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Hydrocortisone enhances total IgE levels—but not the synthesis of allergen-specific IgE—in a monocyte-dependent manner

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SUMMARY

Recently, hydrocortisone (HC), when combined with human IL-4, has been reported to increase IgE levels in supernatants (SN) of *in vitro* cultured leucocytes. In this study we investigated the influence of HC on allergen-specific IgE synthesis. Moreover, we examined the relevance of different cell types in this respect. Peripheral blood mononuclear cells (PBMC), T cell-depleted PBMC, CD14-depleted PBMC and highly purified B cells from 10 allergic (birch pollen and/or grass pollen) patients and five non-allergic individuals were investigated. The cells were incubated with HC and/or recombinant human IL-4 (rIL-4) for 8 days. A considerable increase of total IgE was observed in HC/rIL-4-stimulated cultures compared with rIL-4 alone, HC alone or non-stimulated cultures. We demonstrate that this effect depends on the presence of monocytes in *in vitro* cultures. These results were seen in every experiment, irrespective of healthy or atopic state of the blood donor. The increase of IgE could not be attributed to a rise of birch pollen- and/or grass pollen-specific IgE in patients allergic to these allergens, as shown by IgE-immunoblot. Radio-allergosorbent test (RAST) investigations of HC/rIL-4-stimulated cell cultures from allergic and non-allergic patients confirmed that HC/rIL-4-induced elevated IgE production was also not due to increased production of IgE, specific for important aero-allergens (pollens, house dust mite or animal dander). Therefore we conclude that newly synthesized IgE is not specific for allergens, but that sequential isotype switching in human B cells leads to increased polyclonal IgE production.

Keywords hydrocortisone glucocorticoids IgE synthesis type I allergy

INTRODUCTION

It is well established that atopic individuals become sensitized, including to pollens, mites, spores from moulds or animal dander [1]. Under certain circumstances, these proteins induce the synthesis of allergen-specific IgE in B lymphocytes which causes type I allergic symptoms [2]. Induction of antibody synthesis requires different signals, which *in vivo* are provided during the cognate interaction between an antigen-specific T helper cell (Th) and B cells. Th recognizes highly immunogenic peptides in context with MHC class II molecules [3]. Moreover, co-stimulatory signals activating specific Th are necessary [4–6]. IgE synthesis by B cells requires particular signals. The first signal is given by the cytokine IL-4, which is produced in high amounts by allergen-specific Th2 cells [7,8]. The second signal in this interaction is provided by engagement of the CD40 molecule [9] on B cells by the CD40 ligand [10], which is expressed on the activated T cell [11]. It has been shown in *in vitro* experiments that this T cell-dependent physical interaction can be replaced by antibodies to CD40 or by Epstein–Barr virus (EBV) infection, also leading to mature Cε transcripts in B cells [9,12,13].

In this respect, glucocorticoids (GC) have also been shown to provide a signal which, in combination with IL-4, is able to induce IgE synthesis [14–18]. Because of their strong anti-inflammatory effect, GC are widely used in the treatment of patients with allergic diseases, allergic bronchial asthma in particular. Their efficacy in seasonal allergic rhinitis and in seasonal asthma, associated with their effects on specific IgE levels, has been well documented [19–21]. Conflicting results about the effect of GC on IgE levels *in vivo* have been obtained. Some studies describe an increase of total IgE levels during steroid treatment [22–24], whereas others did not observe significant changes in serum levels [25–27]. The aim of this study was to investigate the influence of hydrocortisone (HC) on the production of allergen-specific IgE *in vitro*. Moreover, particular cell fractions were used in order to elucidate the

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requirement of different leucocyte types on GC-induced IgE synthesis.

MATERIALS AND METHODS

Reagents

HC was obtained from Sigma Chemical Co. (St Louis, MO), diluted in culture medium and used in the concentration range 10^{-3}–10^{-14} molar. Anti-human CD40 MoAb (MoAb 626.1) and human rIL-4 were kindly provided by Sandoz (Vienna, Austria). LE 27 anti-human IgE MoAb for ELISA was provided by Beda Stadler (Institute of Immunology and Allergology, Inselspital Bern, Switzerland).

