0021-972X/99/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 84, No. 11
Printed in U.S.A.

Dissociative Glucocorticoid Activity of Medroxyprogesterone Acetate in Normal Human Lymphocytes*

CHRISTOPH M. BAMBERGER, TOBIAS ELSE, ANA-MARIA BAMBERGER, FRANK ULRICH BEIL, AND HEINRICH M. SCHULTE

Department of Medicine (C.M.B., F.U.B.) and Institute for Pathology (A.-M.B.), University Hospital Eppendorf, 20246 Hamburg; and the IHF Institute for Hormone and Fertility Research, University of Hamburg (C.M.B., T.E., H.M.S.), 22529 Hamburg, Germany

ABSTRACT

The immunosuppressive effects of glucocorticoids (GC) have led to their wide application in the treatment of inflammatory and autoimmune states. However, long term GC treatment is associated with severe side-effects. The development of agents displaying a more favorable ratio of wanted and unwanted GC effects, is, therefore, a major goal of pharmacological and clinical research. In this study, the progesterone receptor agonist medroxyprogesterone acetate (MPA), which also binds to the glucocorticoid receptor (GR), was tested with regard to its immunosuppressive properties. Using a recently established electroporation protocol, we show that MPA (but not progesterone) can suppress a human interleukin-2 (IL-2) promoter-luciferase construct to the same extent as the synthetic GC dexamethasone in normal human lymphocytes. MPA also markedly suppressed IL-2 (as well as IL-1 and IL-6) release, as assessed by specific enzyme-linked immunosorbent assays. In contrast, a highly dexamethasone-inducible glucocorticoid response element-driven promoter construct was only marginally stimulated by MPA in both normal human lymphocytes and HeLa cells. RT-PCR and Western blot analysis of normal human lymphocytes revealed that they do not express progesterone receptor messenger ribonucleic acid and protein, respectively. In contrast, the GR protein was clearly detectable in all samples and was shown to mediate the effects of MPA in transfected Jurkat T lymphoma cells. Our data indicate that 1) MPA can transrepress the human IL-2 gene in normal human lymphocytes in the absence of significant *trans*-activation; and 2) this effect is mediated by GR. Because of its dissociative GC activity, MPA is a highly promising substance for the treatment of inflammatory/autoimmune states. (*J Clin Endocrinol Metab* **84:** 4055–4061, 1999)

ENDOGENOUS glucocorticoids (GC) play an essential role in maintaining body homeostasis and preventing excessive immune responses to antigenic challenges (1, 2). Supraphysiological doses of synthetic GC are used to treat patients with inflammatory or autoimmune diseases (3). However, the desired immunosuppressive effects of GC are accompanied by a large number of side-effects, including weight gain, diabetes, arterial hypertension, and osteoporosis. Therefore, it has been a long-standing goal of pharmacological and clinical research to identify GC that suppress the immune system without causing such pronounced side-effects.

At the molecular level, the effects of GC are mediated by the intracellular glucocorticoid receptor (GR). GR is a ligand-dependent transcription factor, which, upon hormone binding, translocates to the cell nucleus, where it binds to glucocorticoid response elements (GREs) in the promoter regions of target genes, resulting in *trans*-activation of these genes (4–9). *Trans*-activation is probably the predominant mechanism by which GC exert many of their metabolic and cardiovascular side-effects (10–12). In contrast, the antiinflammatory/immunosuppressive effects of GC involve *trans*-repression of target genes not containing any GR-binding sites (2, 3, 9, 13). The human interleukin-2 (IL-2) gene is the prototype of a key immune gene that is repressed by GC (14–16). GC-mediated repression of IL-2 gene expression is thought to be due to direct interaction of GR with other transcriptional enhancers, such as activating protein-1 (AP-1) and nuclear factor-κB (NF-κB) (14–20).

Conventional GC do not dissociate *trans*-activation and *trans*-repression. Strategies to develop improved GC aim to maintain *trans*-repression of immune genes in the absence of significant *trans*-activation of GRE-dependent promoters. In the current study, we tested whether the progesterone receptor (PR) agonist medroxyprogesterone acetate (MPA), which also binds to GR (21, 22), would fulfill these criteria.

Materials and Methods

Cell culture

Peripheral venous blood was drawn from 10 healthy volunteers, in 3 cases repeatedly for different experiments. Peripheral blood leukocytes were isolated using Ficoll-Isopaque density centrifugation (13). Cells were cultured in RPMI 1640 containing 10% FCS, antibiotics and 1 μg/mL phytohemaglutinin to improve transfection efficiency, as recently described by Hughes and Pober (23). After 20 h in culture, the lymphocyte-enriched population of nonadherent cells was collected and used for transfection.

Jurkat human lymphoma T cells and HeLa cells were purchased from American Type Culture Collection (Manassas, VA). Cells were cultured

Received January 28, 1999. Revision received May 20, 1999. Rerevision received July 1, 1999. Accepted July 12, 1999.

Address all correspondence and requests for reprints to: Christoph M. Bamberger, M.D., Department of Medicine, University Hospital Eppendorf, Martinistrasse 52, D-20246 Hamburg, Germany. E-mail: bamberger@uke.uni-hamburg.de.

* This work was supported by the Deutsche Forschungsgemeinschaft (Graduiertenkolleg 336). This work is part of the doctoral thesis of T.E.

in RPMI 1640 or DMEM (Life Technologies, Inc.-BRL, Gaithersburg, MD) with 10% FCS and antibiotics, and passaged twice weekly.

Plasmids

The pGL3-IL-2-luciferase construct (described in Ref. 24) contains a 595-bp fragment of the human IL-2 gene (−548/+47) that has been shown to mediate full induction and repression of IL-2 gene transcription (14–16). The IL-2 promoter contains binding sites for AP-1, NF-κB, and NF-AT (nuclear factor of activated T cells), but no GREs (14–16). pGL3-GRE-tk81-luciferase contains the GRE sequence of the human tyrosine aminotransferase promoter linked to a minimal herpes simplex virus type 1 thymidine kinase (tk) promotor (24).

The hPR-B expression vector (25) was a gift from Dr. P. Chambon. pRShGRα contains the full-length coding region of the human GRα under the control of the constitutively active Rous sarcoma virus promoter. The plasmid pRSv-erbA^{-1} that contains a thyroid receptor complementary DNA in inverse orientation but is otherwise similar to pRShGRα was used as carrier DNA to yield a constant amount of transfected DNA. Both plasmids were donated by Dr. R. Evans (The Salk Institute, La Jolla, CA).

