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In vivo and in vitro effects of glucocorticoids on lectin-induced blastogenesis in atopic dermatitis

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Summary. The effects of glucocorticoids administered in vivo and in vitro on lectin-induced proliferation of lymphocytes sampled from venous blood were investigated in patients with atopic dermatitis (AD) and in normal controls. Stimulation by concanavalin A (Con A), phytohaemagglutinin A (PHA) and pokeweed mitogen (PWM) in patients and controls did not differ significantly under baseline conditions. After in vivo administration of methylprednisolone the decline of Con A-induced blastogenesis of leucocytes was similar in both groups, whereas PHA stimulation caused a significant reduction in the controls only. In vitro addition of different dexamethasone concentrations had a pronounced suppressive effect on Con A- and PHA-induced blastogenesis in both groups, whereas PWM stimulation was unaffected. Pretreatment in vivo with methylprednisolone further decreased the suppression of the Con A and PHA lymphocyte proliferation rate by dexamethasone added in vitro in controls but not in patients. With regard to B-cell proliferation generated by PWM, no consistent glucocorticoid effect could be observed. The impaired effect on lymphocyte blastogenesis of glucocorticoids administered in vivo, in contrast to a normal in vitro reaction to dexamethasone, together with recent findings of an altered glucocorticoid receptor pharmacology in AD, points to a decreased biological in vivo efficiency of methylprednisolone in atopic dermatitis.

Key words: Lectins – Glucocorticoids – Atopic dermatitis

Atopic dermatitis (AD) is accompanied by a variety of disturbed immune [19, 25, 29], hormonal [17, 21] and even psychological [11] factors. In particular, a decrease in suppressors cells [1, 22] as well as normal rates of B lymphocytes have been noted [26, 30, 32]. However, some authors have reported normal T cells and elevated B cells in atopic dermatitis [6, 12].

Results of studies evaluating lymphocyte response in AD patients to different mitogens are variable. Hyporesponsiveness of T- and B-cell proliferation following stimulation with pokeweed mitogen (PWM), concanavalin A (Con A) or phytohaemagglutinin A (PHA) [5, 18, 20, 26, 29] has been reported as well as normal Con A-, PHA- and PWM-induced lymphocyte blastogenesis [13, 23, 32]. Glucocorticoids inhibit lymphocyte proliferation [8, 28] by cytokinetic mechanisms [24] and affect the number of subpopulations of circulating lymphocytes by inducing a redistribution of intravasal cells to other lymphoid compartments, e.g. to the bone marrow [8, 9]. Moreover, glucocorticoids have been shown to inhibit interleukin-1 (IL-1) [31] and interleukin-2 (IL-2) [4] production leading to impaired lectin-induced blastogenesis [8, 18]. T-cell proliferation generated by Con A [8, 10] and PHA [8, 16, 34] is suppressible by glucocorticoids in vivo and in vitro. However, PWM induced B-cell proliferation is relatively resistant to glucocorticoids [2, 15].

In view of the numerous effects of glucocorticoids on the immune system and their frequent use in the treatment of AD, the present study was designed to evaluate the effect of glucocorticoids, administered in vivo and in vitro, on the characteristics of lymphocyte proliferation under lectin-stimulated conditions in AD compared with healthy controls.

Materials and methods

Subjects

Hospitalized patients (3 men and 8 women) suffering from AD and healthy controls (3 men, 7 women), with no history of asthma, hay fever or AD, participated in the study on a voluntary basis having given informed consent. Their mean age was 22.2 ± 5.4 years and 24.3 ± 3.7 years, respectively. All participants were withdrawn from any systemic therapy with corticoids or ACTH at least 2 months prior to the study. Medication, if any, with beta-blocking and/or sleep-inducing drugs was stopped at least 3 days before entering the

