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C Y Wu, … , R Peleman, G Delespesse

Research Article

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Glucocorticoids Increase the Synthesis of Immunoglobulin E by Interleukin 4-stimulated Human Lymphocytes

C. Y. Wu, M. Sarfati, C. Heusser,* S. Fournier, M. Rubio-Trujillo, R. Peleman, and G. Delespesse

*Research Center, Notre-Dame Hospital, University of Montreal, Montreal, Canada H2L 4M1; and *Ciba-Geigy Pharmaceuticals, Basel, Switzerland*

Abstract

This study indicates that hydrocortisone (HC) markedly increases the synthesis of immunoglobulin E (IgE) by interleukin 4 (IL-4)–stimulated human lymphocytes. The effect is glucocorticoid specific and is obtained with low concentrations of HC (0.1–10 μM). In both the early and the late phase of the IL-4–induced response HC exerts its effects which are respectively IL-4 dependent and IL-4 independent. The IgE potentiation cannot be explained by the inhibition of interferon-γ (IFN-γ) production since it is observed in the absence of endogenous secretion of IFN-γ. HC inhibits the production of IgE-binding factors (soluble CD23) and the expression of the low-affinity receptor for IgE, also known as the (FcϵRII) CD23 antigen; however, the residual expression of FcϵRII by IL-4– and HC–treated peripheral blood mononuclear cells (PBMCs) is important since the IgE response of these cells is markedly inhibited by anti–CD23 monoclonal antibody. HC acts mainly by amplifying the cellular interactions between monocytes and lymphocytes; indeed, HC has no effect on monocyte-depleted PBMCs, and moreover, monocytes cannot be replaced by soluble factors. Most importantly, T cells are not required for the induction of IgE synthesis by costimulation with IL-4 and HC. However, the IgE response of rigorously T cell–depleted PBMCs may be further increased by the addition of T cells. Further analysis of the permissive effect of HC on the synthesis of IgE by T cell–depleted PBMCs suggests that HC acts in synergy with IL-4 to trigger the activation and the differentiation of B cells into IgE-producing cells. (*J. Clin. Invest.* 1991. 87:870–877.) Key words: CD23 antigen • estradiol • interferon-γ • progesterone • testosterone

Introduction

Glucocorticoids (GCs)[1] are widely used as anti-inflammatory, immunosuppressive, and antiallergic agents. Their mode of action is very complex since they act at the various steps of the immune response and of the subsequent inflammatory reaction. In addition to a direct effect on immunocompetent cells, GCs markedly influence the migration of leukocytes, the release of inflammatory mediators and the response to these mediators (1, 2). Most studies concur to demonstrate that GCs suppress cell-mediated immune responses whereas their effect on B cell function is more variable (1, 3, 4). The production of polyclonal Ig is significantly increased in the presence of GCs in vitro (5–7). Moreover, as recently reported, GC treatment may differently affect the in vivo IgG and IgA antibody response (8). Treatment of allergic patients with GCs has either no effect or increases the serum IgE levels in spite of clinical improvement (9–11). The effect of GCs on the in vitro synthesis of human IgE has not been documented with the exception of two conflicting studies that examined the influence of GCs on the spontaneous synthesis of IgE by lymphocytes from allergic patients (12, 13). Very recently, Sarfati et al. (14) reported, that upon stimulation with both interleukin 4 (IL-4) and hydrocortisone (HC), the peripheral blood mononuclear cells (PBMCs) of patients with chronic lymphocytic leukemia (CLL) could be induced to synthesize monoclonal IgE. The IgE response of the B cells of CLL patients was strictly dependent upon the presence of HC; indeed in contrast to normal lymphocytes, the PBMCs of CLL patients did not produce any IgE in response to IL-4 alone. In this study we report that HC strongly increases the in vitro synthesis of IgE by IL-4–stimulated human lymphocytes. The analysis of the cellular mechanisms indicates that the IgE-potentiating effect of HC is strictly monocyte dependent. However and most interestingly, the IgE synthesis induced by costimulation with IL-4 and HC does not require the presence of T cells.

Methods

Reagents

HC, testosterone, β-estradiol, progesterone (all from Sigma Chemical Co., St. Louis, MO), and the high-affinity anticorticosteroid RU38486 (kindly provided by Dr. J. P. Pelletier, Notre-Dame Hospital, Montreal) were dissolved in ethanol and stored at $-20°C$. They were diluted in culture medium immediately before use. This resulted in ethanol concentrations in the cultures of < 0.01%. In control experiments, these concentrations of ethanol had no measurable effect on the secretion of IgE and of IgE-binding factors (IgE-BFs), or soluble CD23 (sCD23). In some experiments, water-soluble HC sodium succinate (Upjohn Co., Kalamazoo, MI) was used instead of HC acetate. The monoclonal antibody (MAb) to the low-affinity receptor for IgE (FcϵRII), also known as CD23 antigen (MAb 135), and the isotype-matched control MAb (against LolpI, a grass pollen allergen) have been described previously (15). The neutralizing mouse MAb to human IL-4 (IgG, K) is the same as that used in a previous work (16); its

Address reprint requests to Dr. Delespesse, University of Montreal, Notre-Dame Hospital Research Center, 1560 Sherbrooke Street East, Montreal, Quebec, H2L 4M1 Canada.

