

Glucocorticoid-induced immunoglobulin E synthesis by peripheral blood mononuclear cells from allergic and nonallergic subjects

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Background: Glucocorticoids (GCS) have been shown to induce IgE synthesis in human peripheral blood mononuclear cells (PBMCs) and purified B cells in vitro. However, the differences in immunoglobulin E (IgE) response to GCS between allergic and non-allergic individuals and the mechanism this interaction have not been elucidated.

Objective: We aimed to compare the effect of GCS (budesonide) on interleukin (IL)-4-driven IgE production in vitro in allergic and non allergic subjects and assess the engagement of intracellular mechanisms.

Methods: The study included 22 patients with allergic asthma and/or allergic rhinitis and 24 healthy volunteers. PBMCs were cultured for 11 days with IL-4 and budesonide and IgE concentrations in supernatants were assessed by immunoassays. T and B cell markers were assessed by flow cytometry.

Results: Budesonide enhanced IgE synthesis to higher extent in healthy donors than in allergic patients (mean increase of 16.5 vs 6.3 kU/L, $P < .05$ respectively) acting through glucocorticoid receptor. Budesonide significantly increased lymphoplasmacytoid cells percentage in both media-controlled (2.5-fold increase) and IL-4-stimulated PBMCs (2-fold increase). Added to IL-4 budesonide decreased the percentage of both T cells and CD40L(+) T cells, but strongly increased the percentage of B cells. Protein tyrosine kinase (PTK) inhibitor decreased, but NF- κ B and protein kinase A (PKA) inhibitors expressed modulatory effects on budesonide-induced IgE synthesis.

Conclusions: Budesonide-induced IgE generation in PBMCs differs in magnitude and seems to involve different mechanisms in atopic and non-atopic subjects.

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INTRODUCTION

Immunoglobulin E (IgE) antibody is a key molecule taking part in allergic responses. In vitro studies have shown that glucocorticoids (GCS), which are the most effective treatment for asthma and allergic rhinitis, induced IgE synthesis by human peripheral blood mononuclear cells (PBMCs) and purified B cells in the presence of interleukin (IL)-4.¹⁻⁴ Moreover, oral treatment with prednisone in patients with asthma increased serum total IgE levels as compared with the period before treatment.³ Recently, based on the observation of more than 300 children, Tantisira et al⁵ documented that inhaled GCS led to an increase in serum total IgE concentrations in children, depending on variants of polymorphic Fc ϵ R2 receptor (IgE low-affinity receptor).⁵

Immunoglobulin E is synthesized by B cells and plasma cells.^{6,7} The CD40L-CD40 interaction together with IL-4 receptor (IL-4R) ligation by IL-4 are necessary for IgE synthesis induced by antigen presentation by B cell to T cell. Interleukin-4R activates Janus-activated kinases and causes subsequent phosphorylation of signal transducer and activator of transcription protein 6 molecules, which in turn form homodimers and enter the nucleus.⁸ There they bind to the C ϵ gene promoter encoding sterile messenger RNA (mRNA) transcript. Simultaneously CD40 protein stimulated by CD40L (CD154) from T cells activates tumor necrosis factor receptor-associated factor molecules that subsequently induce nuclear factor-kappa B (NF- κ B), causing its translocation to the nucleus.⁹⁻¹¹ Nuclear factor kappa-B activates transcription of mature mRNA encoding the full IgE protein structure.¹² These data suggest that IL-4 and CD40L play a crucial role in IgE synthesis.

However, the regulation of IgE synthesis and release seems to involve also other stimulatory (IL-13, IL-6, sCD23)¹³⁻¹⁶ as well as inhibitory (IL-12, interferon gamma, IL-15, IL-21, sCD14, CD23) factors.^{6,17-19} The molecular mechanisms of GCS-induced IgE generation are not fully understood. Furthermore, the potential differences in the mechanisms of IgE generation between atopic and nonatopic subjects have not been addressed.

In the current study, we aimed to compare the effect of inhaled glucocorticoid (budesonide) on IgE synthesis, taking

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into consideration the allergic status of patients. We have shown higher responsiveness of nonallergic PBMCs to stimulation with budesonide and described the participation of protein tyrosine kinase (PTK), NF- κ B transcription factor, and protein kinase A (PKA) in budesonide-induced IgE synthesis by PBMCs. The differential engagement of CD40L(+) T cells in budesonide-stimulated IgE synthesis in patients with allergy and healthy individuals was also documented.

