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Regulation of CD23 expression, soluble CD23 release and immunoglobulin synthesis of peripheral blood lymphocytes by glucocorticoids

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SUMMARY

Evidence was obtained that glucocorticoids are capable of modulating the CD23 expression and soluble(s) CD23 release of peripheral blood lymphocytes (PBL). We demonstrate that interleukin-2 (IL-2)- and IL-4-induced CD23 expression are susceptible to glucocorticoids to a different degree. Prednisolone suppressed the spontaneous and IL-2-induced CD23 expression on PBL of healthy donors. The IL-4-induced CD23 expression was influenced much less by prednisolone, but the expression kinetics was altered. The modulation of the expression kinetics appears to be due to a priming effect of prednisolone. Differences were also apparent when the susceptibility of PBL from healthy and atopic donors towards the effect of prednisolone on the IL-4-induced CD23 expression was studied. Preactivation of PBL with *Staphylococcus aureus* strain Cowan I abolished the differences. Prednisolone also suppressed the sCD23 release from unstimulated and IL-2- or IL-4-stimulated PBL and enhanced the immunoglobulin (E,G,A,M) synthesis of PBL. This enhancement appears to be due to a priming effect, since pre-stimulation of PBL with prednisolone was sufficient to enhance the immunoglobulin synthesis. The IL-4-induced IgE synthesis of PBL with or without spontaneous *in vitro* IgE synthesis was synergistically enhanced by glucocorticoids.

INTRODUCTION

Corticosteroids are frequently used as immunosuppressive agents for the therapy of inflammatory or immunologically induced diseases. Many of their effects are due to the binding of the hormone to intracellular receptors. The hormone–receptor complexes bind to specific DNA sequences (Evans, 1988), the glucocorticoid-responsive elements (GRE; Yamamoto, 1985). By this mechanism the gene expression and mRNA concentration are modulated, leading either to an initiation or termination of protein synthesis. The anti-inflammatory action is classically attributed to a class of corticosteroid proteins, the lipocortins. The lipocortins act by inhibiting phospholipase A2, which leads to a suppression of arachidonic acid release from membrane phospholipids and thereby to an altered signal transduction cascade. It has been shown that the synthesis and release of several lymphokines, such as interleukin-1 (IL-2) (Ken *et al.*, 1988), IL-2 and interferon-gamma (IFN-γ) (Arya *et al.*, 1984), IL-3 (Culpepper & Lee, 1985), IL-4 (Daynes & Araneo, 1989) and IL-6 (Helfgott *et al.*, 1987), is regulated via this signal transduction cascade. In addition to the immunosuppressive effects an enhancement of the *in vivo* IgE synthesis after corticosteroid treatment (Posey *et al.*, 1978), as well as an enhanced *in vitro* IgE synthesis from PBL of atopic patients, was reported (Hemady *et al.*, 1985). Recently, it has been shown that B-cell chronic leukaemia cells are capable of synthesizing IgE *in vitro* when the cells are stimulated with corticosteroids (Sarfati *et al.*, 1989).

It has been shown that the low affinity receptor for IgE (FcεRII, CD23) and its soluble cleavage products, the IgE-binding factors (IgE-BF, sCD23), are involved in IgE regulation (Pène *et al.*, 1988). Furthermore, evidence has been provided that the CD23/sCD23 system is involved in IgE mediated allergic responses (Knöller *et al.*, 1989). Differences in the molecular weight pattern of sCD23 in the serum of patients with hyper IgE syndrome, atopic dermatitis and healthy donors have been described (Leung *et al.*, 1986; Bujanowski-Weber *et al.*, 1988).

The IgE synthesis, as well as the CD23/sCD23 synthesis, is induced by IL-4 (Defrance *et al.*, 1987). IL-2 is also capable of inducing sCD23 release (Bujanowski-Weber *et al.*, 1988) and CD23 expression (Knöller *et al.*, 1989; Hivroz *et al.*, 1989). IL-2 selectively induces the expression of CD23 on B cells (Fischer *et al.*, 1990/91). Isolation of two different cDNA for CD23 (Kikutani *et al.*, 1986; Yokota *et al.*, 1988) and the analysis of

Abbreviations: FCS, foetal calf serum; FcεRII, CD23, low affinity receptor for IgE; SAC, *Staphylococcus aureus* strain Cowan I; sCD23, soluble CD23.