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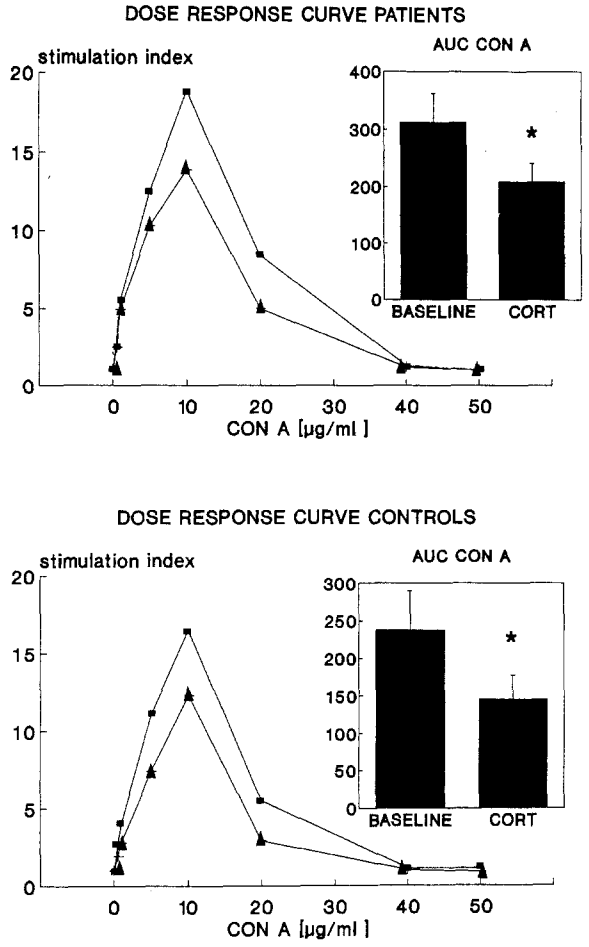

Fig. 1. Mean dose–response curves and AUC for Con A-generated lymphocyte proliferation in patients with AD and in normal controls under baseline conditions (■) and methylprednisolone treatment (▲). SEM are omitted for reasons of presentation. The *inset* shows the mean ± SEM AUC for the baseline (*BASELINE*) and steroid-treated (*CORT*) condition in µg/ml. The difference between baseline and methylprednisolone treatment is significant ($p < 0.04$) in both patients and controls

study. Cutaneous lesions of AD were treated only with corticoid-free ointments for at least 1 week prior to and during the study. Exclusion criteria were prevalence of severe internal illness, and alcohol, drug or nicotine abuse. Diagnosis of AD was based on a history of recurrent flexural eczema, a personal and/or family history of atopy, and clinical criteria of AD as stated by Hanifin and Rajka [14]. Cutaneous involvement, scored according to Costa et al. [7], in the range moderate to marked severity, was 37–67 points (mean ± SD: 46.9 ± 10.2) before therapy and 11–55 points (mean ± SD: 27.6 ± 12.3) at the end of the study.

Test protocol

Venous blood samples were taken at 7 a.m. and 4 p.m. into EDTA-containing plastic tubes on day 1. At 11 p.m. of the same day all patients and controls underwent a 1 mg dexamethasone suppression test to evaluate the integrity of the feedback regulation of the hypothalamus–pituitary–adrenal axis. Post-dexamethasone blood samples were drawn on day 2 as described for day 1. This procedure was followed by daily oral methylprednisolone administration in both groups at 7 a.m.: 8 mg on days 3 to 6, and 4 mg on days 7 and 8. On day 8 blood was drawn at 4 p.m. For determination of lymphocyte proliferation on days 1 and 8, blood samples were collected into prechilled plastic tubes containing EDTA.

Chemicals

^{3}H-Thymidine (specific activity 25 Ci/mmol) was purchased from Amersham (Buckinghamshire, UK) and phosphate buffered saline (PBS) from Boehringer (Mannheim, FRG). Con A, PHA, PWM and sodium metrizoate-Ficoll were obtained from Sigma (St. Louis, Mo., USA), and Rotiszint 22 from Roth (Karlsruhe, FRG). The culture medium consisted of RPMI medium (Boehringer, Mannheim, FRG) containing 2 m*M* L-glutamate (Biochrom, Berlin, FRG) and 0.1 mg/ml gentamicin (Biochrom) with 8% fetal calf serum (Biochrom) added.