Characterization of patients

Fifteen individuals participated in this study. Five patients displayed type I allergy to grass pollens, two patients displayed type I allergy to grass as well as to birch pollens, and three patients to birch pollens alone. Allergy was proved by typical case history, skin prick-test, radioallergosorbent test (RAST) and IgE immunoblot. Moreover, five non-atopic individuals (negative case history, negative skin prick-test and CAP) were included in this study.

Cell preparation and cell culture

Peripheral blood mononuclear cells (PBMC) of allergic and healthy persons were isolated from heparinized blood by Ficoll–Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation. T cells were depleted by rosetting using neuraminidase (Behring, Marburg, Germany)-treated sheep erythrocytes. After saturation of Fc receptors with 20% human AB-serum, PBMC were incubated with anti-CD14 antibody (Janssen & Cilag Pharma, Hallein, Austria) for 30 min at 4°C. After three washing steps, cells were incubated with magnetic beads coated with sheep anti-mouse IgG antibody (Dynabeads M-450; Dynal Inc., Great Neck, NY) for 45 min at 4°C. $CD14^+$ cells were depleted using a magnetic particle concentrator (DYNAL MPC-1, Dynal). Elutriation of monocytes from PBMC was performed according to published techniques [28–30]. In brief, PBMC were loaded into a Beckman elutriator equipped with a JE-6B rotor (Beckman Instruments, Palo Alto, CA) and fractions were recovered at increasing flow rates. This procedure led to an enrichment of $CD14^+$ monocytes to 85–90%. This cell fraction is from now on referred to as 'monocytes'. Magnetic cell sorting was performed according to methods described elsewhere [31]. After this magnetic cell enrichment the cells were stained with an FITC-conjugated anti-CD19 MoAb (Dakopatts, Glostrup, Denmark) as well as PE-labelled anti-CD14 MoAb (Coulter Clone, Hialeah, FL). B cells were then sorted on a FACStar PLUS (Becton Dickinson, Mountain View, CA) as $CD19^+$ cell population. According to flow cytometry the resulting cell population contained >99% $CD19^+$ cells.

All experiments were performed in 24-well tissue culture plates (Costar, Cambridge, UK), using a final volume of 2 ml, for a period of 8 days. PBMC cells (2×10^6/ml) of each fraction were cultured in RPMI 1640 medium (Flow Labs, McLean, VA) supplemented with 10% fetal calf serum (FCS; JRH Biosciences, Lenexa, KS), 2 mM glutamine (Sigma) and 100 μg/ml gentamycin (Sigma). HC was dissolved in culture medium at 10^{-2} molar as stock solution. HC alone or combined with human rIL-4 was added to the cells in the concentration range 10^{-3}–10^{-14} molar. rIL-4 was used at a concentration of 100 U/ml. Cultures stimulated with rIL-4 and anti-CD40 MoAb (0·1 μg/ml), cultures stimulated with rIL-4 alone and unstimulated cultures served as controls. Cell culture supernatants (SN) were collected and immediately analysed for their content of total and specific IgE.

In order to evaluate whether or not monocytes are required for HC/rIL-4-induced IgE synthesis the following experimental approach was used. CD14-depleted PBMC were reconstituted with the elutriated monocytes. To reveal dose-dependency, monocytes were added to $CD14^-$ cells in increasing numbers, and cultures containing 0%, 25%, 50%, 75% and 100% of the starting concentration ($CD14^+$ percentage in PBMC determined by flow cytometry) were investigated for their IgE content in the test system.

Detection of total IgE

Ninety-six-well Maxisorp ELISA plates (Nunc, Roskilde, Denmark) were coated overnight at 4°C with the murine anti-human IgE MoAb LE 27 (2 μg/ml carbonate buffer, pH 9·6). SN and standard solutions (Pharmacia) were incubated overnight at room temperature. Bound human IgE was detected using an alkaline phosphatase-conjugated horse anti-human IgE antibody (Kallestad, Chaska, MN). IgE concentrations were calculated according to appropriate standard curves.

