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# BINDING OF PROGESTINS TO THE GLUCOCORTICOID RECEPTOR

## CORRELATION TO THEIR GLUCOCORTICOID-LIKE EFFECTS ON *IN VITRO* FUNCTIONS OF HUMAN MONONUCLEAR LEUKOCYTES

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**Abstract**—A number of physiological and synthetic progestins were tested for their ability to compete with [$^3$H]dexamethasone for the binding to the glucocorticoid receptor of human mononuclear leukocytes and their ability to elicit glucocorticoid-like effects on the same cells. As compared to the reference compound dexamethasone (relative receptor binding affinity defined as 100%), two potent synthetic progestins with a pregnane-type structure, megestrol acetate and medroxyprogesterone acetate, were found to display a considerable binding affinity towards the receptor (46 and 42%, respectively). The relative binding affinity of the naturally occurring ligand, cortisol, to the receptor was clearly lower (25%). The effective binding of medroxyprogesterone acetate to the glucocorticoid receptor was confirmed by direct binding studies utilizing a tritiated derivative of this steroid. No evidence for the existence of a specific progesterone receptor in human mononuclear leukocytes was obtained as judged by the results of competition experiments where a progesterone receptor-specific ligand [$^3$H]Org 2058 was used. Medroxyprogesterone acetate and megestrol acetate also induced glucocorticoid-like effects on the lymphocyte functions. These included inhibition of the proliferative responses to the T-cell mitogens concanavalin A and phytohaemagglutinin and an enhanced accumulation of immunoglobulin secreting cells in pokeweed mitogen-stimulated cultures. The progestin effect appears to be mediated through a radiosensitive (suppressor) subpopulation of T lymphocytes. In contrast, the synthetic progestins related structurally to 19-nortestosterone, norethisterone and d-norgestrel, were virtually devoid of binding affinity towards the glucocorticoid receptor nor did they measurably influence the *in vitro* lymphocyte functions. These studies demonstrate that certain progestins in common clinical use probably possess inherent glucocorticoid activity and suggest that side effects attributable to this character (e.g. suppression of the pituitary–adrenal axis) might be expected when these compounds are used in pharmacological doses.

The present opinion about the mechanism of steroid action maintains [1–3] that the steroid molecule freely permeates the target cell membrane and binds to a soluble cytoplasmic receptor. The resulting steroid–receptor complex undergoes a process called "transformation" and is then able to bind to nuclear acceptor sites. This interaction triggers an increase in the rate of the transcription of specific DNA sequences, ultimately leading to augmented mRNA and protein synthesis along with alterations in cell functioning.

Progesterone has been shown to display some affinity towards glucocorticoid receptors with various experimental systems [4–7]. This steroid, however, seems to display a peculiar duality since it acts as a glucocorticoid agonist in cultured mouse lymphoma cells [8] and in rat thymocytes [9] while it behaves as an almost pure glucocorticoid antagonist in cultured rat hepatoma cells [10] and in the mouse mammary tumor system [11]. Several authors have reported prolonged allograft survival in experimental animals after treatment with suprapharmacological doses of progesterone and the synthetic progestins medroxyprogesterone acetate, norethindrone and norethynodrel [12–15]. Progesterone and medroxyprogesterone acetate have also been found to suppress the antibody production in rabbits after active immunization [13].

It is possible that also in the human being progestins possess inherent glucocorticoid activity. Thus, progesterone to some degree competes with potent glucocorticoids for the binding to glucocorticoid receptors in human circulating white cells [16–20]. Numerous studies indicate that lymphocytes from pregnant women in the presence of maternal serum [21] show impaired *in vitro* responsiveness to phytohaemagglutinin (PHA) [22–25] and depressed mixed lymphocyte reactivity [26, 27]. The addition of progesterone, 17$\alpha$-hydroxyprogesterone and 20$\alpha$-dihydroprogesterone to lymphocyte cultures has been reported to suppress mitogen and antigen induced blast transformation [28–30].

To conduct a systematic study on the possible glucocorticoid effects of progestins in man, we decided to determine the ability of a number of

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physiological and synthetic progestins to bind to the glucocorticoid receptor of purified human mononuclear leukocytes and to investigate whether these progestins also elicit effects on the *in vitro* lymphocyte activation similar to those induced by the relevant ligands of the receptor, i.e. the glucocorticoids.