Transfection, luciferase assays, and enzyme-linked immunosorbent assays (ELISAs)

Normal human lymphocytes and Jurkat cells were transfected by electroporation (250 V; capacitance, 960 μF). Jurkat cells were electroporated with 10 μg reporter vector and 5 μg total expression vector/8×10^6 cells in a volume of 400 μL. The highest transfection efficiency for normal lymphocytes was achieved by electroporating 50 μg reporter vector/5×10^6 cells in a volume of 250 μL. After transfection, cells were plated in 12-well plates at a density of 500,000 cells/well. After 12 h, cells were stimulated with phorbol ester [tetradecanoyl phorbol acetate (TPA); 0.5×10^{-7} mol/L; Sigma Chemical Co., Deisenhofen, Germany] and ionomycin (1 μg/mL; Sigma Chemical Co.) in the absence or presence of dexamethasone (2.5×10^{-7} mol/L), hydrocortisone (2.5×10^{-7} mol/L), MPA (2.5×10^{-7} mol/L), or progesterone (2.5×10^{-7} mol/L). After 8 h, cells were lysed with a reporter lysis buffer (Promega Corp., Madison, WI), and luciferase activity in the lysate was determined in a luminometer (Lumat LB 9501, Berthold, Wildbad, Germany). HeLa cells were transfected by lipofection as previously described (26).

IL-1, IL-2, and IL-6 concentrations in cell culture supernatants were determined using specific ELISAs (Genzyme, Cambridge, MA) according to the manufacturer's instructions.

All experiments were performed at least twice, in triplicate each time. The transfection experiments in normal lymphocytes were performed at least 10 times. Statistical analysis (unpaired *t* test) was performed using Macintosh StatView software (SAS Institute, Cary, NC).

RT-PCR and Southern hybridization

Total ribonucleic acid (RNA) from normal human lymphocytes, Jurkat cells, HeLa cells (positive control for GR), T47D cells (positive control for PR), and normal human testis [positive control for androgen receptor (AR)] was extracted using standard methods and quantified by UV absorption. Complementary DNA was synthesized from 5 μg total RNA with 100 U SuperScript polymerase (Life Technologies, Inc.), using oligo(deoxythymidine) primers (Life Technologies, Inc.). PCR was carried out with 7 pmol 5′- and 3′-primers, 0.2 mmol/L deoxy-NTPs, and 0.5 U *Taq* polymerase (Genecraft, Germany) in a reaction volume of 50 μL. Initial denaturation was performed for 2 min at 95 C, followed by two cycles each at 68, 66, 64, and 62 C and 35 cycles at 60 C (annealing temperature). Primer sequences were as follows: human GR sense primer, 5′-TGG GGT AAT TAA GCA AGA GA-3′; human GR antisense primer, 5′-AGA TCA GGA GCA AAA CAC AG-3′; human PR sense primer, 5′-AGC CAG AGA TTC ACT TTT TCA C-3′; human PR antisense primer, 5′-TCA TCC GCT GTT CAT TTA GTA TTA-3′; human AR sense primer, 5′-CCC CGA GAG AGG TTG CGT CCC-3′; and human AR antisense primer, 5′-CTC CAA CGC CTC CAC ACC CAG-3′. Glyceraldehyde-3-phosphate dehydrogenase-specific primers were used as a positive control and to exclude contamination with genomic DNA. PCR products were electrophoresed in a 2.0% agarose gel and visualized by UV light.

Southern blotting of the RT-PCR products was performed by standard methods, using a Hybond N^+ membrane (Amersham Pharmacia Biotech, Braunschweig, Germany). Blots were hybridized with a digoxigenin-labeled GR- or PR-specific complementary DNA probe, which was generated by PCR, using the Boehringer DIG-PCR Labeling Kit (Roche Molecular Biochemicals, Mannheim, Germany). Signals were visualized by incubation with antidigoxigenin alkaline phosphatase-conjugated antibodies (Boehringer Mannheim, Mannheim, Germany), subsequent application of CDP-Star luminescence substrate (Boehringer Mannheim), and exposure to Hyperfilm ECL films (Amersham Pharmacia Biotech).

PAGE and Western blotting

Cells were harvested in ice-cold sample buffer b1 [50 mmol/L Tris (pH 6.8), 1% SDS, and 10% sucrose]. Protein concentrations were determined following standard protocols and using BSA protein standards diluted with sample buffer b1. Samples were diluted in a 1:1 mixture of sample buffer b1 and b2 [containing 50 mmol/L Tris (pH 6.80, 3% SDS, 10% sucrose, 10% β-mercaptoethanol, and 0.01% bromophenol blue] in a final volume of 100 μL and a final protein concentration of 300 μg/mL. Electrophoresis was performed in a 10% polyacrylamide separating gel and a 3% stacking gel. Proteins were transferred to a polyvinylidene difluoride membrane (Immobilon P, Millipore Corp., Eschborn, Germany). Membranes were stained with Ponceau S to determine transfer efficiency and homogeneity of protein lanes. After destaining with TBS [Tris-buffered saline: 20 mmol/L Tris-HCl, (pH 7.6), and 137 mmol/L NaCl] for 5 min, membranes were incubated overnight at 4 C in blocking solution [0.1 mol/L maleic acid (pH 7.5), 0.15 mol/L NaCl, 0.005% thimerosal, and 1% blocking reagent; Roche Molecular Biochemicals]. Membranes were washed for 10 min in TBST (TBS plus 0.05% Tween-20) and incubated with the primary antibody. The polyclonal rabbit antihuman GRα antibody was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), and used at a dilution of 1:500 in 9:1 TBST-blocking solution. The monoclonal mouse antihuman PR antibody was obtained from Santa Cruz Biotechnology, Inc., and used at a dilution of 1:500. Blots were incubated for 1 h at room temperature, washed three times for 10 min each time in TBST, and incubated with the IgG-peroxidase-labeled second antibody (1:1000; Sigma Chemical Co.) for 1 h at room temperature. The second antibody was visualized by enhanced chemiluminescence (ECL) reagents (Amersham Pharmacia Biotech) and Hyperfilm ECL films (Amersham Pharmacia Biotech).

