

In vitro and *in vivo* effect of glucocorticoids on IgE and IgG subclass secretion

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Summary

Hydrocortisone (HC) as well as its synthetic derivatives have been shown to strongly enhance interleukin-4 (IL-4)-induced *in vitro* IgE synthesis. To investigate possible effects on IgG subclasses, peripheral blood mononuclear cells (PBMC) were incubated with different glucocorticosteroids in the absence or presence of IL-4. The glucocorticoids alone led to a strongly enhanced secretion of IgG1, IgG2 and IgG3, but not IgG4. The addition of IL-4 induced marked increases in IgG1 and IgG4, no changes in IgG3, but a consistent decrease in IgG2 synthesis. In order to find out whether these profound *in vitro* effects of corticosteroids are also reflected by changes in antibody serum levels during steroid treatment, 10 healthy volunteers took 25 mg prednisone for 7 consecutive days. We could not observe any significant changes of IgE or IgG subclass serum levels during or after this period. However, cell cultures performed after the glucocorticoid treatment revealed a marked decrease in the ability to produce IgG4 and a significantly lower potential to produce IgE in response to IL-4 alone or IL-4 and HC. We conclude that, although strongly implicated by the *in vitro* results, glucocorticosteroid treatment does not result in an increased allergy risk.

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Introduction

Synthetic corticosteroids are widely applied for the treatment of autoimmune disorders, in particular of allergic diseases. Recently, it has been shown that hydrocortisone (HC) and its synthetic derivatives are able to potentiate *in vitro* interleukin-4 (IL-4)-induced IgE production by peripheral blood mononuclear cells (PBMC) [1–4]. To co-stimulate IgE secretion, glucocorticoids can even circumvent the otherwise necessary presence of T cells [2,4,5]. Both enhancing and suppressing effects of glucocorticosteroids on the secretion of IL-4 by lymphocytes have been reported [6–9]. A diurnal variation of endogenous steroid levels has been found to influence *in vivo* IgE production [10]. Considering the enormous *in vitro* potential of corticosteroids to enhance the production of IgE, the present study on their *in vivo* effects was conducted to investigate, whether glucocorticoid therapy leads to the risk of

increased IgE synthesis containing even the concomitant hazard of developing new sensitizations. So far older studies have reported contradictory data on the effect of glucocorticoid treatment on immunoglobulin serum levels of allergic patients [11–19].

HC has also been shown to strongly increase IgA, IgM and IgG production by PBMC [1,20]. However, no data on its effect on human IgG subclasses have been reported. Since the IgG subclass response to most allergens has been found to be restricted to IgG4 and IgG1, occasionally only to IgG4 [21–23], it is important for the understanding of allergies to study possible differential effects of glucocorticoids on the regulation of IgG subclass secretion. In the present study we are able to provide data on the corticosteroid effect on IgG subclasses *in vitro* and *in vivo*.

Materials and methods

Cell cultures

Heparinized venous blood was obtained from healthy

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blood donors. The PBMC were isolated by Ficoll/Hypaque density gradient centrifugation. They were washed three times in RPMI 1640 medium supplemented with 2 mmol/l glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin and 2.5% fetal bovine serum (Gibco, Paisley, UK).

PBMC were cultured in a medium containing 10% in a medium containing 10% fetal bovine serum in 96-well round bottom culture plates (Nunc, Copenhagen, Denmark) in a humidified atmosphere of 5% CO₂ and 95% air. Concentrations of 2×10^6 cells/ml were used. Except for the dose-response studies, recombinant IL-4 (kindly provided by Dr H. C. Kocher) was added at 200 U/ml. The corticosteroids 6- α -methylprednisolone, dexamethasone, fludrocortisone, HC, prednisolone, prednisone and triamcinolone (Sigma, Deisenhofen, Germany) were dissolved in DMSO at a stock concentration of 10^{-1} mol/l and further diluted in culture medium. Glucocorticoids were added at 10^{-6} mol/l, a concentration which has been found in previous experiments to induce optimal antibody production [3]. For the experiments investigating dose-dependence effects on IgG4 and IgE synthesis the steroids were tested at various concentrations ranging from 10^{-3} to 10^{-10} mol/l.

Supernatants were collected after 14 days. The release of cell-associated immunoglobulin performed *in vivo* was determined in cultures containing 100 µg cycloheximide/ml (Sigma). All cultures were done in five identical replicates.