## MATERIALS AND METHODS

*Chemicals.** [1,2-$^3$H]Dexamethasone (specific activity 47.5 Ci/mmole) and [1,2-$^3$H]medroxyprogesterone acetate (specific activity 60 Ci/mmole) were purchased from New England Nuclear (Boston, Mass., U.S.A.). R 5020 was obtained from Roussel Uclaf (Romainville, France). Tritium-labelled (specific activity 25 Ci/mmole) and non-labelled Org 2058 as well as the synthetic steroids lynestrenol, Org 2969 (desogestrel), Org 3236 and 4-pregnene-16$\alpha$,17-dimethyl-3,20-dione were kindly donated by Dr. G. Woods, Organon Scientific Development Group, Lanarkshire, Scotland. Other steroids were from Steraloids (Wilton, N.H., U.S.A.). Ficoll-Isopaque was obtained from Pharmacia AB (Uppsala, Sweden), Dextran T 500 (Macrodex®) from Leiras (Turku, Finland) and RPMI-1640 medium from Orion Diagnostica (Helsinki, Finland).

Pokeweed mitogen (PWM; Gibco) was used at a final dilution of 1:300. Heat-killed Staphylococcus aureus Cowan I bacteria were used at a density of $60 \times 10^6$ organisms/ml. Concanavalin A (ConA; Pharmacia Fine Chemicals) was used at a final concentration of 5 $\mu$g/ml and phytohaemagglutinin (PHA; Difco Laboratories, Detroit, Mich.) at a final dilution of 1:300.

Other chemicals were supplied by Merck (Darmstadt, F.R.G.) and were of the highest purity grade available.

*Isolation of human mononuclear leukocytes.* Blood mononuclear leukocytes were isolated from the buffy coats of 400 ml blood units obtained from healthy male and female volunteers and generously supplied by the Finnish Red Cross Blood Transfusion Service, Helsinki, Finland. The buffy coats were diluted with 2 volumes of 0.1 M NaCl–0.01 M sodium phosphate (PBS), pH 7.4, and the mononuclear cells were separated from granulocytes and erythrocytes by one-step Ficoll-Isopaque (FIP; Pharmacia Fine Chemicals, Uppsala, Sweden; density 1.077) gradient centrifugation at 400 *g* for 40 min at +22° [31]. Contaminating erythrocytes were lysed by treatment with a Tris–HCl buffered 0.84% aqueous solution of $NH_4Cl$, pH 7.6 (Tris–$NH_4Cl$). The purified cell population contained about 80% lymphocytes and 20% monocytes and only insignificant amounts of granulocytes. Cell counts were determined with a Bürker hemocytometer.

*Fractionation of T lymphocytes.* Pure blood T lymphocytes were obtained by passage of the FIP-isolated mononuclear cells over a human Ig-rabbit anti-human Ig column as described [32]. More than 90% of the passed cell population formed rosettes with 2-amino-ethylisothiouronium bromide hydrobromide (AET)-treated sheep erythrocytes [33] and the T-cell population contained less than 1% surface Ig-positive cells.

*Determination of the relative binding affinities of the various steroids towards the glucocorticoid receptor.* The non-labelled steroids were prepared in various dilutions in ethanol and pipetted in duplicate (usually in amounts of 0.1–0.2 ml) to disposable polystyrene tubes. The tubes were evaporated to dryness on a stream of nitrogen whereafter 10 $\mu$l of ethanol was added to augment the solubility of the compounds. The final concentration of ethanol in the incubations (1%) did not affect the binding properties of the receptor. The tritiated ligand ([$^3$H]dexamethasone, [$^3$H]medroxyprogesterone acetate or [$^3$H]Org 2058, as indicated in the text) was added (in 0.2 ml of RPMI-1640 with 50 mmol/l HEPES, pH 7.5; final concentration $2–3 \times 10^{-8}$ mol/l) and the tubes mixed with gentle shaking. Aliquots of 0.8 ml of the mononuclear cell suspension were then pipetted to the tubes followed by their incubation for 45 min at +37°. The extent of nonspecific binding of the tracer was always estimated from incubations containing a 200-fold excess of the corresponding non-radioactive ligand. After incubation, 2 ml of cold PBS (phosphate buffered saline; NaCl 8 g/liter, KCl 0.2 g/liter, $Na_2HPO_4$ 1.15 g/liter and $KH_2PO_4$ 0.2 g/liter) were added to each tube, the cells collected by centrifugation and then washed two times with 3 ml of PBS (at +22°). During each wash, the cells were maintained for 10 min at 22° before centrifugation. The washed cell pellets were suspended in a mixture of 0.5 ml of PBS and 1.0 ml of ethanol and then transferred to liquid scintillation vials for subsequent radioactivity counting. After correction for non-specific binding, competition curves for all the steroids were constructed and the relative binding affinities (relative to dexamethasone = 100%) of the different ligands to the glucocorticoid receptor were calculated at the 50% competition level as described by Korenman [34]. The determination of the dissociation constants ($K_d$s) for the interaction between the various tritiated ligands and the receptor was accomplished as previously described [35] using Scatchard analysis [36] of the binding data.