Results

MPA causes strong trans-repression, but only marginal trans-activation, in normal human lymphocytes

Normal human peripheral blood lymphocytes were transfected with a human IL-2 luciferase construct, using a recently developed electroporation protocol. Stimulation of transfected cells with the phorbol ester TPA and the calcium ionophore ionomycin caused a 267.2 ± 27.5-fold increase in luciferase activity (mean ± SD; Fig. 1a). Both 2.5×10^{-7} mol/L hydrocortisone and 2.5×10^{-7} mol/L dexamethasone caused a significant reduction of TPA/ionomycin-induced luciferase activity in most experiments [22.9 ± 3.6% inhibition ($P = 0.024$) and 38.4 ± 10% inhibition ($P = 0.006$), respectively]. *Trans*-repression of IL-2 promoter activity in response to 2.5×10^{-7} mol/L MPA was at least equal to that produced by dexamethasone (up to 73.3 ± 7.2% repression; $P = 0.0005$), whereas the natural progestin progesterone had no significant effect ($P = 0.47$). The androgen dihydrotestosterone did not cause any significant effect (data not shown). The *trans*-repressive effect of MPA was not influenced by the presence of estradiol (not shown). The *trans*-repressive effects of dexamethasone or MPA could be partially reversed (30–50% reduction of *trans*-repressive activity) by the addition of RU 486, which by itself did not

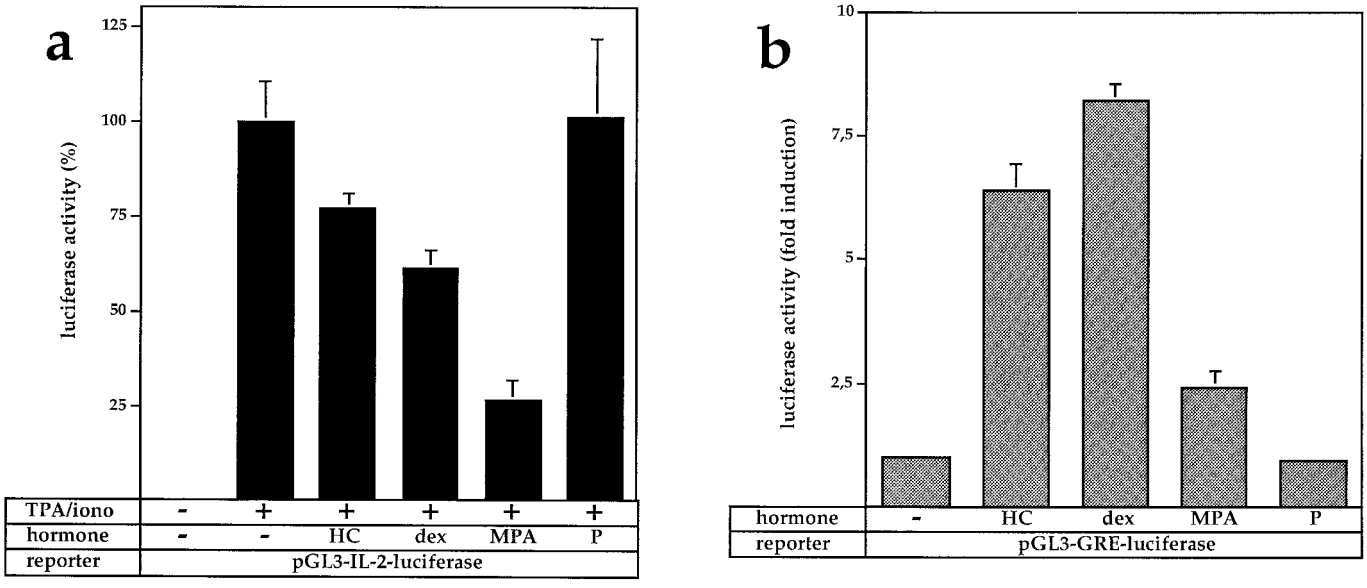

FIG. 1. *Trans*-repression and *trans*-activation in response to MPA in normal human peripheral blood lymphocytes. a, *Trans*-repressive activity of MPA compared to the conventional glucocorticoids hydrocortisone (HC) and dexamethasone (dex) and to the progestin progesterone (P). Leukocytes were isolated from peripheral venous blood of healthy volunteers and cultured in RPMI 1640 containing 10% FCS and 1 μg/mL phytohemaglutinin to improve transfection efficiency. After 20 h, the lymphocyte-enriched population of nonadherent cells was collected, electroporated with the IL-2 luciferase construct, and treated with the substances indicated (concentration for all hormones, 2.5×10^{-7} mol/L). After 24 h, luciferase activity was determined in the cell lysates. b, Similar experiments were performed to analyze *trans*-activation in response to HC, dex, MPA, and P. However, in this case, a luciferase reporter gene under the control of a GRE-containing promoter was transfected. Iono, ionomycin.

significantly suppress IL-2 promoter activity (data not shown).

In 20% of the experiments, stimulated IL-2 promoter activity was not influenced by the addition of any of the steroids used in this study. The reason for this complete steroid resistance is not clear, but may be related to the different sensitivities of the lymphocytes (which were obtained from various donors) to pretreatment with PHA. We only included those experiments with a valid positive control, *i.e.* in which dexamethasone was able to suppress the IL-2 promoter.

Transfection of normal lymphocytes with a GRE-driven luciferase construct in normal human lymphocytes resulted in 6.4 ± 0.5-fold ($P = 0.001$) and 8.2 ± 0.4-fold ($P = 0.0002$) induction of promoter activity in response to 2.5×10^{-7} mol/L hydrocortisone or 2.5×10^{-7} mol/L dexamethasone, respectively (Fig. 1b). In contrast, 2.5×10^{-7} mol/L MPA had only a marginal effect on GRE-dependent promoter activity (2.4 ± 0.4-fold induction; $P = 0.008$). Progesterone did not influence luciferase activity to a significant extent ($P = 0.28$). Therefore, a *trans*-repression/*trans*-activation ratio can be calculated for MPA. This ratio is 1.0 for the standard glucocorticoid dexamethasone (*trans*-repression = 1.0/transactivation = 1.0). As *trans*-repression induced by MPA is 1.91 times stronger than that induced by dexamethasone in a typical experiment, and as MPA-mediated *trans*-activation is only 29% (0.29) of that caused by dexamethasone, the *trans*-repression/*trans*-activation ratio for MPA is 6.6 (*trans*-repression = 1.91/transactivation = 0.29).

