

Hydrocortisone enhances allergen-specific IgE production by peripheral blood mononuclear cells from atopic patients with high serum allergen-specific IgE levels

Y.-J. CHO*, S.-J. HONG†, and H.-B. MOON‡

*Department of Internal Medicine, Ewha Womans University, †Department of Paediatrics and ‡Internal Medicine, University of Ulsan College of Medicine, Seoul, Korea

Summary

Background Although there is convincing evidence that human B cells can be induced to produce IgE by a combination of interleukin 4 (IL-4) and hydrocortisone (HC) in atopic subjects, it is still uncertain if this performs the same functions in allergen-specific IgE synthesis.

Objective This study was designed to investigate the differences of IgE regulation between atopics and nonatopics, interactions of HC with IL-4, and the correlation between *in vitro* total IgE, allergen-specific IgE synthesis and serum IgE levels.

Methods Peripheral blood mononuclear cells (PBMCs) from 16 atopic asthma patients sensitive to *Dermatophagoides farinae* and seven nonatopic controls were cultured with IL-4 and/or HC. Total IgE and *D. farinae*-specific IgE in culture supernatant were measured by ELISA and FAST.

Results IL-4 increased total IgE synthesis in PBMCs from both atopics and nonatopics, whereas, HC had this effect only in some atopics who showed spontaneous IgE production *in vitro*. HC acted synergistically with IL-4 in total IgE synthesis. Their effects were more remarkable in cases with lower total serum IgE levels. PBMCs from eight of 16 atopics produced *D. farinae*-specific IgE *in vitro* either spontaneously or by IL-4 and/or HC. HC had more profound effects than IL-4 in these patients. They also showed higher total IgE synthesis by HC, and higher specific serum IgE levels than the others. IL-4 and/or HC did not induce any *D. farinae*-specific IgE synthesis by PBMCs from nonatopics.

Conclusion HC had a more profound effect than IL-4 on the induction of *D. farinae*-specific IgE synthesis in atopic patients with high serum allergen specific IgE levels. Further studies to determine the causes of these effects, such as the presence of long lived allergen specific B cells as the result of the priming effect of IL-4 *in vivo*, may be needed.

Keywords: asthma, atopics, IgE, allergen specific IgE, *D. farinae*-specific IgE, interleukin-4, hydrocortisone, PBMCs

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Introduction

In the 'two signal hypothesis' for the isotype switching to IgE proposed by Vercelli *et al.* [1], several B-cell activating

signals are necessary in addition to interleukin (IL)-4 [2–4], glucocorticoid hormone is an example of one of these. Human B cells can be induced to produce IgE by the combination of IL-4 and hydrocortisone (HC) in the absence of T cells [5–7]. It was reported that this effect of HC on IgE synthesis was not mediated by IL-4 signals [8].

Corticosteroids are frequently used to treat asthma, which has raised concerns about the potential *in vivo* effect of

Correspondence: H.-B. Moon, Division of Allergy and Clinical Immunology, Department of Medicine, University of Ulsan College of Medicine, 388–1 Pungnap -Dong, Songpa-Gu, Seoul, Korea

corticosteroids on IgE production and the correlation such IgE-enhancement with the clinical aspects on asthma [9].

Another issue to be addressed concerns the differences between the total and allergen-specific IgE regulation in atopics and nonatopics. Although the basic cellular and molecular requirements for the induction and synthesis of human IgE have been extensively investigated [10–12], the mechanisms involved in the regulation of allergen-specific IgE synthesis are not fully understood. The reason for this may be the low frequency of specific IgE-producing cells in the circulation [13] and the lack of appropriate *in vitro* systems for the detection of low concentrations of specific IgE production. Recently allergen-specific IgE was induced by simultaneously activating T cells and B cells with allergen and polyclonally with anti-CD2 and soluble CD40 in the presence of IL-4, in patients with bee venom allergy [14]. However, other studies performed with glucocorticoid provided contradictory evidence upon the synthesis of allergen specific IgE and total IgE [15,16].

In this study, we measured total and allergen-specific IgE production by peripheral mononuclear cells (PBMCs) in the presence of IL-4 and/or HC. We aimed to investigate the differences between atopics and nonatopics, interactions of HC with IL-4, and the correlation between *in vitro* IgE synthesis and serum IgE levels.