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genomic clones of CD23 (Sueter *et al.*, 1987) revealed the existence of two nearly identical receptors, which were regulated differently. CD23 A is a B-cell differentiation marker and CD23 B is the IL-4-inducible FcεRII involved in IgE-mediated allergic responses (Yokota *et al.*, 1988). The analysis of the genomic sequence revealed several putative GRE in the 5′-regulative region of the CD23 transcription unit (A. Fischer, unpublished observation).

These observations prompted us to investigate the influence of the glucocorticoid prednisolone on the spontaneous and cytokine-induced *in vitro* CD23 expression, sCD23 release and Ig(E,G,A,M) synthesis of peripheral blood lymphocytes.

MATERIALS AND METHODS

Cells

Peripheral blood lymphocytes (PBL) were prepared from heparinized venous blood of healthy donors by standard Ficoll–Metrizoate density gradient centrifugation according to the method of Boyum (1968). The cells were washed three times with RPMI-1640 medium.

Culture conditions

All media and supplements were purchased from Gibco Europe Ltd (Karlsruhe, FRG) unless otherwise indicated. Peripheral lymphocytes (1×10^6/ml) were cultured in a water saturated atmosphere containing 5% CO_2. The culture medium consisted of RPMI-1640 medium with 2 mM L-glutamine, 10% foetal calf serum (FCS), 100 μg/ml streptomycin, 100 IU/ml penicillin, 10 mM HEPES and 20 mM sodium hydrogen carbonate.

Stimulation experiments

5×10^6 freshly prepared peripheral lymphocytes were suspended in 5 ml culture medium with various amounts of cytokines, as indicated in the Results. The stimulations were carried out in 6-well culture plates (Costar, Cambridge, MA) over a period of 3–15 days. The following stimuli were used: recombinant human IL-2 (Boehringer, Mannheim, FRG), recombinant human IL-4 (Genzyme, purchased from IC Chemikalien, München, FRG), and prednisolone (1-dehydrocortisone; Sigma Chemie, Deisenhofen, FRG).

Antibodies

The monoclonal antibodies mAb 176 and mAb 135 were a kind gift of G. Delespesse (University of Montreal, Canada). These mAb are specific for different epitopes of the low affinity IgE receptor (Rector *et al.*, 1985) on human lymphocytes (CD23) and its proteolytic fragments (45,000 and 25,000 MW sCD23). Polyclonal goat anti-human Ig(G,E,A,M) (Tago, Burlingame, CA) were purchased from Medac (Hamburg, FRG). ^{125}J-labelled antibodies were prepared by the chloramine T method (Klinman & Taylor, 1969). 0·1 mg (1 mg/ml) of antibodies was labelled with 37 MBq Na125J (specific activity 520·2 GBq/mg of jodine; Amersham-Buchler, Braunschweig, FRG) to a specific activity of 0·296 MBq/μg.

Determination of CD23

The quantification of CD23 on PBL was carried out as previously described (Bujanowski-Weber *et al.*, 1988). Briefly, 1×10^6 non-adherent cells were incubated with ^{125}J-labelled mAb 135 (50 ng/200 μl, 14·8 kBq) for 1 hr. Separation of unbound and bound antibodies was carried out by centrifugation through a 500 μl FCS cushion (500 *g*, 15 min, 4°) in minitubes (Greiner, Nürtingen, FRG). After removal of unbound radioactivity and washing with phosphate-buffered saline (PBS) (0·05% Tween 40), the cell-bound radioactivity was measured with a γ-Counter (Packard Cobra, Packard-Canberra, Frankfurt, FRG). All determinations were made in triplicates at least. The results are given as percentage binding of the total of ^{125}J-labelled mAb 135 applied. The viability of the applied stimulated cells was always higher than 95%, as shown by trypan blue staining. The specificity of the assay for CD23 has been demonstrated by inhibition of mAb 135 binding to stimulated PBL and several cell lines by IgE. Furthermore, the specifity has been demonstrated by the identical binding curves of ^{125}J-labelled IgE (PS) to stimulated PBL and RPMI-8866 cells and by FACS analysis using a sandwich immunofluorescence assay with mAb 135 and FITC-labelled goat anti-mouse Ig (Becton-Dickinson, Heidelberg, FRG).

