

Interleukin-16 Inhibits Immunoglobulin E Production by B Lymphocytes

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Key Words

Interleukin-16 · Immunoglobulin E · Atopy

Abstract

Background: The increased production of IgE is a hallmark of atopic disorders. CD4⁺ T cells regulate the production of Immunoglobulin (Ig) E by B cells. Interleukin (IL) 16, a CD4⁺ specific cytokine, is highly expressed at sites of allergic inflammation. Our aim was to determine the effect of IL-16 on IgE production in atopic subjects. **Methods:** Freshly isolated peripheral blood mononuclear cells (PBMC) from atopic subjects were stimulated with recombinant IL (rIL) 4 and anti-CD40 antibody to promote IgE production in the presence or absence of rIL-16 added at different time intervals prior to stimulation. The levels of IgE in cell culture supernatants collected at day 14 were measured by ELISA. The effect of IL-16 on the expression of the C ϵ transcript was evaluated by reverse-transcription polymerase chain reaction. To evaluate whether the modulatory effect of IL-16 on IgE production was mediated by interferon- γ (IFN- γ), anti-CD40/IL-4-stimulated PBMC were cultured in the presence of rIL-16 and neutralizing concentrations of anti-IFN- γ antibody. **Results:** PBMC stimulated with rIL-4 (400 U/ml) and anti-CD40 monoclonal antibody (0.5 μ g/ml) produced significant amounts of IgE (range: 1.3–46.0 ng/ml). The addition of rIL-16 twenty-

four hours before stimulation significantly reduced the levels of IgE released by anti-CD40/IL-4-stimulated PBMC (0.5–29.6 ng/ml, $p < 0.05$). IL-16 reduced the expression of the C ϵ transcript in stimulated PBMC. IL-16 induced the expression of IFN- γ mRNA. However, the use of anti-IFN- γ antibody did not alter the effect of IL-16 on IgE production. Rescue doses of IL-13 did not restore the production of IgE by PBMC treated with IL-16. IL-16 did not alter IgE production in CD14-depleted cell preparations suggesting that the IL-16-mediated effects on IgE production may be related to CD14⁺ cells. **Conclusion:** These data show that IL-16 inhibits IgE production and therefore may play an important regulatory role in atopic disorders.

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Introduction

Atopic asthma and allergic rhinitis represent a major worldwide health issue. A hallmark of these disorders is the increased production of both total and allergen-specific immunoglobulin (Ig) E. IgE may mediate the pathogenesis of allergic airway diseases through a number of pathways [1]. Firstly, the interaction of Fc ϵ RI-bound IgE on mast cells and basophils in sensitized individuals with the relevant antigen triggers the release of preformed me-

diators, synthesis of prostaglandins and leukotrienes, and transcription of cytokines. In the bronchial mucosa, these mediators of immediate hypersensitivity reactions rapidly induce mucosal edema, mucus production and smooth muscle contraction and eventually elicit an inflammatory infiltrate. Furthermore, IgE interacts with CD23 (FcεRII) on B cells, enhancing the ability of B cells to present antigens and induce immune responses by specific T cells. The genetic analysis of families showed that bronchial hyperresponsiveness and total serum IgE levels are linked [2]. The treatment of subjects with atopic asthma with anti-IgE monoclonal antibodies (mAb) reduces asthma symptoms and acute asthma exacerbations [3–7]. Although parallel pathways of airway responses to antigen exist, the contribution of IgE to the development of allergic airway inflammation has also been demonstrated in animal studies [8–11]. The production of IgE by B cells is highly regulated and involves a complex network of cellular and molecular signals. CD4+ T cells are critically involved in IgE production through the production of interleukin (IL) 4 and IL-13 which stimulate germline Cε transcription, a key feature of the genetic switch of immunoglobulin genes from IgM to IgE, and by providing required signals to B cells mainly through the CD40/CD40L interaction [12].