Materials and methods

Study subjects

Sixteen atopic asthma patients (mean age of 16 years, ranging from 10 to 33), sensitive to the house dust mite, *Dermatophagoides farinae*, and who had not been treated with steroids were chosen for this study. The degree of their asthma ranged from mild intermittent to mild persistent, according to the severity criteria of the NIH Asthma Guidelines [17]. Most of the patients were diagnosed at our hospital. Some of the patients used β_2 -agonist inhalers, which was stopped 2 days before the study. Seven nonatopic controls (mean age of 17, ranging from 10 to 34), who had no history of atopic diseases and had negative skin tests to 50 common aeroallergens, were included as controls.

Cell culture

PBMCs were separated from heparinized venous blood by centrifugation on a Hystopaque (Sigma, Saint Louis, MO, USA) gradient. IMDM culture medium (Gibco, Gaithersburg, MD, USA) was supplemented with 10% fetal calf serum (Gibco), 4.5 mM L-glutamine, 50 μ g/mL of streptomycin and 50 U/mL of penicillin. Cells were cultured in duplicate in 24 well plates (1.5×10^6 /mL), either in medium alone or with 0.01–100 ng/mL of IL-4 (Ono, Osaka, Japan)

and/or 0.01–10 μ M of HC (Sigma) for 14 days in 5% CO₂ at 37°C. To discriminate preformed and/or cytophilic IgE from the newly synthesized IgE, parallel cultures were established in the presence of 100 μ g/mL cycloheximide (Sigma). Supernatants were collected and stored at –20°C.

Assay for *in vitro* IgE synthesis

Total serum IgE was quantified using a Phadebas IgE PRIST (Pharmacia, Uppsala, Sweden). The amount of specific IgE antibodies against *D. farinae* allergen in serum was measured using the radioallergosorbent test (RAST).

IgE in culture supernatant was measured by enzyme-linked immunoabsorbent assay (ELISA), as previously described, with some modification [10]. Briefly, 96-well ELISA plates were coated with rabbit antihuman IgE antibody (Sigma), for 12 h. After incubation, the plates were washed with phosphate buffer saline containing 0.05% Tween 20, a serial dilution of an IgE standard and the supernatants to be tested were then added to each plate. The plates were then incubated overnight. After the first 6 hours incubation with biotin-conjugated rabbit antihuman IgE antibody (6 h at 37°C), they were washed and the incubation continued with streptavidin coupled to peroxidase. The IgE was visualized by adding 1.2-phenylenediamine (DAKO, Denmark) and the plates were read in a micro ELISA reader at 490 nm after stopping the reaction. The sensitivity of this assay was 0.2 ng/mL.

The net increase (delta) in the culture total IgE synthesis was calculated by subtracting the spontaneous IgE production without IL-4 or HC as follows:

Net IgE synthesis = IgE (IL – 4 and/or HC) – IgE (cycloheximide)

Delta (Δ) = Net IgE(IL – 4 and/or HC) – Net IgE (media)

D. farinae-specific IgE in the culture supernatant was measured by the fluorescence allergosorbent test (FAST) kit (3 M Diagnostic System, Saint Paul, MN, USA), as detailed in the manufacturer's instructions. Briefly, *D. farinae* allergen-coated and reference wells were inserted into a FASTDISK carousel. Before measurements were taken, culture supernatants were lyophilized to produce a concentrate, which was stored at –20°C. Concentrated supernatants and calibration standards, were added to the wells and incubated for 10 h at room temperature. Each well was then washed three times with washing buffer. *D. farinae*-specific conjugate was added and the whole incubated for 2 h, which was followed by washing and incubation with fluorescence substrate-A for 30 min at room temperature. The amount of *D. farinae*-specific IgE was expressed in fluorescence signal units (FSU). The sensitivity limit of the assay was 0.15 IU/mL.

	Serum IgE	Spontaneous	IL-4	HC	IL-4 + HC
Normal	11.8 ± 9.7	0.4 ± 0.1	9.2 ± 4.8 (Δ 8.8 ± 4.9)	0.4 ± 0.1 (Δ 0.03 ± 0.1)	23.9 ± 7.1 (Δ 23.6 ± 7.1)
Atopy	921.0 ± 256.0	8.3 ± 2.3*	21.2 ± 6.4* (Δ 12.8 ± 5.7)	12.4 ± 3.1* (Δ 4.0 ± 1.7)	37.7 ± 10.1 (Δ 29.4 ± 9.6)

Δ = Net IgE (IL-4) and/or HC) – net IgE (media). HC, hydrocortisone; IL-4, interleukin-4.

*Significant difference compared with normal controls ($P < 0.05$).

Statistical analysis

Data was expressed as mean ± standard error (SE). Statistical analysis was performed with a Wilcoxon-rank sum test on a Macintosh IIfx computer (Apple Computer, Inc., USA) with the Statview software program. Differences were considered significant at $P < 0.01$.

