CN that require concomitant activation with PMA, such as c-Jun N-terminal kinase (45) and IκB (44), NF-AT can be activated by CN alone (33). Importantly, CN-driven IL-4 promoter activity was efficiently inhibited by Dex in our experiments. Paliogianni et al. (64) reported that the phosphatase activity of CN was not inhibited by Dex in T cells. Consistent with this
Of the four known NF-AT family members, NF-ATp and NF-ATc are thought to predominate in mature T cells (62). Multiple isoforms of these factors have been identified, likely due to alternative splicing of common gene products (66, 67). In both peripheral blood T and Jurkat cells, NF-ATp is constitutively expressed (62, 68). Both the levels and DNA-binding ability of this factor decrease upon continued cell activation (62, 69). In contrast, the expression of NF-ATc is significantly increased by cell activation, and a calcium signal alone can achieve this effect (62). Like other Jurkat lines (62, 68), the cells used in our experiments express both NF-ATp and NF-ATc (Fig. 6, and data not shown). Our observation that the calcium-induced, Dex-sensitive nuclear complex forming on the P1 element in EMSA contained predominantly NF-ATc is likely due to the delayed activation of this factor in calcium-stimulated T cells (62).

Although the nuclear accumulation of NF-ATc was not significantly affected by Dex, its DNA-binding ability was strongly inhibited under these conditions (Figs. 4 and 7). The observation that the GR DBD was unable to displace the highly conserved NF-AT DBD from this region in EMSA (Fig. 5) suggests that this inhibition does not involve competitive DNA binding of the GRα. In contrast, our finding that NF-ATc and the GRα coprecipitated in nuclear extracts of Dex-treated and calcium-stimulated cells supports a model in which the GR interferes with NF-ATc binding to the IL-4 promoter via direct protein-protein interactions (Fig. 9). Current experiments are exploring this possibility. Because we did not detect evidence of interactions between the NF-AT DBD and either the GR DBD (Fig. 5B) or the full-length GR (data not shown) in EMSA, it is possible that interaction with the GRα involves other regions of NF-ATc.

NF-AT domains outside of the DBD are quite divergent, which likely explains the distinct phenotypes of mice rendered deficient for different NF-AT family members. These experiments demonstrated that both NF-ATp and NF-AT4 serve to down-regulate lymphoid responses and Th2 differentiation (34, 37), whereas NF-ATc is essential both during embryogenesis and for cytokine gene expression in Th2 cells (35, 36, 70). Our observation that NF-ATc
cytokines as primary targets. In Inhaled Glucocorticoids in Asthma, R. P. Schleimer, W. Busse, and P. O’Byrne, eds. Dekker, New York, pp. 203–238.