*Lymphocyte cultures and quantitation of the response.* To quantitate the DNA synthesis after PWM, Staphylococcus aureus, ConA or PHA stimulation, $3 \times 10^5$ lymphocytes in 200 $\mu$l of RPMI-1640 tissue culture medium supplemented with 5% fetal calf serum (FCS; Gibco) were cultivated in the presence of various steroids in Falcon Microtest II tissue culture plates (Falcon Labware, Div. Becton, Dick-

* Trivial and systematic names of the synthetic steroids used: dexamethasone, 16$\alpha$-methyl-1,4-pregnadiene-9-fluoro-11$\beta$,17$\alpha$,21-triol-3,20-dione; R 5020, 17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione; Org 2058, 16$\alpha$-ethyl-21-hydroxy-19-nor-4-pregnene-3,20-dione; medroxyprogesterone acetate, 6$\alpha$-methyl-17$\beta$-hydroxy-4-pregnene-3,20-dione acetate; megestrol acetate, 6-methyl-17$\alpha$-hydroxy-4,6-pregnadiene-3,20-dione acetate; cyproterone acetate, 6-chloro-1,2$\alpha$-methylene-17$\alpha$-hydroxy-4,6-pregnadiene-3,20-dione acetate; norethisterone, 17$\alpha$-ethinyl-17$\alpha$-hydroxy-4-estren-3-one; d-norgestrel, 17$\alpha$-ethinyl-18-methyl-17$\beta$-hydroxy-4-estren-3-one; lynestrenol, 17$\alpha$-ethinyl-4-estren-17$\beta$-ol; Org 2969 (desogestrel), 17$\alpha$-ethinyl-11-methylene-18-methyl-4-estren-17$\beta$-ol; Org 3236, 17$\alpha$-ethinyl-11-methylene-18-methyl-17$\beta$-hydroxy-4-estren-3-one.

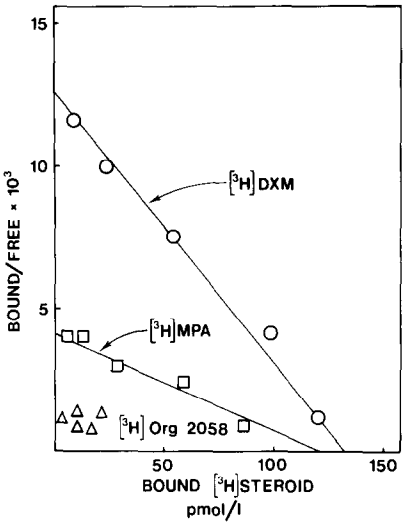


Fig. 1. Scatchard plots of the saturable binding of [$^3$H]-dexamethasone (○) and [$^3$H]medroxyprogesterone acetate (□) to human mononuclear leukocytes. The experimental points for [$^3$H]Org 2058 are also shown (△). In each case there were $17 \times 10^6$ cells/incubation.

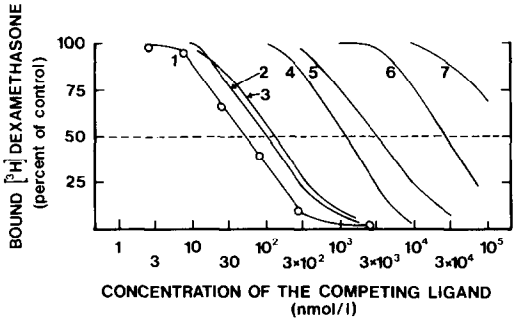


Fig. 2. Competition of the various non-labelled ligands with [$^3$H]dexamethasone for the binding to the human mononuclear leukocytes. Leukocytes ($15 \times 10^6$ cells/incubation) and [$^3$H]dexamethasone (final concentration, $2.5 \times 10^{-8}$ mol/l) were incubated in the presence or absence of increasing amounts of the non-labelled competing ligands indicated. Non-specific binding of [$^3$H]dexamethasone (about 15% of the total binding observed) was always subtracted before calculations. For other details, see Materials and Methods. The steroids shown are as follows: 1, dexamethasone; 2, medroxyprogesterone acetate; 3, megestrol acetate; 4, R 5020; 5, Org 2058; 6, d-norgestrel; 7, norethisterone.

inson & Co., Oxnard, Calif.) in a humidified atmosphere of 5% $CO_2$ in air. The steroids to be tested were first diluted in ethanol at a concentration of 10 mg/ml, and this stock solution was further diluted in RPMI-1640 culture medium to reach the concentrations indicated. The amount of ethanol (0.1%) which was present in the strongest steroid concentrations had no measurable effect on the proliferative response or the generation of Ig-producing cells. The proliferative responses of mitogen-stimulated cultures were measured by addition of 0.4 μCi of tritiated thymidine ([$^3$H]TdR; New England Nuclear, Boston, Mass.; specific activity 6.7 Ci/mmol) per well for 6 hr before terminating the cultures on the third day.