Results

Effects of HC and IL-4 on total IgE synthesis

The mean total IgE levels in sera and culture are shown in Table 1. The spontaneous total IgE synthesis was significantly higher in atopics than nonatopics. IL-4 increased IgE synthesis in a dose-dependent manner (Fig. 1) both in atopics and nonatopics. However, HC induced IgE synthesis, in the absence of IL-4, only in PBMCs from atopic patients who showed spontaneous IgE production. Synergy between IL-4 and HC was noted in both groups of subjects. The optimum concentrations of HC for maximum IgE

synthesis in IL-4-treated PBMCs were in a very narrow range (Fig. 2), level variations were dependent upon the individual donors. Figure 3 illustrates levels of IgE synthesis by representative PBMCs in a control, an atopic, and a hyper-IgE syndrome patient, who was not included in other experiments of this study. No spontaneous total IgE production was detected in the nonatopic individual, levels were moderately high in the atopic patient and very high in the hyper-IgE syndrome patient. However, the maximal percent delta increases in IgE synthesis induced by IL-4 + HC were the exact reverse, e.g. remarkably high in the nonatopic, somewhat moderate in the atopic, and almost none in the hyper-IgE patient.

Effect of HC and IL-4 on *D. farinae*-specific IgE synthesis

In the case of *D. farinae*-specific IgE production, we did not see any spontaneous or IL-4 and/or HC-induced IgE synthesis by PBMCs from nonatopics. Based on the observed response, the atopic patients were divided into two distinct groups. In half (8/16) of the atopics (group 1), there was no

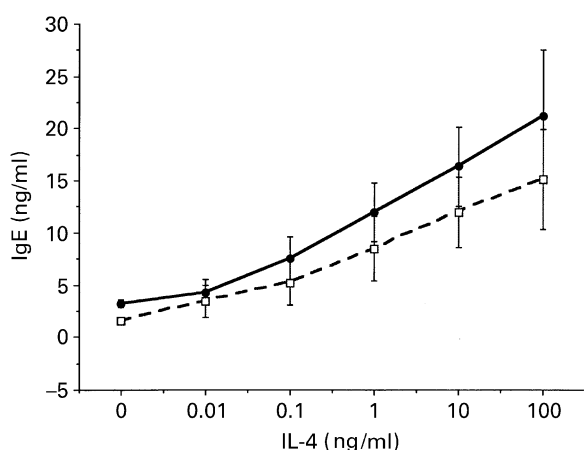


Fig. 1. Dose-dependent effects of interleukin (IL)-4 on IgE synthesis by PBMCs from three normal donors (□) and three atopic patients (●). Results are expressed in ng/mL of net IgE synthesis (mean ± SE).

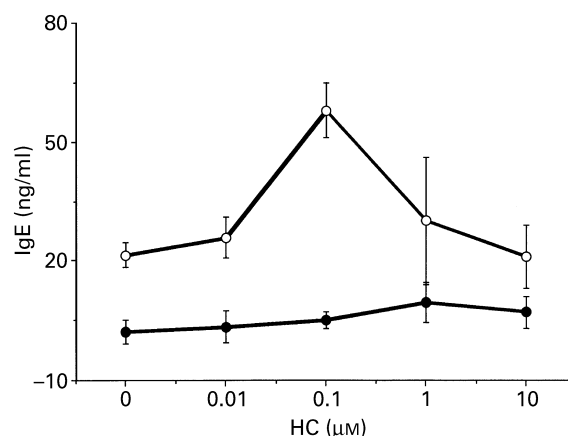


Fig. 2. Dose-dependent effects of hydrocortisone (HC) (●) on IgE synthesis by interleukin (IL)-4 (10 ng/mL)-stimulated PBMCs (○) from three atopic donors. Results are expressed in ng/mL of net IgE synthesis (mean ± SE).

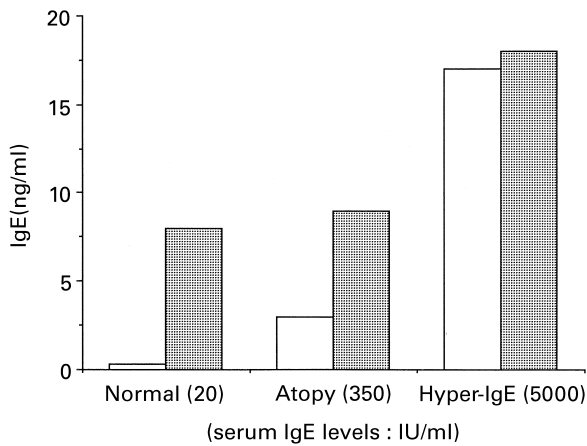


Fig. 3. Spontaneous IgE synthesis (white bars) and effect of interleukin (IL)-4 and hydrocortisone (HC) (hatched bars) on the IgE synthesis by PBMCs from a normal donor, an atopic patient and a patient with high serum IgE levels.