Preparation of cells

A mononuclear cell fraction was prepared under sterile conditions by sodium metrizoate-Ficoll density-gradient centrifugation [3]. Cells were washed twice in 40 ml PBS for 10 min and incubated for 60 min at 37°C to allow sufficient dissociation of endogenous hormone, followed by a third washing procedure. The final concentration of cells was determined using a Coulter Counter (Model S5, Coulter Electronics, UK). The viability of the cells exceeded 95% as judged from their ability to exclude trypan blue. Contamination by erythrocytes was < 10%, and by granulocytes and monocytes < 8%, and did not differ between the test days.

Lymphocyte proliferation tests

Lymphocyte proliferation experiments were performed using plastic microtitre plates with a total volume of 0.22 ml. Cells (25000) were incubated in culture medium at 37°C in an atmosphere containing 5% CO_2 for 60 h to evaluate Con A- and PHA-generated T-cell mitogenesis, and for 130 h to evaluate PWM-generated B-cell proliferation as well as glucocorticoid-induced inhibition of spontaneous mitogenesis. Concentrations of Con A and PWM of 0.5, 1, 5, 10, 20, 40, and 50 µg/ml were used to evaluate the dose–response curves, and concentrations of 0.1, 0.5, 1, 2.5, 5, 10, and 20 µg/ml PHA were used for the determination of the effects on lymphocyte proliferation. In vitro glucocorticoid effects on mitogen-stimulated lymphocyte proliferation were assessed by increasing dexamethasone (DEX) concentrations from 10^{-10} to 10^{-6} M. For determination of glucocorticoid effects in vitro, dose–response curves were obtained for concentrations of 1, 5, 10, and 40 µg/ml Con A and PWM, and for concentrations of 0.5, 1, 5, and 10 µg/ml PHA.

Five hours before ending the incubation period 0.5 µCi ^{3}H-thymidine was added, and incorporated ^{3}H-thymidine was subsequently separated from free nucleotide by rapid filtration through Whatman GF/B filters (Nr. 11731) with a Titertek cell harvester by washing with distilled water for 11 s at room temperature. The filters were transferred into plastic vials and, after the addition of 5 ml of a toluene-based scintillation cocktail (Rotiszint 22 Karlsruhe, FRG), were monitored for tritium in a Beckman LS 5000 TD beta-counter (Munich, FRG) (efficiency about 54%). All samples were assayed in triplicate with a variation coefficient of < 15%.

Data analysis

Lymphocyte proliferation was expressed in terms of a stimulation index determined from the ratio of stimulated to unstimulated (baseline) disintegrations per minute. The area under the dose–response curves (AUC) (stimulation index × lectin concentration) was determined using trapezoidal integration (unit: µg/ml).