In vivo study regimen

In order to study the effect of glucocorticoid treatment on *in vivo* immunoglobulin production, 10 healthy volunteers, three women and seven men (mean age 35, range 24–53 years; mean IgE serum level 61.5 ng/ml, range 1.0–215.8 ng/ml) took 25 mg prednisone for 1 week (days 9–6). The prednisone dose was not corrected for the body surface area. Sera were collected twice before (day –2 and day –1), on day 1, day 4 and day 6 of the treatment period as well as one day (day 7), 1 week (day 14) and 2 weeks (day 21) afterwards. To study, whether the treatment might alter the capacity of PBMC to respond to IL-4 and HC, heparinized blood for cell cultures was obtained on day –1, 7, 14 and 21.

IgE measurement

IgE in the cell supernatants and sera was measured by an enzyme linked immunosorbent assay, which has been described previously [3]: 96-well plates (Nunc) were coated with a rabbit-antihuman-IgE (Behring) for 1 h, then washed three times with buffer, and subsequently the non-specific binding sites were blocked with a 10%

FCS-buffer solution for 30 min. Dilutions of the culture supernatants, sera or purified myeloma protein standards (kindly provided by Dr H.L. Spiegelberg) and a supernatant pool of known IgE content were added and incubated overnight. After washing, the plates were incubated with a biotinylated goat-antihuman-IgE (medac, Hamburg, Germany) for 1 h. Another washing step was followed by the addition of the streptavidin/ peroxidase-complex (Amersham Buchler, Braunschweig, Germany) for 30 min. The plates were washed again and afterwards the substrate o-phenylenediamine-dihydrochloride (Sigma) and H₂O₂ were added. Finally the staining reaction was stopped with 2 mol/l HCl and the optical density (OD) was measured at 492 nm and 630 nm. The concentrations were calculated from appropriate standard curves. Mean values of the replicate cultures were calculated. All data on antibody secretion in all cultures are given as net synthesis after subtraction of preformed immunoglobulin (cycloheximide control).

IgG subclass measurement

IgG subclass concentrations in supernatants and sera were measured by an enzyme linked immunosorbent assay adopted from a radioimmunoassay (RIA) described previously [24]: goat-antimouse-IgG1 (Sigma) was bound on a 96-well plate for 2 h. After washing three times with buffer, the plates were incubated with subclass specific monoclonal mouse-antihuman-antibodies for two hours: anti-IgG1 (clone NL 16, Oxoid, Wesel, Germany), anti-IgG2 (clone HP 6002, Sigma), anti-IgG3 (clone HP 6050, Sigma) or anti-IgG4 (clone RJ 4, Dianova, Hamburg, Germany). Thereafter, they were washed again and non-specific binding sites were blocked by adding a 10% FCS-buffer solution for 30 min. Then dilutions of the supernatants, sera or purified myeloma standards for each IgG isotype (kindly provided by Dr H.L. Spiegelberg) or a standard with known contents of IgG1, IgG2, IgG3 and IgG4 (The Binding Site Ltd, Birmingham, UK) were added and incubated overnight. The plates were washed again and a biotinylated sheep-antihuman-IgG (Amersham Buchler) was added for 1 h. After another washing step the streptavidin/peroxidase-complex was added and the assay was completed as described above for the IgE-ELISA.

Statistical analysis

For statistical analysis the paired Wilcoxon rank sum tested was applied.

Results

In vitro effect of glucocorticoids

As shown in Fig. 1 the incubation of PBMC with HC alone led to a strongly enhanced secretion of IgG1, IgG2 and IgG3, whereas no consistent effect could be observed on IgG4 production. The further addition of IL-4 induced marked increases in IgG1 and IgG4 secretion, no changes in IgG3 production, but a consistent decrease in IgG2 synthesis. Compared with the data obtained on IgE secretion, only the synthesis of IgG4 shows a concomitant regulation by glucocorticoids and/or IL-4.

These effects were also observed, when synthetic derivatives (6- α -methyl-prednisolone, dexamethasone, fludrocortisone, prednisolone, prednisone and triamcinolone) of HC were tested. The data on three representative glucocorticoids are provided in Fig. 1.