METHODS

Patients

The study included 46 subjects: 22 adult patients (12 males and 10 females, mean age 33.2 ± 2.4 years) with bronchial asthma or allergic rhinitis and positive skin prick tests to relevant allergens, and a group of 24 healthy volunteers (6 males and 18 females, mean age, 29.5 ± 1.6 years), with no history of allergy and with negative skin prick test to a panel of inhaled allergens. Patients received neither inhaled glucocorticoids or long-acting β_2 -agonists for 48 hours and antihistamines for 7 days before the study. Each patient gave written agreement for participation in the study. The study was approved by the Local Ethics Committee of the Medical University of Lodz, Poland (Table 1).

Peripheral Blood Mononuclear Cells Isolation and Culture Conditions

Blood obtained by venous puncture and collected in sodium-heparin vacuum tubes was diluted in phosphate-buffered saline (1:3) (Biomed, Lublin, Poland) and centrifuged on Histopaque 1077 density gradient (Sigma-Aldrich, St. Louis, Missouri) at 800g. Interphase cells were washed 3 times in phosphate-buffered saline and resuspended in medium Iscove's Modified Dulbecco's Medium consisting of 2 mM L-glutamine, penicillin (100 U/mL), streptomycin (100 μ g/mL) (Sigma-Aldrich), and 10% fetal bovine serum. As assessed by trypan blue exclusion method, cell viability always exceeded 95%. Cells (2×10^6 cells/mL) were then incubated in tubes (Sarsted, Nümbrecht, Germany) at 37°C in a

5% CO₂ and 95% humidity atmosphere with human recombinant IL-4 (10 ng/mL) and budesonide (10 nM) (Sigma-Aldrich). To assess the molecular mechanisms of budesonide-induced IgE synthesis PBMCs were preincubated separately with glucocorticoid receptor (GR) inhibitor (Ru-486: 1,000 nM), PTK (genistein: 1,000 nM), NF- κ B inhibitor (SN50: 1,000 nM), and PKA inhibitor (4-CM: 100 nM) (all Sigma-Aldrich) for 45 minutes and then cultured with IL-4 and budesonide for 11 days. Optimal concentrations of stimulatory and inhibitory agents were determined in a pilot study. After 11 days of culture, cell-free supernatants were collected and frozen at -70°C to assess IgE concentration.

IgE Measurement

Total IgE concentrations in supernatants from PBMCs cultures and in sera of patients were determined using the fluoroimmunoassay method UniCAP (Pharmacia, Uppsala, Sweden) according to the manufacturer's protocol.

Flow Cytometric Analysis of T Cells and B Cells Expressing CD40L

After 11 days of PBMCs' culture, the percentages of T cells, B cells, and CD40L(+) T cells and CD40L(+) B cells were analyzed with DAKO Galaxy flow cytometer (DAKO Cytomation, Glostrup, Denmark) with the use of antibodies anti-CD3-FITC (T cells), anti-CD22-RPE-Cy5 (B cells), and anti-CD40L-PE (CD40L surface expression) with isotype controls: IgG1-FITC, IgG1-PE-Cy5, IgG1-PE (Becton-Dickinson, Franklin Lakes, New Jersey) according to the manufacturer's protocol. A minimum of 30 thousand cell-gated events were acquired and analyzed with the FloMax program (DAKO Cytomation, Glostrup, Denmark). Before the analysis, attempts of cells staining with propidium iodide to localize dead cells in forward scatter (FCS)/side scatter (SSC) dot-blots were done. Gating strategy excluded debris and necrotic cells to analyze the expression of CD3, CD22, and CD40L only on the surface of living cells. Among PBMCs, cells with high FSC and SSC were analyzed separately (FSC-high/SSC-high cells). The percentages of T cells and B cells as well as both T and B cells expressing CD40L were analyzed in the peripheral blood of individuals.