IL-16 is a cytokine with profound immunoregulatory effects on CD4+ T cells. As such, IL-16 induces the migration of CD4+ T cells [13, 14] and activates CD4+ T cells as demonstrated by the activation of p56lck, the rise in intracellular calcium concentrations, the induction of IL-2 receptor and the upregulation of the HLA-DR [15–17]. Apart from its activation properties, IL-16 exerts certain inhibitory effects, including the inhibition of the mixed lymphocyte reaction and the induction of reversible anergy in CD4+ cells following their activation [18, 19]. We and others have investigated the potential role of this cytokine in T-cell-dependent processes, such as those observed in allergic inflammation [20–27]. The expression of IL-16 is increased in the nasal mucosa of subjects with allergic rhinitis and in endobronchial biopsies of subjects with atopic asthma [22, 23, 27]. Endobronchial segmental challenge with allergen in asthmatic subjects induces the upregulation of airway expression of IL-16, which correlates with the numbers of CD4+ T cells [25]. Allergen challenge is also associated with the release of bioactive IL-16 in the airways [25, 26]. Furthermore, IL-16 is highly expressed in lymphoid tissues which are sites of IgE production [28, 29]. Since IL-16 acts on CD4+ T cells and those cells regulate the production of IgE, we speculated that IL-16 could modulate the production of IgE. In this

study, we investigated the effect of IL-16 on IgE production by peripheral blood mononuclear cells (PBMC) in atopic subjects.

Material and Methods

Reagents

The recombinant human IL (rIL)-16 was generously provided by W.W. Cruikshank and H. Kornfeld (Boston University School of Medicine, Boston, Mass., USA). The rIL-16 was synthesized as previously described [16]. Briefly, the biologically active IL-16 corresponds to the 121 C-terminal amino acid segment, cleaved from the precursor pro-IL-16. It was produced in *Escherichia coli* as a polyhistidine fusion protein using the expression vector pET-30 LIC (Novagen, Madison, Wisc., USA), purified by metal chelation chromatography, cleaved by factor Xa to remove the polyhistidine tag, and passed over a polymyxine B column to remove endotoxin. IL-16 was further purified from the contaminating polyhistidine tag and factor Xa by means of anti-IL-16 affinity chromatography. The bioactivity of rIL-16 was confirmed by means of chemotaxis of human T cells, as previously described [16]. Typical preparation demonstrated a maximal chemotactic response at concentrations of 10–200 ng/ml. rIL-16 preparations have been demonstrated to contain less than 1.0 units/ml of endotoxin, using a limulus amebocyte lysate assay (QCL 1000; Bio-whittaker, Walkerville, Md., USA). rIL-4, anti-interferon-γ (anti-IFN-γ) mAb and mouse IgG2a isotype control were purchased from R&D Systems (Minneapolis, Minn., USA). Anti-CD40 mAb was purchased from Pharmingen International (Mississauga, Canada), rIL-13 from Sigma (St. Louis, Mo., USA) and rIL-5 from BD Biosciences (Mississauga, Canada).

Subject Population and Preparation of Cells

Venous blood was collected from atopic subjects (n = 17) and nonatopic healthy donors (n = 11). Atopic subjects had a history of symptomatic allergies (atopic rhinitis or atopic asthma) with positive skin prick test responses to one or more common aeroallergens. These donors were not using any systemic or topical glucocorticoid therapy and none had undergone previous immunotherapy. PBMC were isolated from blood samples by centrifugation on Ficoll-Paque. For some experiments, B cells were purified by means of positive selection on a magnetic microbead column with magnetic cell sorting CD19 microbeads (Miltenyi Biotec, Auburn, Calif., USA), according to the manufacturer's instructions. The purified B cells were 99% CD19+, with less than 1% detectable CD3+ T cells. In other experiments, PBMC were depleted of CD14+ cells using magnetic cell sorting CD14 microbeads. The study was approved by the institutional Ethics Committee and all participants gave prior informed consent to participation.