As we have been able to demonstrate for IgE previously [3], a shift in the dose-response curve of the effect of glucocorticoids in the presence of IL-4 could also be observed for IgG4 synthesis (Fig. 2). Depending on their anti-inflammatory potential relative to HC, lower concentrations of the more potent derivatives prednisone (fourfold activity) and dexamethasone (30-fold) were sufficient to induce IgG4 synthesis. This dose-response shift was also obtained for 6- α -methyl-prednisolone (fivefold), fludrocortisone (fivefold), prednisolone (fourfold) and triamcinolone (fivefold) (data not shown).

Furthermore, Fig. 2 shows that the dose-effect curves for IgG4 completely paralleled those for IgE. Although variations of the absolute amounts of immunoglobulin induced by the glucocorticoids and IL-4 were noted, this dose-response relation could be observed in all individuals tested.

In order to determine the IL-4 dose at which glucocorticoids are able to potentiate IgE and IgG4 formation, PBMC were incubated with various doses of IL-4 in the presence of an optimal HC concentration. As depicted in Fig. 3, a maximal co-stimulation can be observed at an IL-4 concentration of 10 U/ml. At lower concentrations, a moderate increase of IgG4 but not IgE occurs. In that range IL-4 alone induces some IgG4 but no IgE.

In vivo effect of glucocorticoids

As has been demonstrated for IgE previously [1–5] and shown for the IgG subclasses in the first part of this study, HC and synthetic glucocorticoids exert profound effects on human *in vitro* antibody synthesis. Since synthetic glucocorticoids are often used to control allergic disorders, a study was conducted to investigate,

whether corticoid treatment alters the *in vivo* immunoglobulin production, in particular IgE. Figure 4 summarizes the results on the serum levels of IgE and IgG subclasses measured before, during and after a 7-day treatment period of 10 healthy volunteers with 25 mg prednisone daily. There were no significant changes of any of the immunoglobulin classes tested.

Ex vivo effect of glucocorticoids

In order to investigate, whether taking prednisone induces changes of the reactivity of PBMC to HC and IL-4, PBMC were isolated before and after the prednisone treatment and cultured with IL-4 and HC. Compared with pretreatment values a significant decrease ($P < 0.05$) in their ability to produce IgE in response to IL-4 alone or to IL-4 and HC was observed in cultures performed one day after the prednisone treatment had been completed (Fig. 5). Although PBMC incubated 1 and 2 weeks later still yielded lower IgE levels, these changes were not significant. Changes in the inducibility of IgG4 were similar to those in IgE production, but did not reach significance (data not shown). No marked changes were observed in IgG1, IgG2 and IgG3 secretion or in quantities of preformed immunoglobulins as measured by the cycloheximide control (data not shown).

Discussion

In this study we demonstrate profound effects of HC and its synthetic derivatives on the secretion of IgG subclasses and IgE *in vitro*, implying that the therapeutical administration of glucocorticoids imposes an increased allergic risk. However, despite what would have had to be expected from the *in vitro* experiments, the treatment of healthy volunteers with glucocorticoids taken at a medium dose did not alter IgE or IgG subclass serum levels. In the contrary, after the treatment even a diminished ability of PBMC to respond to steroids and IL-4 to produce IgE in cell cultures was observed.

A stimulatory effect of corticosteroids on *in vitro* production of total IgG has been described [1,20]. Examining the steroid effect on IgG subclass formation for the first time, we were able to show that this is due to an increase in IgG1, IgG2 and IgG3, but not IgG4. However, the addition of IL-4 led to enormous increases in IgE and IgG4 secretion, to a lesser extent of IgG1 and even to a suppression of IgG2 production. Therefore, the co-regulation of IgG4 and IgE by IL-4 reported earlier [24–26] is also maintained in the presence of corticosteroids. It further suggest that, in contrary to the other subclasses, the potentiating effect of steroids on

