Glucocorticoids inhibit IL-4 and mitogen-induced IL-4Rα chain expression by different posttranscriptional mechanisms

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Background: A high level of expression of IL-4Rα chain on the surface of lymphocytes has been described in certain allergic and inflammatory autoimmune diseases. Progression of these diseases are usually controlled by steroid treatment. One mechanism by which these drugs exert their antiinflammatory and immunosuppressive effects is by widely repressing or enhancing the production of cytokines and their receptors.
Objectives: The effect of glucocorticoids on IL-4Rα chain expression has not been previously studied, and this is the aim of the present report. For this purpose, human lymphocytes were induced to express IL-4Rα chain by means of protein kinase C (PKC) activation with phorbol myristate acetate (PMA) or by triggering the Janus kinase-Stat pathway with IL-4 in the presence or absence of pharmacologic doses of dexamethasone.
Methods: IL-4Rα cell surface expression was studied by flow cytofluorometry. The levels and stability of mRNA were assessed by Northern blot analysis. The effect of dexamethasone on the IL-4Rα rate of transcription was determined by nuclear run-on experiments.
Results: Dexamethasone significantly downregulated PMA-induced IL-4Rα mRNA and protein levels in total peripheral blood mononuclear cells and in isolated T cells. The mechanism involved a posttranscriptional regulation of IL-4Rα expression because dexamethasone decreased the PMA-induced IL-4Rα mRNA half-life. However, we found that PMA did not influence the transcription rate of IL-4Rα gene, irrespective of the presence or absence of dexamethasone. This immunosuppressor also diminished the IL-4–induced IL-4Rα expression on the surface of isolated T and B lymphocytes but, interestingly, without modifying mRNA levels that indicates that dexamethasone downregulated IL-4–dependent IL-4Rα expression by acting at a translational or posttranslational level. In fact, we observed that the drug did not affect IL-4–induced IL-4Rα gene transcription rate nor did it shorten mRNA half-life. The effect of dexamethasone on the IL-4Rα was steroid specific because it was totally reversed by the glucocorticoid receptor antagonist RU486.
Conclusion: Our results support that dexamethasone may influence the course of allergic and inflammatory diseases by downregulating the expression of IL-4Rα. (J Allergy Clin Immunol 1998;102:968-76.)

Key words: *Glucocorticoids, dexamethasone, IL-4Rα, phorbol myristate acetate, gene transcription, mRNA half-life, posttranscriptional regulation*

IL-4 is an immunoregulatory cytokine secreted by activated T lymphocytes and mast cells that exerts multiple biologic effects on B and T lymphocytes and on various other nonlymphoid cell types.[1] IL-4 promotes their activities by binding to specific receptors (IL-4R) on the surface of a great variety of responding cells. IL-4R are multichain complexes composed of a 140-kd high-affinity binding chain (IL-4Rα) in association with other accessory molecules. Two types of IL-4R have been described: type I contains the IL-4Rα subunit and the common γ (γ_c) chain,[2,3] whereas type II, which is also a functional receptor for IL-13, uses the IL-13 binding chain (IL-13Rα) as an IL-4Rα–associated polipeptide.[4,5] Binding of IL-4 to both receptors induces tyrosine phosphorylation of the transcription factor Stat6[6,7] and of the insulin receptor substrate 2 (IRS-2).[8,9] Activation of these substrates signals different metabolic pathways, which in turn result in different functional effects. Thus triggering of Stat6 induces the expression of IL-4–responsive genes, such as CD23, Iε, MHCII and IL-4Rα,[10] whereas activation of IRS-2 plays a major role on IL-4–mediated proliferation.[9]

Glucocorticoids are potent antiinflammatory and immunosuppressive agents that exert their function in part by modulating various mechanisms involved in cytokine expression. Glucocorticoids inhibit the expression of various proinflammatory cytokines such as IL-1, IL-2, IL-6, IL-8, IFN-γ, and TNF-α.[11-15] In addition, the expression of certain cytokine receptors is also affected by pharmacologic doses of glucocorticoids. Thus it has been described that these drugs enhance the binding of IFN-γ to human monocytes,[16] upregulate the expression of IL-6R in human hepatoma and epithelial cells,[17] and increase the levels of IL-1R on human polymorphonuclear leukocytes[18] and on human B cells but not on T lymphocytes and other mononuclear cells.[19] Contradictory results have been published on the effect of glucocorticoids on IL-2Rα chain expression. Whereas some authors have described a glucocorticoid-mediated upregulation of IL-

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Abbreviations used
ISR: Insulin receptor substrate
MFI: Mean fluorescence intensity
pBSK: PBluescriptII SK
PKC: Protein kinase C
PMA: Phorbol myristate acetate
T_{H2}: T-helper cells type 2

2Rα chain expression in T-cell lines,[20] other authors[12] have found no effect or even an inhibitory effect in peripheral blood mononuclear cells (PBMCs).[21]

An abnormal regulation of IL-4 has been found to be associated with certain diseases dominated by T_{H2}-mediated immunoresponse, such as allergic reactions,[22] autoimmune diseases,[23] and parasitic infections.[24] Accordingly, an elevated expression of IL-4Rα has been described in T and B cells from patients with rheumatoid arthritis[23] and in PBMCs from subjects with atopic dermatitis.[25] Furthermore, the signal provided by IL-4Rα is responsible for the induction of germ line Cε transcripts that leads to an enhanced IgE production.[26] Recently it has been found that a polymorphism for the IL-4Rα subunit segregates with atopy and that this mutation may predispose to allergic diseases by altering the signaling function of the receptor.[27] Progression of inflammatory and allergic processes are usually controlled by steroid therapy. One mechanism by which steroids may control these diseases may be by regulating IL-4Rα expression, a hypothesis that has not been previously studied. We have found that dexamethasone diminishes IL-4Rα mRNA and protein expression in phorbol myristate acetate (PMA)-stimulated T cells by affecting a posttranscriptional mechanism that involves mRNA destabilization. Similarly, IL-4-dependent IL-4Rα expression was also downregulated by dexamethasone in both T and B lymphocytes but, in this case, by acting at a translational or posttranslational level.