CD20(+)CD38(+) Cells Analysis in Immunofluorescence Microscopy

In PBMCs, after 11 days of culture, CD20(+)CD38(+) cells were assessed using antibodies anti-CD20-PE (orange fluorescence) and anti-CD38-FITC (green fluorescence) according to the manufacturer's protocol (Becton-Dickinson).

sCD40L Measurement

Serum soluble CD40L (sCD40L) concentrations were assessed by enzyme-linked immunosorbent assay (ELISA) using ELISA plate reader ELISA LP 400 (450 and 620 nm) according to the manufacturer's protocol (Pasteur Diagnostics, France).

Statistical Analysis

The results are presented as mean $\pm$ SD for variables with normal distribution of values or expressed in median of

Table 1. Clinical Characterization of Patients Included in the Study

	Allergic patients	Healthy subjects	P
Number (n)	22	24	> .05
Sex			
Woman	10	18	> .05
Men	12	6	> .05
Age (yrs):			
Mean	33.2 ± 2.4	29.5 ± 1.6	> .05
Range	(23–60)	24–57	
FEV1 (%)			
Mean	88.6 ± 2.4	99.9 ± 1.8	< .0001
Range	(57–106)	85–121	
Serum t IgE (kU/L)			
Mean	210.6 ± 75.0	21.8 ± 5.8	< .0001
Range	(1–1,690)	2–123	

quartile range for the remaining cases. The distribution of particular variables was verified by the W Shapiro-Wilk test, whereas the Levene test was performed to test homogeneity of variances. If there was a normal distribution and homogeneous variance, the significance of differences between 2 groups was estimated using the Student t test for independent trials or analysis of variance with post-hoc Tukey's test. However, if any of these criteria were not fulfilled, a Wilcoxon test for independent trials within the group or Mann-Whitney *U* test for analysis of differences between 2 groups was used, as well as Kruskal-Wallis with post-hoc Conover-Inman test for variance analysis. Association between variables was expressed by Spearman rank correlation coefficient. $P < .05$ was significant. For statistical evaluation, the Statistica (StatSoft, Inc., Tulsa, Oklahoma) program was used.

RESULTS

Differential In Vitro Budesonide-Induced IgE Synthesis by PBMCs of Allergic Patients and Healthy Subjects

Spontaneous IgE synthesis by PBMCs cultured without IL-4 was detectable in patients with allergy ($n = 22$), but not in healthy donors ($n = 24$) (Fig 1A). Interleukin 4-induced IgE production was observed in both groups; however, in allergic responders it was slightly but significantly lower than in nonallergic individuals (Fig 1A). In allergic patients, both spontaneous and IL-4-driven IgE synthesis strongly correlated with serum total IgE levels ($R = 0.8$, $P < .001$; $R = 0.46$, $P < .05$, respectively).

Budesonide added to IL-4 (10 ng/mL) strongly induced IgE synthesis by PBMCs (Fig 1A). Budesonide-induced IgE production in the presence of IL-4 was significantly lower in allergic patients than in healthy donors (means, 7.4 ± 1.9 vs 17.6 ± 4.2 kU/L, respectively; $P < .05$). Budesonide alone (10 nM) did not influence IgE generation (data not shown).

The Effect of Budesonide on B Cells and T Cells: Correlation with IgE Synthesis

The possible mechanisms of IgE synthesis enhancement by budesonide were investigated. Budesonide added to IL-4 increased the percentage of B cells in both groups as compared with IL-4 alone (Fig 2A). No difference was found between allergic patients and healthy donors. Large cells expressing CD20 and CD38 markers, indicating their lymphoplasmacytoid origin, were identified within PBMCs (CD20[+]CD38[+]) cells (Fig 2D,E). Budesonide added to IL-4 significantly increased (doubled) lymphoplasmacytoid CD20(+)CD38(+) cells percentage as compared with media-controlled cultures in both allergic patients and healthy subjects (Fig 2B). A positive correlation between CD20(+)CD38(+) cells and B cells numbers ($R = 0.78$, $P < .0001$, $n = 20$) was observed. The percentages of both CD20(+)CD38(+) cells (Fig 2B) and CD40L(+) B cells (Fig 2C) among media-controlled PBMCs were significantly higher in allergic patients than in healthy donors.

