J. steroid Biochem. Vol. 23, No. 5A, pp. 553–555, 1985
Printed in Great Britain.

0022-4731/85 $3.00 + 0.00

TRANSCORTIN MODULATES THE EFFECT OF CORTISOL ON MITOGEN-INDUCED LYMPHOCYTE PROLIFERATION AND IMMUNOGLOBULIN PRODUCTION

DIRK FAICT, JAN L. CEUPPENS and PIETER DE MOOR
Laboratorium voor Experimentele Geneeskunde en Endocrinologie and Laboratorium voor Klinische Immunologie (J.L.C.), Campus Gasthuisberg, B-3000 Leuven, Belgium

(*Received* 7 *March* 1985)

Summary—In the present study we investigated whether transcortin modulates the *in vitro* effects of cortisol on the proliferation of human peripheral blood mononuclear cells (PBMC) stimulated by different mitogens and on mitogen-induced polyclonal immunoglobulin production. Physiological doses of cortisol (10–1000 nM) strongly inhibited the proliferation of PBMC stimulated by the monoclonal antibody OKT3 or by phytohemagglutinin. Addition of pure cortisol-free transcortin significantly reduced these inhibitory effects in a dose dependent manner. Transcortin (1 μM) caused a 3- to 4-fold reduction of the effects of 100 nM cortisol. Transcortin alone had no influence on the proliferation of stimulated PBMC. Polyclonal immunoglobulin production by PBMC stimulated with pokeweed mitogen was enhanced by physiological doses of cortisol. A concentration of 100 nM cortisol caused an increase of immunoglobulin G and M production of 81 and 55% respectively. This effect was abolished by addition of 1 μM transcortin to the cultures, whereas transcortin alone had no effect. These results indicate that an evaluation of the effect of corticoids on lymphoid tissues should be based on the free cortisol level rather than on the total cortisol concentration.

INTRODUCTION

Glucocorticoid hormones have striking, but diverse effects on lymphoid tissues and cells [1]. *In vivo*, the greater fraction of these hormones is bound to transcortin and to albumin and only a small amount circulates in the free form [2]. Both *in vivo* [3] and *in vitro* [4] corticoids and corticoid–transcortin complexes had similar effects on lymphoid cells, whereas in many other systems [2, 5] the corticoid–transcortin complexes were biologically inactive. On the other hand, it has been shown that a corticoid-resistant human lymphoid cell line becomes corticoid sensitive after incubation with transcortin [6]. These experiments prompted us to investigate the effect of cortisol and of transcortin on human PBMC. We examined the *in vitro* effects of both compounds on mitogen-induced proliferation of PBMC and on antibody production.

EXPERIMENTAL

Materials

Transcortin was purified as described previously [7]. This pure preparation contained 0.002 mol cortisol per mol transcortin and could bind 0.74 mol cortisol per mol transcortin, as measured by gel filtration [7]. The culture medium (RPMI 1640 medium, containing 10% heat-inactivated foetal calf serum, 100 μg/ml streptomycin, 100 U/ml penicillin and 2 mM L-glutamine) had a cortisol concentration of 0.1 nM and could bind 0.03 nmol cortisol/ml.

Mitogens used in this study were phytohemagglutinin (PHA; Wellcome Reagents, Beckenham, U.K.), pokeweed mitogen (PWM; Calbiochem, Lucerne, Switzerland) and the monoclonal antibody OKT3 (Ortho Pharmaceuticals, Raritan, NJ). The characteristics of the antibody OKT3 have been described in [8].

Cell cultures

PBMC were cultured as in [7] with minor modifications. Briefly, venous blood from 6 adult men (22–45 years) was collected on acid-citrate-dextrose and mononuclear cell suspensions were prepared by centrifugation on Ficoll-Hypaque gradients. The cell preparations contained about 80% lymphocytes and 20% monocytes. For proliferation studies different amounts of cortisol and/or transcortin and PHA (0.125 μg/ml), OKT3 (10 ng/ml) or PWM (1 μg/ml) were added to 0.5×10^6 cells in a final volume of 1 ml culture medium. Four aliquots (0.2 ml) of each tube were distributed in microtiter plates and incubated for 72 h (cells stimulated with OKT3 or PHA) or 96 h (cells stimulated with PWM). Proliferation was estimated by adding 1 μCi [^{3}H]thymidine for the last 8 h of the culture period and measuring the uptake. For the determination of immunoglobulin production, PWM was added in an optimal concentration of 1 μg/ml to 0.5×10^6 cells in a 1 ml vol. The cells were incubated in duplicate for 7 days. Then the cell suspensions were centrifuged and immunoglobulin G and M were measured in the culture supernatant by ELISA [9].