METHODS

Reagents

Human recombinant IL-4 (rIL-4) was purchased from Genzyme Corp (Cambridge, Mass). PMA and dexamethasone were from Sigma Chemical Co (St Louis, Mo). PMA was dissolved in dimethyl sulfoxide, and dexamethasone was water soluble. The glucocorticoid antagonist RU486 was provided by Roussel Uclaf (Romainville, France) and was dissolved in ethanol. PMA and RU486 were diluted in culture medium, and the final concentration of dimethyl sulfoxide and ethanol in cultures was less than 0.01%. Actinomycin D was obtained from Sigma Chemical Co. Mouse anti-human IL-4Rα mAb (M57) was a gift from Dr S. Gillis from Immunex Corp (Seattle, Wash). FITC-F(ab)′$_2$ goat antimouse IgG antibody was obtained from Tagoimmunologicals (Camarillo, Calif).

Cell preparation and culture

Cells were obtained from peripheral blood by venipuncture of healthy volunteers and from children with chronic tonsillitis. PBMCs were prepared by Ficoll-Hypaque density centrifugation. Tonsils were dispersed into single cell suspensions, washed, and resuspended in RPMI 1640 medium with antibiotics. B cells were purified from tonsils by removing T cells that were rosetted with aminoethylisothiouronium-treated sheep red blood cells. Unrosetted cells in the interphase were collected and washed. The B-cell population obtained was typically more than 98% surface $CD19^+$ and less than 1% $CD3^+$, as estimated by flow cytometry. T cells were obtained from the rosetted cells by sheep red blood cell lysis. Contaminating B cells were eliminated by cytotoxic treatment with Lymphokwik-T (One Lambda Co, Los Angeles, Calif). When required, magnetic-bead depletion with anti-CD19–coated Dynabeads (Dynal, Oslo, Norway) was also used to eliminate residual B cells. The T-cell population contained less than 1% of $CD19^+$ cells and was more than 98% positive for CD3.

Cell cultures of either PBMCs or T and B cells were carried out at a concentration of 2×10^6/mL in RPMI 1640 medium containing 2 mmol/L L-glutamine and Hepes (Bio Whittaker, Verviers, Belgium) and supplemented with 10% heat-inactivated FCS (ICN Flow, Costa Mesa, Calif) and antibiotics. Cells were incubated at 37°C and 5% carbon dioxide, with the reagents added to the concentration indicated in each experiment. After the incubation period, cells were collected for RNA extraction or binding studies as indicated later.

Northern blot analysis

Total cellular RNA was isolated at different incubation periods by acid guanidinium thiocyanate-phenol-chloroform extraction.[28] Ten-microgram RNA/samples were denatured at 65°C for 15 minutes in an electrophoresis buffer (20 mmol/L MOPS, 5 mmol/L sodium acetate, 1 mmol/L EDTA; pH 7.0) containing 5% formaldehyde and 50% formamide and ethidium bromide at 30 μg/mL. RNA was then size-fractionated by electrophoresis through 1.2% agarose gels containing 2% formaldehyde. RNA integrity and equivalent amounts of total RNA/gel were assessed by monitoring 28S and 18S under ultraviolet irradiation. RNA was capillary transferred to nylon membranes (Zetaprobe; BioRad, Hercules, Calif) and fixed by baking for 2 hours at 80°C.

The probes used were a 830–base pair *Not1-Cla1* insert of a IL-4Rα cDNA cloned in pBluescriptII SK (pBSK; provided by Dr P. Beckmann, Immunex Corp, Seattle, Wash) and an 800–base pair *Pst1- Xba1* insert of a GAPDH cDNA (ATTC, Rockville, Md) subcloned into pBSK. The DNA was labeled with [α-^{32}P] deoxycytidine triphosphate (3000 Ci/mmol; ICN) by the random hexamer priming method (Stratagene, La Jolla, Calif). Hybridization was performed at 42°C for 42 hours in 50% deionized formamide, 5 × Denhardt's solution, 5 × sodium saline phosphate EDTA buffer (SSPE), 1% SDS, 5% Dextran sulfate, and 100 μg/mL salmon sperm DNA. The membranes were rinsed and washed 3 times with 0.2 × sodium saline citrate buffer (SSC) and 0.5% SDS for 30 minutes at 65°C and exposed to radiograph film at –70°C. Bands on the autoradiograms were integrated by scanning densitometry with the Bio Image Intelligent Quantifier program (Ann Arbor, Mich), and values were normalized as a percentage of the control GAPDH signal.