1. *Abbreviations used in this paper:* CLL, chronic lymphocytic leukemia; CSN, culture supernatant; FcϵRII, low-affinity receptor of IgE, also known as CD23 antigen; GCs, glucocorticoids; HC, hydrocortisone; IgE-BFs, IgE-binding factors or soluble CD23; sCD23, soluble CD23 antigen.

production and characterization will be reported separately. Recombinant human IL-4 was obtained from Genzyme Corp., Boston, MA; HB101 culture medium was from Hana Biologies, Inc., Berkeley, CA. RPMI 1640 culture medium, fetal calf serum (FCS), penicillin, and streptomycin were purchased from Flow Laboratories, Rockville, MD. MO_2 MAb (against CD14A8) was obtained from Beckton-Dickinson, Mountain View, CA.

Mononuclear cell preparation and culture conditions

PBMCs were isolated from the heparinized blood of healthy volunteers by density gradient centrifugation on Sepracell-MN (Sepratech Co., Oklahoma City, OK) according to the instruction manual. For monocyte depletion, PBMCs were applied on a second Sepracell MN gradient from which two fractions were collected, i.e., monocyte depleted and monocyte enriched. Cells from the monocyte-depleted fractions (which still contained 2–5% MO2-positive cells) were washed and incubated for 45 min at room temperature with 4 mM L-leucine methyl ester (Sigma Chemical Co.) in Hepes-buffered RPMI 1640 culture medium as described (17). The resulting cells contained no detectable MO2-positive cells. Adherent cells were isolated from the monocyte-enriched fraction by adherence to plastic dish. To this end, cells were resuspended in 5 ml of RPMI 1640 medium supplemented with 10% FCS (3 × 10^6 cells/ml) and incubated for 45 min at 37°C in a 100 × 15-mm plastic dish (Nunclon Delta SI, Gibco/BRL, Burlington, Ontario). Adherent cells were collected by incubation of the flask for 15 min at 4°C with cold PBS containing 1 mM EDTA, pH 7.2, followed by vigorous washing of the adherence surface by repeated pipetting of the PBS-EDTA solution. After washing twice with HBSS, > 80% of the resulting cells were MO2 positive. T cell–depleted PBMCs were prepared by two-cycle rosetting with 2-aminoethylisothiouronium bromide-treated sheep red blood cells (SRBC) and by separating the rosette-forming cells on Ficoll-Metrizoate gradient. The residual T cells were further removed by means of anti–CD3-coated magnetic beads (Dynabeads M-450, Dynal Inc., Great Neck, NY) according to the instruction manual. Briefly, the beads were coated with purified anti–CD3 MAb (OKT3, Ortho Pharmaceutical, Raritan, NJ; 50 μg of antibody for 4 × 10^8 beads), and reacted for 1 h with the T cell–depleted preparation (assumed to contain 5% residual CD3-positive cells) at a bead/target cell ratio of 40:1. After removal of the Dynabeads with a magnet, the resulting cells contained no CD3-positive cells.

Cells were cultured at the concentration of 1.5 × 10^6 cells/ml in HB101 culture medium supplemented with 5 mM L-glutamine, 5% FCS, 50 IU penicillin, and 50 μg/ml streptomycin. Cultures were carried out in a 1-ml volume in 48-well tissue culture plates (Costar Data Packaging, Cambridge, MA) for a period of 12–14 d. In all the experiments reported in this study, IL-4 was used at 10 ng/ml, which was found to be optimal in our culture conditions.

Analysis of CD23 expression

FcεRII/CD23 expression was detected by indirect immunofluorescence analysis using flow cytometry. Briefly, 1 × 10^6 cells were washed twice in PBS, supplemented with 3% (wt/vol) BSA and 0.1% sodium azide (PBN). Cell pellets were incubated overnight on ice, with 50 μl of biotinylated anti–CD23 MAb 208.25.A.3/135 (MAb 135) at a final concentration of 3 μg/ml. This MAb recognizes the low-affinity FcεRII on monocytes, B and T lymphocytes. As a negative control, the cells were incu-

bated with 50 μl of a biotinylated unrelated isotype matched mouse MAb (anti-Lol P1) of the same subclass (IgG1, K). In all samples, 50 μl of normal human IgG (300 μg/ml) was added to prevent passive adsorption of the MAb on FcγR. Cells were then washed three times in PBN and incubated for 30 min in the dark at 4°C with 25 μl of FITC-conjugated avidin. After two washes in PBN and one in PBS-azide, cells were resuspended in 1 ml of PBS-azide and analyzed by using a FACScan® and C30 software (Becton, Dickinson & Co.). CD3-positive cells were detected by staining with FITC-conjugated Leu 4; an FITC-mouse IgG was used as negative control; monocytes were identified by using FITC-Leu M3 and one FITC-mouse IgG2b as negative control (all from Becton, Dickinson & Co.).