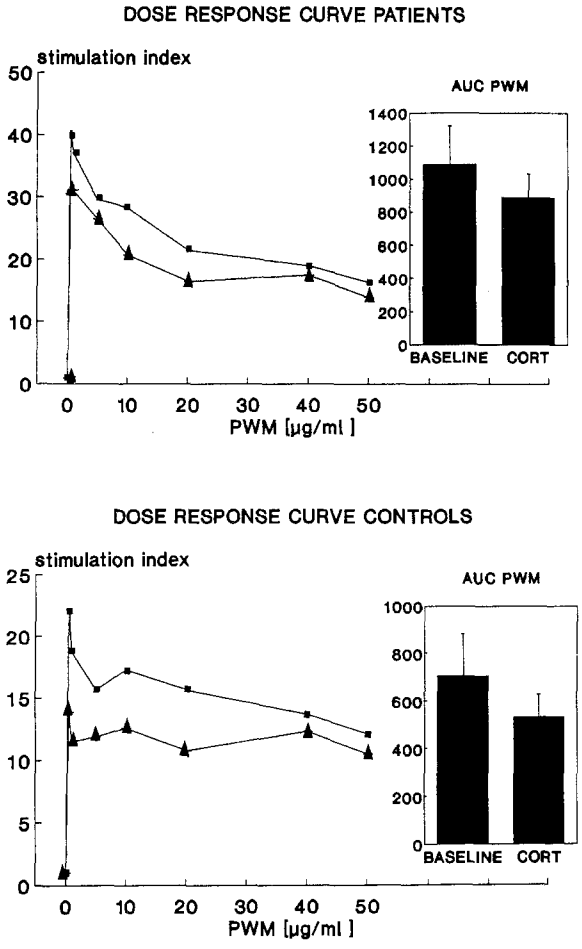

Fig. 2. Mean dose–response curves and AUC of PWM-generated lymphocyte proliferation in patients and controls under baseline conditions (■) and methylprednisolone treatment (▲). SEM are omitted for reasons of presentation. The *inset* shows the mean ± SEM AUC for the baseline (*BASELINE*) and methylprednisolone treated (*CORT*) conditions in µg/ml. There is no significant difference in the reaction to methylprednisolone treatment in patients and controls

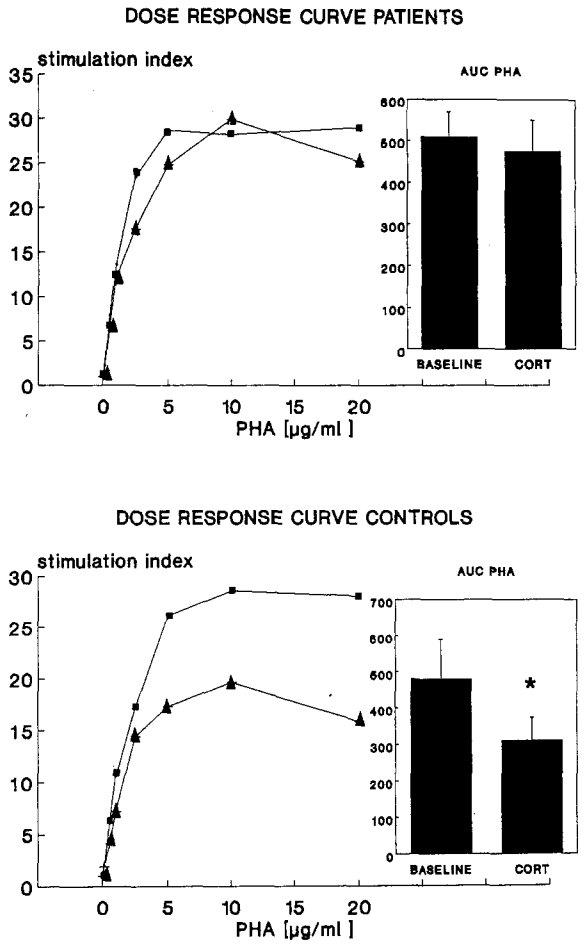

Fig. 3. Mean dose–response curves and AUC of PHA-generated lymphocyte proliferation in patients and controls under baseline conditions (■) and methylprednisolone treatment (▲). SEM are omitted for reasons of presentation. The *inset* shows the mean ± SEM AUC for the baseline (*BASELINE*) and methylprednisolone treated (*CORT*) condition in µg/ml. Baseline and methylprednisolone conditions differ significantly in controls only

The results are expressed as means ± SD, and as means ± SE in the figures. Comparisons between patients and controls were made using Student's *t*-test, the intra-individual comparisons by the *t*-test for paired samples. All significance levels are two-tailed. Statistical significance was taken as $p < 0.05$.

Results

Increasing concentrations of mitogens added to lymphocytes from patients and controls resulted in dose–response curves with maximal stimulation of lymphocyte proliferation at 10 µg/ml Con A (Fig. 1) and at 1 µg/ml PWM (Fig. 2). PHA-generated mitogenesis was maximal at concentrations of 10–20 µg/ml (Fig. 3). There was no shift of the maxima in the dose–response curves in patients and controls after glucocorticoid administration (Figs. 1–3). The AUC after stimulation with Con A, PHA or PWM did not differ significantly between patients and controls (Figs. 1–3) under baseline conditions.