Nuclear run-on transcription assay

Transcription of the IL-4Rα gene was measured by the nuclear run-on assay as described elsewhere.[29] Briefly, 10^8 PBMCs were lysed for 5 minutes in 4 mL of ice-cold 10 mmol/L Tris-HCl, pH 7.5, 3 mmol/L $MgCl_2$, 10 mmol/L NaCl, and 0.5% NP-40. The lysate was centrifuged at 500*g* for 5 minutes; the nuclear pellet was washed once with lysis buffer, recentrifuged, and resuspended in 100 μL glycerol-buffer (50 mmol/L Tris-HCl pH 8.0, 40% glycerol,

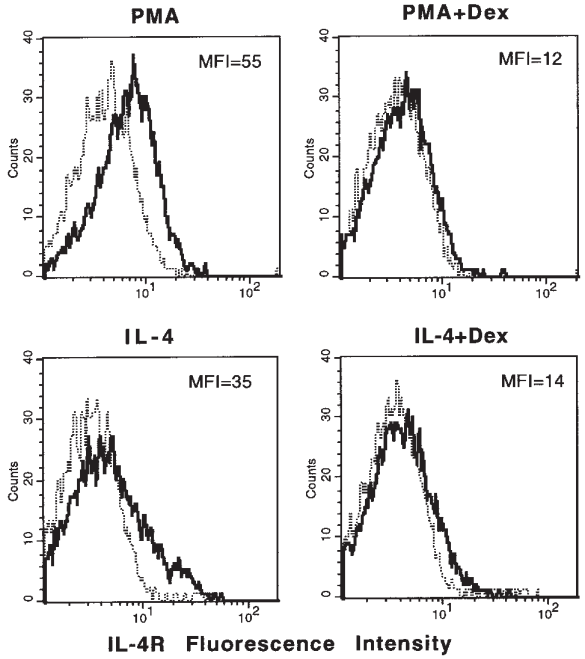

FIG 1. Downregulation of IL-4Rα cell surface expression by dexamethasone *(Dex)*. PBMCs (2×10^6 cells/mL) were cultured for 24 hours with PMA (10 ng/mL) or IL-4 (100 U/mL) in the presence or absence of dexamethasone (10^{-7} mol/L). IL-4Rα expression was analyzed by flow cytometry with the M57 mAb (*solid lines*). MFI on a logarithmic scale was calculated by substracting the background fluorescence signal (*dotted lines*). Profiles from a single experiment are shown as representative of 5 similar experiments.

5 mmol/L $MgCl_2$, 0.1 mmol/L EDTA). For nuclear run-on analysis, a mixture containing the nuclei and 80 μL transcription buffer (12.5 mmol/L Tris-HCl, pH 8.0, 6 mmol/L $MgCl_2$, 125 mmol/L KCl, 2 mmol/L dithiothreitol, 1 mmol/L each of ATP, CTP, and GTP) was incubated at 26°C for 20 minutes in the presence of 125 μCi [α-^{32}P] UTP (3000 Ci/mmol; ICN). The reaction was terminated by incubation at 37°C for 20 minutes with 200 μL stop-buffer (10 mmol/L Tris-HCl, pH 7.4, 0.5 mmol/L NaCl, 50 mmol/L $MgCl_2$, 2 mmol/L $CaCl_2$), 40 U DNAse I, and 150 U RNAsin. Then a proteinase K digestion (200 μg/mL) in 1% SDS was performed at 37°C for 45 minutes. The samples were phenol/chloroform–extracted and precipitated twice in 2.5 mol/L ammonium acetate. Then the isolated nuclear RNA was resuspended in 50% formamide. Equal numbers of counts per minute of ^{32}P-RNAs were hybridized for 24 hours on nylon filters slot-blotted with 5 μg of linearized plasmids. The plasmids were pBSK alone, as a negative control, or containing the cDNAs for IL-4Rα, TNF-α, and GAPDH, washing and quantification were performed as described earlier.

Cytofluorometric analysis of IL-4Rα expression

For binding studies, 500,000 cells resuspended in 100 μL of PBS were incubated for 30 minutes at 4°C with 0.5 μg of mouse anti-human IL-4Rα mAb M57 or with an isotype matched mouse anti-keyhole limpet hemocyanin as an irrelevant control antibody. The cells were washed 3 times and incubated for another 30 minutes with FITC-goat antimouse IgG antibody. After the second staining period, cells were washed 3 times, resuspended in PBS, and analyzed on a cytofluorograph FACScan (Becton Dickinson, San Jose Calif). Dead cells were excluded from the analysis by staining with propidium iodide (5 μg/mL; Becton Dickinson), and 5000 cells were then analyzed with a logarithmic scale. The specific fluorescence intensity was quantified as median fluorescence intensity (MFI) and calculated by subtracting the background fluorescence signal.

TABLE I. Dexamethasone-mediated downregulation of IL-4Rα protein expression induced by PMA

	IL-4Rα MFI		
	24 h	48 h	72 h
PBMCs			
Medium	7.00 ± 2.16	16.00 ± 2.16	14.33 ± 6.18
PMA	24.00 ± 6.24	28.00 ± 4.35	36.33 ± 15.04
PMA + DEX	6.00 ± 1.73*	15.66 ± 0.57*	9.33 ± 3.51
T cells			
Medium	5.66 ± 4.18	7.00 ± 4.30	
PMA	30.00 ± 4.35	24.33 ± 4.50	
PMA + DEX	12.33 ± 9.01*	10.33 ± 2.08†	

Cells were cultured at 2×10^6 cells/mL in medium alone or with PMA (10 ng/mL) in the presence or absence of dexamethasone *(DEX)* (10^{-7} mol/L), and the IL-4Rα expression was analyzed by flow cytometry with the M57 mAb. Results from 3 experiments are expressed as mean ± SD of MFI. Significant differences between cultures incubated in the absence and presence of dexamethasone are indicated.
*$P < .05$.
†$P < .01$.