Radioimmunoassays

Measurement of Ig. These assays were performed as described previously (18). Briefly, 96-well polyvinyl microtiter plates (Dynatech Laboratories, Inc., Alexandria, VA) were coated with either mouse MAb specific to human IgE (clone 89) or with IgG fraction of sheep antisera specific to α, γ, or μ chains. After a 2-h blocking with Hanks' supplemented with FCS, the samples (75 μl of buffered culture supernatant [CSN]) were incubated overnight at room temperature in a humid atmosphere. The wells were then washed and reacted overnight with 75 μl of radiolabeled, affinity-purified antibodies to human μ, γ, or α chains or with MAb to human IgE (clone 4.15, a gift from Dr. A. Saxon, University of California at Los Angeles, Los Angeles, CA). The results are expressed as the net Ig synthesis, obtained after subtraction of the Ig levels measured in cycloheximide-treated parallel cultures. The IFN-γ levels in cell-free CSNs were determined by a commercially available solid-phase radioimmunoassay (RIA) kit (Centocor Co., Malvern, PA).

IgE-BFs. IgE-BFs were measured by a solid-phase RIA as described previously (19). Briefly, 96-well microtiter plates
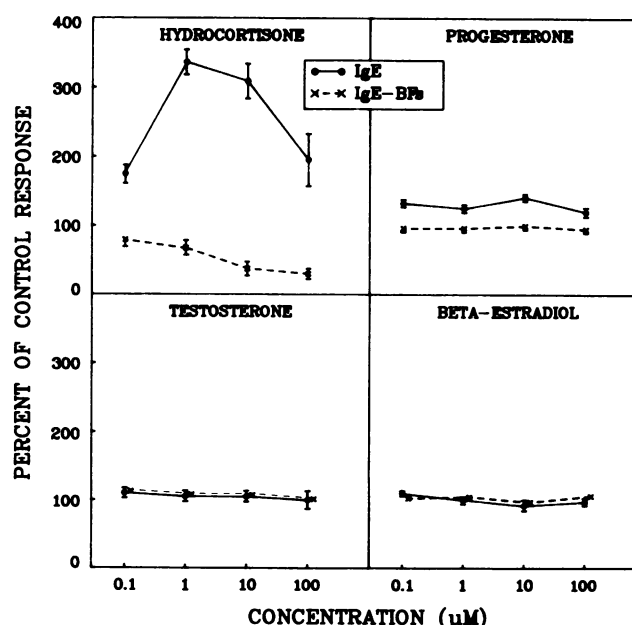

Figure 1. Effect of steroid hormones on IgE and IgE-BF production by IL-4-stimulated PBMCs from four normal donors. Results are expressed in percentage (mean±1 SEM) of the response to IL-4 alone which was of 20±3.2 ng/ml of IgE and 360±27 U/ml of IgE-BFs.
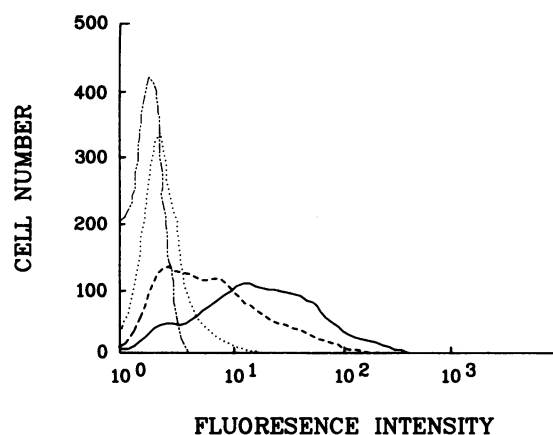
Figure 2. Effect of HC on FcεRII expression by IL-4–stimulated tonsillar B lymphocytes. Cells (1 × 10⁶/ml) were cultured 48 h as indicated, washed, and analyzed by flow cytometry. Shown is the expression of FcεRII on unstimulated cells (· · · ·) or IL-4–stimulated cells in the absence (—) or in the presence of 10 μM HC (---). Also shown is the autofluorescence determined by an isotype-matched unrelated mouse MAb (-·-·-).

were coated with 100 μl of anti–CD23 MAb 176 at a concentration of 15 μg/ml. After overnight incubation, plates were blocked for 2 h with Hanks' supplemented with FCS, and after washing, 75 μl of the sample was allowed to incubate for 4 h. The plates were then washed and 75 μl of ¹²⁵I-anti–CD23 MAb 135 (2–3 × 10⁴ cpm/ng) was added to the wells. After overnight incubation, the wells were washed and counted in a gamma counter. The total radioactivity was±300,000 cpm/well and the blank was determined by H-FCS (±160 cpm).

Measurement of [³H]thymidine incorporation

Cells (3 × 10⁵ cells in 0.2 ml of culture medium) were cultured in four replicates in 96-well flat-bottom plate (catalogue no. 3599, Costar Data Packaging) for the indicated periods of time. 6 h before harvesting, 0.1 ml of CSN was collected from each well (for the measurement of IgE) and replaced by culture medium containing 1 μCi of [³H]thymidine (sp act 2 μCi/mmol; Amersham Corp., Arlington Heights, IL). Cells were collected by a cell harvester (Cambridge Technology, Cambridge, MA) and the radioactivity (counts per minute) was counted in

scintillation fluid Econofluor (New England Nucleur, Boston, MA).

