EXPERIMENTAL GENETICS

Dexamethasone Suppresses Human Interleukin-5 Gene Promoter

V. A. Mordvinov, C. C. Kok*, E. Arthaningtyas*, G. T. F. Schwenger*, A. Cristow*, C. J. Sanderson*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 7, pp. 91-94, July, 2005
Original article submitted April 1, 2005

Synthetic glucocorticoid dexamethasone suppressed interleukin-5 gene expression in PER-117 human T cells at the level of transcription. The conserved lymphokine element 0 in the interleukin-5 gene promoter context served as a target for dexamethasone.

Key Words: *interleukin-5; cell activation; dexamethasone; promoter*

The T cell-derived cytokine interleukin-5 (IL-5) regulates proliferation, differentiation, and function of eosinophils. It is mainly produced by reactive T helper 2 cells that play a role in the pathogenesis of allergic diseases accompanied by eosinophilia [14].

Glucocorticoids are effective drugs for the therapy of these diseases. These preparations decrease IL-5 concentration in the lungs and peripheral blood of patients [1,3]. Despite extensive use of glucocorticoids in the therapy of allergic diseases, the mechanism of their effect on IL-5 gene expression remains unknown.

Here we studied the mechanism underlying the effects of synthetic glucocorticoid dexamethasone on human IL-5 gene expression. Experiments were performed on PER-117 human T cells characterized by inducible production of several cytokines, including functionally active IL-5 [6].

Our results indicate that dexamethasone suppresses transcription of IL-5, which depends on conditions of cell activation.

Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences, Novosibirsk; *School of Biomedical Sciences, Curtin University and Western Australian Institute for Medical Research, Pert, Australia. ***Address for correspondence:*** mordvin@bionet.nsc.ru. V. A. Mordvinov

MATERIALS AND METHODS

PER-117 cells were grown as described elsewhere [6]. The cultures were activated with 10 ng/ml phorbol-12-myristate-13-acetate (PMA, Sigma), 1 mM cAMP (Sigma), 0.25 μM calcium ionophore (CaI, A23187, Sigma), and 0.2 μg/ml α-Leu-CD28 (αCD28, Becton Dickinson). Functionally active IL-5 was assayed by means of ELVA [2]. The cells were treated with dexamethasone immediately before activation.

RNA was isolated using RNeasy® Mini Kit (Qiagen) and treated with RQ1 RNase-free Dnase (Promega). Complementary RNA for on-line polymerase chain reaction (PCR) was synthesized using AMV reverse transcriptase (Promega) according to manufacturer's protocol.

On-line PCR was performed using LightCycler (Roche Diagnostics Australia). The reaction mixture contained 2 μl cDNA, 0.5 μM primers, 800 μM deoxynucleotide triphosphates, 1.5 mM $MgCl_2$ (for IL-5) or 2.5 mM $MgCl_2$ (for glyceraldehyde-3-phosphate dehydrogenase, GAPDH), 1.25 mg/ml bovine serum albumin, 0.5x SYBR green (Sigma), and 1 U Platinum *Taq* DNA polymerase (Invitrogen) in 1x reaction buffer (total volume 20 μl). We used the following primers: IL-5 direct primer 5'-TATGCCATCCCCACA GAAAT-3', IL-5 reverse primer 5'-CAGTACCCCC

0007-4888/05/1401-0080

TTGCACAGTT-3', GAPDH direct primer 5'-ACCA CAGTCCATGCCATCAC-3', and GAPDH reverse primer 5'-TCCACCACCCTGTTGCTGTA-3'. The mixture was incubated at 95°C for 2 min and then 40 amplification cycles were performed: 1 sec at 95°C, 5 sec at 62°C, and 10 sec at 72°C.