Cell Stimulation for IgE Synthesis

PBMC (1.5×10^6 cells/ml) or purified B cells (0.5×10^6 cells/ml) were cultured at 37°C with 5% CO₂ in flat-bottomed 48-well or curved-bottomed 96-well culture plates, respectively, in the presence of anti-CD40 antibody (0.1–2 µg/ml) and rIL-4 (50–400 U/ml). On day 7, cell cultures were supplemented with new medium for half the volume of the well. IgE production was as-

essed by measuring IgE levels by ELISA in supernatants collected on day 14 and by reverse-transcription polymerase chain reaction (RT-PCR) analysis of C ϵ mRNA transcripts in RNA extracted from cells harvested on day 5 in the course of the culture. To assess the effect of IL-16 on IgE production, cells were incubated for 14 days in the presence of rIL-16 (100 ng/ml) or an equivalent concentration of β -galactosidase produced in an identical fashion in *E. coli*, added at different time intervals prior to stimulation. The viability of cell cultures on day 14 was assessed by determining the number of propidium-iodide-positive cells by flow cytometry and fluorescence-activated cell sorting analysis. In selected experiments, the effect of anti-IFN- γ mAb on the IL-16-mediated inhibition of IgE synthesis was studied by adding 10 μ g/ml anti-IFN- γ mAb, an amount capable of neutralizing 100% of the bioactivity due to 50 U/ml of recombinant human IFN- γ . In other experiments, anti-CD40/IL-4-stimulated PBMC were incubated with IL-16 in the presence of rescue doses of rIL-13 (25 ng/ml) or rIL-5, used as controls, added to cell cultures on days 1 and 7. Based on previous experiments done in our laboratory, this dose of rIL-13 is at least 2.5 times in excess of that needed to induce STAT-6 phosphorylation in B cells.

ELISA for IgE Production

The IgE protein levels in cell culture supernatants were measured in duplicate using a noncommercial ELISA kit. Ninety-six-well Maxisorp Nunc plates (Nalge Nunc International, Rochester, N.Y., USA) were coated with 100 μ l rat anti-human IgE mAb (Biosource International, Medicorp Inc., Montreal, Canada), diluted in PBS 1 $\times$ at a final concentration of 5 μ g/ml and incubated overnight at 4°C. Then they were washed with a solution of PBS 1 $\times$ containing 0.1% Tween 20. The wells were blocked for 2 h at room temperature with a 0.5% gelatin in PBS 1 $\times$ blocking solution. After washing off the blocking solution, the wells were filled with 100 μ l of culture supernatant and incubated at room temperature for 2 h. A standard curve was run simultaneously with normal human polyclonal IgE (Cedarlane Laboratories, Hornby, Canada) at concentrations ranging from 0 to 50 ng/ml. The wells were washed and then incubated at room temperature for 2 h with affinity-purified biotinylated goat anti-human IgE (Biosource International, Medicorp Inc.), diluted in blocking solution at a final concentration of 0.04 μ g/ml. The plates were washed and then incubated for 1 h at room temperature with 0.15 μ g/ml streptavidin-horseradish-peroxidase-conjugated enzyme (Biosource International, Medicorp Inc.), diluted in blocking solution. The plates were washed and developed with tetramethyl benzidine (Zymed Laboratories Inc., San Francisco, Calif., USA) and the optical density was read at 450 nm with an absorbance microplate reader (Spectra Rainbow; Tecan, Salzburg, Austria).

RT-PCR Detection of C ϵ mRNA and IFN- γ mRNA

Total RNA was isolated from PBMC using a Genelute Mammalian Total RNA kit (Sigma Chemical Co., Oakville, Canada). The RNA suspension was digested with DNase I to remove any DNA contamination. RT-PCR determination for IFN- γ mRNA was performed using commercially available primers (Biosource International, Medicorp Inc.) and the Qiagen one-step RT-PCR kit according to the manufacturer's instructions. For C ϵ mRNA detection, total RNA was reverse transcribed to cDNA using hexanucleotides at a $\times 10$ concentration (Roche, Laval, Canada) with an Omniscript RT kit (Qiagen). C ϵ primers were designed