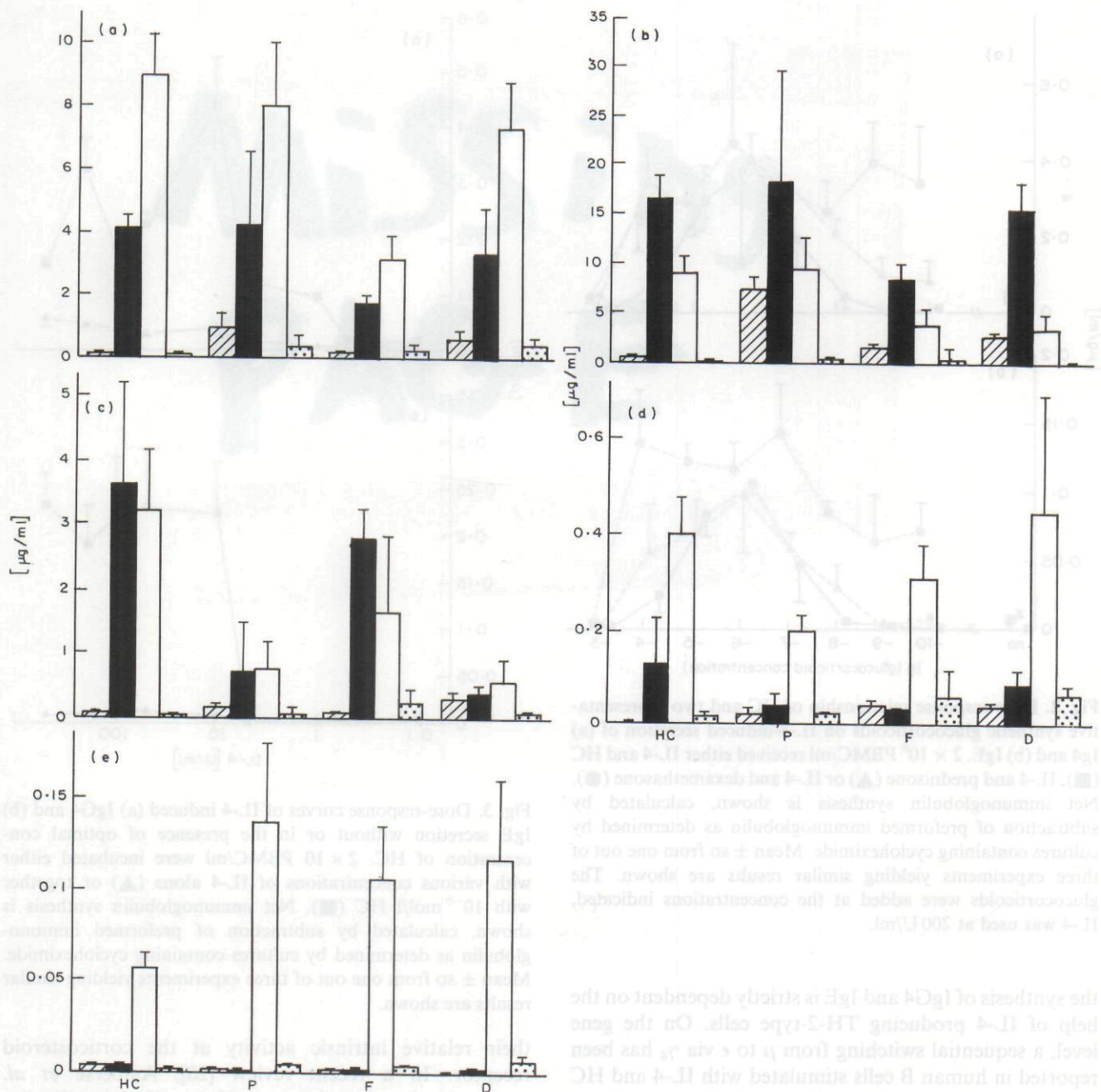


Fig. 1. Effect of hydrocortisone (HC), prednisone (P), fludrocortisone (F) and dexamethasone (D) on the secretion of IgG subclasses and IgE by PBMC in the presence of or without IL-4. 2×10^6 PBMC/ml were either incubated alone (□), with glucocorticoid (■), with glucocorticoid and IL-4 (▨) or with IL-4 (▤). Net immunoglobulin synthesis is shown, calculated by subtraction of preformed immunoglobulin as determined by cultures containing cycloheximide. Mean $\pm$ sd from one out of three experiments yielding similar results are given. Glucocorticoids were used at 10^{-6} mol/l, IL-4 was added at 200 U/ml. (a) IgG1. (b) IgG2. (c) IgG3. (d) IgG4. (e) IgE.