Systemic glucocorticoid administration resulted in a significant reduction in Con A-induced blastogenesis (AUC_{ConA}) in both groups ($p < 0.04$) (Fig. 1), whereas PHA-stimulation of lymphocytes resulted in a significant reduction in blastogenesis (AUC_{PHA}) after glucocorticoid administration in the controls only ($p < 0.03$) (Fig. 3). The differences in AUC_{PWM} in patients and controls prior to and after glucocorticoid treatment did not reach statistical significance (Fig. 2).

Dexamethasone administered in vitro had a pronounced suppressive effect on Con A and PHA dose–response curves in patients and controls at baseline and under corticoid treated conditions (Fig. 4a, b). However, in vivo pretreatment with methylprednisolone further decreased the in vitro suppression of $AUC_{Con\,A}$ and AUC_{PHA} by dexamethasone in the controls ($p < 0.05$), but not in the patients (Fig. 4a, b). In contrast, no consistent effect of the corticoid pretreatment on the in vitro response to dexamethasone could be observed with regard to PWM-generated B-cell proliferation (Fig. 4c).

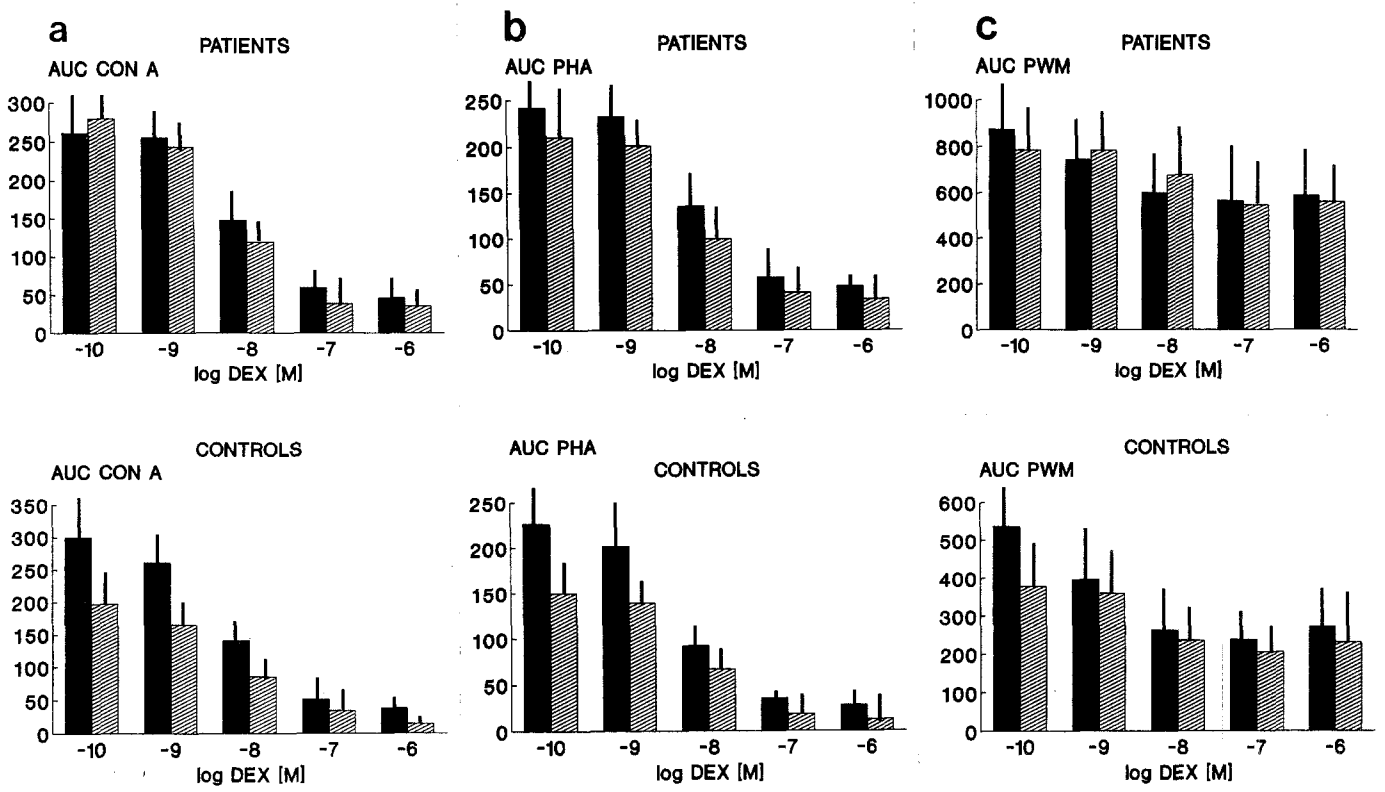

Fig. 4a–c. Mitogen stimulation after administration of different dexamethasone (*DEX*) concentrations in vitro in untreated (*solid bars*) and corticoid-treated (*hatched bars*) patients and controls. **a** Mean ± SEM AUC of Con A-generated lymphocyte proliferation in μg/ml. A significantly greater suppression occurred in controls than in patients after methylprednisolone pretreatment. **b** Mean ± SEM AUC of PHA-generated lymphocyte proliferation in μg/ml. A significantly greater suppression occurred in controls than in patients after methylprednisolone pretreatment. **c** Mean ± SEM AUC of PWM-generated lymphocyte proliferation in μg/ml. No significant effect of methylprednisolone pretreatment was found in patients and controls

Discussion

The normal response to Con A, PHA and PWM found in our patients with AD is in accordance with previous reports [23, 32] which could not confirm findings of impaired leucocyte proliferation in AD [5, 18, 20]. The decreased Con A-induced blastogenesis in patients and controls after the administration of glucocorticoids in vivo and in vitro may be due to the suppressive effects of glucocorticoids on T-cell mitogenesis [8, 16, 28, 34].