and then purchased from Alpha DNA (Montreal, Canada). The sense primer led in the first exon of the C ϵ mRNA coding sequence (CH1 domain) and consisted of 5'-CTCCCTCAACGGGACAATA-3'. The antisense primer was 5'-TCTGACAGC-CAGTGCTTCTG-3' and attached itself to a sequence in the second exon of the coding sequence (CH2 domain) of the C ϵ cDNA. The C ϵ probe corresponded to a 426-nucleotides fragment of the cDNA. The PCR reaction was performed with a Hotstar Taq DNA polymerase kit (Qiagen), as recommended by the manufacturer. All PCR assays were run in a 25- μ l reaction, with 25 ng of RNA, 2.5% MgCl₂, 2.5 mM deoxyribonucleotide triphosphate, 0.5 μ M primers and 1 U Hotstar Taq polymerase. First, Hotstar Taq DNA polymerase was activated by a 15-min incubation at 95°C. To detect the C ϵ transcripts, the PCR reactions were conducted at 94°C for 45 s, 62°C for 45 s and then 72°C for 45 s, for 45 cycles. The β -actin housekeeping gene was used as a control band (Biosource International, Medicorp Inc.).

Statistical Analysis

Results are expressed as means $\pm$ SD. The statistical analysis was performed using one-way ANOVA for repeated measurements followed by the Newman-Keuls test for multiple comparisons. Statistical significance was established at a 5% level of confidence.

Results

Effect of IL-16 on in vitro IgE Production

Initial experiments ($n = 3$) were conducted to determine the optimal culture conditions for IgE production. PBMC isolated from subjects with allergic rhinitis were put in culture for 14 days in the presence of different concentrations of anti-CD40 antibody (0–2 μ g/ml) and rIL-4 (50–400 U/ml). After 14 days, cell culture supernatants were collected and the levels of IgE protein were measured by ELISA. PBMC isolated from subjects with atopic rhinitis and/or atopic asthma do not secrete significant levels of IgE spontaneously. Significant synthesis of IgE over baseline levels was only observed in 1 subject when PBMC were stimulated with IL-4 in the absence of anti-CD40 antibody. During this time interval, the highest IgE levels were measured when cells were incubated with anti-CD40 antibody at a concentration of 0.5 μ g/ml and rIL-4 at a concentration of 400 U/ml. Therefore, all subsequent experiments were performed using these concentrations. To evaluate the effect of rIL-16 on IgE production, PBMC were incubated with rIL-16 (100 ng/ml) added 24 h before stimulation with anti-CD40 antibody and rIL-4 ($n = 17$). IL-16 significantly reduced IgE levels in anti-CD40/IL-4-stimulated cell culture, as evaluated by ELISA (fig. 1). rIL-16 alone, in the absence of stimulation with anti-CD40 antibody and rIL-4, did not significantly alter IgE production. As a control, stimulated PBMC were cultured with an

Fig. 1. Effect of IL-16 on IgE production. PBMC were stimulated with rIL-4 (400 U/ml) and anti-CD40 mAb (0.5 µg/ml) in the presence or absence of rIL-16 (100 ng/ml) added 24 h prior to stimulation. The levels of IgE in the culture supernatants were assessed by ELISA. Results are expressed as means $\pm$ SD (n = 17). NS = Unstimulated cells. * p < 0.001 compared to unstimulated cells; ** p < 0.05 compared to anti-CD40/IL-4-stimulated cells (ANOVA).