Results

HC potentiates the synthesis of IgE by IL-4–stimulated PBMC. The synthesis of IgE by IL-4–stimulated PBMCs is significantly increased in the presence of 0.1–10 μM HC (Fig. 1). At optimal concentrations (1 or 10 μM) the IgE-potentiating effect of HC is constant but its magnitude is highly variable (from 2- to 100-fold increase) as determined in a very large number of experiments. At 100 μM, the effect of HC is irregular and in some cases the IgE response is suppressed. Although the data are not shown, it is of note that HC significantly increases the production of IgA but not of IgG or IgM by IL-4–stimulated PBMCs. In the absence of IL-4, HC does not elicit IgE synthesis whereas, as expected (7), it augments the production of all the other Ig classes. As seen, other steroid hormones, including testosterone, progesterone, and estradiol, do not influence the synthesis of IgE. These data suggesting that the IgE-potentiating activity of HC is glucocorticoid specific are supported by two additional observations: (*a*) it is inhibited by 70–80% in the presence of equimolar concentrations of RU38486, a potent inhibitor of steroid hormone receptors; (*b*) dexamethasone, a glucocorticoid analogue binding to GC receptor with an affinity 20 times greater than that of HC is also at least 10 times more active than HC in potentiating IgE synthesis (not shown).

Role of FcεRII or of its soluble fragments in the IgE-potentiating effect of HC. Since FcεRII or its soluble IgE-binding fragments (IgE-BFs) were shown to play a role in the IL4-induced synthesis of IgE (20–22), we have examined the effect of HC on the expression of these molecules by IL-4–stimulated PBMCs. HC significantly inhibits both the release of IgE-BFs (Fig. 1) and the expression of FcεRII (Fig. 2) by IL-4–stimulated lymphocytes; this effect is GC specific. At concentrations of 1 or 10 μM, which are optimal for potentiating IgE synthesis, HC suppresses the production of IgE-BFs by 40–70%. The residual expression of FcεRII (and/or the release of its soluble fragments) is required for an optimal IgE response to IL-4 and HC. Indeed, the IgE response obtained in the presence of HC is markedly suppressed by anti–FcεRII MAb but not by the isotype-matched control MAb (Fig. 3). In four such experiments, FcεRII MAb (1 μg/ml) inhibited the IgE response to IL-4 alone by 60±15% and the IgE response to IL-4 + HC by 80±12%.

IgE (ng/ml)

ADDED TO CULTURES			
—			
IL4			
IL4 + mAbER			
IL4 + mAbLolP₁			
IL4 + HC			
IL4 + HC + mAbER			
IL4 + HC + mAbLolP₁			