Detection of specific IgE

Phleum pratense (timothy grass) and *Betula verrucosa* (white birch)-specific IgE was detected using IgE immunoblotting as described previously [32]. Birch and grass pollens were obtained from Allergon AB (Engelholm, Sweden) and extracted as described [32,33]. Proteins were separated by SDS–PAGE and transferred to nitrocellulose (NC). After saturating with buffer A (50 mM sodium phosphate pH 7·5; 0·5% Tween 20; 0·5% bovine serum albumin (BSA); 0·05% sodium azide) for 30 min at room temperature, cell culture SN were incubated overnight at 4°C with NC strips. Plasma of patients (1:4 diluted in buffer A) were used as positive controls, buffer A alone as negative control. Bound IgE was detected with a ^{125}J-labelled rabbit anti-human IgE (Pharmacia). The IgE content specific to important aero-allergens of cell culture SN of T cell-depleted PMBC with HC/rIL-4-induced elevated total IgE levels was assessed using the RAST system (Pharmacia). Perennial allergens such as cat dander (RAST antigel e1) and house dust mite (RAST antigen d1) and seasonal allergens such as grass pollen (RAST antigen g3) and birch pollen (RAST antigen t3) were investigated.

RESULTS

Analysis of cell fractions by flow cytometry

Characterization of different cell fractions (five non-allergic, 10 allergic individuals) revealed for PBMC: $CD3^+$, 59·9 ± 10·8%; $CD19^+$, 8·09 ± 4·7%; $CD14^+$, 14·8 ± 7·5%; and $CD16^+$, 13·2 ± 5·1%; for T cell-depleted PBMC: $CD3^+$, 1·4 ± 1·2%; $CD19^+$, 22·9 ± 12·7%; $CD14^+$, 44·5 ± 20·5%; and $CD16^+$, 18·4 ± 9·97%.

CD14 depletion was formed in PBMC of seven patients (three non-allergic, four allergic individuals) and flow cytometry revealed the following data: for PBMC: $CD3^+$,

57·0 ± 10·7%; $CD19^+$, 8·05 ± 3·8%; $CD14^+$, 12·2 ± 6·3%; and $CD16^+$, 9·6 ± 4·2%; for T cell depleted PBMC: $CD3^+$, 1·4 ± 1·2%; $CD19^+$, 23·4 ± 8·0%; $CD14^+$, 42·7 ± 12·5%; and $CD16^+$, 13·2 ± 1·7%; for CD14-depleted PBMC: $CD3^+$, 69 ± 6·04%; $CD19^+$, 7·75 ± 3·6%; $CD14^+$, 2·3 ± 1·5%; and $CD16^+$, 9·77 ± 1·7%.

Influence of HC on total IgE synthesis

The stimulation of PBMC with a combination of HC and rIL-4 led to a significant increase of total IgE synthesis in each experiment. This effect was most pronounced at HC concentrations of 10^{-4}–10^{-7} molar (Fig. 1). HC alone had no effect on IgE synthesis. rIL-4 displayed a weak effect on IgE synthesis in PBMC. The influence of HC/rIL-4 on polyclonal IgE synthesis was even more pronounced in this cell fraction. This phenomenon may be attributed to the relative enrichment of B cells and monocytes in this culture. No total IgE was observed in $CD14^-$ PBMC, neither HC/rIL-4 nor anti-CD40 MoAb enhanced IgE levels. (Fig. 1c). Using highly purified B cells we did not detect IgE levels comparable to those in SN of PBMC or T cell-depleted PBMC. Although the observed levels were very low, a weak effect of HC combined with rIL-4 was visible (Fig. 1d). Medium controls were in all cases negative. No difference in production of total IgE between allergic and non-allergic donors could be observed (Table 1).

HC/IL-4-induced increase of total IgE is monocyte-dependent

Addition of elutriated monocytes to CD14-depleted PBMC reconstituted HC/rIL-4-dependent IgE synthesis (Fig. 2).This effect was particularly evident when monocytes were added in the concentration present in the freshly isolated PBMC fraction. No IgE was detected, either in SN of the CD14-depleted PBMC or in SN of elutriated monocytes. Lack of IgE synthesis in CD14-depleted PBMC could not be attributed to a relative enrichment of $CD16^+$ cells, as the percentage of natural killer (NK) cells (9·77 ± 1·7%) was not significantly higher than in PBMC (9·6 ± 4·2%).