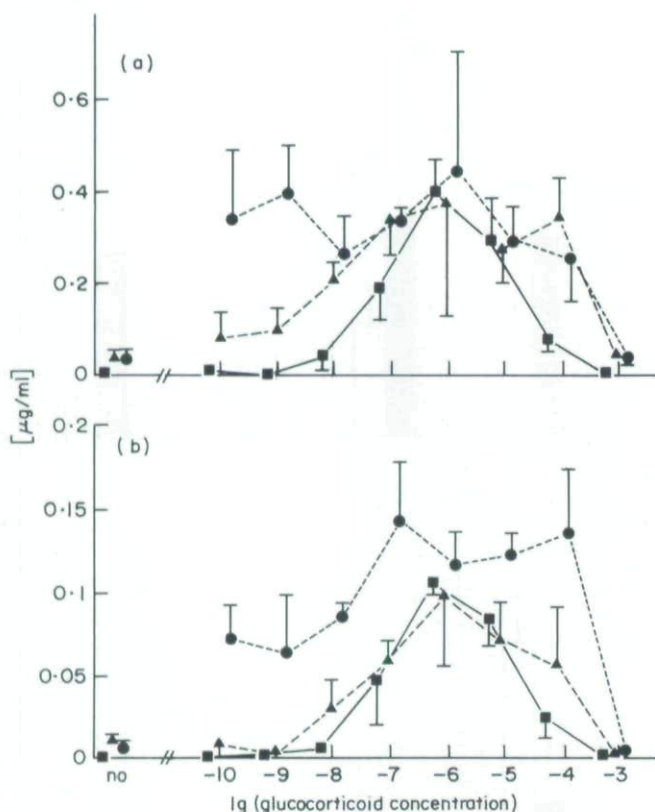


Fig. 2. Dose-response relationship of HC and two representative synthetic glucocorticoids on IL-4-induced secretion of (a) Ig4 and (b) IgE. 2×10^6 PBMC/ml received either IL-4 and HC (■), IL-4 and prednisone (▲) or IL-4 and dexamethasone (●). Net immunoglobulin synthesis is shown, calculated by subtraction of preformed immunoglobulin as determined by cultures containing cycloheximide. Mean $\pm$ SD from one out of three experiments yielding similar results are shown. The glucocorticoids were added at the concentrations indicated, IL-4 was used at 200 U/ml.

the synthesis of IgG4 and IgE is strictly dependent on the help of IL-4 producing TH-2-type cells. On the gene level, a sequential switching from μ to ϵ via γ_4 has been reported in human B cells stimulated with IL-4 and HC [27]. The strong regulatory linkage is in concordance with clinical data on allergic patients, where, after exposure to allergens, besides the formation of IgE a restriction of IgG subclass responses to IgG1 and IgG4 is frequently found [21–23]. IgG4 is also induced by immunotherapy and is believed to be associated with a beneficial outcome of this treatment [23,28,29]. Furthermore, patients with parasitic infections have elevated IgE and IgG4 serum levels [30–32]. As we have already shown for IgE [3], the steroid effect on IgG subclasses was not only exerted by HC, but also by its synthetic derivatives, showing a dose-effect relation depending on