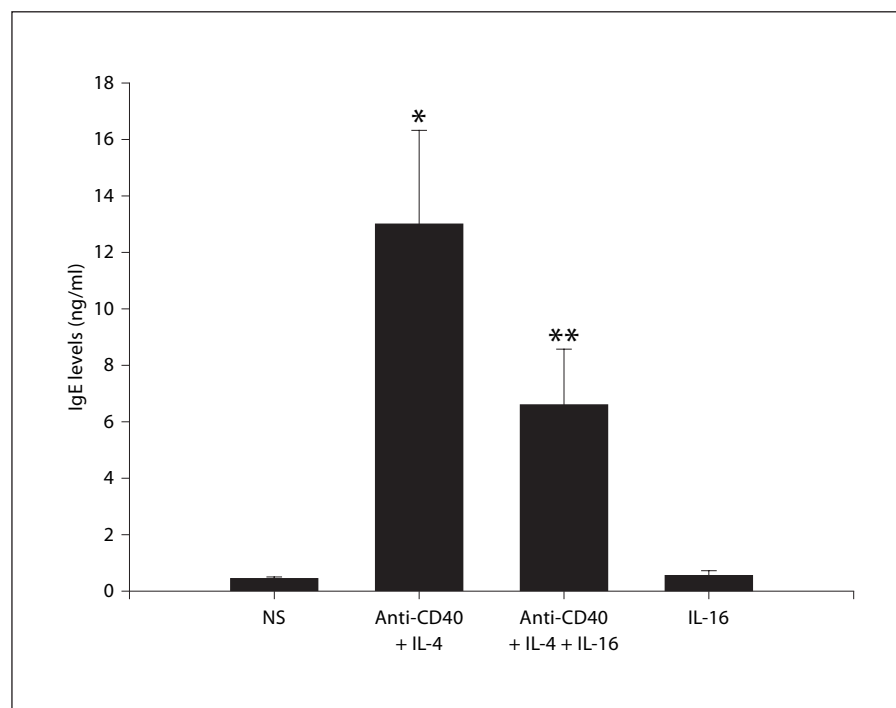


Table 1. Effects of rIL-16 and β -galactosidase on IgE production by anti-CD40/IL-4-stimulated PBMC

Experimental conditions	IgE (ng/ml)
Control	2.34 $\pm$ 2.86
Anti-CD40/IL-4	20.72 $\pm$ 6.25 ^a
Anti-CD40/IL-4 + IL-16	9.72 $\pm$ 8.36 ^b
Anti-CD40/IL-4 + β -galactosidase	19.94 $\pm$ 5.46 ^a

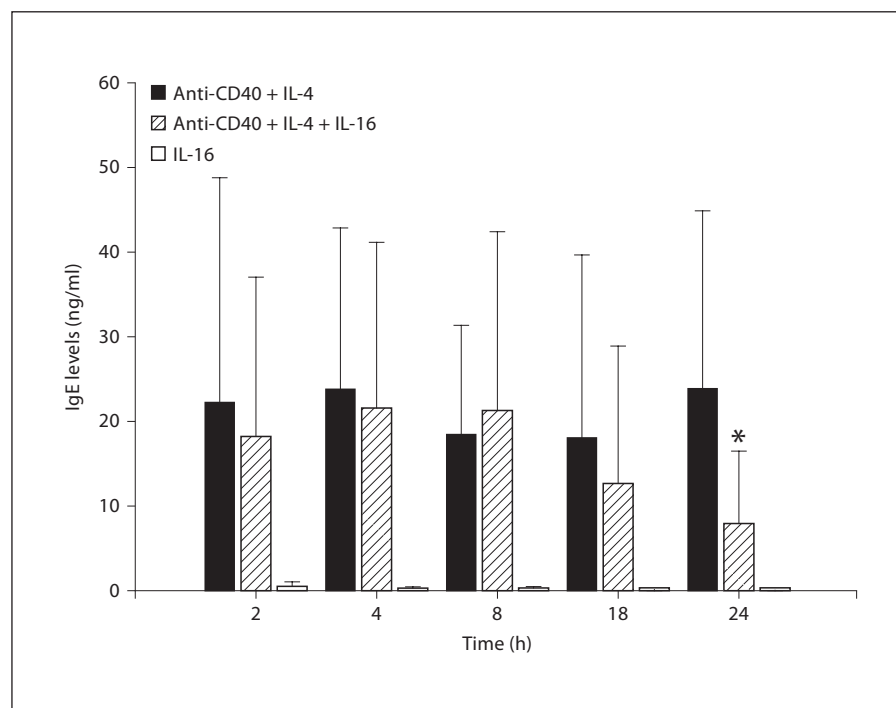
^a p < 0.001 compared to control.