Determination of sCD23

The quantification of sCD23 was carried out by a sandwich RIA as described in detail elsewhere (Bujanowski-Weber *et al.*, 1988). Briefly, microtitre plates with removable round-bottomed wells (Dynatech, Denkendorf, FRG) were precoated with mAb 176 (1 μg/ml). The unoccupied protein binding capacity of the plastic surface was saturated with 0·05% Tween 40 (Serva, Heidelberg, FRG) and with 1% bovine serum albumin (BSA; Boehringer) in PBS; incubation with culture supernatants of the stimulated cells was then performed and the bound sCD23 was detected with ^{125}J-labelled mAb 135 (12·5 ng/100 μl, 3·7 kBq). Results are given in U/ml sCD23. 100 U/ml was defined as the concentration of sCD23 in the supernatant of a serum-free 1-day culture of RPMI-8866 cells at a density of 1×10^6 cells/ml.

Analysis of immunoglobulins

The Ig(E,G,A,M) content of the culture supernatants was determined by a solid-phase sandwich RIA. Microtitre plates with removable round-bottomed wells were precoated for 4 hr with polyclonal anti-human Ig(E,G,A,M) (1:1000 dilution). One-hundred microlitres of an appropriate dilution of the cell-free supernatants [IgE undiluted, Ig(G,A,M) 1:5–1:50 dilution] were applied overnight at 4° (IgE) or at room temperature (IgG, IgM, IgA). In parallel, standard curves for IgE (0·2–200 ng/ml) and Ig(G,A,M) (0·3–300 ng/ml) were performed for the calibration of the RIA. ^{125}J-labelled class-specific antibodies (12·5 ng/100 μl, 3·7 kBq) were added for additional 4 hr and the bound activity was measured. The detection limit for IgE was 0·2 ng/ml and for Ig(G,A,M) less than 0·3 ng/ml. The specificity of the assays was demonstrated by adding immunoglobulins of other isotypes.

Analysis of data

All experiments were made at least three times. Because of the great inter-individual variance of the quantitative measurements, the results of one typical experiment are shown. Therefore, the standard deviations given in the figures are the deviations calculated from triplicate cultures out of a single experiment. The significancies given in the result section were calculated by Student's *t*-test for the mean values of three independent experiments.

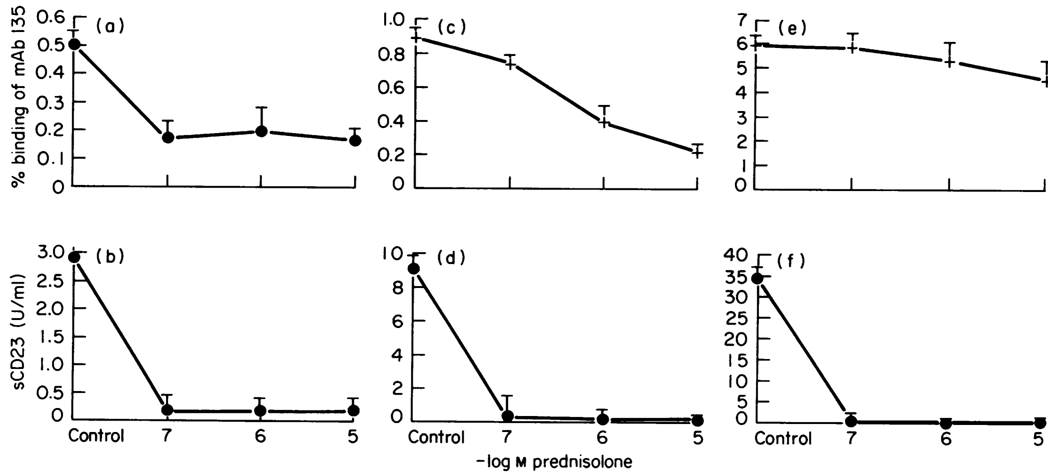

Figure 1. Effect of prednisolone on the spontaneous and cytokine-induced CD23 expression and sCD23 release from PBL. PBL of healthy donors were stimulated with different concentrations of prednisolone and IL-2 (20 U/ml) or IL-4 (10 U/ml). The CD23 expression was measured after 3 days of stimulation (IL-4 co-stimulations) or 7 days (IL-2 co-stimulations). The sCD23 content of the cell-free supernatants was analysed at Day 7. Effect of prednisolone on the (a) spontaneous CD23 expression; (b) the spontaneous sCD23 release; IL-2-induced (c) CD23 expression and (d) sCD23 release; IL-4-induced (e) CD23 expression and (f) sCD23 release.