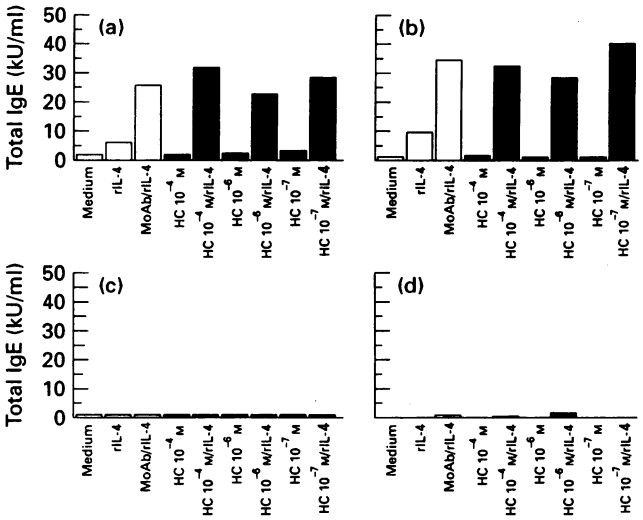

Fig. 1. Representative experiment: different cell fractions of a grass pollen-allergic patient were stimulated with different concentrations of hydrocortisone (HC) with and without rIL-4. Medium, rIL-4 alone and anti-CD40 MoAB were used as controls. (a, b) Total IgE levels in supernatants (SN) of peripheral blood mononuclear cells (PBMC) (a) and T cell-depleted PBMC (b). (c) Total IgE levels in SN of CD14-depleted PBMC. (d) Total IgE levels of purified B cells.

Influence of HC on specific IgE synthesis

Allergen-specific IgE was detected in cell culture SN of seven out of 10 patients allergic to grass pollens or to birch pollens (Table 1: patients 2, 3, 6, 7, 8, 9 and 10). In immunoblots IgE binding patterns to proteins in complete grass or birch pollen extract varied considerably. To overcome this problem, representative experiments using PBMC and T cell-depleted PBMC from allergic individuals were performed. Ten parallel cultures were established using medium, rIL-4, HC and HC/rIL-4. HC

Table 1. Hydrocortisone/rIL-4-stimulated and non-stimulated culture supernatants (SN) of T cell-depleted peripheral blood mononuclear cells (PBMC) with induced elevated total IgE levels were assessed for specific IgE content to important aero-allergens by radioallergosorbent test (RAST)

		Cat, PRU/ml		Dust mite, PRU/ml		Grass, PRU/ml		Birch, PRU/ml		Total IgE, kU/l	
	Patient	Medium	HC/rIL-4	Medium	HC/rIL-4	Medium	HC/rIL-4	Medium	HC/rIL-4	Medium	HC/rIL-4
Birch pollen-allergic, Grass pollen-allergic	1	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	0	2·0
	2	<0·35	<0·35	<0·35	<0·35	0·545	<0·35	<0·35	<0·35	1·99	25·6
	3	N.D.	<0·35	<0·35	<0·35	<0·35	0·457	ND	<0·35	4·72	186·7
	4	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	0·36	1·82
	5	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	0·98	28·2
	6	<0·35	<0·35	<0·35	<0·35	0·436	<0·35	1·18	0·509	18·2	74·6
	7	<0·35	<0·35	<0·35	<0·35	<0·35	0·391	<0·35	<0·35	0·19	6.89
	8	<0·35	<0·35	<0·35	<0·35	<0·35	0·553	<0·35	<0·35	8·02	119·74
	9	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	0·487	0·495	1·34	5·64
	10	<0·35	<0·35	<0·35	<0·35	ND	<0·35	0·438	0·640	ND	ND
Non-allergic	11	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	1·9	40·2
	12	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	0	9·2
	13	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	0·85	12·89
	14	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	0	1·4
	15	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	1·39	4·69