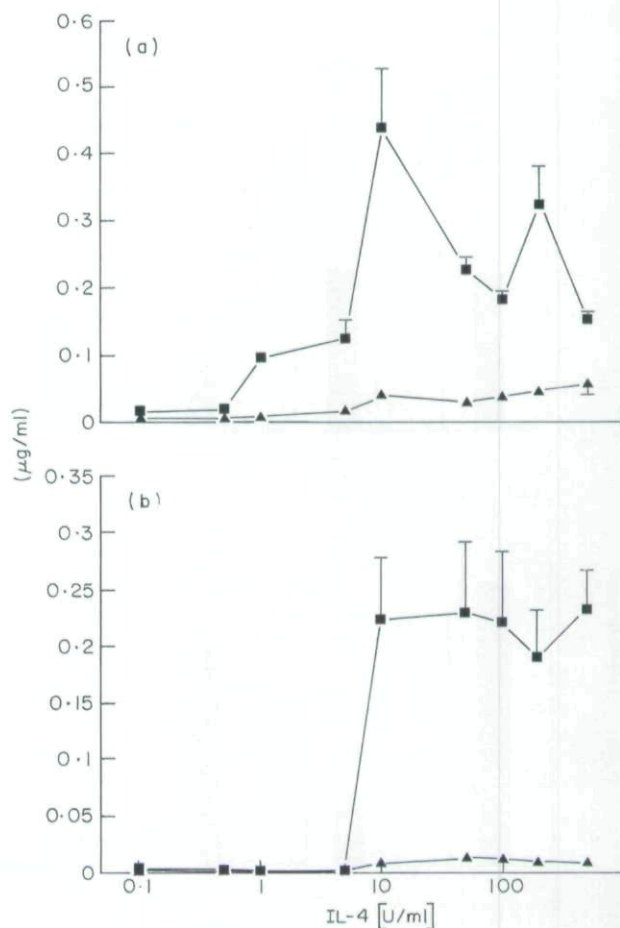


Fig. 3. Dose-response curves of IL-4 induced (a) IgG4 and (b) IgE secretion without or in the presence of optimal concentration of HC. 2×10^6 PBMC/ml were incubated either with various concentrations of IL-4 alone (▲) or together with 10^{-6} mol/l HC (■). Net immunoglobulin synthesis is shown, calculated by subtraction of preformed immunoglobulin as determined by cultures containing cycloheximide. Mean $\pm$ SD from one out of three experiments yielding similar results are shown.

their relative intrinsic activity at the corticosteroid receptor. In a recent review [28], Aalberse *et al.* summarize that the epitope specificity of IgG4 antibodies is often different from that of IgE antibodies implying rather the involvement of two different B cells than a sequential switch of one B cell, which has been observed *in vitro* in the presence of HC [27]. Therefore glucocorticoid treatment might not only influence the amount but also the specificity of the IgG4 antibodies produced. Since the role of IgG4 for the clinical manifestation of allergy is still not well defined, it is currently unclear, whether such a shift towards antibodies with the same specificity as IgE would be a favourable or an adverse effect of steroid therapy.