In addition, budesonide added to IL-4 decreased the percentage of T cells and CD40L(+) T cells as compared with IL-4 alone in both allergic patients and healthy subjects (Fig 2A). Interestingly, in patients with allergy, a negative correlation between the percentage of CD40L(+) T cells and budesonide-induced IgE synthesis was found ($R = -0.71$, $P = .03$) (Fig 3A). In contrast, in healthy donors, this correlation was positive ($R = 0.96$, $P = .00002$) (Fig 3B).

With regards to the in vivo relevance, both the percentage of CD40L(+) T cells in peripheral blood as well as serum concentrations of soluble sCD40L were significantly higher in patients with allergy than in healthy subjects (Fig 4). However, no correlation was found between CD40L(+) T cells percentage and sCD40L concentrations and serum total IgE levels within either allergic or nonallergic subjects.

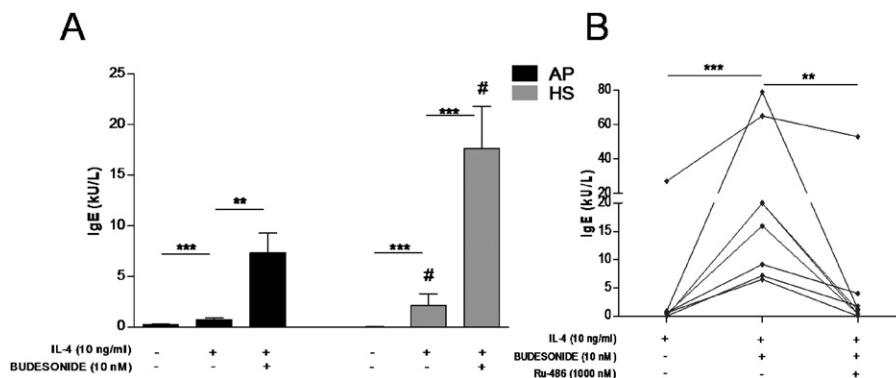


Figure 1. IgE synthesis by PBMCs in vitro. A, The effect of IL-4 and budesonide on IgE synthesis by PBMCs from allergic patients (AP; $n = 22$) (black bars) and in healthy subjects (HS; $n = 24$) (gray bars); (means $\pm$ SEM). B, The effect of glucocorticoid receptor inhibitor (Ru-486) on budesonide-induced IgE synthesis in both allergic patients and healthy subjects (AP; $n = 3$; HS; $n = 4$) (B). ** $P < .01$, *** $P < .001$, # $P < .05$ HS versus AP in the same condition.