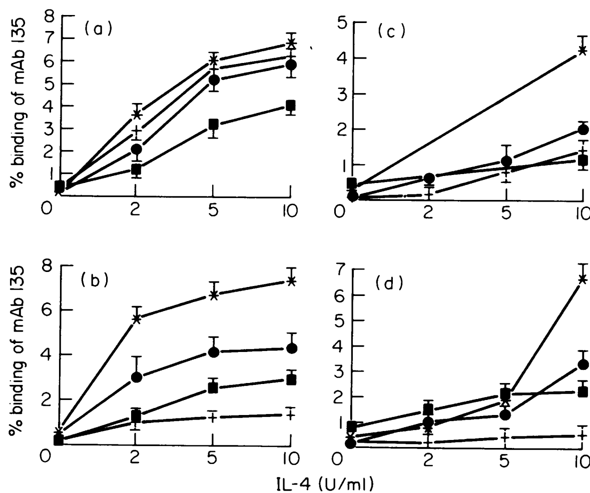

Figure 2. Effect of prednisolone on the CD23 expression of PBL from normal and atopic donors. Freshly prepared PBL of healthy (a) and atopic donors (b) were incubated with different concentrations of IL-4 in the presence or absence of prednisolone. The CD23 expression was analysed after 3 and 7 days of stimulation with IL-4. The same experiments were carried out after SAC pre-stimulation of PBL from normal (c) and atopic (d) donors. (●) Prednisolone co-stimulation (Day 3); (*) control (Day 3); (+) prednisolone co-stimulation (Day 7); (■) control (Day 7).

RESULTS

Effect of prednisolone on the spontaneous and cytokine-induced CD23 expression and sCD23 release of PBL

Freshly isolated PBL of healthy donors were incubated with different concentrations of prednisolone over a period of 3 days. The cells were then assayed for CD23 expression and the culture supernatants analysed for the content of sCD23 at Day 7. As is apparent, CD23 expression (Fig. 1a) significantly decreased ($P<0.05$) and the sCD23 release (Fig. 1b) from PBL decreased to the detection limit ($P<0.005$).

To evaluate the effect of glucocorticoids on the cytokine-induced CD23 expression, PBL of healthy donors were incubated with IL-2 or IL-4 in the presence of different concentrations of prednisolone. The cell cultures were assayed for CD23 expression at Day 3 or Day 7. This is due to the different CD23 expression maxima after IL-2 and IL-4 stimulation. The sCD23 content of the supernatants was measured in both stimulation protocols at Day 7 (Fig. 1d and f).

The IL-2-induced CD23 expression and sCD23 release was decreased by prednisolone in a dose-dependent manner. Even at a concentration of 10^{-7} M prednisolone, the IL-2-induced CD23 expression ($P<0.05$), as well as the IL-2-induced sCD23 release ($P<0.005$), was suppressed.

The IL-4-induced CD23 expression was not affected by prednisolone at concentrations from 10^{-7} to 10^{-6} M. A slight, but significant, decrease of CD23 expression ($P<0.05$) was obtained with higher concentrations of prednisolone (10^{-5} M). In contrast, the IL-4-induced sCD23 release was completely inhibited at all prednisolone concentrations analysed ($P<0.005$).

Effect of prednisolone on the CD23 expression of pre-activated and non-preactivated PBL from normal and atopic donors

Freshly isolated PBL from normal and atopic donors were stimulated with different concentrations of IL-4 in the presence or absence of 10^{-6} M prednisolone. A second series of experiments was carried out with PBL which were pre-stimulated with SAC and subsequently triggered in the same way. The CD23 expression of the cells was assayed at Days 3 and 7 after the addition of the stimuli. Figure 2a shows the results for donor cells from non-atopics. With IL-4 alone a dose-dependent increase of CD23 expression with a maximum at Day 3 was obtained. Addition of prednisolone to low amounts of IL-4 (2 U/ml) slightly decreased the CD23 expression ($P<0.05$). At

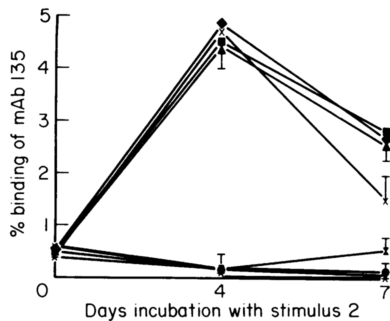

Figure 3. Priming effects of IL-4 and prednisolone on CD23 expression. PBL of healthy donors were pre-stimulated with IL-4 (10 U/ml) or prednisolone (10^{-6} M) for 1 day. After 1 day the cells were cultured with stimulus 2, as indicated below. The CD23 expression was analysed at Day 3 and Day 7 of stimulation period 2. (●) 0/0; (+) 4/0; (*) 4/P; (■) P/4; (×) 0/4; (◆) 4P/4; (▲) 4P/4P; ( ) 4P/0. P, prednisolone; 4, IL-4. The alpha-numerical combination in front of the slash refers to stimulus 1, the combination after the slash refers to stimulus 2.