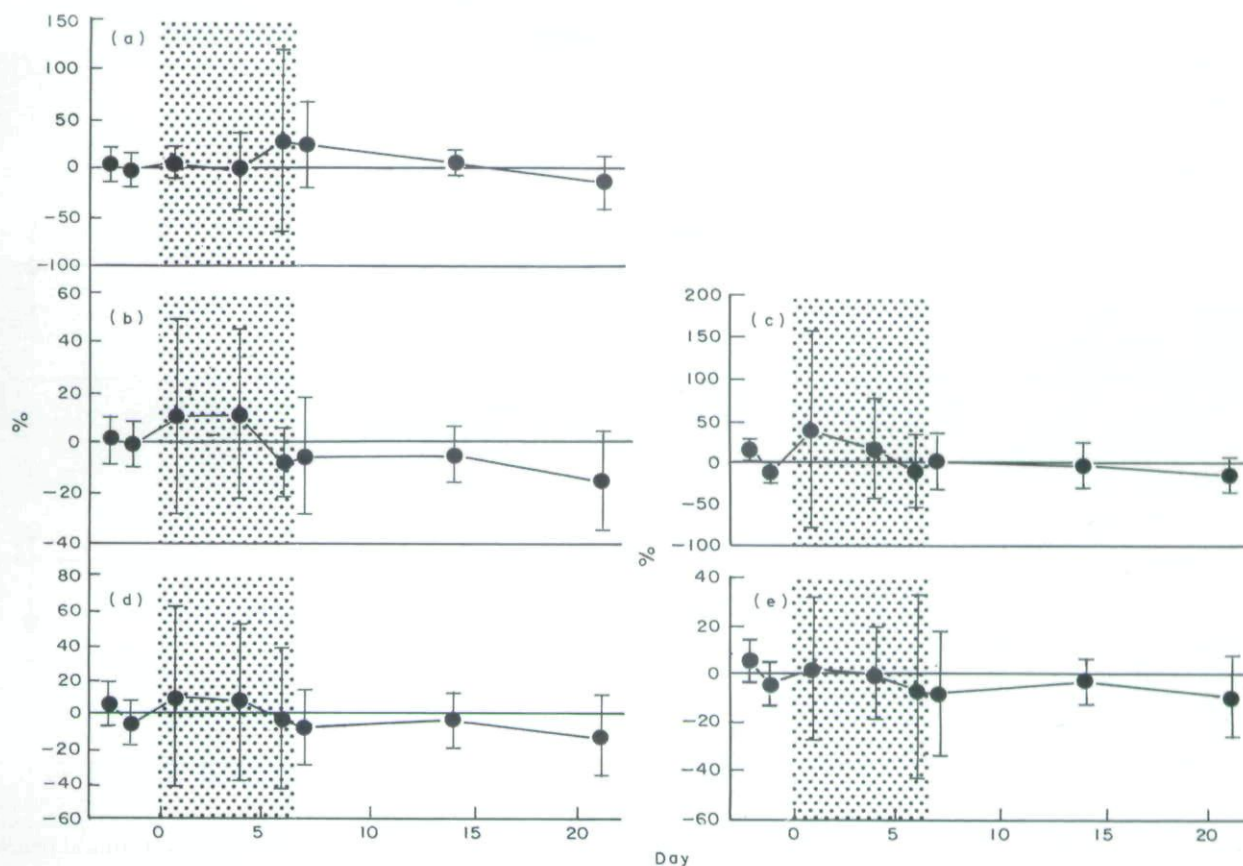


Fig. 4. Monitoring of IgE and IgG subclass serum levels before, during and after prednisone treatment. Mean $\pm$ SEM from 10 healthy donors (% changes compared with pretreatment levels). The period of prednisone treatment is indicated by the shaded area. The mean of the two measurements obtained 1 and 2 days before treatment was defined as zero. (a) IgE. (b) IgG1. (c) IgG2. (d) IgG3. (e) IgG4.

To investigate possible *in vivo* effects of glucocorticoids on immunoglobulin secretion 10 volunteers took 25 mg prednisone for 7 consecutive days. This dose regimen was chosen because corticosteroids at medium doses are often administered for the management of various diseases, including the control of allergic disorders. Our results show that in contrary to the *in vitro* experiments no significant changes of IgE or IgG subclass levels were induced during an observation period of 3 weeks. Considering the steep increase in IgG4 and IgE secretion at a certain IL-4 concentration *in vitro* (Fig. 3), one explanation for the outcome of the trial might be the dependence of IgE and IgG4 production on the presence of IL-4, i.e. cortisone enhances IgE and IgG4 only in such individuals where a sufficient amount of endogenous IL-4 is available. This has been suggested for atopic individuals [25,33] and may be the case during parasitic infection [31,33,34]. An initial increase of serum IgE in allergic patients under glucocorticoid treatment has been reported in two older studies [18,19]. However, two others did not find any significant changes in IgE

serum levels [13,14]. Glucocorticoids themselves may be able to influence IL-4 secretion. Whereas in one study an enhanced capacity of T cells to produce IL-4 in response to glucocorticoids has been reported [6], three others completely contradict these findings by demonstrating a glucocorticosteroid induced suppression of IL-4 production by lymphocytes [7–9]. Even if the latter data reflect the human *in vivo* situation and the lack of increased IgE and IgG4 synthesis under corticosteroid treatment is only due to reduced quantities of endogenous IL-4, prednisone should have increased IgG1, IgG2 and IgG3 serum levels. Given the fact that physiologic serum levels of HC are in the range of 10^{-6} mol/l, a major reason for the inability of prednisone to alter immunoglobulin serum levels may be the existence of an already sufficient amount of endogenous HC inducing optimal IgE and IgG subclass secretion, which cannot be further increased by additional exogenous cortisone. This hypothesis is supported by the dose-response curves obtained *in vitro* (see Fig. 2). It also implies that HC plays a major if not indispensable role in