MPA suppresses IL-2 and IL-6 production by normal human lymphocytes in a dose-dependent manner

TPA/ionomycin-induced IL-2 release in normal human lymphocytes, as determined by an IL-2-specific ELISA, was strongly reduced by 2.5×10^{-7} mol/L hydrocortisone or 2.5×10^{-7} mol/L dexamethasone (69.4 ± 4.4% and 78.8 ± 2.3% inhibition, respectively) and somewhat less by 2.5×10^{-7} mol/L MPA (62.4 ± 6.9% inhibition), indicating more pronounced posttranscriptional effects of the classic GC (not shown). Progesterone inhibited IL-2 release by 9 ± 11.7% (not shown). In dose-response experiments, both dexamethasone and MPA significantly suppressed IL-2 release at concentrations of 10^{-7} mol/L or more (Fig. 2a). Similar results were obtained for IL-6 (Fig. 2b) and IL-1 (not shown).

The trans-activating effect of MPA is enhanced in cells transfected with a GR expression vector

To determine whether the dissociative effect of MPA depends on the GR expression level, normal lymphocytes were cotransfected with the GRE reporter construct and the GRα expression vector. The results of these experiments are shown in Fig. 3a. *Trans*-activation in response to MPA was significantly stronger in GRα-transfected compared to mock-transfected lymphocytes [hydrocortisone, 17.2 ± 2.1-fold *vs.* 6.4 ± 0.5-fold stimulation ($P = 0.005$); dexamethasone, 18.7 ± 2.3-fold *vs.* 8.2 ± 0.4-fold ($P = 0.007$); MPA, 12.5 ± 2.1-fold *vs.* 2.4 ± 0.4-fold ($P = 0.006$); progesterone, 2.8 ± 0.7-fold *vs.* no effect ($P = 0.02$)]. This effect was observed at all hormone concentrations tested (10^{-9}–10^{-5} mol/L; not shown).

Similar results were obtained in HeLa cells, which, like

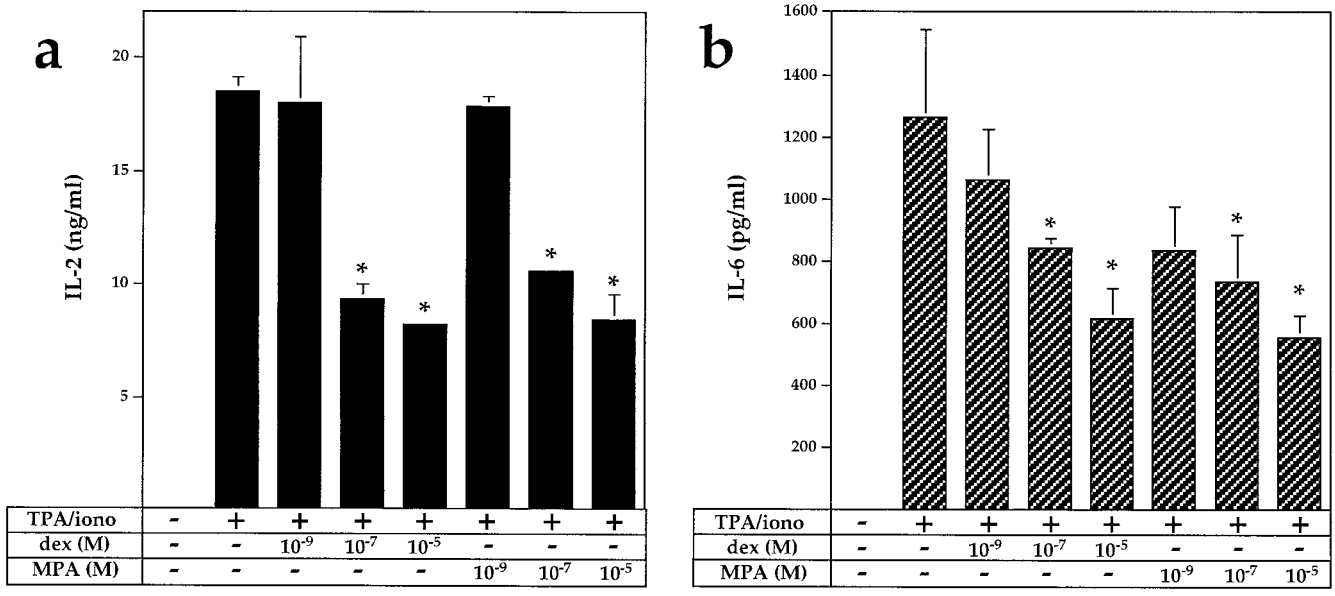

FIG. 2. Modulation of IL-2 (a) and IL-6 (b) release by MPA in normal human lymphocytes. Leukocytes were isolated from peripheral venous blood of healthy volunteers and cultured overnight in RPMI 1640 containing 10% FCS. The lymphocyte-enriched population of nonaderent cells was collected and stimulated with phorbol ester (TPA) and ionomycin (iono) in the presence of either dexamethasone (dex) or MPA at the concentrations indicated. After 24 h, cytokine concentrations in the supernatants were determined by IL-2- and IL-6-specific ELISAs, respectively. *, $P < 0.05$.