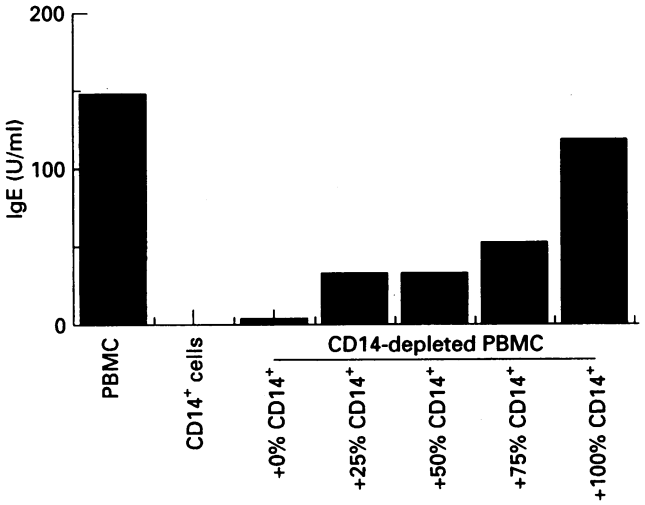

Fig. 2. Representative experiment: different cell fractions from a grass pollen-allergic patient were stimulated with hydrocortisone (10^{-5} M) and recombinant IL-4 (100 U/ml). The requirement for monocytes on IgE production was evaluated by adding increasing amounts (0–100%) of elutriated monocytes to CD14-depleted peripheral blood mononuclear cells (PBMC). $CD14^+$ percentage in freshly isolated PBMC was considered 100%.

was used at a concentration of 10^{-6} molar, which had previously been found to induce a pronounced increase of total IgE. As shown in Fig. 3, SN revealed heterogeneous specific IgE contents. Even SN from unstimulated or rIL-4-stimulated cells were positive (Table 1). IgE-binding to grass pollen major allergens in the molecular mass range 28–45 kD [33] was evident in every SN, though quantitative differences could be observed. IgE binding to minor allergens in the lower or higher molecular mass range (13 kD, 60–90 kD) was randomly distributed, irrespective of whether cells were stimulated (Fig. 3b, c, d) or not (Fig. 3a). However, analysing pools produced from these SN, no significant difference in IgE binding to grass pollen extract could be observed (Fig. 3). In contrast to total IgE levels, neither HC alone nor HC combined with rIL-4 significantly affected allergen-specific IgE production.

To investigate whether the elevated total IgE synthesis was due to increased production of allergen-specific IgE to which patients were not sensitized, cell culture SN of T cell-depleted PBMC were assessed for specific IgE to important aeroallergens by the RAST system. As shown in Table 1, SN of non-stimulated and HC/rIL-4-stimulated T cell-depleted PBMC derived from five out of eight grass pollen allergics contained specific IgE to grass pollen. SN from three out of five birch pollen allergics contained specific IgE to birch pollen. In contrast to total IgE levels, HC/rIL-4 did not significantly enhance specific IgE levels compared with non-stimulated cultures. In these SN, no IgE specific for allergens to which patients were not already sensitized was detectable. In SN of non-allergic individuals no specific IgE was observed (Table 1).

DISCUSSION

GC display strong anti-inflammatory potency, and are widely used in diseases where inflammation is of pathogenic importance. Type I allergy, and in particular allergic bronchial asthma, presents indications for local application of GC. The beneficial effects of this treatment are well established [21,34,35]. In the past, depot injections were administered for treatment of allergic symptoms (e.g. during the pollen season). Recently it was demonstrated that HC, in doses which are similar to physiological serum levels (10^{-6} M), is able to enhance IgE production in PBMC *in vitro* [14–18]. This effect was also evident in our experiments (Fig. 1a,b). No difference was observed when PBMC obtained from the blood of either birch or grass pollen-allergic patients or healthy non-allergic donors were used. However, in previous reports the requirement of particular cell types of the PBMC fraction for this effect remained a matter of controversy. Wu *et al.* [14] and Nüsslein *et al.* [16] reported an absolute requirement of monocytes in their culture system, whereas Jabara *et al.* [15,38] observed an increase of IgE also in purified B cells. In our hands, no increased levels of total IgE were detected in SN of monocyte-depleted PBMC (Fig. 1c), whereas re-addition of elutriated monocytes to these cultures led to reconstitution of IgE synthesis (Fig. 2). The lack of IgE production in monocyte-depleted cultures cannot be attributed to an inhibiting effect of