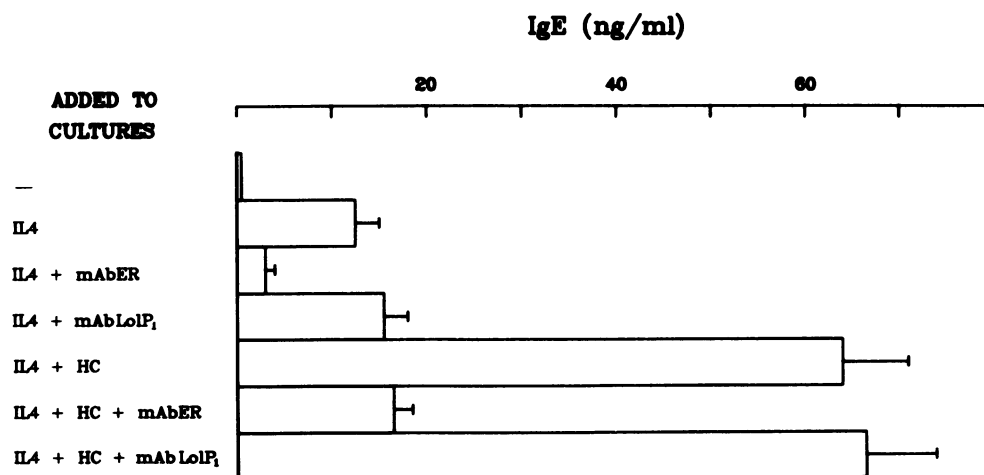*Figure 3.* Effect of MAb against FcεRII (MAb 135) on the synthesis of IgE by PBMCs stimulated with IL-4 or with IL-4 and 10 μ MHC. MAbER 135 and the isotype-matched control MAb (anti-Lol pI, an antigen from Rye grass pollen) were used at 1 μg/ml; HC was used at 10 μM. Shown are the mean values±1 SD of three replicates, with one representative experiment out of four.
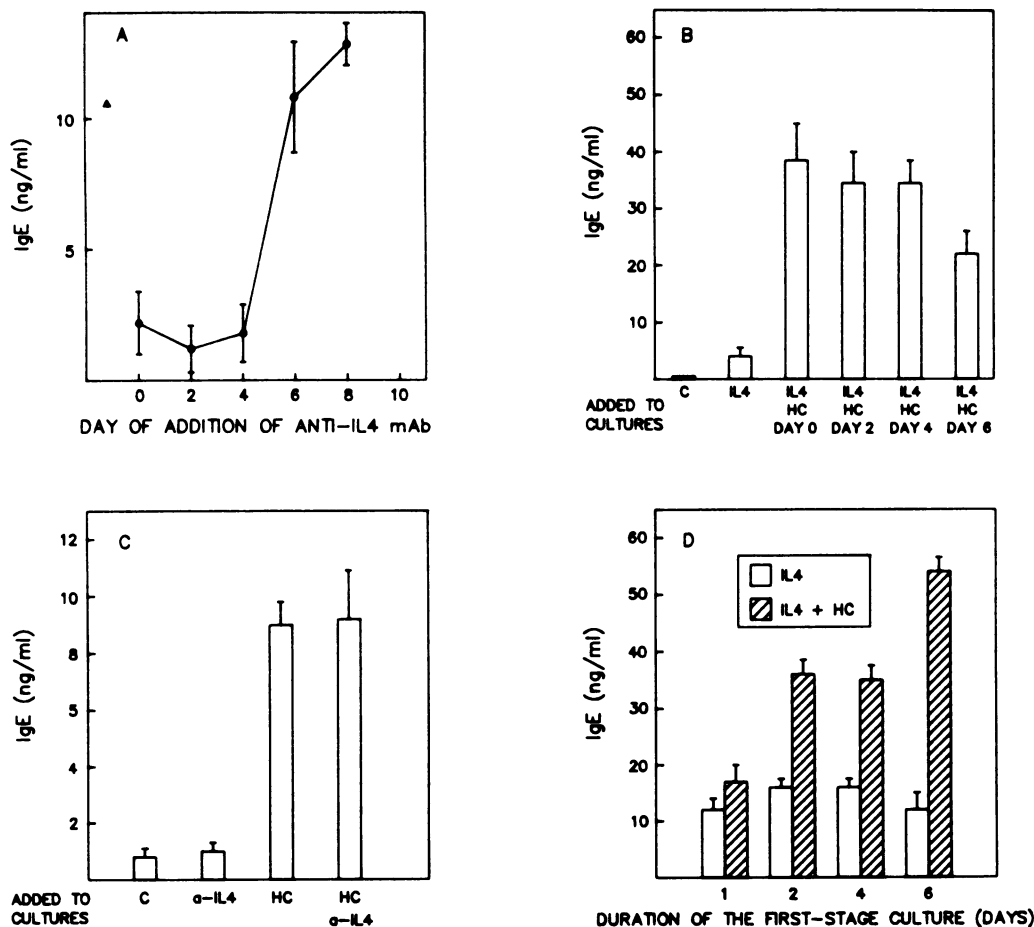
Figure 4. Kinetic studies. Delayed addition of (*A*) anti–IL-4 MAb (10 μg/ml) or (*B*) HC (10 μM) to IL-4–stimulated cultures of PBMCs. In *A*, the solid triangle (▲) refers to the control IgE response observed in the absence of anti–IL-4 MAb. (*C*) Secondary cultures where PBMCs have been preactivated for 1 wk with IL-4, washed, and cultured for another week in culture medium alone (column marked *C*) or in the presence of either anti–IL-4 MAb (10 μg/ml), HC (10 μM), or both HC and anti–IL-4 MAb. (*D*) Cells were first preincubated for the indicated periods either with IL-4 alone or with IL-4 and HC (10 μM), washed, and then recultured in the presence of IL-4 for a total culture period of 12 d. In each case one representative of at least four experiments is shown; mean±1 SD of triplicates.

HC acts at multiple levels in the cellular interactions leading to the synthesis of IgE by IL-4–stimulated PBMC. The data in Fig. 4 *A* indicate that a neutralizing MAb to IL-4 suppresses the IL-4–induced IgE synthesis only when added during the first 4 d of the 12-d culture; at day 6 or after, this antibody has no effect. In contrast, HC (10 μM) still increases the production of IgE when added at day 6 (Fig. 4 *B*), suggesting that HC may still act during the IL-4–independent stage of the IgE response. This was confirmed in two-stage cultures where PBMCs are first incubated for 7 d with IL-4, extensively washed, and recultured for another week in the absence of IL-4. The addition of HC to the secondary cultures consistently increases IgE synthesis (Fig. 4 *C*). The possible effect of HC during the early stage of the IL-4 stimulation was next examined in washout experiments. In these assays PBMC are first cultured for various periods of time with either IL-4 alone or with IL-4 and HC, the cells are then washed and recultured with IL-4 alone. As seen (Fig. 4 *D*), PBMCs exposed to both IL-4 and HC during the first 1–4 d of the culture produce more IgE than control cells exposed to IL-4 alone.

HC potentiates the synthesis of IgE even in the absence of endogenous production of IFN-γ. An obvious explanation of the IgE-potentiating effect of HC is that it suppresses the production of IFN-γ (3) a natural antagonist of IgE synthesis (22). However, additional mechanisms must also be involved since HC strongly increases the IgE response of neonatal lymphocytes which do not produce IFN-γ when cultured in the absence of HC. In four such experiments, umbilical cord blood

lymphocytes were cultured in culture medium alone or in the presence of either IL-4 or of IL-4 and HC. At day 4, 250 μl of CSN was collected for IFN-γ measurement and replaced by fresh culture medium. IFN-γ was not detectable in any of these cultures, whereas the mean (±1 SEM) values of IgE in these four experiments were 2.4±0.8 ng/ml for IL-4–stimulated cultures and 15.5±3.9 ng/ml for cultures stimulated with both IL-4 and 1 μM HC.