The basic reporter construct was synthesized from the pGL3 basic vector (Promega) using IL-5 gene fragment located between positions -2050 and +44. Other constructs were obtained by consecutive deletions of the 5' region in the initial fragment of the IL-5 gene cloned in the basic construct. Transfection of PER-117 cells and study of luciferase activity were performed as described previously [8]. Each experiment included transfection of cells with the construct in 5 repetitions.

We studied the interaction between nuclear proteins and synthetic double-chain oligonucleotide CLE0o [8]. The primary sequence of CLE0o (5'-GAAATTA TTCATTTCCTCAAAG-3') is identical to the sequence of the conserved lymphokine element 0 (CLE0) in the human IL-5 gene promoter located between positions -59 and -38.

RESULTS

Dexamethasone can suppress IL-5 production by PER-117 cells. The effect of glucocorticoid on IL-5 gene expression in this culture depends on the conditions of cell activation. Dexamethasone completely blocked IL-5 production by cells activated with PMA and cAMP. However, this glucocorticoid produced little effect on IL-5 expression induced by PMA and CaI [6].

Dexamethasone in doses >50 nM suppressed IL-5 production by PER-117 cells activated with PMA alone or in combination with αCD28. Under these conditions IL-5 production decreased by more than 80%. Dexamethasone in these doses did not cause apoptosis and was nontoxic for cultures stimulated with various combinations of cell-activating agents. These data suggest that the inhibitory effect of dexamethasone on IL-5 gene expression in PER-117 cells induced by PMA, PMA/cAMP, or PMA/αCD28 is mediated by specific mechanisms.

Dexamethasone can activate the mechanisms reducing the efficiency of gene expression at the transcriptional, posttranscriptional, and posttranslational level [4,10,11]. For identification of the target level for the effect of dexamethasone during suppression of IL-5 production in PER-117 cells we evaluated the effect of this glucocorticoid on IL-5 mRNA content.

IL-5 mRNA synthesis was susceptible or resistant to dexamethasone (similarly to IL-5 production) depending on the conditions of cell activation. Dexamethasone completely blocked mRNA accumulation in cells activated with PMA alone or in combination with cAMP and αCD28. However, dexamethasone only slightly decreased mRNA content in cells treated with PMA and CaI (Fig. 1).

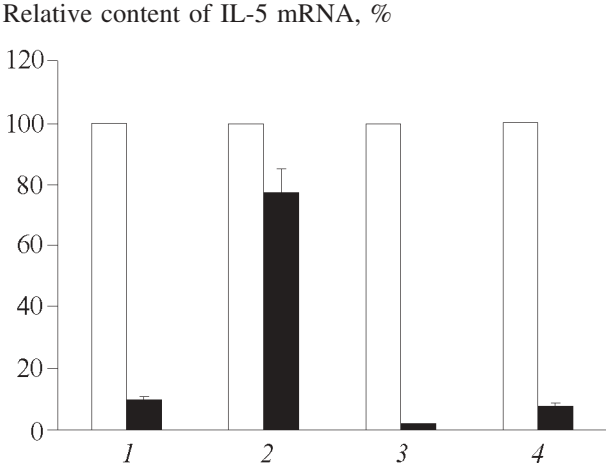

Fig. 1. Content of IL-5 mRNA in PER-117 cells stimulated with cell activators in various combinations. Here and in Fig. 2: light bars, control (no dexamethasone); dark bars, with dexamethasone. IL-5 mRNA content was measured by on-line PCR. The data were standardized to glyceraldehyde-3-phosphate dehydrogenase mRNA content. PMA (*1*), PMA/CaI (*2*), PMA/cAMP (*3*), and PMA/αCD28 (*4*).

The inhibitory effect of dexamethasone on mRNA synthesis can be associated with suppression of gene transcription or activation of mRNA degradation. It was interesting to evaluate which mechanism mediates the decrease in IL-5 mRNA content in PER-117 cells. We studied the effect of dexamethasone on activity of the reporter constructs expressing the luciferase gene under control of the IL-5 promoter.