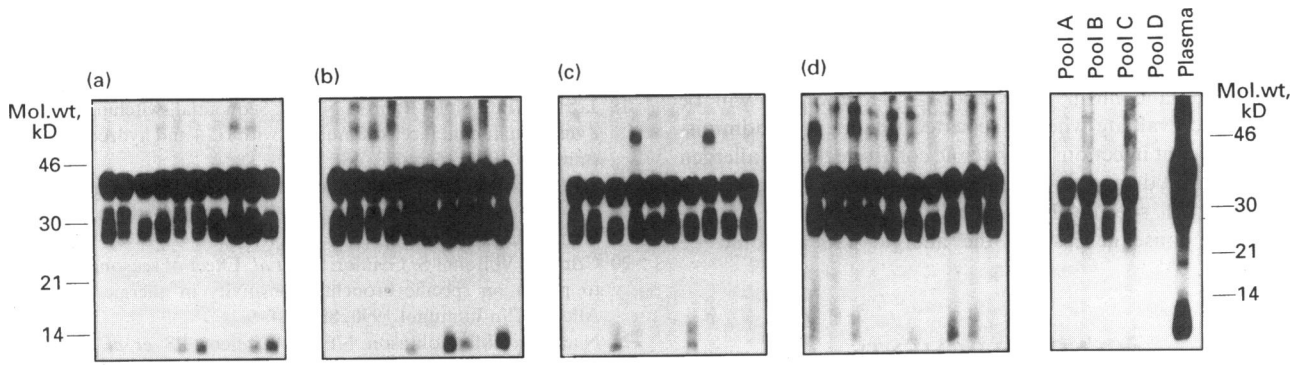

Fig. 3. Representative IgE immunoblot: IgE binding to grass pollen extract of supernatants (SN) of 10 parallel experiments. Cells were isolated from a grass pollen-allergic patient. (a) T cell-depleted peripheral blood mononuclear cells (PBMC) + medium. (b) T cell-depleted PBMC + rIL-4 (100 U/ml). (c) T cell-depleted PBMC + hydrocortisone (HC) (10^{-6} M). (d) T cell-depleted PBMC + HC/rIL-4 (10^{-6} M). On the right, SN pools established from parallel cultures a, b, c and d are compared. Positive control: patients' plasma.

$CD16^+$ NK cells, as the percentage of $CD16^+$ cells in this fraction was not significantly increased. On the other hand, IgE synthesis of T cell-depleted PBMC was increased in comparison with PBMC.

This phenomenon is obviously due to an enrichment of B cells and monocytes (but also of NK cells) in this cell fraction. Total IgE production in purified B cells was very low (Fig. 1d). Although the absolute number of B cells was much higher, IgE levels never reached those present in HC/rIL-4-stimulated PBMC or non-T cultures. Investigating the production of allergen-specific IgE, we observed quantitatively and qualitatively different patterns of IgE binding to allergen extracts. Even SN of unstimulated cultures of allergic patients frequently contained detectable levels of allergen-specific IgE (e.g. patients 2, 6, 9 and 10, Table 1). This phenomenon is obviously due to the distribution of long-living IgE-producing cells in culture [36]. To overcome this problem, SN of 10 parallel cultures from allergic individuals were pooled and analysed for specific IgE in immunoblot experiments (Fig. 3). PBMC and T cell-depleted PBMC cultures stimulated with HC/rIL-4 (strong increase of total IgE) revealed no evidence for increased production of IgE with specificity for grass and birch pollen allergens, to which the patients were sensitized. Analysis of these SN for other important atopic allergens (to which patients were not allergic) again revealed negative results before and after HC stimulation (Table 1). Moreover, increased IgE levels in SN of non-allergic individuals could not be identified as specific for a panel of important atopic allergens, including pollen allergens, allergens from house dust mite and animal dander. We conclude that increased IgE synthesis results from isotype switching rather than from expansion of a precommitted B cell population.

Only a few studies addressed the effect of steroid treatment on IgE levels in humans. Moreover, these studies revealed quite contradictory results. Early reports by Bargatze & Katz demonstrated that IgE synthesis was temporarily related to diurnal variation in endogenous steroid production in the mouse system [37]. Furthermore, there is evidence that treatment of allergic patients with corticosteroids leads to an increase of IgE levels *in vivo* [22–24]. On the other hand, it has been shown that oral treatment of healthy individuals with glucocorticoids during a period of 7 days did not increase the risk of development of type I allergy [27]. A recent report from Zieg *et al.* demonstrates that orally administered prednisone leads to a rise of serum IgE which is due to polyclonal activation of IgE synthesis, and concluded that GC were not clinically deleterious when administered for a short-term period [24]. Our data confirm these findings. However, it will be necessary to evaluate whether repeated treatment or administration of depot injections of systemic cortisone during allergen exposure (e.g. during a pollen season) would influence the production of specific IgE. If this were the case, future deleterious consequences for the allergic patient could be excluded.

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