Table I. IgE-potentiating Activity of HC

Added to cultures	IgE-potentiating activity in cells in culture			
	PBMCs	MO-depleted*	MO-depleted + MO[‡]	MO[§]
	ng/ml			
—	0.1	0.2	0.2	0.1
Il-4	8.7±1.2	1.2±0.4	12.6±2	0.2
HC (10 μM)	0.1	0.1	0.3	0.1
Il-4 + HC	32±3.8	1.4±0.2	35±4.1	0.1

* The monocyte-depleted cells contained no detectable MO₂-positive cells.
[‡] These cultures contained 1.5 × 10⁶ monocyte-depleted PBMCs + 0.3 × 10⁶ adherent cells; the adherent cells contained 82% MO₂-positive cells, 5% CD20-positive cells, and 1% CD3-positive cells.
[§] These cells were cultured at 1.5 × 10⁶ cells/ml. Identical results were obtained in three additional experiments.

Table II. Effect of HC on IgE Synthesis by T Cell-depleted PBMCs

Cells in culture	Added to cultures	IgE (ng/ml)			
		Expt. 1	Expt. 2	Expt. 3	Expt. 4
		ng/ml			
PBMCs	—	0.4	0.4		0.5
	Il-4	26.0	5.6	ND	5.8
	IL-4 + HC§	43.0	10.6		26.0
T cell–depleted PBMCs*	—	0.3	0.2	0.6	2.3
	IL-4	0.3	0.2	0.8	2.1
	IL-4 + HC	40.0	50.0	31.0	14.0
T cell–depleted PBMC‡ + T cells	—			0.5	0.3
	IL-4	ND	ND	16.0	12.9
	IL-4 + HC			58.0	31.2

* The T cell–depleted PBMCs contained no detectable CD3-positive cells. ‡ These cultures contained 1.5 × 10⁶ T cells–depleted PBMCs and 0.6 × 10⁶ T cells. Shown are the mean values of triplicate cultures. ND, not done. § 10 μM.

The IgE-potentiating activity of HC is monocyte dependent. Previous studies have indicated that monocytes are required for an optimal IgE response to IL-4 (23). The complete removal of monocytes from PBMCs (by a two-step procedure described in Methods) strongly reduces the IgE response to IL-4 and completely abrogates the IgE-potentiating activity of HC (Table I). Both are reconstituted by the addition of adherent cells to the monocyte-depleted PBMCs. Monocytes cannot be replaced by the culture supernatant of adherent cells (cultured for 24 or 48 h in culture medium alone, or in the presence of either IL-4, HC (1 or 10 μM) or of both IL-4 and HC) tested at concentrations ranging from 5% to 40% (not shown). The addition of either IL-1 (0.5–5 ng/ml), IL-6 (50–300 U/ml) or a combination of both also fails to restore the IgE-potentiating effect of HC on monocyte-depleted PBMCs (not shown).

T lymphocytes are not required for the synthesis of IgE by lymphocytes costimulated with IL-4 and HC. As expected, rigorously T cell–depleted PBMCs do not synthesize IgE in response to IL-4 alone, whereas the same cells secrete large amounts of IgE in response to IL-4 and HC (Table II). Reconstitution with T cells restores the responsiveness to IL-4 and further increases the response to IL-4 and HC. The amplifying effect of T cells is completely abrogated by cyclosporine A (Table III). It is of note that the ability of HC to support T cell–independent IgE synthesis may be observed with very low concentrations (1 nM) of HC (not shown).

As a first approach to analyze the permissive effect of HC on the synthesis of IgE by T cell–depleted PBMCs, we have

Table III. Effect of Cyclosporine A on the IgE Response of B Cells Co-stimulated with Il-4 and HC

	Stimulants	IgE in cells in culture	
		B cells‡	T + B cells§
		ng/ml	
Expt. 1	—	0.1	0.1
	IL-4	0.1	8±2
	IL-4 + CyA‖	0.1	1±0.5
	IL-4 + HC¶	20.2±1.4	29±3
	IL-4 + HC + CyA	17.6±0.9	14±2.8
Expt. 2*	—	3.3±0.5	5.3±1.8
	IL-4	2.2±0.4	15.8±1.2
	IL-4 + CyA	3.9±0.3	3.1±0.4
	IL-4 + HC	14.4±0.9	31.2±1.2
	IL-4 + HC + CyA	12.4±1.5	13.0±0.9