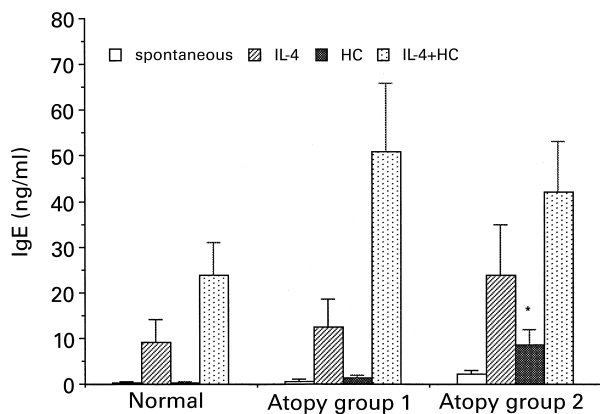


Fig. 4. Characteristics of total IgE synthesis by interleukin (IL)-4 and/or hydrocortisone (HC)-stimulated PBMCs from normal controls, atopy group 1 and atopy group 2. Results are expressed in ng/mL of net IgE synthesis (mean $\pm$ SE).

significant *D. farinae*-specific IgE production at all, but in the other half (group 2), specific IgE production was induced, either spontaneously or by IL-4, HC, or by IL-4 with HC (Table 2). Interestingly, HC had a more profound effect than IL-4 upon the induction of specific IgE in group 2 patients.

Relationship between total and specific IgE synthesis

We examined the *in vivo* and *in vitro* features of total and *D. farinae*-specific IgE synthesis in atopics and found that the amount of specific IgE produced *in vitro* significantly correlated with that of the total IgE induced by IL-4 + HC, but not with IL-4 alone (data not shown). Group 2 patients had a higher total IgE response to HC (Fig. 4), and higher serum RAST scores to *D. farinae* antigens than group 1 patients (3.61 ± 0.28 vs 2.50 ± 0.19 , $P < 0.05$). However, their total serum IgE levels were similar.

Discussion

We have demonstrated in the present study that IL-4 increased total IgE synthesis by PBMCs in both atopics and nonatopics, whereas HC had this effect only in some atopics who showed spontaneous IgE production *in vitro*. HC acted synergistically with IL-4 in terms of total IgE synthesis. We also found that PBMCs from atopics with higher allergen-specific serum IgE levels also produced allergen-specific IgE *in vitro*.

HC might act directly on B cells in the presence of IL-4 to induce isotype switching of IgE synthesis. This hypothesis was proven by Jabara *et al.* [4], who demonstrated that the HC induced S Ψ /Se switch recombination in IL-4-treated B cells. However, conflicting results were reported on the effect of HC in the absence of IL-4. Prete *et al.* [18] reported that 10^{-5} and 10^{-6} molar concentrations of HC were inhibitory, whereas lower concentrations of HC could

Table 2. *D. farinae*-specific IgE levels in atopy group 2 patients in serum (RAST) and *in vitro* (IU/ml)

Patients no.	RAST	Spontaneous	IL-4	HC	IL-4 + HC
1	4.20	<0.20	0.27	0.97	0.94
2	4.20	0.24	2.13	0.67	2.50
3	4.20	0.40	6.10	1.09	7.73
4	2.30	2.00	0.36	6.20	4.00
5	3.60	0.33	5.30	1.25	13.99
6	4.00	<0.20	2.44	0.86	2.50
7	4.00	0.22	4.00	1.00	4.20
8	3.10	<0.20	0.40	0.22	0.68
Mean $\pm$ SE	3.61 ± 0.28	0.5 ± 0.21	1.92 ± 0.78	2.97 ± 0.78	4.3 ± 0.60

HC, hydrocortisone; IL-4, interleukin-4. Atopy group 2 represents atopic patients with inducible *D. farinae*-specific IgE synthesis *in vitro*.

stimulate IgE synthesis. Other groups have contradicted this, by reporting that HC alone could not induce IgE synthesis without monocytes in the culture system [7].

HC treatment induced IgE production by PBMCs only in atopic patients who showed spontaneous IgE production, in our study. The discrepancy between this and previous reports is believed to be due to the different characteristics of the cell donors or the concentrations of HC used in the cultures.