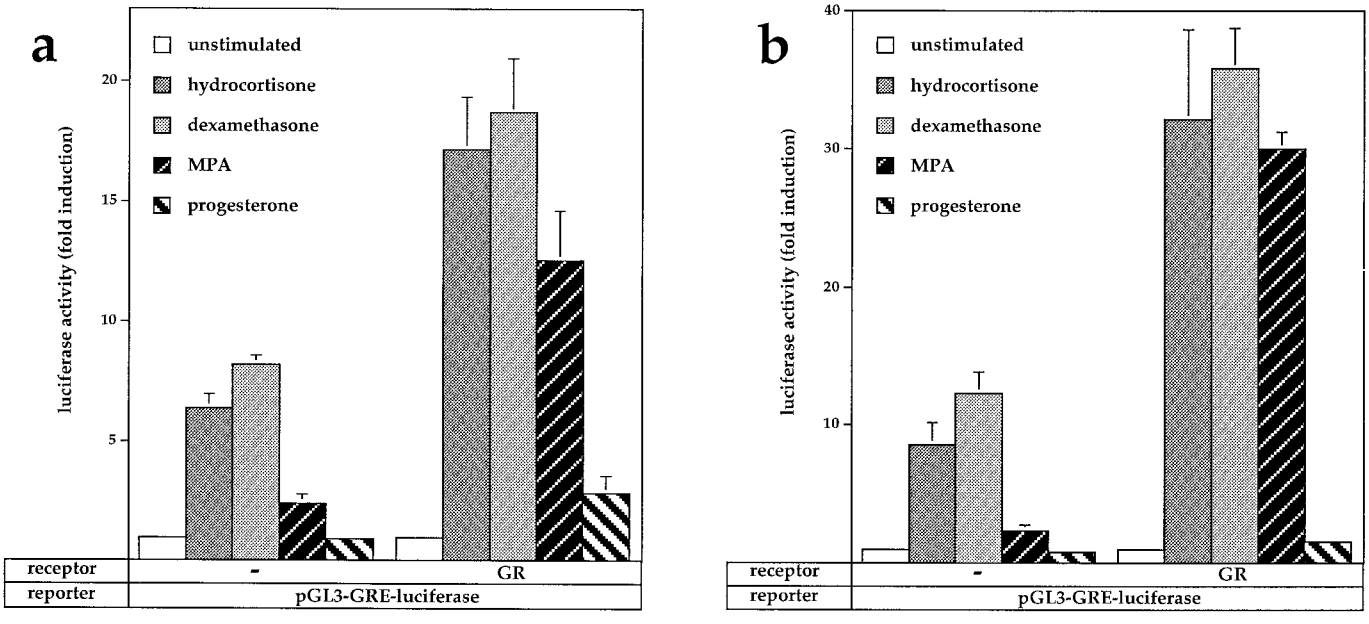

FIG. 3. Effects of a cotransfected GR expression vector on MPA-induced *trans*-activation. a, Leukocytes were isolated from peripheral venous blood of healthy volunteers and cultured in RPMI 1640 containing 10% FCS and 1 μg/mL phytohemaglutinin. After 20 h, lymphocytes were collected and electroporated with the GRE luciferase construct and either mock or GR expression vector. The transfected cells were treated with the hormones indicated (concentration for all hormones, 2.5×10^{-7} mol/L), and luciferase activity was determined in the cell lysates after 24. b, HeLa cells were cultured in DMEM containing 10% FCS. Cells were transfected with the GRE luciferase construct and either mock or GR expression vector using the lipofection method. Cells were then treated with the hormones indicated, and luciferase activity was measured in the cell lysates after 24 h.

normal lymphocytes, express GR endogenously (Fig. 3b). In HeLa cells transfected with the GRE reporter plasmid only, MPA-induced *trans*-activation was weak. Again, the *trans*-activating effect of MPA (and the other steroids) was dramatically enhanced in cells cotransfected with GRα [hydrocortisone, 35.8 ± 6.2-fold *vs.* 7.8 ± 1.4-fold stimulation (P = 0.0099); dexamethasone, 39.9 ± 3.1-fold *vs.* 11.1 ± 1.4-fold (P = 0.00045); MPA, 33.4 ± 1.4-fold *vs.* 2.1 ± 0.1-fold (P = 0.00006); progesterone, 1.7 ± 0.2-fold *vs.* no stimulation (P = 0.0016)].

To determine whether the effects of receptor cotransfection are related to the level of GR expression, Jurkat T lym-

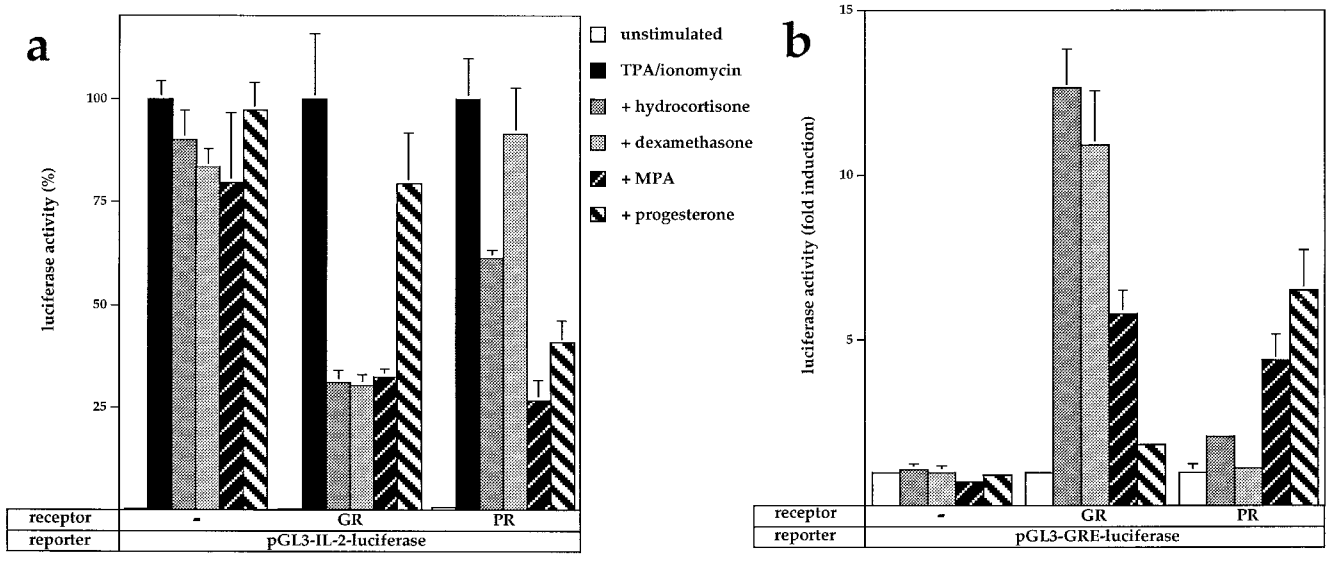

FIG. 4. MPA-induced *trans*-repression and *trans*-activation in Jurkat T lymphoma cells cotransfected with a GR or a PR expression vector. a, Jurkat cells were cultured in RPMI 1640 containing 10% FCS. Cells were electroporated with the IL-2-luciferase reporter construct and either mock (−), human GR, or PR-B (PR) expression vectors. Cells were stimulated with phorbol ester (TPA) and ionomycin (iono) and treated with the hormones indicated (2.5×10^{-7} mol/L). Luciferase activity was measured in the cell lysates after 24 h. b, To analyze *trans*-activation, Jurkat cells were transfected with the GRE-luciferase construct and treated with the hormones indicated (2.5×10^{-7} mol/L).

phoma cells, which do not express functional GR endogenously, were transfected with the above reporter plasmids and with different amounts of GRα expression vector. In these experiments, the maximum *trans*-activating effect of MPA reached that of dexamethasone only at maximum GRα concentrations (10 μg expression vector/8×10^6 cells), but not at 2 and 5 μg transfected GRα expression vector, where it was below 50% of maximum dexamethasone-induced *trans*-activation (not shown).

These results confirm the importance of using cells that express physiological levels of GR when analyzing potentially dissociative GC (see also *Discussion*).