Statistical analysis

The Kolmogorov-Smirnov test was used to verify the normal distribution of the data. Then, paired data obtained in the absence and in the presence of dexamethasone were analyzed with the Student *t* test (parametric distribution) from SPSS (SPSS, Inc, Chicago, Ill) statistical program . A *P* value of less than .05 was considered a significant difference between groups.

RESULTS

Decreased IL-4Rα protein expression by dexamethasone

The effect of dexamethasone on the IL-4Rα expression induced by means of protein kinase C (PKC) by PMA was analyzed by flow cytometry with the IL-4Rα–specific mAb M57 in both freshly isolated PBMCs and in highly purified T cells (Table I, Fig 1). When PBMCs were activated with PMA (10 ng/mL) in the presence of pharmacologic doses of dexamethasone (10^{-7} mol/L), a statistically significant downregulation of the induced IL-4Rα protein levels was observed at 24 and 48 hours. This inhibitory effect of dexamethasone was also seen at 72 hours, being, in this case, almost statistically significant. Similar significant results were found in isolated T cells that indicated that the diminished IL-4Rα-specific MFI observed in PMA-treated PBMCs was due to a direct effect of dexamethasone in activated T cells. The dexamethasone-induced downregulation of IL-4Rα chain in PMA-activated PBMCs was dose dependent as shown in Fig 2. As may be observed, pharmacologic doses of dexamethasone (10^{-6} to 10^{-7} mol/L) reduced the IL-4Rα protein expression by approximately 60%.

Next, we studied the effect of dexamethasone on IL-4–mediated IL-4Rα expression. For this purpose,

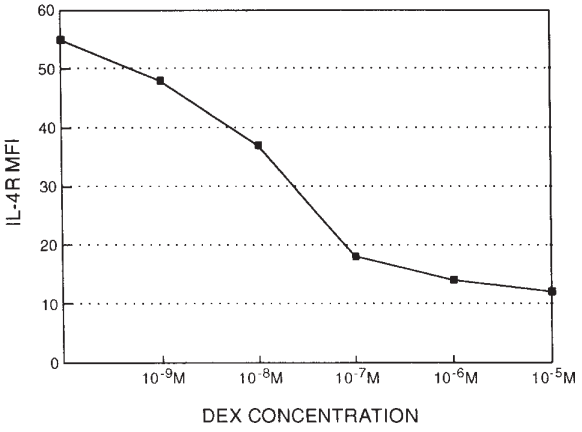

FIG 2. Dose-dependent downregulation by dexamethasone *(Dex)* of IL-4Rα cell surface expression. PBMCs (2×10^6 cells/mL) were cultured in the presence of PMA (10 ng/mL) and various concentrations of dexamethasone. After 24 hours of culture, IL-4Rα MFI was measured by flow cytometry with the M57 mAb. Results shown are representative of 3 experiments.

TABLE II. Dexamethasone-mediated downregulation of IL-4Rα protein expression induced by IL-4

	IL-4R MFI		
	PBMCs	**T Cells**	**B Cells**
Medium	5.40 ± 2.33	3.33 ± 1.24	29.33 ± 12.11
IL-4	26.40 ± 6.91	26.33 ± 3.05	47.66 ± 10.59
IL-4 + DEX	11.70 ± 3.36*	17.00 ± 5.29*	29.66 ± 10.69†

Cells were cultured at 2×10^6 cells/mL in medium alone or with IL-4 (100 U/mL) in the presence or absence of dexamethasone *(DEX)* (10^{-7} mol/L), and the IL-4Rα expression was analyzed by flow cytometry with the M57 mAb. Results from 3 experiments are expressed as mean ± SD of MFI. Significant differences between cultures incubated in the absence and presence of dexamethasone are indicated.
*$P < .05$.
†$P < .01$.

PBMCs and purified T and B lymphocytes were cultured for 24 hours in the presence of 100 U/mL of IL-4 with or without dexamethasone at a concentration of 10^{-7} mol/L. As shown in Table II and Fig 1, dexamethasone also diminished the synthesis of the surface IL-4Rα protein in all cell populations studied because the MFI was significantly lower in cultures where this drug was added.

This downregulation of IL-4Rα cell surface expression is not due to a deleterious effect of dexamethasone on cell viability because we analyzed only viable cells selected by propidium iodide exclusion, and in our cell culture conditions, glucocorticoids did not induce apoptosis as assessed by DNA fragmentation analysis (data not shown).