Corticosteroid effects on PHA-induced blastogenic response have been found to be variable [8]. This variability has been explained by the observation that glucocorticoids can shift the peak of the PHA dose–response curve to higher concentrations [8], but this could not be confirmed in the present study. Since the suppression of PHA responsiveness by glucocorticoids is dose related, it could be hypothesized that the suppression only in the controls of AUC_{PHA} after methylprednisolone therapy is due to a higher sensitivity of normal lymphocytes to glucocorticoids. This hypothesis is supported by the observation that higher glucocorticoid concentrations, as obtained in vitro, also produced a sufficient suppression of AUC_{PHA} in patients. Thus, the significantly greater suppression of Con A- and PHA-induced blastogenesis in vitro in the controls after methylprednisolone treatment points to a subtle in vivo resistance of leucocytes to glucocorticoids in patients with AD.

Elevated IL-2 receptor levels in AD have recently been described [4] and glucocorticoids are known to reduce both IL-2 receptor sites and IL-2 production [4, 33]. Therefore, impaired suppression of these receptors by glucocorticoids in AD may be the reason for the higher lectin-induced proliferation rate of lymphocytes following glucocorticoid therapy in comparison with the controls.

PWM-induced blastogenesis measured after 5 days of culture has been shown mainly to reflect B-cell proliferation [2, 8, 15] and has been observed to be relatively refractory to the suppressive effect of glucocorticoids [2, 8, 15]. These findings are in accordance with our results in patients and controls. Our data show a lowered suppression of some proliferation parameters of mononuclear leucocytes after in vivo administration of methylprednisolone, while the in vitro effects of dexamethasone appear to be normal. These findings together with recently reported alterations in glucocorticoid receptor pharmacology in AD [27] suggest a decreased biological efficiency of methylprednisolone on lymphocytes in vivo, the origin of which remains to be elucidated.

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