To quantitate the number of Ig-secreting cells, $5 \times 10^6$ lymphocytes in 2 ml of FCS containing RPMI-1640 medium/well were cultivated with PWM or Staph. aureus in Nunclon® "macroplates" (Nunc, Roskilde, Denmark) and the response measured by the reverse protein A (pA; Pharmacia Fine Chemicals) plaque assay modified from the method of Gronowicz *et al.* [37]. Sheep red blood cells (SRBC) were coupled with pA as follows: one part of pA (0.5 mg/ml) and one part of packed SRBC were added to ten parts of $2.5 \times 10^{-4}$ mol/l chromium chloride in 0.9% NaCl. The mixture was incubated at room temperature for 5 min and then washed once with PBS and twice with Hank's balanced salt solution (HBSS). For the plaque assay, 25 μl of 30% pA-coupled SRBC and 25 μl of 1:30 diluted polyvalent rabbit anti-human Ig were incubated at room temperature for 5 min. After incubation, 50 μl of guinea pig complement, diluted 1:6 and preabsorbed with SRBC, and 100 μl of lymphoid cell suspension ($0.25–0.5 \times 10^6$ cells/ml) were added. After mixing, Cunningham chambers [38] were filled with the cell suspension, sealed with paraffin, incubated at +37° for 2 hr, and the plaque-forming cells (PFC) were counted in an indirect light microscope.

*X-Ray irradiations.* FIP-isolated lymphoid cells diluted in 1 ml of RPMI-1640 culture medium were irradiated with a Stabilipan (Siemens AG, Erlangen, F.R.G.) X-ray irradiator at a dose rate of 711 rad/min.

## RESULTS

### *Receptor binding studies*

Human mononuclear cells were incubated with increasing concentrations of [$^3$H]-dexamethasone, [$^3$H]-medroxyprogesterone acetate or [$^3$H]-Org 2058 and the resultant specific binding plotted according to Scatchard (Fig. 1). Linear binding curves were obtained for [$^3$H]-dexamethasone and [$^3$H]-medroxyprogesterone acetate with $K_d$s of $1.0 \times 10^{-8}$ mol/l and $3.1 \times 10^{-8}$ mol/l, respectively, and there were about 4500 saturable binding sites/cell for both steroids. On the contrary, no significant amount of saturable binding was found when [$^3$H]Org 2058 was used as the ligand (Fig. 1), no matter whether the experiment was performed at +4° or +22° (data not shown) instead of +37° as routinely; the extent of nonspecific binding of [$^3$H]Org 2058 amounted to about 0.2% of the total steroid concentration used.

Competition curves for selected compounds are illustrated in Fig. 2. In cases where significant competition by the compounds with [$^3$H]dexamethasone occurred, the resultant competition curves were roughly parallel. The relative binding affinities of the compounds tested, as calculated on the molar basis, are listed in Table 1. The highest relative binding affinities were recorded for the two potent synthetic progestins, megestrol acetate and medroxyprogesterone acetate (46 and 42%, respectively, as compared to dexamethasone = 100%). Among the naturally occurring steroids, the most

Table 1. Relative binding affinities of the various pregnane and 19-nortestosterone derivatives towards the glucocorticoid receptor of the human mononuclear leukocytes

| Compound | $n$* | Relative† binding affinity |
|---|---|---|
| *Pregnane derivatives* | | |
| Progesterone | 4 | 6.3 ± 1.6 |
| 17-Hydroxyprogesterone | 3 | 7.1 ± 3.0 |
| Deoxycorticosterone | 3 | 4.6 ± 0.8 |
| 4-Pregnene-11$\beta$-ol-3,20-dione | 2 | 2.4 (2.2–2.6) |
| 11-Deoxycortisol | 2 | 5.1 (4.8–5.3) |
| 4-Pregnene-11$\beta$,17$\alpha$-diol-3,20-dione | 2 | 3.6 (2.6–4.6) |
| 4-Pregnene-11$\beta$,21-diol-3,20-dione | 2 | 4.1 (4.0–4.1) |
| Cortisol | 4 | 25 ± 6 |
| 17$\alpha$-Hydroxyprogesterone acetate | 2 | 6.7 (5.0–8.3) |
| R 5020 | 2 | 4.2 (3.3–5.1) |
| Org 2058 | 2 | 1.9 (1.6–2.2) |
| Medroxyprogesterone acetate | 4 | 42 ± 9 |
| Megestrol acetate | 2 | 46 (39–52) |
| Cyproterone acetate | 2 | 5.1 (3.5–6.7) |
| 4-Pregnene-16$\alpha$,17-dimethyl-3,20-dione | 2 | 14 (11–16) |
| *Nortestosterone derivatives* | | |
| Norethisterone | 2 | 0.1 (0.06–0.08) |
| d-Norgestrel | 3 | 0.4 ± 0.3 |
| Lynestrenol | 2 | 0.1 (0.04–0.01) |
| Org 2969 | 2 | 0.1 (0.04–0.2) |
| Org 3236 | 2 | 1.6 (1.2–2.0) |