Differential effect of dexamethasone on the IL-4Rα mRNA levels induced by PMA or IL-4

To determine whether dexamethasone exerts its inhibitory effect on induced IL-4Rα protein expression by modulating mRNA levels, we used the Northern blot analysis to quantify the specific IL-4Rα mRNA in cells cultured for 12 hours with PMA (10 ng/mL; Fig 3) or IL-4 (100 U/mL; Fig 4) in the presence or absence of dexamethasone (10^{-7} mol/L). The hybridization signal was measured by optical scanning, and the values were normalized to those obtained by hybridization with a constitutive GAPDH probe. When PBMCs and T cells were stimulated with PMA, a significantly diminished amount of IL-4Rα transcript levels was obtained in the cultures where dexamethasone was present (Fig 3), indicating that dexamethasone downmodulated the PKC-mediated IL-4R protein synthesis by decreasing IL-4Rα mRNA expression. In contrast, as shown in Fig 4, the IL-4Rα mRNA levels induced by IL-4 in PBMC and T and B cells were not affected by dexamethasone. Similarly, dexamethasone did not diminish the IL-4Rα mRNA levels in B cells after 12 hours of culture in medium alone (data not shown). These observations indicate that dexamethasone exerts its inhibitory effect on IL-4Rα protein expression by different mechanisms, which may depend on the cell activation signals.

Effect of dexamethasone on the rate of IL-4Rα gene transcription and on the mRNA stability

We analyzed whether the dexamethasone-induced inhibitory effect on IL-4Rα expression involved transcriptional or posttranscriptional mechanisms. For this purpose, we performed nuclear run-on transcription assays in PBMCs and T cells cultured with PMA (10 ng/mL) and with IL-4 (100 U/mL) in the presence or absence of dexamethasone (10^{-7} mol/L). The nuclei were isolated after 2, 4, 8, and 12 hours in PBMC cultures and after 8 hours in T-cell cultures. We found that activation by means of PKC with PMA did not increase the IL-4Rα baseline transcription rate in both populations at any time studied, irrespective of the presence or absence of dexamethasone. Under the same experimental conditions, PMA was able to significantly increase the transcription rate of TNF-α gene, returning to baseline levels on the addition of dexamethasone (Fig 5, *A*). In contrast, IL-4 increased by approximately 8-fold the transcription rate of the IL-4Rα gene at 8 hours of culture, and it was not modified by adding dexamethasone (Fig 5, *B*).

We also considered the possibility that dexamethasone modified a PMA-dependent increased IL-4Rα mRNA stability. To this end, PBMCs were cultured in medium alone or in the presence of PMA (10 ng/mL) for 8 hours, then actinomycin D (5 μg/mL) was added and the mRNA was extracted after additional periods of time. The IL-4Rα mRNA half-life was calculated by plotting the normalized hybridization value versus time decay in minutes. Our results indicate that PMA upregulated the IL-4Rα mRNA expression by prolonging the mRNA half-life from 45 minutes in medium alone to 150 minutes in the presence of PMA (Fig 6, *A* and *B*). When dexamethasone was added to the cell cultures simultaneously with actimomycin D, the IL-4Rα mRNA half-life decayed to levels similar to those obtained in medium alone (approximately 45 minutes; Fig

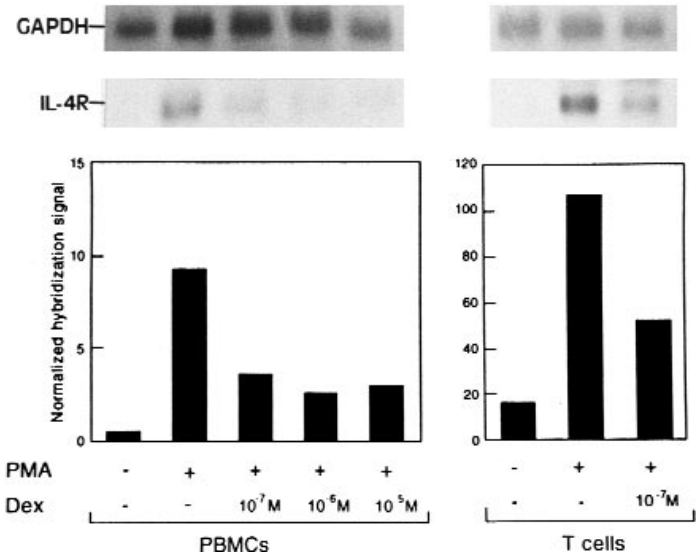

FIG 3. Dexamethasone *(Dex)*-mediated inhibition of IL-4Rα mRNA expression induced by PMA. PBMCs and highly purified T cells were cultured at 2×10^6 cells/mL and stimulated with PMA (10 ng/mL) in the presence or absence of various doses of dexamethasone. After 12 hours of culture, Northern blot analysis was performed by hybridization with an IL-4Rα probe and, subsequently, with a GAPDH probe as a constitutive standard *(upper panel)*. The IL-4Rα hybridization signal was integrated by scanning densitometry and normalized as a percentage of the GAPDH signal *(lower panel)*. Results shown are representative of 2 separate experiments.