Statistical analysis

Data were analyzed by Student's *t*-test.

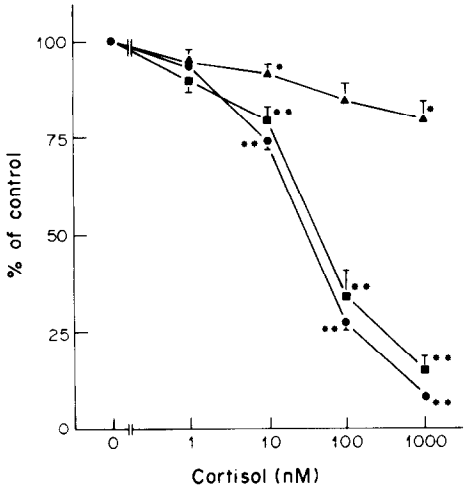

Fig. 1. Influence of cortisol on [^{3}H]thymidine incorporation in human PBMC, stimulated with OKT3 (■), PHA (●) or PWM (▲). Values are expressed as percent of stimulated cultures without cortisol (controls, 100%). The [^{3}H]-thymidine incorporation for these control cultures was 15683, 18821 and 24938 cpm/well for cells stimulated with OKT3, PHA and PWM respectively. Each point represents the mean of six cultures (±SE). Statistical significance of the difference with control cultures is indicated by * ($P < 0.05$) and ** ($P < 0.01$).

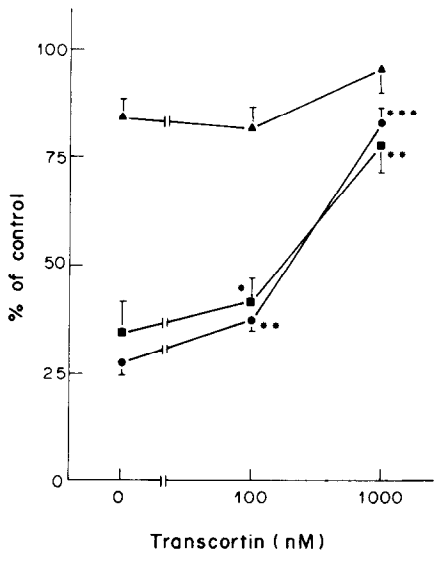

Fig. 2. Influence of transcortin on the [^{3}H]thymidine incorporation in human PBMC, cultured in the presence of 100 nM cortisol and stimulated with OKT3 (■), PHA (●) or PWM (▲). Values are expressed as percent of stimulated cultures without transcortin or cortisol (controls). Each point represents the mean of six cultures (±SE). Statistical significance of the difference with cultures with 100 nM cortisol, but without transcortin is given by * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$).

RESULTS

Effect of cortisol and transcortin on the proliferation of PBMC

Cortisol inhibited the proliferation of mitogen-stimulated human PBMC in a dose-dependent manner (Fig. 1). The effective doses (10–1000 nM) were in the physiological range. The cortisol concentration present in the medium (only 0.1 nM) was well below this effective dose. The degree of the inhibition by cortisol was dependent on the mitogen: cortisol highly inhibited the [^{3}H]thymidine incorporation in PBMC stimulated with the specific T lymphocyte mitogen OKT3 [8] and with PHA. At a concentration of 1 μM, cortisol caused an inhibition of 90%. The proliferation of PWM-stimulated lymphocytes was much less inhibited (Fig. 1). A dose of 1 μM caused only a 20% inhibition of the [^{3}H]thymidine incorporation. This was not due to the rather high dose of PWM used, since similar results were obtained when 10 to 100-fold lower concentrations of PWM were used (results not shown).