* Number of experiments.
† Relative to dexamethasone = 100 (mean of two experiments including the range of observations, or mean ± S.E.M.).

effective competitors were cortisol (25%), 17-hydroxyprogesterone (7.1%) and progesterone (6.3%). The five progestins of the 19-nortestosterone series were only weak competitors in this system (Table 1).

In order to more thoroughly characterize the nature of the binding component for medroxyprogesterone acetate in the mononuclear leukocytes, the cells were incubated in the presence of [$^3$H]-medroxyprogesterone acetate and varying amounts of non-labelled steroids. Owing to the remarkably high non-specific binding of [$^3$H]medroxyprogesterone acetate to the cells (about 45–75% of the total binding with the concentrations of the tracer used), reproducible competition curves were difficult to obtain and, therefore, numerical values for relative binding affinities (relative to medroxyprogesterone acetate) were not calculated. Despite these drawbacks, the following order of competing potency (ability to compete with [$^3$H]medroxyprogesterone acetate for the binding to the human mononuclear leukocytes) was repeatedly demonstrated for a group of selected steroids (Fig. 3): dexamethasone > medroxyprogesterone acetate > cortisol > progesterone > Org 2058 >> norethisterone.

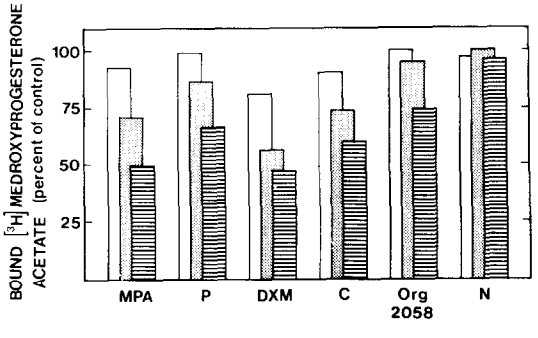


Fig. 3. Competition of the various non-labelled ligands with [$^3$H]medroxyprogesterone acetate for the binding to human mononuclear leukocytes. Leukocytes ($15 \times 10^6$ cells/incubation) and [$^3$H]medroxyprogesterone acetate (final concentration, $3.0 \times 10^{-8}$ mol/l) were incubated in the absence (bound [$^3$H]medroxyprogesterone acetate defined as 100%) and presence of the competing ligands indicated. The concentrations of the steroids tested were $3 \times 10^{-8}$ mol/l (□), $3 \times 10^{-7}$ mol/l (▨), and $3 \times 10^{-6}$ mol/l (▤). MPA, medroxyprogesterone acetate; P, progesterone, DXM, dexamethasone, C, cortisol and N, norethisterone.

*The effect of the different progestins on mitogen-induced DNA synthesis*

Progesterone caused a dose-dependent inhibition of [$^3$H]thymidine incorporation in mitogen-stimulated cultures of peripheral blood mononuclear leukocytes and purified T lymphocytes. A 50% inhibition was obtained with a hormone concentration of approximately $3 \times 10^{-6}$ mol/l (Fig. 4).

At concentrations of up to $3 \times 10^{-8}$–$3 \times 10^{-6}$ mol/l norethisterone and d-norgestrel did not induce any significant stimulation or inhibition of the mitogen-induced DNA synthesis, whether tested with mononuclear leukocytes or with purified T lymphocytes (Fig. 4). On the other hand, medroxyprogesterone acetate and megesterol acetate showed an effect comparable to that of dexamethasone both with mononuclear leukocytes and pure T cells. When mononuclear leukocytes were stimulated with PWM