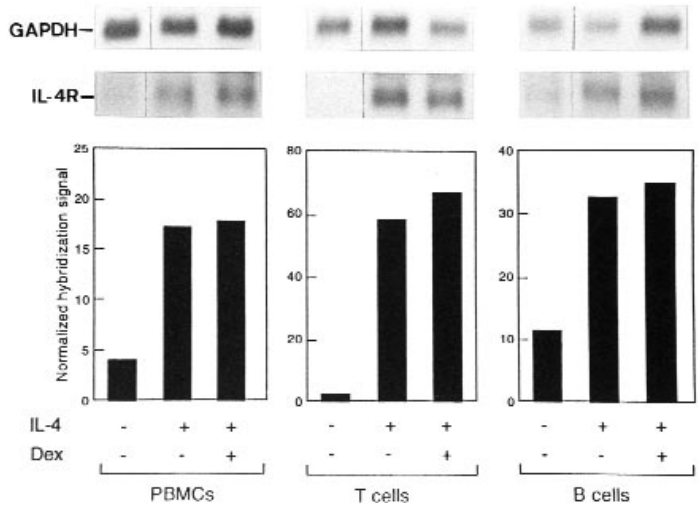

FIG 4. Dexamethasone *(Dex)* does not affect IL-4Rα mRNA expression induced by IL-4. PBMCs and highly purified T and B cells were cultured at 2×10^6 cells/mL with IL-4 (100 U/mL) in the presence or absence of dexamethasone (10^{-7} mol/L). After 12 hours of culture, Northern blot analysis was performed *(upper panel)* and the IL-4Rα hybridization signal was normalized *(lower panel)* as indicated in Fig 3. Results shown are representative of 2 separate experiments.

6, *C*), indicating that dexamethasone reduces the PMA-inducible IL-4Rα levels by altering the mRNA stability.

IL-4 also prolonged the IL-4Rα mRNA half-life (120 minutes) as compared with that obtained in medium alone (Fig 6, *D*). However, in this case the addition of dexamethasone to the cell cultures did not affect the mRNA half-life (110 minutes; Fig 6, *E*). This result was expected because dexamethasone did not modify the IL-4Rα transcript levels induced by IL-4 (Fig 4).

Inhibition of IL-4Rα expression by dexamethasone is steroid specific

To further support the specific effect of dexamethasone in lowering the IL-4Rα levels, the glucocorticoid receptor antagonist RU486 was used. This drug antagonizes the glucocorticoid function by competing for binding to the glucocorticoid receptor. PBMCs were cultured with PMA at 10 ng/mL alone or with dexamethasone

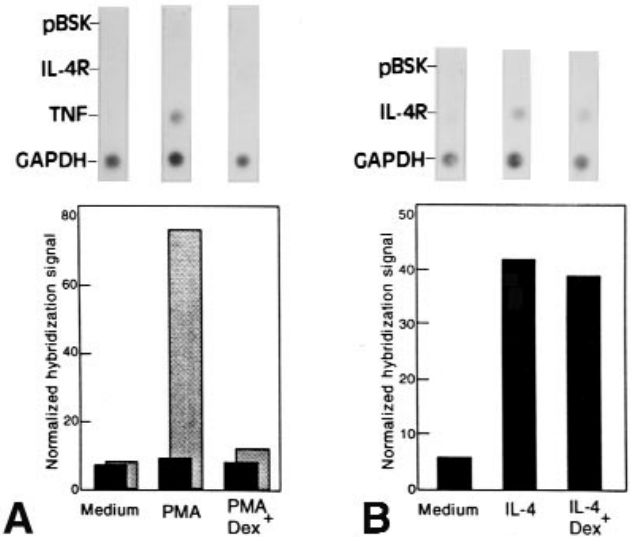

FIG 5. Effect of dexamethasone *(Dex)* on IL-4Rα gene transcription rate. 10^8 PBMCs (2×10^6 cells/mL) were stimulated for 8 hours (**A**) with PMA (10 ng/mL) or (**B**) IL-4 (100 U/mL) in the presence or absence of dexamethasone (10^{-7} mol/L). Cultures in medium alone were established as a control for the basal transcription rate. After nuclear run-on assays were performed, the hybridization signal obtained for the IL-4Rα mRNA (*solid bars*) and TNF-γ (*dotted bars*) was normalized by scanning densitometry to that obtained for the GAPDH mRNA. Bluescript plasmid (*pBSK*) was used as an internal control. Similar results were obtained in PBMCs cultured for 2, 4, and 12 hours and in T cells cultured for 8 hours.

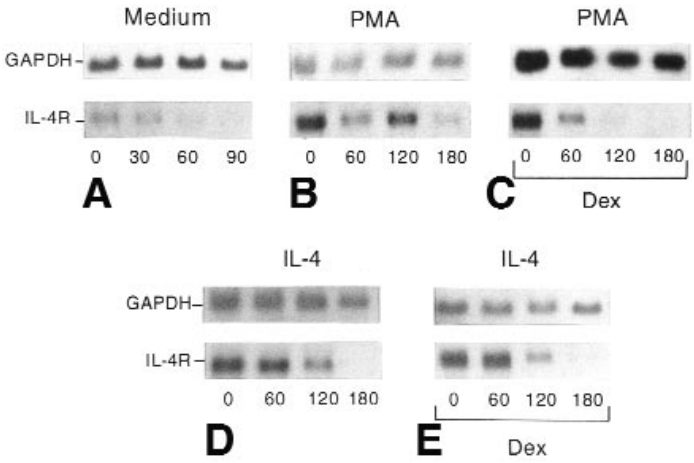

FIG 6. Effect of dexamethasone *(Dex)* on IL-4Rα mRNA half-life. PBMCs (2×10^6 cells/mL) were cultured in medium alone and stimulated with PMA (10 ng/mL) or IL-4 (100 U/mL). After 8 hours of culture, actinomycin D (5 μg/mL) was added to halt further transcription, and dexamethasone was added simultaneously where indicated. Northern blot analysis was performed after additional periods of culture (time indicated in minutes), and the IL-4Rα hybridization signal was normalized as indicated in Fig 3. The IL-4Rα mRNA half-life was 45 minutes in medium alone (**A**), increased to 150 minutes with PMA (**B**), and decayed to 45 minutes in the presence of dexamethasone (**C**). When the cells were incubated with IL-4 in the presence or absence of dexamethasone (**D**, **E**), the mRNA half-life was prolonged to 120 and 110 minutes, respectively. Results from 1 of 2 similar experiments are shown.