Addition of cortisol-free, biologically active transcortin reduced the effect of a constant amount (100 nM) of cortisol (Fig. 2). In the absence of transcortin, 100 nM cortisol caused an inhibition of the [^{3}H]thymidine incorporation in PBMC stimulated with OKT3 or PHA of 66 and 73% respectively. In the presence of 1 μM transcortin, the same dose of cortisol caused an inhibition of only 21 and 17%.

Transcortin alone had no effect. Mean values (±SE, $n = 6$) obtained for the [^{3}H]thymidine incorporation in PBMC cultured in the presence of 1 μM transcortin and stimulated with OKT3, PHA and PWM were 109.1 (±8.4), 100.7 (±3.5) and 90.4 (±2.8) percent of the control values respectively.

Effect of cortisol and transcortin on antibody production

Antibody production by PWM-stimulated PBMC was enhanced by physiological doses of cortisol

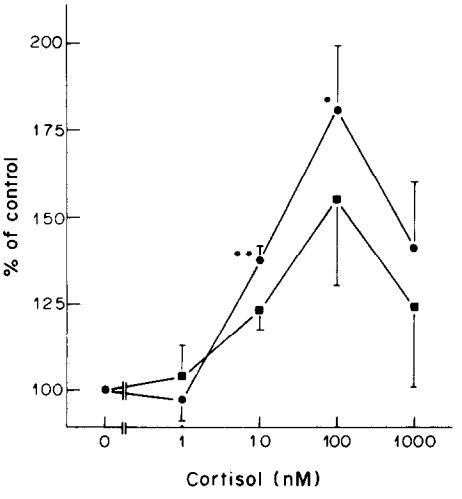

Fig. 3. Influence of cortisol on the production of immunoglobulin G (●) and M (■) by human PBMC, stimulated with PWM. Values are expressed as percent of stimulated cultures without cortisol (controls, 100%). The IgG and IgM production of these control cultures were 2066 and 1457 ng/tube respectively. Each point represents the mean of six cultures (±SE). Statistical significance of the difference with control cultures is indicated by * ($P < 0.05$) and ** ($P < 0.01$).

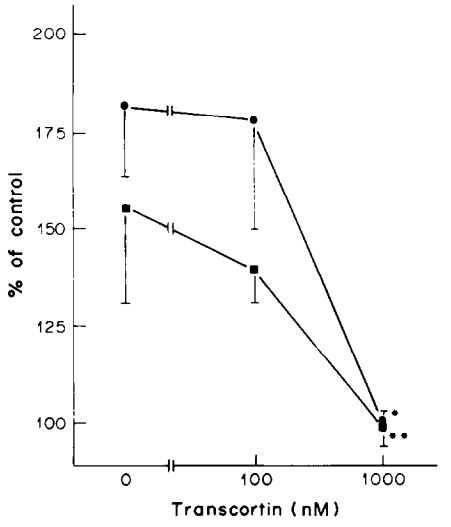

Fig. 4. Influence of transcortin on the production of immunoglobulin G (●) and M (■) by human PBMC cultured in the presence of 100 nM cortisol and stimulated with PWM. Values are expressed as percent of stimulated cultures without transcortin or cortisol. Each point represents the mean of six cultures (±SE). Statistical significance of the difference with cultures with 100 nM cortisol, but without transcortin is given by * ($P < 0.05$) and ** ($P < 0.01$).

(Fig. 3). At a concentration of 100 nM, cortisol caused a 81 and 55% increase of immunoglobulin G and M production respectively. This effect was reduced by addition of 100 nM transcortin to the cells and abolished by 1 μM transcortin (Fig. 4). Transcortin alone had no effect. Immunoglobulin G and M production in the presence of 1 μM transcortin were 98.0 (±5.7) and 91.1 (±6.6) percent of the controls respectively.