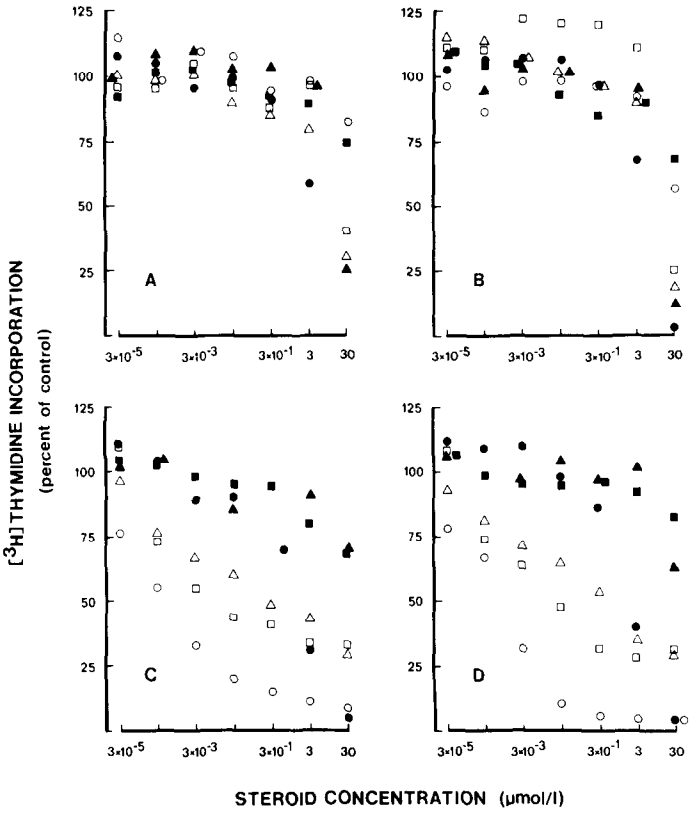


Fig. 4. The effect of various concentrations of dexamethasone (○), medroxyprogesterone acetate (□), megestrol acetate (△), progesterone (●), norethisterone (▲), and d-norgestrel (■) on [$^3$H]TdR incorporation in PWM- (A) and Staph. aureus- (B) stimulated cultures of human mononuclear leukocytes and on ConA- (C) and PHA- (D) stimulated cultures of purified T cells. Each symbol represents the mean of 3–6 different experiments.

or Staph. aureus in the presence of dexamethasone, medroxyprogesterone acetate or megestrol acetate, no inhibition of DNA synthesis was noticed until an apparently toxic steroid concentration of $3 \times 10^{-5}$ mol/l was reached (Fig. 4). When purified T cells were stimulated with the T cell mitogens ConA and PHA in the presence of dexamethasone, medroxyprogesterone acetate or megestrol acetate, a 50% inhibition of DNA synthesis was reached at a concentration of $3 \times 10^{-9}$–$3 \times 10^{-8}$ mol/l (Fig. 4).

*The effect of the different progestins on the PWM-induced Ig synthesis* in vitro

Medroxyprogesterone acetate and megestrol acetate showed an effect similar to that of dexamethasone in the PFC assay when mononuclear leukocytes were simulated with PWM (Fig. 5): in concentrations of $3 \times 10^{-9}$–$3 \times 10^{-7}$ mol/l, medroxyprogesterone acetate and megestrol acetate induced a significant increase in the number of PFC. No significant enhancement in the number of PFC was obtained when mononuclear leukocytes were stimulated with Staph. aureus or when irradiated (1000 rad) cells were used in the PWM-assay (Fig. 5). Progesterone, norethisterone or d-norgestrel brought about no enhancement of the Ig synthesis in any of the systems measured as PFC response (Fig. 5). At very high concentrations ($3 \times 10^{-6}$–$3 \times 10^{-5}$ mol/l) all the progestins tested inhibited the PFC responses by about 50%.

## DISCUSSION

We demonstrate here that certain progestins efficiently compete with [$^3$H]dexamethasone for the binding sites in human mononuclear cells. Previous studies have indicated that dexamethasone is virtually specific for the glucocorticoid receptor with no significant affinity to the progesterone receptor [39]. That the competition of the progestins indeed occurs at the glucocorticoid receptor sites is further supported by two facts. First, we did not find any evidence for the presence of progesterone receptor in the mononuclear leukocytes as judged by the experiments where [$^3$H]Org 2058, a highly specific steroid for the progesterone receptors [40], was used as the radioligand (Fig. 1). Second, tritiated medroxyprogesterone acetate was more efficiently displaced from the binding sites in the leukocytes by potent glucocorticoids than by progestins (Fig. 3). It is especially noteworthy that norethisterone, which binds almost equally as compared to medroxyprogesterone acetate to the human progesterone receptor [41], was not able to displace [$^3$H]medroxypro-

