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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 cotransfected 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. The Journal of Immunology, 2000, 164: 825–832.

Glucocorticoids (GC)³ 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-kB) 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 been previously 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’-GGAAAA-3’) (33). The precise roles of individual NF-AT proteins in regulation of 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-AT 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 SacI 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 XbaI 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 IIL-2 1.25CAT 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 GRo expression vector (pRS hGRo, see Ref. 52) was a kind gift of Dr. Ron Evans (Salk Institute, La Jolla, CA). The plasmid ΔCaMAl 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 pREPA2-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 GC expression was identified on the basis of different IL-4 promoter constructs (26, 39), pLuc 372 was maximally expressed with a full-length promoter construct that contains the P1 NF-AT element as the site of an activation-induced and GC-inhibited nuclear protein complex that contains NF-ATc.

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 GC-deficient Jurkat T cells (see Materials and Methods). Fig. 1 shows the results of experiments using a full-length promoter construct that contains 327 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 (pRSSHGRo) in transient cotransfection experiments. As shown in Fig. 1, Dex strongly inhibited calcium-induced pLuc 372 activity in a GR-dependent manner.

Electrophoretic mobility shift assays

The following 30-bp oligonucleotides and their complements were synthesized: 5′-ATCTGGTGTAACGAAAATTTCCACTGTAAAAC-3′ (P1/OAP -92 to -60); 5′-TGGTAAAGGAAATTCTCCTAAATTTAC-3′ (P1/SOS -86 to -60); and 5′-ATGCCTGAACCCGAGGAAAATGATGTTTACATTG-3′ (P1/MAR -100 to -80). The canonical GRE oligonucleotide was purchased (Promega). The −35 to +65 promoter fragment used for competition EMSA was generated by gel purifying the HindIII-XhoI restriction fragment from pCR Script 35. Nuclear extracts were obtained from 5 × 10⁶ Jurkat cells using the method of Schreiber et al. (56). EMSAs were performed using 5 μg nuclear protein, 0.8 μg poly(dG-dC) (Pharmacia, Piscataway, NJ), and 5 pg [γ-32P]-end-labeled probe in a final volume of 10 μl. Free probes and protein-DNA complexes were resolved by 5% PAGE with 0.5 × Tris-borate-EDTA (TBE). In some experiments, extracts were incubated with 1 μl of the following antisera for 30 min at 4°C before addition of probe: 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

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 downregulate 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 further 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
inhibited the promoter in combination with PMA. Importantly, Dex did not inhibit pCAT 95 or other promoter constructs in control 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 nuclear 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 containing only the P0 element and downstream sequences (pLuc 65, see Materials and Methods). In these experiments, we found that pLuc 65 was un inducible by calcium ionophore in transiently transfected Jurkat T cells (0.95 ± 0.11-fold induction, n = 4, data not shown). These data are consistent with the observation that multiple P elements are needed for maximal promoter inducibility (24) and strongly suggest that the P1 element harbors a Dex-sensitive regulatory element.

In keeping with the uninducibility of pLuc 65, we found that the P0 element binds a constitutive, but not activation-induced, complex in EMSA (T.F.B., R.C., and S.N.G., unpublished observations). Therefore, we next studied the regulation of nuclear protein complex formation on the P1 element by Dex. Fig. 4 shows that a broad complex formed using nuclear extracts isolated from calcium 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 constitutively 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 supported 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 compete 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 containing 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 conditions (Fig. 6, lanes 6 and 10). Thus, the contiguous 134 bp downstream 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 activated 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 nondenaturing conditions (see Materials and Methods). As shown in Fig. 7A, multiple isoforms of NF-ATc were immunoprecipitated under these conditions. A predominant band of ~86 kDa was observed (Fig. 7A, band III) together with two more slowly migrating species corresponding to the ~110-kDa (Fig. 7A, band II) and ~140-kDa (Fig. 7A, band 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 by 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).
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-AT itself is a target of the current 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

FIGURE 5. The GR DBD interacts weakly with the P1 element, but is unable to displace the NF-AT DBD. A, A recombinant fragment of the GR DBD was incubated with radiolabeled oligonucleotides encompassing the P1 element (lane 1) or a canonical GRE (lane 3) and was analyzed by EMSA. The GRE supported both strong monomeric and dimeric complexes, but only a weak monomeric complex formed on the P1 oligonucleotide. A total of 100 ng/lane of the GR were used. B, A recombinant fragment of the NF-ATp DBD was incubated with a radiolabeled oligonucleotide encompassing the P1 element without (lane 1) or with the GR DBD (lanes 2–4) and was analyzed by EMSA. As expected, the P1 element readily supported NF-AT binding. Even extremely high concentrations of this factor were unable to displace the bound NF-AT DBD (lane 2). Concentrations of proteins used were ~500 ng/lane of the murine NF-ATp DBD and ~200/20 ng/lane of the rat GR DBD. C, The GR DBD (2 and 20 ng/lane) was incubated with radiolabeled oligonucleotides encompassing the P0 element or P1 elements as indicated and was analyzed by EMSA. The GR interacted weakly only with the P1 element.