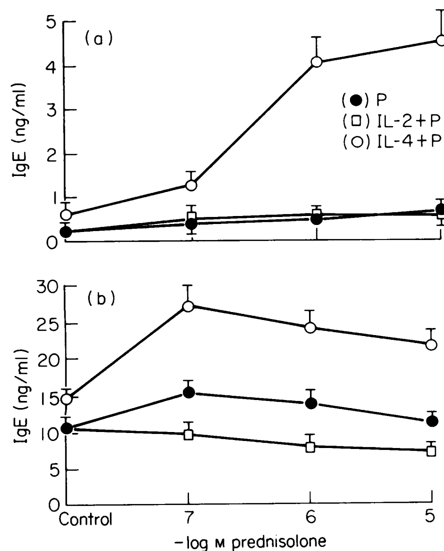

Figure 4. Effect of prednisolone on the IgE synthesis of PBL from donors without (a) or with (b) spontaneous IgE synthesis. PBL were incubated with different concentrations of prednisolone (P) in the presence or absence of IL-2 (20 U/ml) or IL-4 (10 U/ml). The cell-free supernatants were assayed for the IgE contents at Day 15.

higher IL-4 concentrations prednisolone had no significant effects. At Day 7 an enhancement of the IL-4 induced CD23 expression in the presence of prednisolone was observed. Further experiments were carried out with PBL of atopic donors (Fig. 2b). It is apparent that the IL-4 induced CD23 expression of atopic donors is decreased by prednisolone (Day 3 $P < 0{\cdot}05$; Day 7 $P < 0{\cdot}005$).

SAC preactivated PBL from healthy donors showed the following results (Fig. 2c): At Day 3 the CD23 expression induced by IL-4 was inhibited by prednisolone ($P < 0{\cdot}005$). At Day 7 the expression of CD23 by low amounts of IL-4 (0–2 U/ml) was inhibited by prednisolone; no significant modulation of CD23 was detected at higher IL-4 concentrations. When SAC-preactivated PBL of atopic donors was studied, it was apparent that the dose-dependent increase of CD23 expression by IL-4 was suppressed by prednisolone ($P < 0{\cdot}05$).

Priming effects of prednisolone on the IL-4-induced CD23 expression

Experiments were then carried out to elucidate whether prednisolone or IL-4 primes the cells for a subsequent stimulus. PBL of healthy donors were preincubated for 1 day with the first stimulus, washed and then stimulated with the subsequent stimulus. The cells were assayed for CD23 expression at Days 0, 4 and 7 (Fig. 3).

At Day 4 a pronounced increase of the CD23 expression was obtained under the following conditions: (i) no pre-stimulation, post-stimulation with IL-4; (ii) pre-stimulation with IL-4 and prednisolone, post-stimulation with IL-4 or IL-4 and prednisolone; (iii) prestimulation with prednisolone, post-stimulation with IL-4.

Under all conditions (i–iii) the amount of CD23 expression was similar.

At Day 7 a significant enhancement of the CD23 expression was obtained under the following conditions: (i) no pre-stimulation, post-stimulation with IL-4; (ii) pre-stimulation with prednisolone, post-stimulation with IL-4; (iii) pre-stimulation with IL-4 and prednisolone, post-stimulation with IL-4 or IL-4 and prednisolone. The enhancement of the IL-4-induced CD23 expression was significantly higher ($P < 0{\cdot}05$) when the cells were incubated with prednisolone, independent of the stimulation period in which the glucocorticoid was applied.