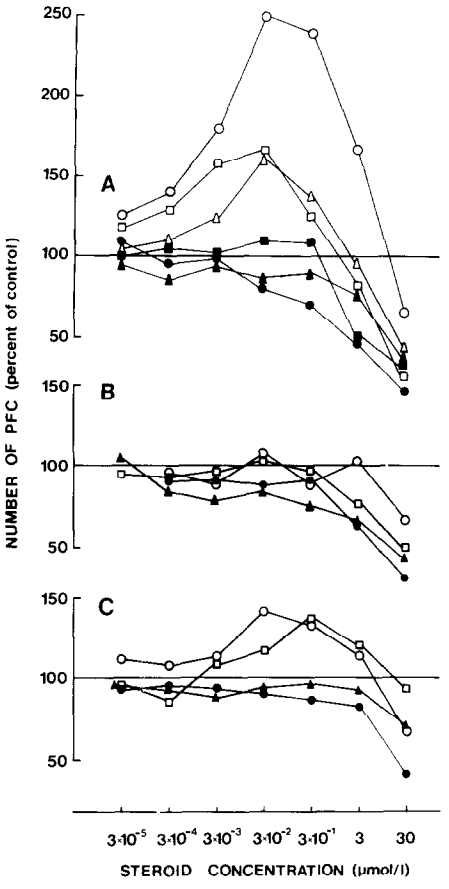


Fig. 5. The effect of various concentrations of dexamethasone (○), medroxyprogesterone acetate (□), megestrol acetate (△), progesterone (●), norethisterone (▲), and d-norgestrel (■) on the PFC response of PWM-stimulated (A), X-irradiated (1000 rad) and PWM-stimulated (B) and Staph. aureus-stimulated (C) cultures of human mononuclear leukocytes. Each symbol represents the mean of 3–6 different experiments.

gesterone from the receptor sites in the mononuclear cells (Fig. 3).

Certain caveats should be born in mind when interpreting results of this type of competition study. One might suspect some metabolism of the steroids to occur during the incubation period. We tested this possibility (data not shown) by subjecting a selected group of tritiated compounds (dexamethasone, progesterone, cortisol, Org 2058, medroxyprogesterone acetate, d-norgestrel) to thin-layer chromatography on silica gel plates before and after 45 min incubation at +37° in the presence of human mononuclear leukocytes. No metabolic conversions took place as judged by the co-chromatography of each test compound before and after the incubation. Another word of caution concerns the unavoidable possibility that some serum steroid binding proteins may have remained adherent to the mononuclear leukocytes after their isolation. In this respect it is of importance that the reference compound dexamethasone does not bind [42] to the relevant serum binder, transcortin, which suggests that the relative binding affinity data are not liable to any systematic bias.

We conclude that, as far as the receptor aspect is concerned, two subgroups among biologically active progestins [41] can be categorized. One group includes compounds with a pregnane structure (e.g. progesterone, megestrol acetate, medroxyprogesterone acetate) which bind efficiently, not only to the progesterone receptor [41] but also to the glucocorticoid receptor (Table 1). The other group comprises steroids with a structure derived from 19-nortestosterone (e.g. norethisterone, d-norgestrel) which bind avidly to the progesterone receptor [41] without remarkable affinity to the glucocorticoid receptor (Table 1). The progestin specificity of the latter group is, however, limited by the fact that these compounds possess intrinsic androgenic activity, probably because they to some extent bind to the androgen receptor [43].

The present data suggest that hydrophobic interactions play an important role in the interactions of steroids with the human glucocorticoid receptor as they do in the steroid–progesterone receptor interactions [41]. Thus, the introduction of 17$\alpha$-acetoxy and 6$\alpha$-methyl groups to the progesterone molecule (Table 1) resulted in an increase in the relative binding affinity from 6.3 to 42% (medroxyprogesterone acetate) or 46% (megestrol acetate, bearing an additional double bond at the carbon 6). Furthermore, the methylation of the progesterone molecule at the positions 16 and 17 (4-pregnene-16$\alpha$,17-dimethyl-3,20-dione; Table 1) approximately doubled the receptor binding affinity. In contrast, the introduction of polar (hydroxyl) substituents to the steroid molecule led to less pronounced effects on the relative binding affinity. Thus, progesterone derivatives with one (17-hydroxyprogesterone, deoxycorticosterone, 4-pregnene-11$\beta$-ol-3,20-dione) or two (11-deoxycortisol, 4-pregnene-11$\beta$,17$\alpha$-diol-3,20-dione, 4-pregnene-11$\beta$,21-diol-3,20-dione) hydroxyl groups had relative binding affinities of 2.4–7.1% (Table 1). The simultaneous presence of three hydroxyl groups at the carbons 11, 17 and 21 (cortisol) increased the relative binding affinity up to 25%.