DISCUSSION

Corticoids have diverse effects on lymphoid cells [1]. In accordance with previous reports we observed both inhibitory and stimulatory effects. The proliferation of PBMC stimulated with the monoclonal antibody OKT3, a mitogen specific for T lymphocytes [8], was strongly inhibited by cortisol, indicating that T lymphocytes were very sensitive to this corticoid. A similar degree of inhibition was observed for PHA. Proliferation of PBMC stimulated with PWM was less inhibited. Antibody production by these cells on the other hand was enhanced by corticoids. Since, *in vivo*, the greater fraction of corticoids is bound to transcortin [2], it is important to know whether this protein modulates the action of cortisol. As expected [4, 7, 10] transcortin had no effect on the proliferation of PBMC stimulated with PHA. Proliferation induced by OKT3 or by PWM and the PWM-induced immunoglobulin production were also not affected by transcortin. However, transcortin did modulate the effect of cortisol. The biological activity of cortisol was significantly reduced by addition of biologically active, cortisol-free transcortin. The activity of cortisol in the presence of transcortin corresponded better with the free cortisol levels than with total cortisol concentrations. Assuming an affinity constant of the transcortin–cortisol association at 37°C of 3×10^7 [2] free cortisol in cultures with 10^{-7} M cortisol and 10^{-6} M transcortin was calculated to be $3.5\ 10^{-9}$ M. The inhibition of the [^{3}H]thymidine incorporation and the stimulation of antibody production by PBMC cultured in the presence of 10^{-7} M cortisol and 10^{-6} M transcortin corresponded indeed with the effect of $3.5\ 10^{-9}$ M cortisol alone (compare Figs 1–2 and Figs 3–4). This effect is physiologically relevant since both the absolute concentrations of cortisol and transcortin and the cortisol–transcortin ratio used in this study were in the physiological range [11].

These results clearly indicate that for the estimation of the effect of corticoids on lymphoid tissues and cells, the free cortisol level rather than the total cortisol concentration should be used.

Acknowledgements—The authors thank Dr W. Heyns for stimulating discussions, Mrs S. Vertessen for skillful assistance and Mrs M. Vander Auwera and B. Minten for the preparation of the manuscript.

REFERENCES

1. Parillo J. E. and Fauci A. S.: Mechanisms of glucocorticoid action on immune processes. *A. Rev. pharmac. Tox.* **19** (1979) 179–201.
2. Westphal U.: *Steroid–Protein Interactions.* Springer, New York (1971) pp. 213, 434.
3. Rosner W. and Hochberg R.: Corticosteroid-binding globulin in the rat: isolation and studies of its influence on cortisol action *in vivo*. *Endocrinology* **91** (1972) 626–632.
4. Rosner W., Ong K. S. and Khan M. S.: The effect of corticosteroid binding globulin on the *in vitro* incorporation of thymidine into human lymphocytes. *J. clin. Endocr. Metab.* **54** (1982) 201–203.
5. Ballard P. L.: Delivery and transport of glucocorticoids to target cells. In *Glucocorticoid Hormone Action* (Edited by J. D. Baxter and G. G. Rousseau). Springer, New York (1979) pp. 25–48.
6. Amaral L. and Werthamer S.: Cortisol resistant RPMI-1788 lymphocytes become sensitive to cortisol subsequent to a 24 h incubation period in medium containing purified human transcortin. *Experientia* **37** (1981) 903–904.
7. Faict D., De Moor P., Van Baelen H., Heyns W., Stevens E. and Ceuppens J.: Cortisol-free transcortin: preparation and effect on mitogen-stimulated lymphocytes. *J. steroid Biochem.* **19** (1983) 1397–1401.
8. Van Wauwe J. P., De Mey J. R. and Goossens J. G.: OKT3: a monoclonal anti-human T lymphocyte antibody with potent mitogenic properties. *J. Immun.* **124** (1980) 2708–2713.
9. Ceuppens J. L. and Goodwin J. S.: Regulation of immunoglobulin production in pokeweed mitogen-stimulated cultures of lymphocytes from young and old adults. *J. Immun.* **128** (1982) 2429–2434.
10. Ogawa K., Sueda K. and Matsui N.: The effect of cortisol, progesterone, and transcortin on phytohemagglutinin-stimulated human blood mononuclear cells and their interplay. *J. clin. Endocr. Metab.* **56** (1983) 121–126.
11. Hamanaka Y., Manabe H., Tanaka H., Monden Y., Uozumi T. and Matsumoto K.: Effects of surgery on plasma levels of cortisol, corticosterone and non-protein-bound cortisol. *Acta endocr., Copenh.* **64** (1970) 439–451.