Expression of constructs containing the first 2000, 1300, 509, and 60 nucleotide pairs in the IL-5 gene promoter (CLE0/TATA plasmid) was resistant to the effect of this glucocorticoid in cells stimulated with PMA and CaI. However, dexamethasone completely blocked expression of these plasmids in cultures activated by PMA alone or in combination with cAMP and αCD28. In stimulated cultures dexamethasone had no effect on activity of the control reporter plasmid (pGL3-Control, Promega) expressing luciferase gene under control of the SV40 promoter. These data indicate that under specific conditions of cell activation dexamethasone suppresses transcription of the reporter constructs carrying human IL-5 gene promoter.

Dexamethasone produced a similar effect on the expression of the reporter constructs. It can be hypothesized that the regulatory element CLE0, which provides expression of the CLE0/TATA minimal plasmid, serves as a target for dexamethasone in the IL-5 gene promoter context (Fig. 2).

CLE0 acts as a key regulatory element in the IL-5 promoter [7]. Its positive role in the regulation of gene transcription is mainly determined by transcription factor AP1 [15], whose DNA-binding activity can be

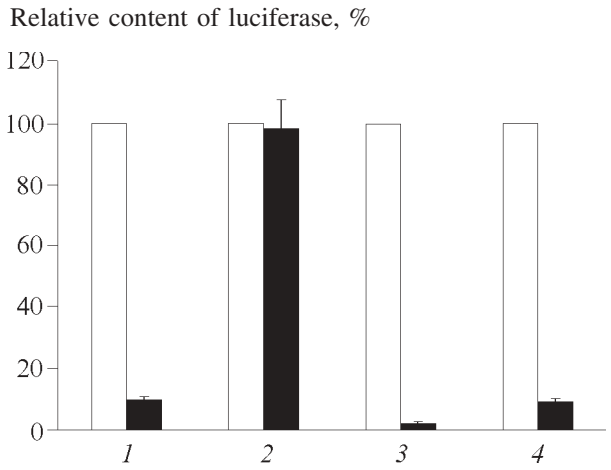

Fig. 2. Luciferase activity in PER-117 cells transfected with the CLE0/TAT construct and stimulated with cell activators in various combinations.

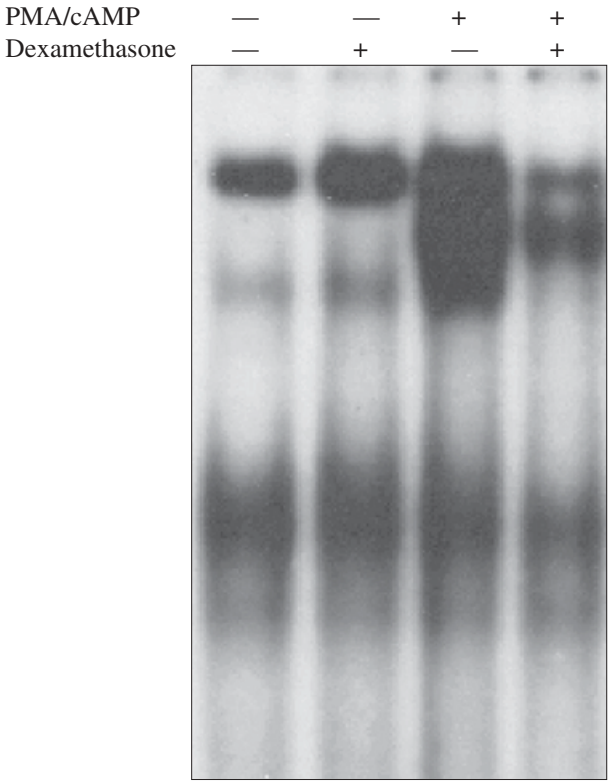

Fig. 3. Binding of nuclear proteins from PER-117 cells to a synthetic double-chain oligonucleotide CLE0o including human IL-5 gene promoter sequence between positions -59 and -38.

suppressed by glucocorticoids [5,12]. Therefore, suppression of IL-5 expression with dexamethasone is mediated by a decrease in the production of the transcription complex CLE0.