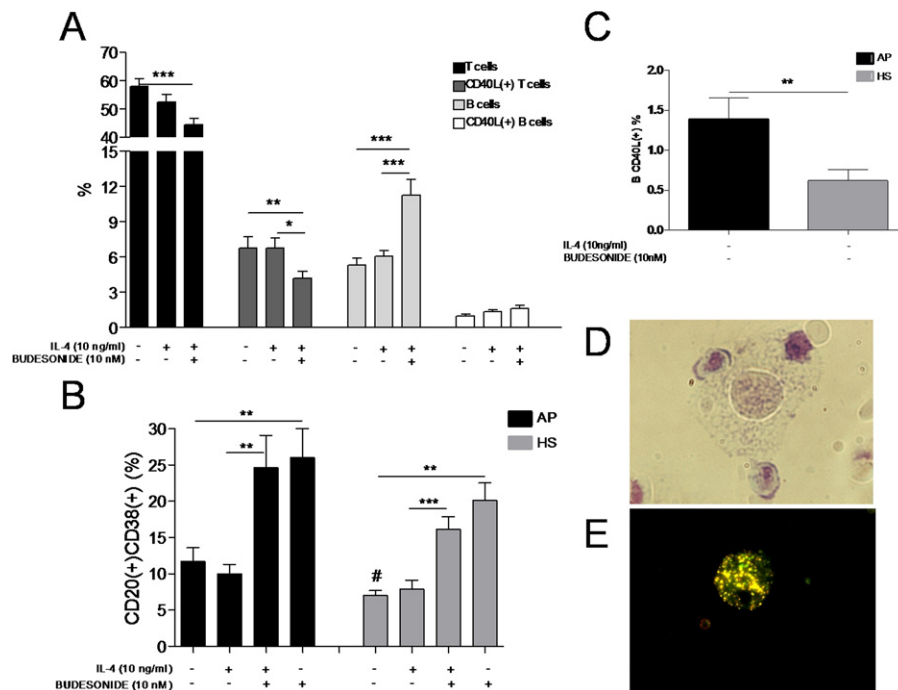


Figure 2. The influence of budesonide on PBMCs in vitro. A, The effect of budesonide on T cells, CD40L(+) T cells and B cells and CD40L(+) B cells among PBMCs from allergic patients (AP, $n = 10$) and healthy subjects (HS, $n = 10$) taken together assessed by flow cytometry. B, The effect of budesonide on lymphoplasmacytoid cells populations from allergic patients (AP, $n = 10$) (black bars) and healthy subjects (HS, $n = 10$) (gray bars) assessed by flow cytometry. C, B CD40L(+) cells' percentage in media-controlled PBMCs after 11 days of culture in allergic patients (AP) and healthy subjects (HS); * $P < .05$, ** $P < .01$, *** $P < .001$, # $P < .05$. D, Large lymphoplasmacytoid cell in PBMCs population after 11-day stimulation with IL-4 and budesonide (light microscope, 1,000 $\times$, immersion). E, CD20(+)CD38(+) large cell in PBMCs' population after 11-day stimulation with IL-4 and budesonide - lymphoplasmacytoid cell (green: anti-CD38- FITC, orange: anti-CD20-PE, fluorescent microscope, 400 $\times$, immersion).

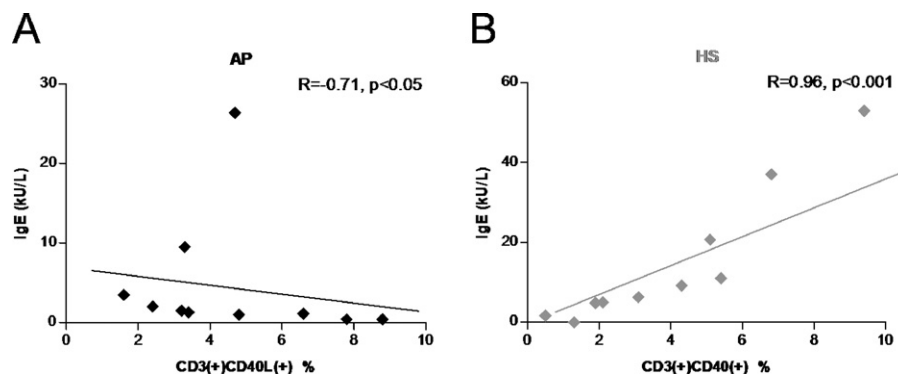


Figure 3. The association between budesonide-induced IgE synthesis and CD40L(+) T cells' percentages. A, The correlation between percentage of CD40L(+) T cells and budesonide-induced IgE synthesis by PBMCs of allergic patients after 11 days of culture in vitro (Spearman $R = -0.71$, $P < .05$). B, The correlation between percentage of CD40L(+) T cells and budesonide-induced IgE synthesis by PBMCs of healthy donors after 11 days of culture in vitro. (Spearman $R = 0.96$, $P < .001$) (individual data).

Signaling Pathways Involved in Budesonide-Induced IgE Synthesis

In the next step, signaling pathways possibly involved in IgE generation stimulated by budesonide were investigated. We confirmed that budesonide induced IgE synthesis in PBMCs through GR in both allergic patients and healthy subjects (Fig

1B). Because IgE synthesis requires the activation of IL-4 receptor (IL-4R) and CD40 on B cells, we checked the engagement of PTK and NF- κ B as well as PKA (structurally bound to the p65 unit of NF- κ B) in budesonide-induced IgE synthesis. In both groups, budesonide-induced IgE production was inhibited by PKT inhibitor (genistein) (Fig 5A).