HC had more profound effects than IL-4 on the induction of *D. farinae*-specific IgE synthesis in some atopic patients. This differed from the relationships found in total IgE synthesis. Because PBMCs were used instead of pure B cells in this study, the possibility of differences being due to the use of IL-4 rather than IL-4 producing T cells could not be excluded. However, the finding that HC alone produced IgE only in some atopics suggests that PBMCs might be primed with IL-4 *in vivo* in these patients. This means that if B cells were stimulated sufficiently by IL-4 *in vivo*, it would be easier for these cells to differentiate and expand into IgE secreting cells *in vitro*. The fact that T cells from atopic patients produce larger amount of IL-4 than those of non-atopics [19–21] supports the above findings. This explanation is supported by our data and one other report [22] which shows that *in vitro* IL-4 had no additional effect on the IgE producing B cells from patients with hyper-IgE syndrome. The explanation could be that if B cells had been over-stimulated by an excess of IL-4 *in vivo*, they would no longer respond to IL-4 *in vitro*.

A few studies have addressed the effect of steroid and IL-4 treatment on allergen-specific IgE production *in vitro* [14–16,23–25]. Dolecek *et al.* [24] reported that spontaneous allergen-specific IgE was produced *in vitro* from atopic patients, however, they could not find any enhancing effect of IL-4, IL-13 and/or anti CD40 antibody on allergen-specific IgE. On the contrary, allergen-specific IgE was induced by simultaneously activating T and B cells with allergen, polyclonally with Anti-CD2 and with soluble CD40 ligand in the presence of IL-4 [14]. This report also suggested the differential regulation of allergen specific and total IgE by variations in antigen concentration and highlighted the pivotal role of T cells in controlling the synthesis of IgE antibody isotypes. The effect of glucocorticoid on allergen-specific IgE was reported to be different from its effect upon total IgE production. Bohle *et al.* [15] demonstrated that the newly synthesized IgE, induced by HC/IL-4, is not allergen-specific IgE. Other study by Akdis *et al.* [16] reported opposite regulatory effects of glucocorticoid on antigen-specific and total IgE formation *in vitro*. Characteristics of allergen-specific IgE production were not totally in accord with those of total IgE synthesis in our study, too. However, the reasons why these studies demonstrate different allergen-specific IgE production *in vitro* may be due to

the sensitivity of the detecting system and the individual characteristics of the patients. As the amount of allergen-specific IgE found *in vitro* was minimal, we concentrated the supernatant, and used FAST to detect allergen-specific IgE. *In vivo* (serum) and *in vitro* IgE production are not always in correlation either. Although those patients who showed *D. farinae*-specific IgE production *in vitro* had higher levels of total IgE synthesis by HC and higher *D. farinae*-specific IgE serum levels, total IgE synthesis *in vitro* by IL-4 is different.

Glucocorticoids are the most effective drugs available today for the treatment of allergic disease. It is therefore of concern that *in vitro* glucocorticoids increase the synthesis of IgE. Klebl *et al.* [26] confirmed that a 7 day treatment with 25 mg prednisolone dose not significantly increase IgE levels *in vivo*, although, they did notice a slightly increasing trend toward the end of the treatment period. This may have become more apparent if the treatment was prolonged, as has been reported by Posey *et al.* [27] and Settupane *et al.* [28]. More importantly, PBMCs isolated *ex vivo* after the treatment period showed reduced the inducibility of IgE by IL-4 in the presence of hydrocortisone, when compared with the pretreatment levels [29]. PBMCs obtained from patients before and after administration of prednisolone revealed a significant decrease in interferon- γ synthesis [30], and a reduced numbers of eosinophils, T cells and macrophages in the bronchial wall [31]. These reports suggest that the observed rise in IgE production associated with prednisolone treatment is not clinically deleterious, but reflects the immunomodulatory effect of corticosteroids.

In conclusion, HC had more profound effects than IL-4 on the induction of *D. farinae*-specific IgE synthesis in atopic patients with high serum allergen-specific IgE levels. IL-4 and/or HC did not induce any *D. farinae*-specific IgE synthesis by PBMCs in nonatopic patients.

Our results suggest the possibility of the presence of long-lived allergen-specific B cells as the result of the priming effect of IL-4 *in vivo*. Further studies to determine the causes of these effects are needed. Though the effect of systemic corticosteroid on an asthma attack is probably due to its anti-inflammatory effect, we need to evaluate whether repeated treatment with systemic corticosteroid influences the production of specific IgE in atopics.

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