We studied the interaction of the CLE0o oligonucleotide comprising the CLE0 sequence with nuclear proteins from nonactivated and activated PER-117 cells treated with dexamethasone. Dexamethasone significantly decreased the formation of DNA-protein complexes in PMA/cAMP-stimulated cells (Fig. 3). Similar results were obtained in experiments with proteins from PMA/αCD28-stimulated cells. However, dexamethasone had little effect on protein-binding activity of CLE0o in nonactivated cells or cultures stimulated with PMA and CaI. The effect of dexamethasone on protein complexes of CLE0 depended on the conditions of cell activation (similarly to IL-5 production, IL-5 mRNA synthesis, and activity of the IL-5 promoter in reporter plasmids). The interaction between CLE0 and transcription factors was impaired in cells stimulated with PMA alone or in combination with cAMP and αCD28. However, dexamethasone did not modulate DNA-binding activity of CLE0 in PMA/CaI-activated cultures. These data indicate that dexamethasone suppresses transcription of the IL-5 gene under specific conditions of cell activation. CLE0 in the IL-5 gene promoter context serves as the target for dexamethasone.

Our findings are consistent with the mechanism of action of dexamethasone on IL-5 expression in other cells [9,13]. We identified the target for dexamethasone in the IL-5 promoter context. The mechanism underlying the effect of the suppressor should be evaluated in further studies of cytokine expression regulated by glucocorticoids. The results of this work can be used to synthesize new pharmacological preparations for the therapy of allergic diseases accompanied by eosinophilia.

REFERENCES

1. E. N. Charlesworth, A. Kagey-Sobotka, R. P Schleimer, *et al.*, *J. Immunol.*, **146**, 671-676 (1991).
2. D. R. Coombe, A. M. Nakhoul, S. M. Stevenson, *et al.*, *J. Immunol. Methods*, **215**, 145-150 (1998).
3. S. Greenfeder, S. P. Umland, F. M. Cuss, *et al.*, *Respir. Res.*, **2**, 71-79 (2001).
4. J. Han, T. Brown, and B. Beutler, *J. Exp. Med.*, **171**, 465-475 (1990).
5. H. Konig, H. Ponta, H. J. Rahmsdorf, *et al.*, *Embo J.*, **11**, 2241-2246 (1992).
6. V. A. Mordvinov, S. E. Peroni, M. L. De Boer, *et al.*, *J. Immunol. Methods*, **228**, 163-168 (1999).
7. V. A. Mordvinov and C. J. Sanderson, *Arch. Immunol. Ther. Exp. (Warsz.)*, **49**, 345-351 (2001).
8. V. A. Mordvinov, G. T. Schwenger, R. Fournier, *et al.*, *J. Allergy Clin. Immunol.*, **103**, 1125-1135 (1999).
9. A. Mori, O. Kaminuma, M. Suko, *et al.*, *Blood*, **89**, 2891-2900 (1997).
10. R. Newton, *Thorax*, **55**, 603-613 (2000).
11. K. Peppel, J. M. Vinci, and C. J. Baglioni, *Exp. Med.*, **173**, 349-355 (1991).
12. H. Ponta, A. C. Cato, and P. Herrlich, *Biochim. Biophys. Acta*, **1129**, 255-261 (1992).
13. F. G. Rolfe, J. M. Hughes, C. L. Armour, *et al.*, *Immunology*, **77**, 494-499 (1992).
14. C. J. Sanderson, *Blood*, **79**, 3101-3109 (1992).
15. G. T. Schwenger, C. C. Kok, E. Arthaningtyas, *et al.*, *Biol. Chem.*, **6**, 47,022-47,027 (2002).