^b p < 0.01 compared to anti-CD40/IL-4-stimulated cells.

equivalent concentration of β -galactosidase, an irrelevant protein produced in an identical fashion to IL-16 (n = 9). The production of IgE was not altered in the presence of β -galactosidase (table 1). Maximal biological effects of IL-16 on target cells have been observed at concentrations ranging from 10^{-9} to 10^{-10} mol/l [13–19]. Accordingly, in the present study, we have done a dose-response curve of the effect of rIL-16 on IgE production at doses ranging from 50 to 200 ng/ml, which fall into that range. A significant inhibition of IgE production by IL-16 was observed when IL-16 was used at concentrations of 50, 100 and

200 ng/ml (data not shown). To determine the time course of the IL-16-mediated inhibition of IgE production, PBMC were incubated with rIL-16 added 2–24 h prior to stimulation by anti-CD40 antibody and rIL-4 (n = 6). Figure 2 shows that the effect of IL-16 was time dependent, showing an effect after 18 h of preincubation and maximizing at 24 h. IL-16 did not affect the viability of PBMC as assessed by propidium iodide staining and flow cytometry analysis on day 14 (less than 2% propidium-iodide-positive cells). Thus, the decreased viability of cells did not account for the inhibitory effects of IL-16 on IgE production in unfractionated PBMC. To investigate whether the inhibitory effect of IL-16 was related to atopy, similar experiments were conducted in PBMC cultures prepared from nonatopic donors (n = 11). In contrast to the effects observed in PBMC from atopic donors, IL-16 did not modify IgE synthesis in anti-CD40/IL-4-stimulated PBMC isolated from nonatopic donors (unstimulated: 2.4 ± 1.6 ng/ml; anti-CD40/IL-4 stimulated: 26.2 ± 4.1 ng/ml; anti-CD40/IL-4 stimulated + IL-16: 24.3 ± 3.8 ng/ml; p > 0.05). To determine whether the inhibition of IgE synthesis reflected a general downregulation of immunoglobulin synthesis, the effects of IL-16 on IgG production were investigated. IL-16 did not modify IgG synthesis in stimulated PBMC (anti-CD40/IL-4 stimulated: 2.09 ± 1.53 µg/ml; anti-CD40/IL-4 stimulated + IL-16: 2.19 ± 1.55 µg/ml; p > 0.05).

Fig. 2. Time course of IL-16-mediated inhibition of IgE production. PBMC were incubated with rIL-16 (100 ng/ml) added at different time points prior to anti-CD40/IL-4 stimulation. IgE levels in the supernatants were measured by ELISA. Results are expressed as means $\pm$ SD (n = 6). * p < 0.05 compared to anti-CD40/IL-4-stimulated cells (ANOVA).



Effect of IL-16 on the Expression of the C ϵ mRNA Transcript

Having determined that IL-16 reduced IgE production in PBMC from atopic donors, we next investigated whether the inhibitory effect of IL-16 on IgE production reflected an inhibition of the anti-CD40/IL-4-mediated induction of C ϵ transcripts. C ϵ transcripts were detected at a very low level in unstimulated cells (fig. 3). Whereas C ϵ mRNA expression was upregulated as expected in anti-CD40/IL-4-stimulated PBMC after 5 days of culture, IL-16 reduced the expression of the C ϵ transcript (fig. 3).

Effect of IL-16 on IgE Synthesis in Human B Cells

Although IL-16 has no reported direct effect on B cells, we next investigated the effect of IL-16 on the IgE production by B cells in the absence of T cells. To this end, B cells were purified from PBMC isolated from atopic donors (n = 7) and stimulated with anti-CD40 mAb in the presence of IL-4. Consistent with previous observations, IgE synthesis in stimulated purified B cells was lower than that seen in unfractionated PBMC [30]. Treatment with IL-16 did not modify IgE levels in supernatants collected on day 14 (anti-CD40/IL-4 stimulated: 5.9 ± 2.2 ng/ml; anti-CD40/IL-4 stimulated + IL-16: 4.1 ± 1.1 ng/ml; p > 0.05). These observations raised the possibility that the inhibitory effect of IL-16 on IgE production by PBMC involves T cells.