GR, PR, and AR mediate the effects of MPA in Jurkat T lymphoma cells

To test whether the dissociative GC-like effects of MPA (*i.e.* strong *trans*-repression, weak *trans*-activation) observed in normal human lymphocytes could also be mediated by other steroid receptors, such as PR and AR, Jurkat T lymphoma cells were transfected with the respective expression vectors and the luciferase constructs indicated above (Fig. 4).

In Jurkat cells transfected with the reporter plasmids only, both IL-2- and GRE-promoter activities were marginally, but not significantly, altered by the four agents, indicating that these cells do not contain significant amounts of functional GR or PR.

In Jurkat cells transfected with the GRα plasmid, IL-2 promoter repression rates were as follows (hormone concentration = 2.5×10^{-7} mol/L in all experiments): hydrocortisone, 69 ± 6.9% ($P = 0.007$); dexamethasone, 69.4 ± 6.2% ($P = 0.007$); MPA, 67.6 ± 5.2% ($P = 0.008$); progesterone, 30.6 ± 11.4% ($P = 0.08$; Fig. 4a). In some experiments, the repressive effect of progesterone on IL-2 promoter activity reached significance. The GRE construct was induced 12.6 ± 0.8-fold by hydrocortisone ($P = 0.002$), 10.9 ± 1.7-fold by dexamethasone ($P = 0.004$), 5.8 ± 0.7-fold by MPA ($P = 0.004$; $P_{(\text{dexamethasone } vs. \text{ MPA})} = 0.0095$), and 1.8 ± 0.1-fold by progesterone ($P = 0.0008$) in GRα-transfected Jurkat cells (Fig. 4b).

In PR-transfected Jurkat cells, hydrocortisone repressed the IL-2 promoter by 38.6 ± 7.3% ($P = 0.0086$), dexamethasone by 8 ± 18% ($P = 0.19$), MPA by 73.1 ± 0.6% ($P = 0.0007$), and progesterone by 59 ± 8.5% ($P = 0.001$; Fig. 4a). The GRE reporter plasmid was induced 2.1 ± 0.1-fold by hydrocortisone ($P = 0.007$), 1.1 ± 0.1-fold by dexamethasone ($P = 0.25$), 4.4 ± 0.6-fold by MPA ($P = 0.006$), and 6.5 ± 1.7-fold by progesterone ($P = 0.007$; Fig. 4b).

In summary, *trans*-repression of the IL-2 construct by MPA was similar in normal human lymphocytes and in GR-expressing Jurkat cells. MPA also suppressed the IL-2 construct in PR-transfected Jurkat cells, indicating that both GR and PR are able to mediate this effect. Compared to normal lymphocytes, the dissociative effect of MPA was less pronounced, yet still highly significant in GRα-expressing Jurkat cells (*trans*-repression/*trans*-activation ratio, 1.83). Finally, the AR also mediated the effects of MPA in Jurkat cells (not shown).

GR, but not PR, messenger RNA (mRNA) and protein are expressed in normal human lymphocytes

To confirm that the dissociative effects of MPA on *trans*-repression and *trans*-activation in normal human lymphocytes are mediated by GR, we studied the expression of GR, PR, and AR in these cells at both the mRNA (RT-PCR) and protein (Western blot) levels. As shown in Fig. 5a, GR mRNA was expressed in normal lymphocytes of male and female donors and in Jurkat cells. The correct sequence of the amplification products was confirmed by Southern hybridization. Using a highly sensitive touchdown protocol, no band was obtained with the PR-specific primers. When the number of cycles was increased to 40 or more, a faint and inconsistent band was obtained in human lymphocytes and Jurkat

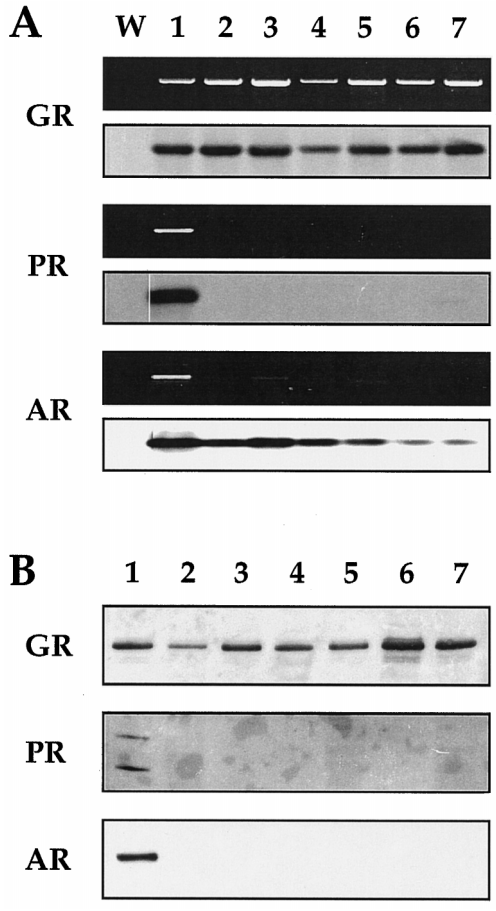

FIG. 5. Expression analysis of GR, PR, and AR mRNA and protein in human lymphoid cells. a, RNA was extracted, reverse transcribed, and amplified by PCR, using human GR-, PR-, and AR-specific primers. The PCR products were run on an agarose gel and visualized by ethidium bromide. The correct sequence of the amplification products was verified by nonradioactive Southern hybridization. b, Protein extracts were run on a polyacrylamide gel, blotted on a polyvinylidene difluoride membrane, and incubated with specific polyclonal antihuman GR-, PR-, and AR-specific antibodies. Blots were then incubated with a secondary, digoxigenin-labeled antibody. Note that the antibody against the common region of the human PR detects both the PR-A and the PR-B isoform. W, Water; 1, positive control cells (HeLa for GR, T47D breast cancer cells for PR, normal human testis for AR); 2, unstimulated female lymphocytes; 3, TPA/ionomycin-stimulated female lymphocytes; 4, unstimulated male lymphocytes; 5, TPA/ionomycin-stimulated male lymphocytes; 6, unstimulated Jurkat cells; 7, TPA/ionomycin-stimulated Jurkat cells.

cells, indicating extremely low, if any, expression of PR mRNA in these cells. AR mRNA was weakly expressed in all samples tested.