FIGURE 6. The P1 element and downstream sequences are unable to compete for GR binding to a GRE. The ability of oligonucleotides containing the P1 element (P/OAP and P, see Materials and Methods) and a larger promoter fragment (~35 to +65, see Materials and Methods) to inhibit binding of the GR to a canonical GRE was determined by EMSA. In the absence of competitor oligonucleotides, the GRE readily supported monomeric and dimeric GR complexes (lane 2). Increasing molar concentrations of the GRE effectively blocked GR binding (lanes 3 and 7), but this was unaffected by up to 50-fold molar excess of both the P element oligonucleotides (lanes 4, 5, 8, and 9) and the larger promoter fragment (lanes 6 and 10).
Dex is lane 6 lane 5. A nonspecific band noted with the control Ab (with calcium ionophore for 4 h increased the nuclear accumulation of each multiple isoforms of NF-ATc were detected under these conditions. Treatment using enhanced chemiluminescence with an anti-NF-ATc Ab (7A6). Mucipitated proteins were analyzed by SDS-PAGE and Western blot analysis.

FIGURE 7. Expression of NF-AT proteins and the GR detected by immunoprecipitation. A, A11 cells were stimulated for 1 h (lane 1) or 4 h (lanes 2–6) with A23187 (0.5 μM, A), DMSO control ( — ), or Dex (1 μM, D) before extraction of nuclear proteins and immunoprecipitation under non-denaturing conditions (see Materials and Methods) with an anti-NF-ATc (αN), an anti-GR (αG), or control Abs (C) as indicated. Immunoprecipitated proteins were analyzed by SDS-PAGE and Western blot analysis using enhanced chemiluminescence with an anti-NF-ATc Ab (7A6). Multiple isoforms of NF-ATc were detected under these conditions. Treatment with calcium ionophore for 4 h increased the nuclear accumulation of each isoform (compare lanes 2 and 4), which was not significantly affected by Dex (lane 6). A nonspecific band noted with the control Ab (lane 5) is indicated by the asterisk. B, When the same membrane was stripped and analyzed for expression of the GR, a single band of ~94 kDa was detected in proteins immunoprecipitated by the GR Ab in Dex-treated extracts (lane 3), corresponding to the known expression of the GRα isoform in A11 cells. Note that the GRα also coprecipitated with NF-ATc in cells coincubated with A23187 and Dex (lane 6). Nonspecific bands immunoprecipitated with the control Ab (lane 5) are indicated by the asterisks.

observation, we found that Dex did not inhibit calcium ionophore-induced NF-ATc nuclear accumulation in A11 cells as determined by immunoprecipitation and Western blot analysis (Fig. 8) or by immunofluorescence (our unpublished observations). These data suggest that GC might directly interfere with NF-AT-dependent promoter transactivation. Studies to date of the effects of GC on NF-AT-driven IL-2 promoter activity have not reached a consensus. In one study, transcription driven by the IL-2 promoter-distal NF-AT site was partially inhibited by Dex in transfected Jurkat T cells (47). The same investigators also found that Dex inhibited the binding of nuclear factors from Jurkat and primary T cells to this element in EMSA (65). 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 in these studies. In contrast, other investigators found that the inhibition of IL-2 transcription by GC did not involve NF-AT (48, 49). Reasons for these discrepancies are currently unknown, but may be due to differences in experimental conditions or cell lines used.

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
is inhibited by GC is especially relevant in this regard. The molecular basis for these differential effects of NF-AT proteins are currently not well understood. Our observation that overexpressed NF-ATc increased calcium-driven IL-4 promoter activity is consistent with a direct role for this factor in enhancing IL-4 gene expression. The modest increase in transcriptional activation of NF-ATc-overexpressing cells may be due to the lack of posttranslational modification of NF-ATc or of heteromorphic cofactors in our system. Importantly, however, IL-4 promoter activity was rendered Dex-sensitive under these conditions.

In summary, we conclude that GC inhibit IL-4 gene expression by interfering directly with NF-ATc-driven promoter activity (Fig. 9). These observations help explain the potent inhibitory effects of GC on Th2-driven inflammatory responses such as allergic asthma. Because Dex and cyclosporin A appear to inhibit NF-AT by different mechanisms, it will be interesting to study possible synergistic effects of these agents on IL-4 expression in T cells. The expression of IL-4 in a subset of patients with steroid-resistant asthma is not sensitive to GC (71). Studying the molecular pathway described in this report in subjects with this syndrome should be insightful. Finally, our results also provide evidence for a novel transactivating effect of the GR. It will be interesting to determine whether repressive interactions are responsible for many of the transactivating effects of the GR. Finally, our results also provide evidence for a novel way described in this report in subjects with this syndrome should be insightful. Finally, our results also provide evidence for a novel transactivating effect of the GR. It will be interesting to determine whether repressive interactions are responsible for many of the transactivating effects of the GR.

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