Effects of Depletion of CD14⁺ Cells on IL-16-Induced Reduction in IgE Synthesis

The putative receptor for IL-16 is CD4. Previous studies have shown that IL-16 modulates the function of CD4-expressing monocytes and dendritic cells, raising the possibility that the effects of IL-16 on IgE production may be related to functional effects of IL-16 on monocytes in our system. To test this hypothesis, PBMC cultures were depleted of CD14⁺ cells and stimulated for IgE production. Our results show that IL-16 did not inhibit IgE production in CD14-depleted cell cultures (unstimulated: 0.5 ± 0.1 ng/ml; anti-CD40/IL-4 stimulated: 6.8 ± 1.7 ng/ml; anti-CD40/IL-4 stimulated + IL-16: 8.3 ± 2.3 ng/ml; n = 5). These data suggest that the inhibitory effect of IL-16 on IgE production may be mediated by CD14⁺ cells, presumably monocytes.

Role of IFN- γ in Mediating the Effect of IL-16 on IgE Production

IFN- γ is a strong inhibitor of IgE production. We therefore investigated whether IL-16 induced IFN- γ production and found that IL-16 upregulated IFN- γ mRNA expression in PBMC from atopic donors using RT-PCR (fig. 4a). To further assess whether the inhibition of IgE production may be mediated by IFN- γ , anti-CD40/IL-4-stimulated PBMC were incubated with IL-16 and neutral-

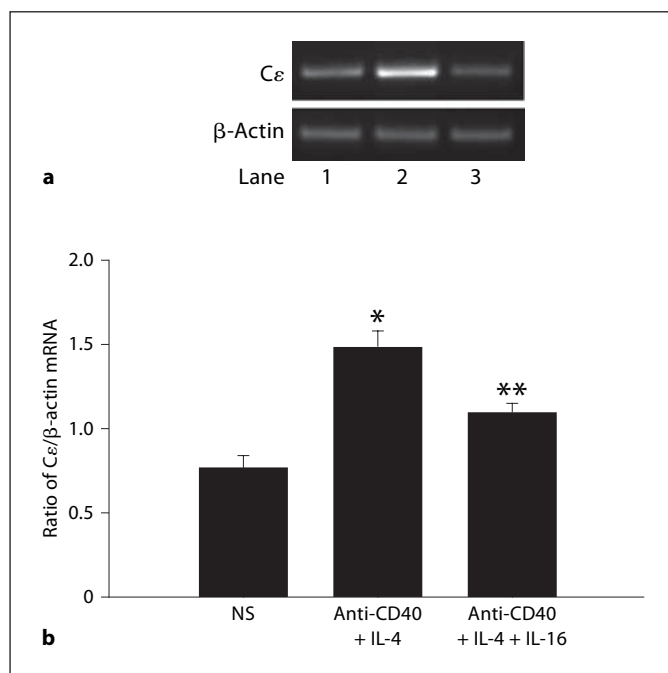


Fig. 3. Effect of IL-16 on the expression of the Cε mRNA transcript. Stimulated PBMC were incubated for 5 days in the presence or absence of rIL-16 (100 ng/ml) added 24h prior to stimulation. Cε mRNA expression was assessed by RT-PCR. **a** One representative experiment of 6 independent experiments is shown. Lane 1 = unstimulated cells; lane 2 = anti-CD40/IL-4-stimulated cells; lane 3 = anti-CD40/IL-4-stimulated cells in the presence of IL-16. The amplified cDNA fragments have the expected lengths of 426 bp for Cε and 417 bp for β-actin. **b** A semiquantitative densitometric analysis was performed on 6 independent experiments. NS = Unstimulated cells. * $p < 0.001$ compared to unstimulated cells; ** $p < 0.05$ compared to anti-CD40/IL-4-stimulated cells (ANOVA).

izing concentrations of anti-IFN-γ mAb or IgG2a isotype control ($n = 12$). Figure 4b shows that the inhibitory effect of IL-16 on IgE production was not altered by the addition of anti-IFN-γ mAb used at a concentration that neutralizes the effect of IFN-γ on allergen-induced responses in PBMC [31].