The results of the Western blot experiments are shown in Fig. 5b. GR protein was detectable in all protein extracts from normal human lymphocytes. Surprisingly, untransfected Jurkat cells expressed more immunoreactive protein than normal lymphocytes. As untransfected Jurkat cells do not respond to GC, and transfection with a GR expression vector can reestablish GC responsiveness (see above), the GR protein present in these cells must be defective. Consistent with the PCR experiments, no PR protein was detectable in any of the lymphocyte preparations. As opposed to AR mRNA, no AR protein was detectable in human lymphocytes.

Discussion

In the present study we show for the first time that the PR and GR agonist MPA exerts dissociative GC activity in normal human lymphocytes. While MPA treatment caused pronounced *trans*-repression of IL-2 gene expression, MPA-mediated *trans*-activation of a highly dexamethasone-inducible GRE-dependent promoter was marginal. Furthermore, we demonstrate that GR, PR, and AR can mediate this effect, as Jurkat cells, which are devoid of functional steroid receptors, were rendered MPA sensitive by transfection of the respective expression vectors. However, our expression studies clearly indicate that the observed effects of MPA are mediated by GR, as human lymphocytes only express GR, but not PR. AR mRNA was found to be expressed in normal lymphocytes; however, AR protein expression was below the detection limit of our Western blot experiments.

As early as 1965, Hulka *et al.* reported that progestins can exert immunosuppressive effects (27). Later, it became clear that progesterone can inhibit T cell responsiveness, and this effect was thought to be important in tolerance of the fetal allograft (28). However, the immunosuppressive action of progesterone itself is rather weak, as indicated by the massive doses that are needed to exert this effect (29). There are few reports analyzing the effects of the synthetic progestin MPA on the immune system. Mallmann *et al.* observed decreased T cell numbers in breast cancer patients who received high doses of MPA (30). Another group showed that MPA could inhibit lymphocyte proliferation *in vitro* (21). Our study is the first to analyze the effects of MPA on IL-2 gene expression and thus represents the first quantitative approach to the immunosuppressive action of MPA at the molecular level. Surprisingly, we found that the suppressive effect of MPA on IL-2 gene expression is as strong as that of the classic glucocorticoids, dexamethasone and hydrocortisone. IL-1 and IL-6 were also strongly suppressed by MPA. In contrast, progesterone did not exert any immunosuppressive effect at physiological concentrations in our study.

Whether the immunosuppressive effects of progestins are mediated by PR or other steroid receptors has been a matter of dispute. Indeed, PR expression in human lymphocytes, which would be a prerequisite for any PR-mediated effect, is controversial (29, 31). Using a highly sensitive touchdown PCR protocol, we did not detect any PR mRNA in unstimulated or stimulated human lymphocytes under the conditions described. Furthermore, we did not detect any PR protein in these cells by Western blotting. We conclude that if PR is expressed in human lymphocytes, the levels are far too low to exert any significant effect. In contrast, we and others have shown that GR mRNA and protein are clearly expressed in human lymphocytes. It has also been shown that MPA can bind to GR (21, 22). Finally, Jurkat cells, which are devoid of functional GR, are rendered MPA sensitive by transfection of a GR expression vector. AR does not play a role in mediating the effects of MPA in human lymphocytes, as its expression is below the detection limit of our Western blot, and dihydrotestosterone, which is a much stronger AR agonist than MPA, has no effect on IL-2 promoter activity in these cells.

The most important finding in our study is that MPA can dissociate between *trans*-repression and *trans*-activation in

normal human lymphocytes. It is noteworthy that this effect is most pronounced in lymphocytes expressing normal levels of GR endogenously. MPA-induced *trans*-activation was much stronger in both lymphocytes and HeLa cells transfected with a GRα expression vector. These data are consistent with findings reported by Szapary *et al.*, who showed that the percentage of maximal GC-induced *trans*-activation is dramatically increased in transiently GR-transfected cells (32). Furthermore, antiglucocorticoids can be converted to GC under these conditions. Both our data and the study by Szapary *et al.* underline the importance of using cells that express normal levels of GR when analyzing potentially dissociative GC and/or antiglucocorticoids.

It has been the long-standing goal of pharmacological research to develop GC that dissociate *trans*-repression and *trans*-activation, because many of the side-effects of conventional GC can be attributed to *trans*-activation (10–12). In 1997, Vaysierre *et al.* reported that a novel class of synthetic GC could differentiate between *trans*activation and AP-1 *trans*-repression (33). We now demonstrate similar effects of MPA. As opposed to the novel agents described by Vaysierre *et al.*, the clinical experience with MPA is extensive; it has been used for the treatment of metastatic endometrial, breast, and renal cancer. Consistent with our *in vitro* findings, the development of Cushingoid features is less pronounced than that in patients treated with conventional GC and only occurs at excessive doses (34, 35). It has even been shown that MPA can reverse one of the most devastating side-effects of conventional GC, the development of osteoporosis (36). We conclude that MPA is a highly promising substance for the treatment of autoimmune/inflammatory diseases, and that our data justify further studies in animals and humans affected with these diseases.

Acknowledgments

We are grateful to Dr. R. Jung, Department of Clinical Chemistry, University Clinic Eppendorf, for the measurements of IL-1 and IL-6. We are also grateful to Dr. Sylvia Asa, University of Toronto (Toronto, Canada), for helpful suggestions on the manuscript.