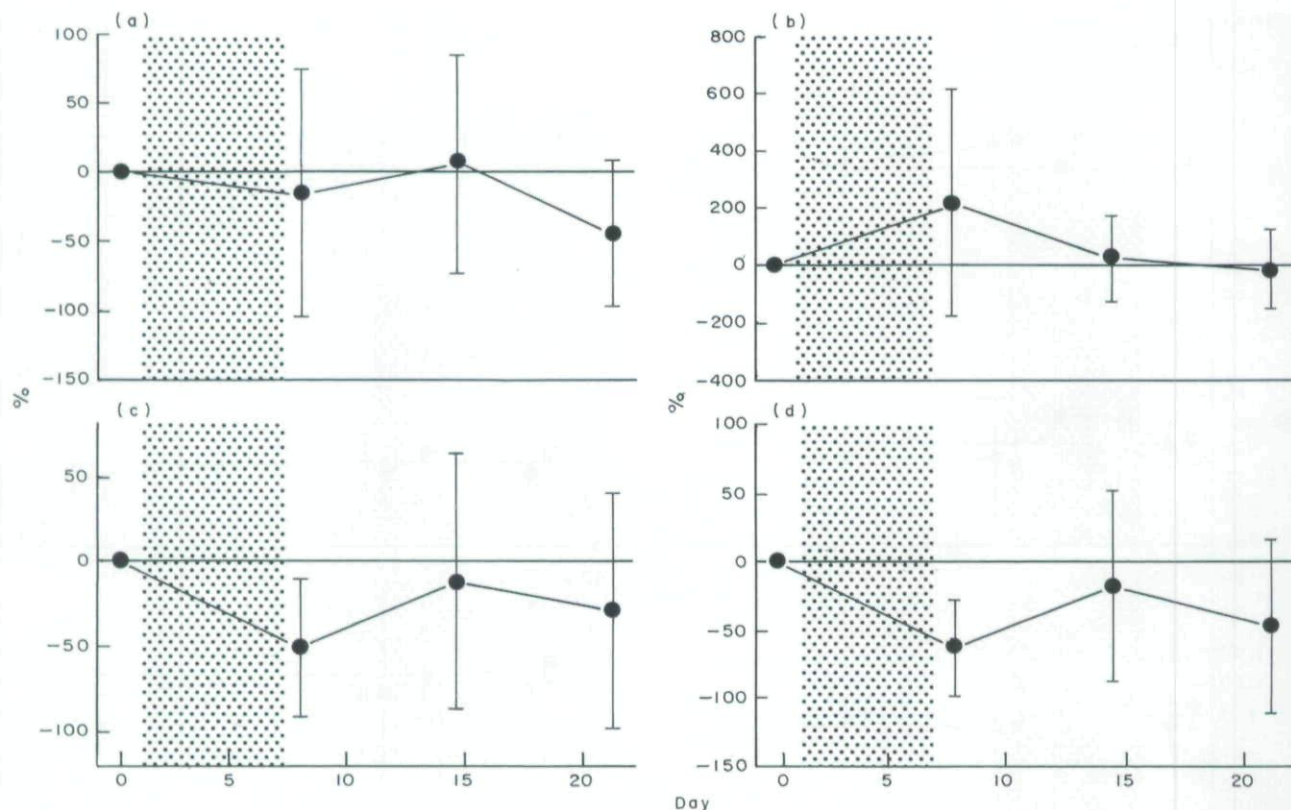


Fig. 5. Effect of HC and IL-4 on IgE secretion by PBMC from healthy volunteers isolated before or after a 1 week treatment period with 25 mg prednisone daily. Mean changes compared with pretreatment secretion $\pm$ SEM. The period of prednisone treatment is indicated by the stippled area. PBMC were cultured (a) alone, (b) with HC, (c) with IL-4, (d) with HC and IL-4.

physiological IgE production. In mice, the *in vivo* IgE synthesis has been found to be temporally related to diurnal variation in endogenous steroid production [10].

On the other hand, a significantly decreased ability of PBMC to produce IgE in response to IL-4 alone or IL-4 and HC indicates that the prednisone treatment might even induce a long-term suppression of IgE synthesis. The underlying mechanism is not understood and might depend on a decreased availability of IL-4, since the production of IgE, and most likely IgG4, but not of the other IgG subclasses was affected. This hypothesis is supported by the *in vitro* studies, which show an active suppression of IL-4 synthesis by steroids [7–9].

The study presented here does not rule out the possibility that higher corticosteroid doses or a long-term steroid treatment might eventually lead to a significant rise of the production of IgE or IgG subclasses. However, older studies dealing with that subject reported rather a decreased than an increased immunoglobulin secretion. Volunteers receiving 96 mg methylprednisone for 3–5 days had decreased IgG serum levels after 2 weeks [11]. Allergic patients on a sustained low-dose therapy with glucocorticoids had lower serum IgE than

untreated controls [15,16]. Taking together the data from previous reports, which are almost exclusively on allergic subjects, a cortisone-induced increase of serum IgE was observed in two out of six studies [13–16,18,19]. This was only mild and compared with the potent effect obtained *in vitro* minor. Elevated serum total IgG was not detected in five out of five studies [11,12,17–19]. Our results confirm these studies and show that this is also true for healthy individuals.

We conclude that glucocorticoid treatment does not contain a substantially enhanced risk for increased IgE synthesis and the development of sensitizations in non-allergic persons receiving steroids. However, whether this conclusion can be readily transferred to allergic patients, needs to be established by a separate study.

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