Modulation of the cytokine (IL-2, IL-4)-induced IgE synthesis by prednisolone

PBL of donors with or without spontaneous IgE synthesis were incubated with different concentrations of prednisolone in the presence of IL-4 or IL-2. The cell-free supernatants were analysed for their IgE content at Day 15. Figure 4a shows the results obtained with the cells of a healthy donor. It is apparent that stimulation with prednisolone led to an enhanced IgE synthesis (10^{-5} M prednisolone, $P < 0{\cdot}05$). The IL-4-induced increase in IgE synthesis was synergistically enhanced by prednisolone (10^{-5} M prednisolone, $P < 0{\cdot}005$). No effect of the prednisolone-induced IgE synthesis was obtained with IL-2.

When PBL from donors with spontaneous IgE synthesis were studied, the following results were obtained (Fig. 4b): prednisolone led to an enhanced IgE synthesis with a maximum at 10^{-7} M prednisolone ($P < 0{\cdot}05$). The induction of IgE synthesis by IL-4 was synergistically enhanced by prednisolone with an optimum at 10^{-7} M prednisolone ($P < 0{\cdot}005$). In the presence of IL-2, prednisolone induced a slight, dose-dependent reduction of IgE synthesis ($P < 0{\cdot}05$).

Modulation of the Ig(G,A,M) release from cytokine-stimulated PBL

Experiments were carried out to analyse the Ig(G,A,M) content of the supernatants from PBL of healthy donors which were stimulated for 7 days with 10^{-6} M prednisolone in the presence or absence of IL-2 or IL-4.

Figure 5 shows that prednisolone also induced an increased synthesis of IgG, IgA and IgM. Co-stimulations with IL-4

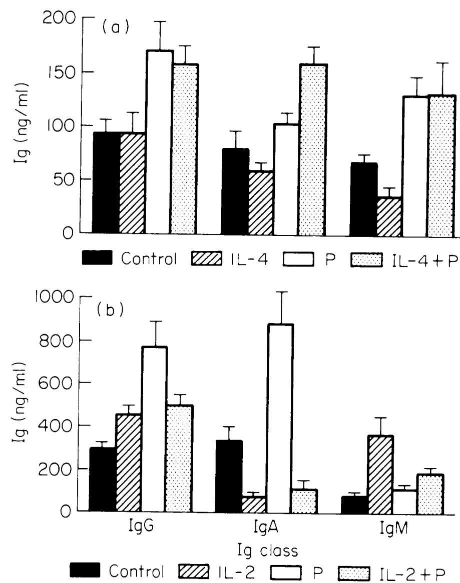

Figure 5. Ig(G,A,M) synthesis of cytokine-stimulated PBL by prednisolone. PBL from healthy donors were stimulated for 7 days with different concentrations of prednisolone in the presence or absence of (b) IL-2 (20 U/ml) or (a) IL-4 (10 U/ml). The Ig contents of the culture supernatants were analysed at Day 7.

(Fig. 5a) did not affect the prednisolone-induced IgG and IgM synthesis. The prednisolone-induced IgA synthesis was synergistically enhanced by IL-4 ($P<0.05$). Co-stimulation with IL-2 revealed that the prednisolone-induced IgG and IgA synthesis was decreased (IgG, $P<0.005$; IgA, $P<0.05$), whereas the synthesis of IgM was enhanced by IL-2 ($P<0.05$).

Priming effects of prednisolone on the IL-4-induced Ig(E,G,A,M) synthesis (Fig. 6)

PBL of healthy donors were incubated with IL-4 or prednisolone for 3 days. Subsequently, the cells were washed and stimulated with IL-4 or prednisolone. After IL-4 pre-stimulation, the additional stimulation with IL-4 or prednisolone or a combination of both stimuli led to an enhanced IgE synthesis ($P<0.05$).

Pre-stimulation of PBL with prednisolone led to an enhanced IgE synthesis ($P<0.05$). Subsequent stimulation with IL-4 or prednisolone during the second stimulation period did not induce a further enhancement of the IgE synthesis.

With regard to the IgG synthesis no significant differences were obtained independently whether the cells were pre-stimulated with IL-4 for 1 day or whether IL-4 was present over the whole culture period. A slight, but insignificant suppression of the IgG synthesis was observed when prednisolone was present during the second culture period. After prednisolone pre-stimulation the IgG synthesis was enhanced independently of the applied stimuli during the second culture period ($P<0.005$).

After IL-4 pre-stimulation, the IgM synthesis was suppressed when the cells were subsequently stimulated with IL-4 ($P<0.05$). No significant effects were obtained when the cells were post-stimulated with prednisolone. Co-stimulation with IL-4 and prednisolone led to an enhanced IgM synthesis ($P<0.05$). Pre-stimulation with prednisolone induced an enhanced IgM synthesis which was not modulated by post-stimulation with IL-4 or prednisolone. However, the combined stimuli increased synthesis of IgM ($P<0.005$).