The two experiments are representative of a series of six.
* Allergic donor.
‡ Rigorously T cell–depleted PBMCs containing no detectable CD3-positive cells.
§ 1.5 × 10⁶ B cells + 0.6 × 10⁶ T cells.
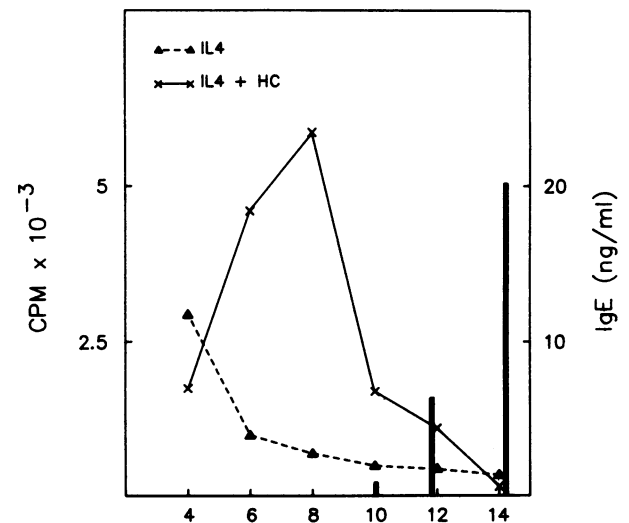‖ CyA, cyclosporine A (1 μg/ml).
¶ 10 μM.

Figure 5. Kinetic study of [³H]thymidine incorporation and IgE secretion (solid bars) by T cell–depleted PBMCs cultured in the presence of IL-4, or of IL-4 and HC (10 μM). There was no IgE in the cultures stimulated with IL-4 alone. This experiment was repeated four times with similar results. Note that, the [³H]thymidine uptake by IL-4–stimulated cells at day 4 is not different from that of either IL-4 and HC-stimulated cells or of control unstimulated cells. This was determined by statistical analysis of the pooled data from five experiments.

examined the effect of HC on the proliferation of these cells. As seen (Fig. 5) HC significantly increases the [³H]thymidine uptake by the IL-4–stimulated cultures and this occurs before the onset of the IgE secretion. Using a two-stage culture system, we have next shown that IL-4 and HC must be present at the same time in order to induce IgE synthesis by T cell–depleted PBMCs. In these assays, cells are first incubated for 7 d in the presence of either IL-4 alone or IL-4 and HC, washed, and cultured for another week (in the absence of IL-4) with or without HC. The results, contrasting with those observed on unfractionated PBMCs, clearly indicate that: (a) IgE is produced only when HC is present together with IL-4 during the primary cultures; (b) HC has no effect in the secondary cultures. For example in one (out of four) such experiment, the presence of both IL-4 and HC in the primary cultures, induced the synthesis of 160±2 mg/ml of IgE in the secondary culture (irrespective of the presence of HC in the secondary culture). No IgE was detected in the secondary cultures when IL-4 was used alone in the primary cultures even when HC was added to the secondary cultures.

Discussion

It was recently reported that GCs increase the in vivo production of IL-4 and suppress that of IL-2; most interestingly this was observed with pharmacological as well as with physiological levels of GCs (24). The present study indicates that GCs markedly increase the synthesis of IgE by human lymphocytes cultured in the presence of IL-4. The significance of these two observations is twofold. First, they explain why the treatment of allergic patients with GCs does not suppress, and may even increase, their IgE antibody production (9–11). Secondly, they suggest that stress may favor the production of IgE. Hence, these findings probably explain the significant and transient elevation of serum IgE levels after myocardial infarction (25). This may also account for earlier observations in rodents that the magnitude of the IgE antibody response is temporally related to diurnal variation in endogenous steroid production (26). Two observations indicate that the effect of hydrocortisone on IgE synthesis is GC specific and most likely mediated by the HC–GC receptor complex. First, the steroid sex hormones have no influence on the IL-4–induced synthesis of IgE. This finding is of interest since it is known that serum IgE levels are influenced by age and sex (27, 28). Secondly, the IgE-potentiating activity of HC is inhibited by RU38486, a potent steroid hormone antagonist (29). The augmenting effect of HC on IgE synthesis by IL-4–stimulated cells is not isotype-specific, and moreover in the absence of IL-4, HC does not induce IgE synthesis whereas it increases the production of the other isotypes. The latter phenomenon has been extensively analyzed and shown to involve both cellular interactions between monocytes, CD4⁺ T cells and B lymphocytes as well as the release of T cell–derived soluble factors induced by GCs (30, 31).

The mechanisms leading to the synthesis of IgE after the addition of IL-4 to cultures of human PBMCs have been extensively analyzed. Briefly, IL-4 triggers cognate interactions between T and B cells, as a result of which B cells are primed to respond to soluble factors including IL-4 itself as well as endogenous IL-2, IL-6, and the low molecular weight B-cell growth factor (23, 32–35). Another important mechanism is the IL-4–induced suppression of the production of natural antagonists of IgE synthesis such as PGE₂ and IFN-γ (36, 37). The present study further indicates that IL-4 needs to be present only during the first 4–5 d of the 12-d culture in order to elicit the synthesis of IgE which is most apparent at the end of the culture period. Moreover, the results show that HC potentiates the IgE response by acting both in the early (IL-4 dependent) and in the late stage (IL-4 independent) of the IL-4–driven stimulation of PBMCs. This indicates that, as expected from the ubiquity of GC receptors (38), HC acts at multiple steps of the IgE response to IL-4. As a first approach to analyze the IgE-potentiating effect of HC we have examined whether it may be explained by (a) the inhibition of IFN-γ production, (b) a change in the expression of FcεRII, and (c) an alteration of the cellular interactions required to induce IgE synthesis by IL-4–stimulated PBMCs. To our knowledge, IFN-γ, IFN-α, and PGE₂ are the only known natural antagonists of the IL-4–induced synthesis of IgE (22). The inhibitory effect of GCs on the production of IFN-γ and of PGs are well known whereas the influence of GCs on IFN-α production has not been reported (39, 40). Since IL-4 by itself strongly inhibits IFN-γ (36) and PGE₂ (37) production, it is unlikely that a major mechanism accounting for the IgE-potentiating effect of HC resides in the suppression of the small residual endogenous production of these agents. Indeed, HC markedly increases the synthesis of IgE by IL-4–stimulated neonatal lymphocytes in the absence of endogenous production of IFN-γ.