References

1. **Chrousos GP.** 1995 The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. N Engl J Med. 332:1351–1362.
2. **Wick G, Hu, Y, Schwarz S, Kroemer G.** 1993 Immunoendocrine communication via the hypothalamo-pituitary-adrenal axis in autoimmune disease. Endocr Rev. 14:539–563.
3. **Boumpas DT, Paliogianni F, Anastassiou ED, Balow JE.** 1991 Glucocorticosteroid action on the immune system: molecular and cellular aspects. Clin Exp Rheumatol. 9:413–423.
4. **Evans RM.** 1988 The steroid and thyroid hormone receptor superfamily. Science. 240:889–895.
5. **Truss M, Beato M.** 1993 Steroid hormone receptors: interaction with deoxyribonucleic acid and transcription factors. Endocr Rev. 14:459–479.
6. **Tsai M-J, O'Malley BW.** 1994 Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem. 63:451–486.
7. **Beato M, Herrlich P, Schütz G.** 1995 Steroid hormone receptors: many actors in search of a plot. Cell. 83:851–857.
8. **Mangelsdorf DJ, Thummel C, Beato M, et al.** 1995 The nuclear receptor superfamily: the second decade. Cell. 83:835–839.
9. **Bamberger CM, Schulte HM, Chrousos GP.** 1996 Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. Endocr Rev. 17:245–261.
10. **Becker PB, Gloss B, Schmid W, Strähle U, Schütz G.** 1986 *In vivo* protein-DNA interactions in a glucocorticoid response element require the presence of the hormone. Nature. 324:686–688.
11. **Imai E, Miner JN, Mitchell JA, Yamamoto KR, Granner DK.** 1993 Glucocorticoid receptor-cAMP response element-binding protein interaction and the response of the phosphoenolpyruvate carboxykinase gene to glucocorticoids. J Biol Chem. 268:5353–5356.
12. **Brasier AR, Li J.** 1996 Mechanisms for inducible control of angiotensinogen gene transcription. Hypertension. 27:465–475.
13. **Bamberger A-M, Erdmann I, Bamberger CM, Jenatschke S, Schulte HM.** 1997 Transcriptional regulation of the human leukemia inhibitory factor gene: modulation by glucocorticoids and estradiol. Mol Cell Endocrinol. 127:71–79.
14. **Northrop JP, Crabtree GR, Mattila PS.** 1992 Negative regulation of interleukin 2 transcription by the glucocorticoid receptor. J Exp Med. 175:1235–1245.
15. **Vacca A, Felli MP, Farina AR, et al.** 1992 Glucocorticoid receptor-mediated suppression of the interleukin 2 gene expression through impairment of the cooperativity between nuclear factor of activated T cells and AP-1 enhancer elements. J Exp Med. 175:637–646.
16. **Paliogianni F, Raptis A, Ahuja SS, Najjar SM, Boumpas DT.** 1993 Negative transcriptional regulation of human interleukin 2 (IL-2) gene by glucocorticoids through interference with nuclear transciption factors AP-1 and NF-AT. J Clin Invest. 91:1481–1489.
17. **Jonat C, Rahmsdorf HJ, Park KK, Cato AC, Gebel S, Ponta H, Herrlich P.** 1990 Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. Cell. 62:1189–1204.
18. **Schüle R, Rangarajan P, Kliewer S, et al.** 1990 Functional antagonism between oncoprotein c-jun and the glucocorticoid receptor. Cell. 62:1217–1226.
19. **Yang-Yen H-F, Chambard JC, Sun YL, Smeal T, Schmidt TJ, Drouin J, Karin M.** 1990 Transcriptional interference between c-Jun and the glucocorticoid receptor: mutual inhibition of DNA binding due to direct protein-protein interaction. Cell. 62:1205–1215.
20. **Scheinman RI, Gualberto A, Jewell CM, Cidlowski JA, Baldwin ASJ.** 1995 Characterization of mechanisms involved in transrepression of NF-κB by activated glucocorticoid receptors. Mol Cell Biol. 15:943–953.
21. **Kontula K, Paavonen T, Luukkainen T, Andersson LC.** 1983 Binding of progestins to the glucocorticoid receptor. Correlation to their glucocorticoid-like effects on *in vitro* functions of human mononuclear leukocytes. Biochem Pharmacol. 32:1511–1518.
22. **Selman PJ, Wolfswinkel J, Mol JA.** 1996 Binding specificity of medroxyprogesterone acetate and proligestone for the progesterone and glucocorticoid receptor in the dog. Steroids. 61:133–137.
23. **Hughes CCW, Pober JS.** 1996 Transcriptional regulation of the interleukin-2 gene in normal human peripheral blood T cells. J Biol Chem. 271:5369–5377.
24. **Bamberger CM, Else T, Bamberger A-M, Beil FU, Schulte HM.** 1997 Regulation of the human interleukin-2 (IL-2) gene by the α and β isoforms of the glucocorticoid receptor. Mol Cell Endocrinol. 131:233–240.
25. **Meyer ME, Pornon A, Ji J, Bocquel MT, Chambon P, Gronemeyer H.** 1990 Agonistic and antagonistic activities of RU486 on the functions of the human progesterone receptor. EMBO J. 9:3923–3932.
26. **Bamberger CM, Bamberger A-M, Wald M, Chrousos GP, Schulte HM.** 1997 Inhibition of mineralocorticoid activity by the β-isoform of the human glucocorticoid receptor. J Steroid Biochem Mol Biol. 60:43–50.
27. **Hulka JF, Mohr K, Liebermann MW.** 1965 Effect of synthetic progestational agents on allograft rejection and circulating antibody production. Endocrinology. 77:897–901.
28. **Clemens LE, Sitteri PK, Stites DP.** 1979 Mechanism of immunosuppression of progesterone on maternal lymphocyte activation during pregnancy. J Immunol. 122:1978–1985.
29. **Schust DJ, Anderson DJ, Hill JA.** 1996 Progesterone-induced immunosuppression is not mediated through the progesterone receptor. Hum Reprod. 11:980–985.
30. **Mallmann P, Dietrich K, Krebs D.** 1990 Effect of tamoxifen and high-dose medroxyprogesterone acetate (MPA) on cell-mediated immune functions in breast cancer patients. Methods Find Exp Clin Pharmacol. 12:699–706.
31. **Paldi A, d'Auriol L, Mishari M, Bakos A, Chaouat G, Szekeres-Bartho J.** 1994 Expression of the gene coding for the progesterone receptor in activated human lymphocytes. Endocr J. 2:317–319.
32. **Szapary D, Xu M, Simons Jr SS.** 1996 Induction properties of a transiently transfected glucocorticoid-responsive gene vary with glucocorticoid receptor concentrations. J Biol Chem. 271:30576–30582.
33. **Vaysierre BM, Dupont S, Choquart A, et al.** 1997 Synthetic glucocorticoids that dissociate transactivation and AP-1 transrepression exhibit antiinflammatory activity *in vivo*. Mol Endocrinol. 11:1245–1255.
34. **Leis D., Bottermann P, Ermler R, Henderkott U, Gluck H.** 1980 The influence of high doses of oral medroxyprogesterone acetate on glucose tolerance, serum insulin levels and adrenal response to ACTH. A study of 17 patients under treatment for endometrial cancer. Arch Gynecol. 230:9–13.
35. **Lang I, Zielinski CC, Templ H, Spona J, Geyer G.** 1990 Medroxyprogesterone acetate lowers plasma corticotropin and cortisol but does not suppress anterior pituitary responsiveness to human corticotropin releasing hormone. Cancer. 66:1949–1953.
36. **Grecu EO, Weinshelbaum A, Simmons R.** 1990 Effective therapy of glucocorticoid-induced osteoporosis with medroxyprogesterone acetate. Calcif Tissue Int. 46:294–299.