The IgA synthesis of IL-4-stimulated PBL was not altered after subsequent post-stimulation with IL-4. Post-stimulation with prednisolone led to a reduced synthesis of IgA ($P<0.05$). This effect was not detectable when the cells were co-stimulated with IL-4 and prednisolone in the second incubation period.

Pre-stimulation with prednisolone induced an enhanced IgA synthesis ($P<0.05$), which was not significantly affected by post-stimulation with IL-4, while with prednisolone a further increase was obtained ($P<0.05$). Co-stimulation with IL-4 and prednisolone in the second incubation period reduced the IgA synthesis compared to the experiments in which the cells were post-stimulated with prednisolone alone ($P<0.05$).

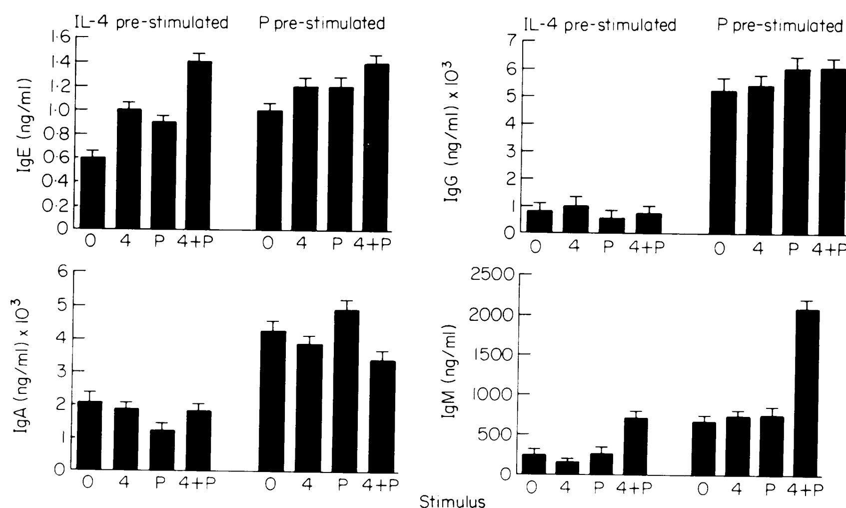

Figure 6. Influence of staging of the stimuli on Ig(E, G, A, M) synthesis. PBL of healthy donors were stimulated with IL-4 (10 U/ml) or prednisolone (10^{-6} M). After 3 days the cells were washed and incubated with a combination of IL-4 and prednisolone for further 7 days. After this time the cell-free supernatants were assayed for IgE (a), IgG (b), IgM (c) and IgA content (d).

DISCUSSION

It has been shown that CD23 acts as a regulatory molecule for B cells and that CD23 is involved in inflammatory reactions induced by monocytes, eosinophils and platelets. In this study we evaluated the influence of the corticosteroid prednisolone on CD23 expression and sCD23 release of PBL from normal and atopic donors. Our results show that the spontaneous expression of CD23 type A on B cells (Yokota *et al.*, 1988) and the sCD23 release are suppressed by prednisolone. The IL-2-induced synthesis of CD23, as well as the IL-2-induced sCD23 release, are also suppressed by prednisolone. The IL-4-induced production of CD23, which is mainly due to an increase of CD23 type B on T cells, B cells and monocytes was only slightly affected by high doses of prednisolone. In contrast, the IL-4-induced sCD23 release was inhibited completely. The differences in the susceptibility of the IL-2- and IL-4-induced CD23 expression towards prednisolone might be due to different modes of action for the IL-2 and IL-4 induction of CD23. This view is supported by the fact that IL-2, in contrast to IL-4, solely enhances the CD23 expression on B cells.

It has been described that the sCD23 release from PBL occurs via proteolysis (Nakajima *et al.*, 1987). Since the sCD23 release from PBL was decreased by glucocorticoids under all stimulation conditions tested, it is likely that the IL-2- and IL-4-induced sCD23 release was achieved by the same proteolytic mechanism which was inhibited by glucocorticoids. This assumption is strengthened by the expression kinetics of CD23 after co-stimulation with IL-4 and prednisolone, which led to a suppression of sCD23 release and an accumulation of cell-surface bound CD23 receptor protein.