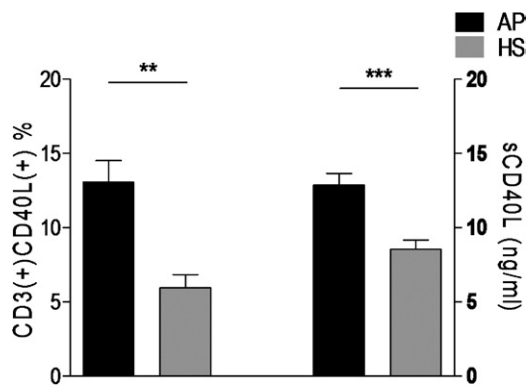


Figure 4. Levels of T CD40L(+) cells and sCD40L in peripheral blood of allergic patients (AP, $n = 22$) (black bars) and healthy subjects (HS, $n = 24$) (gray bars); (means $\pm$ SEM) ** $P < .01$, *** $P < .0001$.

Surprisingly, genistein significantly enhanced IL-4-driven IgE synthesis only in allergic patients (Fig 5B).

Inhibition of NF- κ B and PKA gave diverse results regardless of atopic or nonatopic state. Preincubation of PBMCs with NF- κ B inhibitor decreased budesonide-induced IgE release in 12 of 23 patients, whereas in 9 subjects IgE synthesis was increased (Fig 5C). Similarly, PKA blockade inhibited IgE production induced by budesonide in 16 of 23 patients; however, in 7 subjects IgE synthesis was not significantly enhanced (Fig 5D). In addition, the strong correlation of budesonide-induced IgE synthesis on preincubation of PBMCs with NF- κ B and PKA blockers was observed ($R = 0.96$, $P < .0001$) (Fig 5E).

DISCUSSION

Our study demonstrates that budesonide, the GCS commonly used in the treatment of IgE-mediated diseases such as bronchial asthma and allergic rhinitis, may strongly induce total IgE synthesis by PBMCs in vitro. Although the stimulatory effect of hydrocortisone and prednisone on IgE synthesis was previously proved in healthy individuals,^{1,3} this is the first study showing inhaled GCS to induce IgE production in both healthy subjects and patients with allergy. Tantisira's recent study⁵ reporting that long-term treatment with inhaled GCS led to an increase in serum total IgE levels in children suggests that the effect of budesonide observed in our study may have a potential clinical relevance.⁵

Immunoglobulin E production requires CD40 ligation on B cells by CD40L (CD154) expressed mostly on T cells. Hydrocortisone may increase mRNA CD40L expression in PBMCs and CD40L surface expression on T and B cells after 3 and 6 hours of incubation, thus increasing IgE synthesis.²⁰ We found that budesonide enhanced the percentage of B cells and, furthermore, doubled the number of cells expressing CD20 and CD38 molecules, which was accompanied by total IgE synthesis increase. Both CD20 and CD38 are markers of lymphoplasmacytoid cells.^{21,22} In addition, the strong positive correlation between B cells and lymphoplasmacytoid cells

CD20(+)CD38(+) percentages on PBMC stimulation with budesonide was observed.

Our results indicate that after 11 days of culture budesonide decreased CD40L(+) T cells' percentage of PBMCs in the IL-4 condition in both allergic patients and healthy donors. The decrease of CD40L(+) T cells' percentage could reflect the decrease of whole T cells' population, because glucocorticoids can decrease T cell numbers in vitro.³ Conversely, the decrease of CD40L(+) T cells' percentage could result from the increase of B cells within PBMCs. Interestingly, although budesonide decreased CD40L(+) T cells' percentage in both groups, in patients with allergy the negative correlation between CD40L(+) T cells' percentage and IgE synthesis was observed, whereas in healthy subjects this association was strongly positive, which suggests possible differences in the mechanism of IgE synthesis depending on atopic state. This result indicates that in allergic patients the lower the CD40L(+) T cells' percentage, the higher the budesonide-induced IgE synthesis. However, in healthy subjects, the higher the CD40L(+) T cells' percentage, the higher the budesonide-induced IgE synthesis. As it was shown, the intensity of CD40 ligation on B cells may activate different pathways of IgE synthesis involving NF- κ B1 or NF- κ B2.²³