(10^{-7} mol/L) in the presence or absence of RU486 at 10^{-5} and 10^{-6} mol/L. The total RNA was extracted at 12 hours, and the IL-4Rα–specific signal was analyzed by Northern blot. As expected, the hybridization signal obtained in the culture with dexamethasone was lower than in its absence, and this inhibitory effect was reversed by RU486 (Fig 7). Next, we investigated whether RU486 antagonized the action of dexamethasone on IL-4Rα surface expression in PBMCs cultured for 24 hours with PMA (10 ng/mL) or IL-4 (100 U/mL) with or without 10^{-7} mol/L of dexamethasone. Various concentrations of RU486, ranging from 10^{-5} to 10^{-8} mol/L, were added at the initiation of cultures. As shown in Fig 8, the IL-4Rα MFI returned to dexamethasone-untreated levels in both

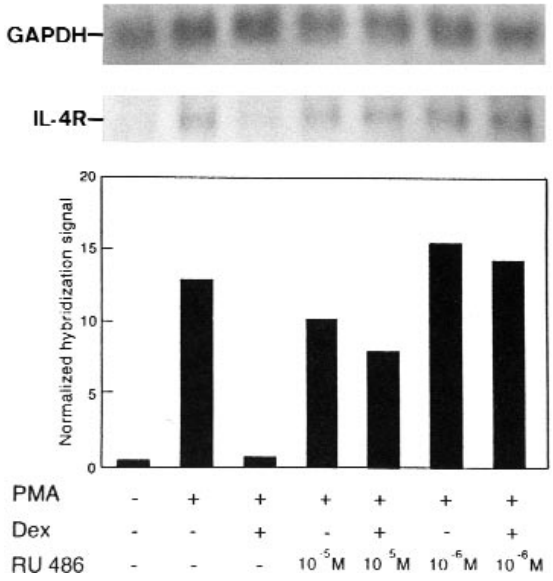

FIG 7. Effect of the glucocorticoid antagonist RU486 on the dexamethasone *(Dex)*-mediated downregulation of the IL-4Rα mRNA expression induced by PMA. PBMCs (2×10^6 cells/mL) were stimulated with PMA (10 ng/mL) and dexamethasone (10^{-7} mol/L) in the presence of RU486 at different concentrations. After 12 hours of culture, a Northern blot was performed *(upper panel)* and the IL-4Rα hybridization signal was normalized *(lower panel)* as indicated in Fig 3. Results shown are representative of 2 experiments.

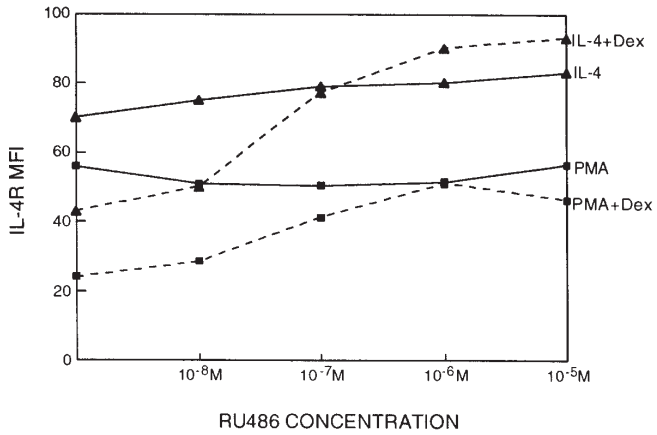

FIG 8. Effect of the glucocorticoid antagonist RU486 on the dexamethasone *(Dex)*-mediated downregulation of IL-4Rα cell surface expression induced by PMA and IL-4. PBMCs (2×10^6 cells/mL) were treated with PMA (10 ng/mL) or IL-4 (100 U/mL) in the presence or absence of dexamethasone (10^{-7} mol/L). Simultaneously, RU486 was added to the cultures at different concentrations. After 24 hours, IL-4Rα MFI was analyzed with the M57 mAb. Results are representative of 3 experiments.

PMA- and IL-4–activated cells treated with RU486. This reagent almost completely abolished the inhibitory effect of dexamethasone at concentrations as low as 10^{-7} mol/L. The dose dependency indicates that the effect was not caused by direct toxicity of RU486.

DISCUSSION

Regulation of immunoresponses by glucocorticoids is widely due to their capacity to enhance or repress the production of cytokines and their receptors. In this study a dexamethasone-mediated downregulation of PMA- or IL-4-induced IL-4Rα expression is described, and evidence is also shown that the glucocorticoid modulates IL-4Rα levels by different regulatory mechanisms, depending on the nature of the activation signal.