FcεRII and its soluble fragments were reported to play a role in the synthesis of human IgE by IL-4–stimulated lymphocytes and by unstimulated lymphocytes from allergic donors (18, 20, 21, 41). The present results indicate that GCs reduce the expression of FcεRII and the subsequent release of FcεRII-soluble fragments (IgE-BFs) by IL-4–stimulated PBMCs. These data are in agreement with earlier studies on the expression of FcεRII by the U937 macrophage cell line (42). At optimal concentrations for IgE potentiation (1–10 μM) HC suppresses the release of IgE-BFs by only 40–70%. Most importantly, the IgE response to IL-4 and HC is dependent upon the expression of FcεRII inasmuch as it is suppressed almost completely by anti–FcεRII MAb. All the previous studies on the enhancing effect of GCs on the Ig production by human lymphocytes concur to indicate that it is strictly dependent upon the presence of monocytes (43, 44). The present results further suggest that a direct cell–cell contact between lymphocytes and monocytes is required in order to observe the IgE-potentiating effect of GCs. Indeed, monocytes cannot be replaced by monocyte-derived soluble factors nor by a cocktail of IL-1 and IL-6. An intimate contact between lymphocytes and monocytes was shown to be necessary for the induction of (a) Ig synthesis by PBMCs in response to GCs (43) and (b) the synthesis of IgE by monoclonal B cells from CLL patients in response to IL-4 and HC (M. Sarfati, G. Delespesse, and M. Rulio, manuscript submitted for publication).

The addition of IL4 to highly purified resting B cells is not sufficient to induce IgE secretion nor class switching to IgE, unless additional activation signals like lipopolysaccharides (LPS) for mouse lymphocytes or Epstein-Barr virus (EBV) for human cells are delivered to the cells (45, 46). Hence, B cell activation and proliferation is required for the IL-4–induced isotype switching and for the transformation of precursor B cells into Ig-producing cells (47). When unfractionated PBMC are cultured with IL-4, the B cell activation signals are delivered by the T cells (32–34). We here report that after rigorous depletion of T cells, a strong IgE response is induced by costimulation with IL-4 and GCs, even when the latter are employed at physiological concentrations (10 nM hydrocortisone). Since

GCs are known to inhibit the proliferation of both B and T lymphocytes, it was important to examine whether the IL-4 and HC-induced synthesis of IgE by T cell–depleted PBMCs was accompanied by a wave of cellular proliferation. The data clearly indicate that HC induces a modest but significant increase of DNA synthesis by the IL-4–stimulated T cell–depleted PBMCs. This effect spans from days 6 to 10 and precedes the secretion of IgE which is first detectable at day 10. The enhancing effect of GCs on DNA synthesis by IL-4–stimulated cells appears to be rather unique since GCs were shown to inhibit the proliferative response of B cells to various stimulants (1). However, more recent studies have shown that CGs interfere mainly with the early rather than with the late stages of B cell activation (4). In contrast to the present observations, Emilie et al. (44, 48) reported that the enhancing activity of GCs on the IgM/IgG production by *Staphylococcus aureus* Cowan I and IL-2 activated human T cell–depleted PBMCs was accompanied by a significant albeit incomplete inhibition of DNA synthesis.

Unlike unfractionated PBMCs, T cell–depleted mononuclear cells must be in contact with both IL-4 and HC, at least for 48 h, in order to synthesize IgE. Hence, in the two-stage cultures, HC must be present together with IL-4 during the primary cultures, and moreover addition of HC to the secondary cultures has no effect. The cellular and the molecular mechanisms whereby HC and IL-4 stimulate T cell–depleted PBMC to synthesize IgE are currently analyzed in more detail. Since IL-4 is capable to induce a T cell–independent synthesis of IgE in the presence of physiological concentrations of HC, it is possible that this mechanism may also operate in vivo. It may be postulated that during stress, which increases the secretion of cortisol and favors the production of IL-4 (24), bystander B cells (that are not involved in MHC-restricted interactions with T cells) may be induced to differentiate into IgE-producing cells. Finally, it is worth noting that the IgE response of T cell–depleted PBMCs may be further increased by the addition of T cells. The amplifying effect of T cells is abrogated by cyclosporine A, suggesting that it is mediated by T cell–derived lymphokines rather than by direct cell–cell interactions between T and B lymphocytes. Supporting this view are the recent observations indicating that IL-2 and T cell–derived low molecular weight B cell growth factor play an important role in the IL-4–induced IgE response (34).

Acknowledgments

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