In the next step, we wanted to assess the signaling pathways' involvement in budesonide-induced IgE generation. First, we confirmed that budesonide generated IgE synthesis through the GR. As shown previously, IgE production requires activation of some intracellular signal transducers, such as PTK and NF- κ B signal transducers from IL-4 receptor (IL-4R) and CD40, respectively.^{12,24} Our results show the involvement of PTK in budesonide-induced IgE production, because genistein, a PTK inhibitor, decreased budesonide-induced IgE generation. Although genistein is widely known as the PTK inhibitor, it also can affect other signal transducers such as NF- κ B, Akt, COX-2, ErbB2, bcl-2, and the mitogen-activated protein kinase pathway,²⁵⁻²⁷ mostly involved in the regulation of cell cycle and apoptosis. It can bind to estrogen and insulin growth factor receptors as well. One cannot exclude that the inhibitory effect of genistein on the IgE synthesis could occur through NF- κ B inhibition, AP-1/CREB binding suppression, as well as the cell cycle arrest. The influence of genistein on the other factors with regard to budesonide-induced IgE production is less probable, because they are not involved in IgE synthesis. Moreover, most of the data showing genistein influence on signaling pathways come from studies on cancer cells, which additionally hinders the interpretation of our results.

The involvement of NF- κ B in budesonide-induced IgE synthesis turned out to be ambiguous. In some individuals, NF- κ B inhibition led to the decrease and in the others to the enhancement of budesonide-induced IgE synthesis. The inhibition of PKA either decreased or increased budesonide-induced IgE synthesis. The c subunit of PKA, which role in IgE synthesis has been unknown, is structurally combined with NF- κ B. In addition, the strong positive correlation between changes in budesonide-induced IgE synthesis observed in our study on NF- κ B and PKA inhibition supports the