It has been discussed that CD23 is involved in allergic responses (Yokota *et al.*, 1988). Furthermore, it has been shown that PBL from atopic donors are more susceptible to IL-4 than PBL from normal donors (Pfeil *et al.*, 1989) and that CD23 expression is enhanced by allergen (Prinz *et al.*, 1987; Pfeil *et al.*, 1989). Therefore, we investigated the response pattern of IL-4-stimulated PBL from atopic and healthy donors towards prednisolone. It is obvious that the IL-4-induced CD23 synthesis on PBL of atopic in comparison to normal donors is much more susceptible towards prednisolone. Recently, it has been shown that SAC-pre-stimulated PBL change the response pattern towards cytokines. SAC-pre-stimulated PBL do not generate IgE after IL-4 stimulation, whereas SAC-pre-stimulated cells were required for the IgE induction by IL-2 (Knöller *et al.*, 1989). Interestingly, after SAC pre-stimulation, both PBL from normal and atopic donors were susceptible towards the suppressive effect of prednisolone for IL-4-induced CD23 expression. Apparently, the modulation of the cytokine-induced CD23 expression is due to a priming effect of glucocorticoids. Obviously, it is sufficient that prednisolone was present at the beginning of the co-stimulation or that the cells were pre-stimulated with prednisolone.

It is known that IL-4 (Defrance *et al.*, 1987) and, under certain conditions, IL-2 (Knöller *et al.*, 1989, Chan & Dosch, 1989) are capable of enhancing IgE synthesis. Therefore, we investigated the effects of prednisolone and IL-2/IL-4 on the IgE synthesis in co-stimulation assays. It was apparent that IL-4 as well as prednisolone was capable of enhancing IgE synthesis from PBL of donors with and without spontaneous IgE synthesis. In both cases prednisolone and IL-4 acted synergistically on the IgE synthesis. Daynes & Araneo (1989) showed that glucocorticoids are capable of enhancing the production of IL-4 from T cells, whereas IL-2 production is decreased. One might speculate that the effect of glucocorticoids on IgE synthesis is mediated by the stimulation of IL-4 production. This view is supported by the fact that the co-stimulation of PBL from donors with spontaneous *in vitro* IgE synthesis with IL-2 and prednisolone led to an inhibition of the prednisolone-induced IgE synthesis, which has been shown to be a characteristic feature of the IL-4-induced IgE synthesis (Fischer *et al.*, 1990/91).

Inconsistent results have been published as to the effect of glucocorticoids on Ig synthesis (Posey *et al.*, 1978; Hemady *et al.*, 1985). Sarfati *et al.* (1989) demonstrated that BCLL cells and activated B cells are capable of synthesizing IgE after corticosteroid treatment. Our data demonstrate the ability of peripheral lymphocytes to synthesize IgE *in vitro* after prednisolone treatment. It was shown that glucocorticoids enhanced the synthesis of all Ig classes and that IL-2 and IL-4 showed a complex modulatory pattern on the glucocorticoid-induced immunoglobulin synthesis. It is known that glucocorticoids modulate the expression of various cytokines and cytokine receptors. Glucocorticoids also exert inhibitory effects on B-cell activation and proliferation without suppressing the B-cell differentiation. Therefore, glucocorticoids may have an enhancing effect on the differentiation of B cells via either direct action on B cells or by the rearrangement of the T-cell or monocyte-derived cytokine expression pattern. Interestingly, glucocorticoids are capable of generating Ig^+/Ia^- B cells from normal and pre-activated murine B cells (McMillan *et al.*, 1988). The fact that glucocorticoids decrease the CD23 expression on PBL provides further evidence that CD23 and MHC class II are most likely co-ordinately regulated (Rousset *et al.*, 1988). Glucocorticoids synergistically enhanced the IL-4-induced IgE synthesis. This is due to a priming effect, because pre-stimulation of PBL with glucocorticoids was sufficient to enhance the IL-4-induced IgE synthesis. In this regard, it has been described that the glucocorticoid-mediated alteration of IFN-γ, IL-2 and lymphotoxin synthesis by T cells occurs already after pre-stimulation of the cells (Cesario *et al.*, 1986).

In conclusion our data provide evidence for the differential effects of glucocorticoids on the spontaneous and IL-2/IL-4-induced CD23 expression, sCD23 release and immunoglobulin synthesis. Whether these effects reflect an alteration of the cytokine expression pattern or a direct effect on the B-cell differentiation is currently under study.

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