We have also investigated the influence of progesterone and some selected synthetic progestins *in vitro* functions of human lymphocytes. The synthetic pregnane derivatives, medroxyprogesterone acetate and megestrol acetate, were selected due to their considerable binding affinity towards the glucocorticoid receptor, while the synthetic 19-nortestosterone derivatives, norethisterone and d-norgestrel, where chosen since they did apparently not bind to the glucocorticoid receptor. All these model steroids are known to bind avidly to the human progesterone receptor and also to exert potent progestin activity *in vivo* [41]. Dexamethasone was used as a reference steroid for the glucocorticoid activity. Four different mitogens were selected to study the regulatory influence of progestins to human lymphocytes *in vitro*. The T cell dependent B lymphocyte differentiation induced by PWM has been regarded as a model of the *in vivo* immune response [44]. ConA and PHA are selective T cell mitogens [45] while protein A-carrying Staph. aureus strain Cowan I bacteria

directly activates B lymphocytes independently of T cells [46].

Progesterone itself was found to display a general suppressive activity on the lymphocyte functions, whether tested with mitogens activating only T cells, T + B cells or B cells. The synthetic progestins with a pregnane-type structure showing affinity for the glucocorticoid receptors, medroxyprogesterone acetate and megestrol acetate, gave a different response pattern. While the DNA synthesis of purified T cells stimulated with the T cell mitogens ConA and PHA were very sensitive to the inhibitory activity of these two steroids, the PWM response activating both B cells and T cells was inhibited to a lesser degree and the response induced by the B cell mitogen, Staph. aureus, was even slightly enhanced (Fig. 4). In addition, the PFC response of the mononuclear cells after PWM stimulation in the presence of $3 \times 10^{-9}$–$3 \times 10^{-7}$ mol/l of medroxyprogesterone acetate or megestrol acetate was significantly increased (Fig. 5). This kind of dual activity on the *in vitro* lymphocyte functions was similar to that obtained with dexamethasone. In contrast, the 19-nortestosterone derivatives, norethisterone and d-norgestrel, which did not show any significant binding affinity to the glucocorticoid receptor, were also devoid of the enhancing effect of the PFC generation induced by dexamethasone (Fig. 5).

The mechanism by which medroxyprogesterone acetate and megestrol acetate promote enhanced B cell maturation (Fig. 5) *in vitro* is still to be elucidated. However, as these steroids efficiently bind to the glucocorticoid receptor it is reasonable to assume that this induction operates through molecular mechanisms similar to those of glucocorticoids. Human suppressor T cells have been reported to be especially sensitive *in vitro* to the action of steroid hormones, including glucocorticoids [47–49] and estradiol [50]. Thus, it is tempting to suggest that inhibition of the suppressor cell activity by dexamethasone, medroxyprogesterone acetate or megestrol acetate leads to an enhanced B cell differentiation and an increased generation of PFC.

X-Irradiation with a dose of 1000 rad is known to rather selectively inhibit the T suppressor cell activity [51]. The enhancement of the PWM induced PFC response obtained with dexamethasone, medroxyprogesterone acetate and megestrol acetate was not seen when these steroids were added to cultures of X-irradiated cells. Moreover, the presence of medroxyprogesterone acetate did not induce significant enhancement of the PFC response induced by the T-cell independent [45] mitogen Staph. aureus (Fig. 5). These findings further support the concept that the principal target for hormonal influence is a radiosensitive (suppressor) T cell population.

Under physiological conditions in man the prevailing progesterone concentrations are not likely to reach such levels as to permit an effective competition with cortisol for glucocorticoid receptor sites to occur, with the possible exception of pregnancy. Progesterone, present in very high concentrations at the maternal–fetal interphase, has been proposed to constitute a physiological locally-acting immunosuppressant to prevent fetal rejection [52], although there is no direct evidence that this would occur via a glucocorticoid receptor-mediated mechanism.

We believe, however, that during pharmacological interventions the cross-reactions demonstrated in this study may reach clinical significance. High doses of medroxyprogesterone acetate have been reported to impair glucose tolerance in diabetics [53], depress growth hormone release induced by hypoglycemia [54] and strongly suppress the function of the pituitary–adrenal axis [55]. Medroxyprogesterone acetate alone also maintains adrenalectomized patients without signs of hypocortisolism [56] and shows immunosuppressive activity in dogs [14]. In view of the occurrence of glucocorticoid receptors in uterine tissue [57] even the possibility remains that a part of the antineoplastic effect of medroxyprogesterone acetate in endometrial cancer [58] is mediated via the glucocorticoid receptor instead of the progesterone receptor. To our knowledge, there is no data to indicate that progestins of the 19-nortestosterone type would possess any glucocorticoid-like *in vivo* activity in the human being.

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