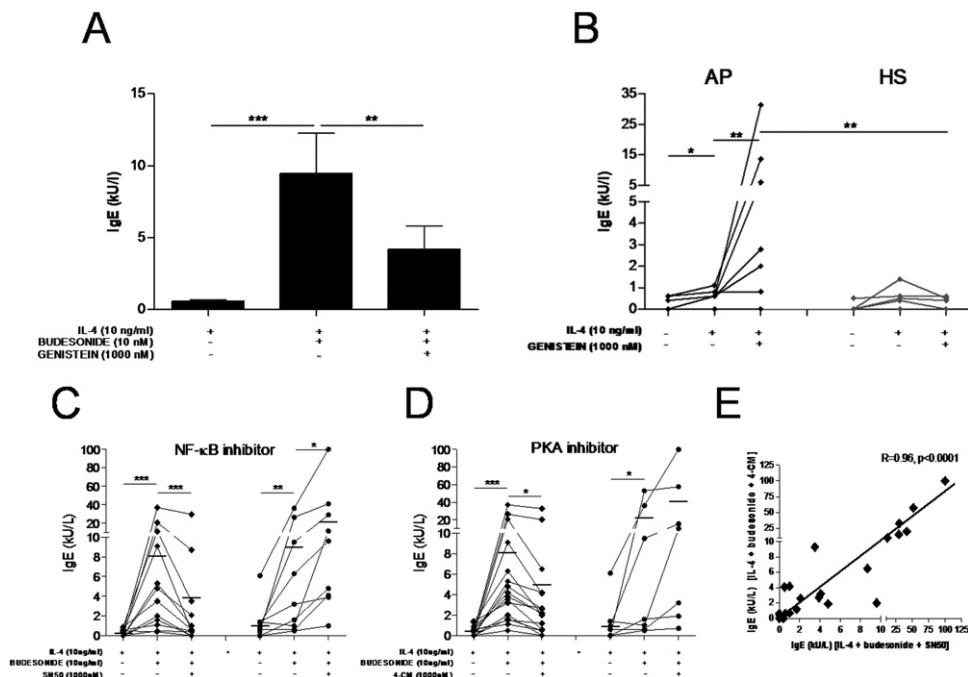


Figure 5. The engagement of intracellular transducers: protein tyrosine kinase (PTK), NF- κ B, and protein kinase A (PKA) in IgE synthesis by PBMCs in vitro. A, The effect of PTK inhibitor (genistein; Gen) on budesonide-induced IgE synthesis by PBMCs from both allergic patients and healthy subjects ($n = 14$). B, The effect of genistein on IL-4-induced IgE synthesis in allergic patients (AP, $n = 7$) and healthy subjects (HS, $n = 7$). C, NF- κ B inhibitor (SN-50) decreased budesonide-induced IgE synthesis in 12 patients (rhombs) and increased in 9 patients (dots) in both allergic and healthy subjects taken together. D, PKA inhibitor (4-CM) decreased budesonide-induced IgE synthesis in 16 patients (rhombs) and increased in 9 patients (dots) in both allergic and healthy subjects taken together. E, The positive correlation of budesonide-induced IgE synthesis on preincubation of PBMCs with NF- κ B (SN-50) and PKA (4-CM) blockers was observed ($R = 0.96$, $P < .0001$); (* $P < .05$, ** $P < .01$, *** $P < .001$. ns, nonsignificant; A: mean $\pm$ SEM; B, C, D: individual data and mean).

assumption of dependent involvement of both NF- κ B and PKA in IgE generation triggered by GCS.

One can explain these ambiguous results if one considers the complexity of NF- κ B forms (NF- κ B1 [p65/p50], NF- κ B2 [p52/RelB], p65/p65, p50/p50), which were shown to act on the promoter of the $C\epsilon$ gene.^{28,29} Nuclear factor-kappa beta p50-specific SN50 molecule used in our study might have inhibited IgE generation through the blockade of NF- κ B1 translocation to the nucleus in certain group of individuals.³⁰ Conversely, in other subjects it might have enhanced budesonide-induced IgE synthesis either *via* free p65/p65 homodimers and NF- κ B2 or by blocking p50/p50 homodimers' repressive activity.

Nuclear factor-kappa B 1p65 activation requires phosphorylation by catalytic subunit of PKAc. That suggests its engagement in IgE production.^{31,32} Therefore, we might have observed the decrease of budesonide-induced IgE synthesis on PKAc blockade in some of individuals, which possibly resulted from NF- κ B1 indirect inhibition. However, because NF- κ B2 does not contain the p50 subunit, it might not have been affected by PKA inhibitor (4-CM) used in experiments, thus leading to the IgE synthesis increase in other subjects.

Based on our results, one cannot conclude whether budesonide induced IgE synthesis by direct effect on B cells or

rather by stimulating T cell-B cell or B cell-B cell interactions. However, budesonide increased total, but not specific, IgE synthesis, which indicates total, unspecific activation of B cells. Nevertheless, IL-4 remains a key factor, because although budesonide alone enhanced the number of B cells and lymphoplasmacytoid cells, it was not able to induce IgE synthesis without the presence of exogenous IL-4.

Apart from the mechanism of budesonide-induced IgE synthesis, we describe several differences between patients with allergy and nonallergic subjects with regard to *in vitro* IgE generation by PBMCs. Firstly, but not surprisingly, media-controlled IgE production was detectable only in allergic patients, and it positively correlated with total serum IgE concentrations. Spontaneous IgE generation was accompanied by a higher percentage of both CD40L(+) B cells and lymphoplasmacytoid cells as compared with healthy individuals. However, IgE present in media of PBMCs from allergic patients could also derive from the degradation of B cells and plasma cells already producing IgE *in vivo* as well as from the effector cells expressing Fc ϵ R receptors among PBMCs. Secondly, PBMCs from allergic patients seemed to be less susceptible to exogenous IL-4 and budesonide stimulation, because IgE synthesis was significantly lower as compared with healthy donors. Moreover, PTK inhibitor increased IgE synthesis in the

IL-4 condition in allergic patients, but not in healthy donors. Interestingly, in patients with allergy, the negative correlation between CD40L(+) T cells' percentage and IgE synthesis was observed, whereas in healthy subjects this correlation was strongly positive. This suggests possible differences in the mechanism of IgE synthesis depending on atopic state.

Finally, with regards to the in vivo relevance, we also found that both CD40L(+) T cell percentage and serum soluble CD40L molecule (sCD40L) concentrations were higher in allergic patients than in healthy donors, suggesting higher T cell activity in this group, probably because of ongoing inflammation.

In summary, our study demonstrates that budesonide may induce IgE synthesis by PBMCs through GR with an engagement of PTK, NF- κ B, and PKA, and possibly through the influence on CD40L-CD40 interaction and stimulation of lymphoplasmacytoid cells' development. Furthermore, differences in cellular and molecular mechanisms of the IgE generation between atopic and nonatopic patients seem to exist.

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