Pharmacologic doses of dexamethasone clearly exerted a downmodulatory effect on PMA-induced IL-4Rα expression, decreasing both the protein and mRNA levels. Dexamethasone diminished IL-4Rα mRNA levels by altering mRNA stability, because in the presence of dex-

amethasone rapid degradation of the accumulated IL-4Rα mRNA occurred. The mechanism by which dexamethasone decreased IL-4Rα mRNA stability is not known. Instability of mammalian mRNA encoding oncoproteins and lymphokines has been largely related to the presence of adenosine and uridine (AU)–rich sequences in the 3′ untranslated region. Such sequences may be the target of nucleases that, on binding to these nucleotidic regions, induce an accelerated mRNA degradation. Although glucocorticoids may promote mRNA destabilization by activating the synthesis of such ribonucleases, as occurs in other systems,[30] we do not think that this is the mechanism by which dexamethasone destabilizes the PMA-induced IL-4Rα mRNA because AU-rich sequences have not been described in the noncoding region of the IL-4Rα message.[31] It is well known that the presence of other sequences in the 3′ and 5′ untranslated region may also influence mRNA stability. Thus it has been shown that glucocorticoids induce an accelerated degradation of IL-1β mRNA by a mechanism independent of the presence of $A(U)_nA$ sequences,[32] probably by inhibiting or inducing the transcription of proteins that exert a selective effect on mRNA stability. In fact, protein synthesis was shown to be required for the glucocorticoid-mediated stabilization of beta-casein mRNA.[33]

We have also shown by run-on assay that PMA, independent of the presence or absence of dexamethasone, did not modify the IL-4Rα gene transcription rate in PBMC and in T cells. In contrast to this observation, a previously published report showed that PKC activation induced an increased rate of IL-4Rα gene transcription.[29] The reason for this discrepancy is unknown, but a loss of transcriptional activity of PMA in our cell culture conditions could be excluded because the TNF-α gene transcription rate is enhanced by approximately 7-fold in the presence of this phorbol ester. Similar to our finding, it has been found that this mitogen increases the expression of other molecules, such as ICAM-1, IFN-γ, and CD40L,[34-36] by stabilizing mRNA levels rather than by increasing the transcription rate.

In agreement with previous publications,[29,37] we have also observed that cell activation by IL-4 led to an accumulation of IL-4Rα mRNA in both T and B lymphocytes, which was due to an increased gene transcription rate and to a prolonged mRNA half-life.[29] Addition of dexamethasone downmodulated the IL-4–dependent IL-4Rα protein expression in T and B cells without altering IL-4–induced IL-4Rα mRNA levels, which indicates that dexamethasone did not affect IL-4–dependent induction of IL-4Rα gene transcription nor mRNA stability as we have confirmed by subsequent studies. Because activation of the transcription factor Stat6 is essential to promote the transcription of IL-4Rα gene,[10] our results indicate that dexamethasone did not affect activation of Stat6. Rather, it seems that this drug probably modulates the IL-4–dependent IL-4Rα protein levels by regulating certain events, such as the rate of translation, the proteolytic processing, and the intracellular protein trafficking, involved in the translational and posttranslational steps leading to protein synthesis and cell surface expression. In fact, these are the mechanisms by which dexamethasone downregulated the production of IL-1β and of TNF induced by LPS.[11,38]

The downmodulation of the PMA or IL-4–induced IL-4Rα expression by dexamethasone was steroid specific and required the presence of an intact glucocorticoid receptor because this effect was totally reversed at both, protein and mRNA levels, by the glucocorticoid-receptor antagonist, RU486. Because the dexamethasone-induced inhibition of IL-4Rα expression occurred at a posttranscriptional level and an intact glucocorticoid receptor was necessary, it is plausible that the decreased IL-4Rα expression may be secondary to a glucocorticoid-induced inhibition of the transcription of genes that regulate mRNA stability, the rate of translation, or other posttranslational functions. In fact, it has been described that glucocorticoids may inhibit the transcription of important regulatory genes by functionally interfering with the PMA-activated transcription factors AP-1 and NFκB.[39-41]

Similarly to our findings, it has been reported that dexamethasone decreases the activity of the IL-2Rα chain gene in PHA- and PMA-activated PBMCs.[12] The authors attributed this phenomenon to an indirect effect resulting from the inhibition of IL-2 production induced by the steroid. However, in another report, dexamethasone inhibited IL-2Rα chain expression in PMA blasts in the presence of exogenous IL-2.[42] The possibility that glucocorticoids downregulate IL-4Rα expression by inhibiting the PMA-induced production of IL-4 was excluded because PMA alone did not deliver sufficient intracellular signals to induce IL-4 production.[43] Moreover, we were unable to detect IL-4 in the culture supernatant of activated cells by a highly sensitive enzymoimmunoassay (data not shown). In pathologic processes characterized by an in vivo overstimulation of the IL-4–IL-4R pathways, this inhibitory effect of dexamethasone on the IL-4–induced IL-4R cell surface expression may have physiologic relevance. In fact, the IL-4–mediated upregulation of IL-4Rα levels was almost completely abrogated by dexamethasone in B cells. By decreasing IL-4Rα levels, glucocorticoids may indirectly control other IL-4–induced functions. For example, the downregulation by dexamethasone of IL-4–dependent CD23 expression in B cells[44] may be executed by decreasing the amount of surface IL-4Rα. Consistent with our findings, the inability of IL-4 to enhance the specific IL-4Rα fluorescence intensity in rat T_{H2} cells treated with dexamethasone has also been reported.[45] We have also observed a tendency towards a decreased IL-4Rα expression in B cells from patients with rheumatoid arthritis who are undergoing steroid treatment.[23] Further studies will help to elucidate the implication of the present findings in the outcome of pathologic processes.

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