Effect of Rescue IL-13 on IL-16-Mediated Inhibition of IgE Synthesis in PBMC

Because IL-16 inhibited IgE production in PBMC isolated from atopic donors but not from nonatopic donors, we asked whether the IL-16 effects might have been related to diminished signals derived from T cells from atopic donors. We previously observed that unstimulated PBMC from atopic donors but not from nonatopic donors spontaneously release IL-13 in culture medium over time

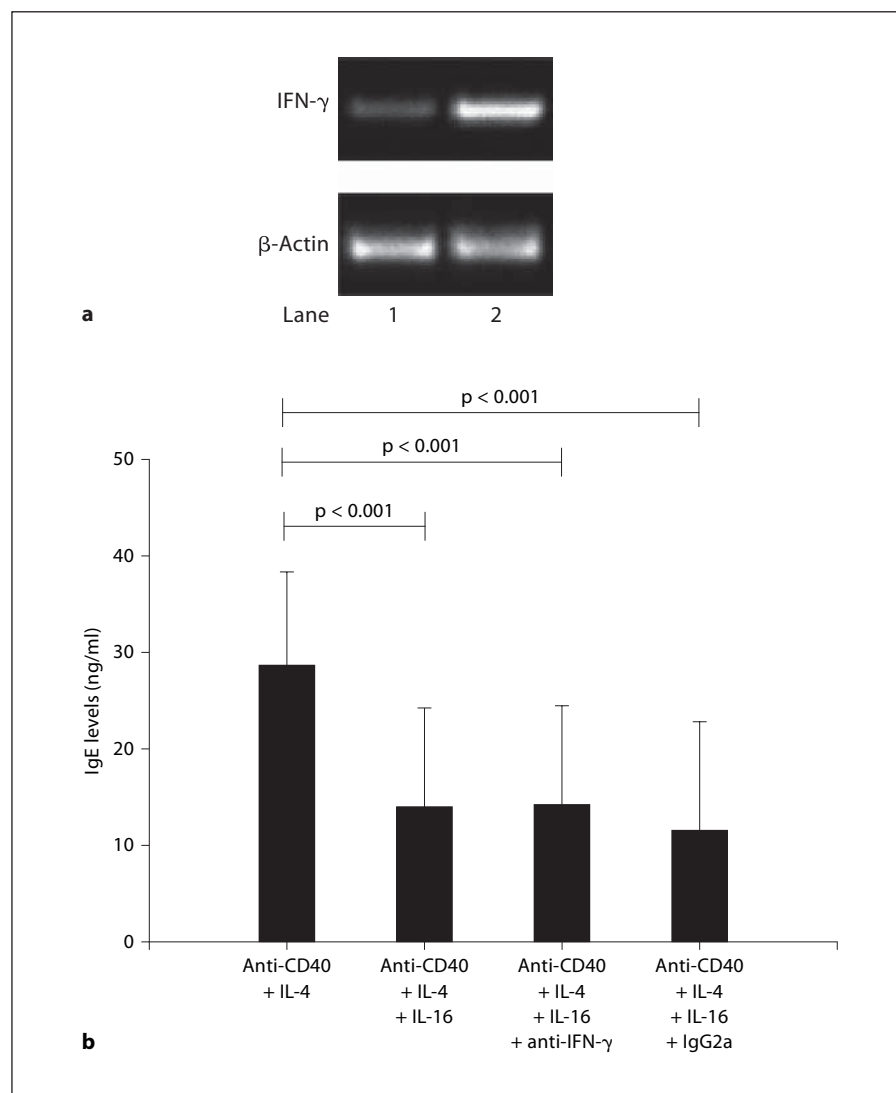
and that IL-16 reduces this basal secretion. We therefore investigated whether adding back IL-13 would restore the IgE production by anti-CD40/IL-4-stimulated PBMC pretreated with IL-16. Our data show that the inhibition of IgE synthesis by IL-16 was not overcome by rescue doses of rIL-13 (anti-CD40/IL-4 stimulated: 27.1 ± 2.2 ng/ml; anti-CD40/IL-4 stimulated + IL-16: 17.6 ± 2.7 ng/ml; anti-CD40/IL-4 stimulated + IL-16 + rescue rIL-13: 18.1 ± 2.9 ng/ml; $p > 0.05$).

Discussion

IL-16 may have a role in diverse immunologic responses and participate in various forms of T-cell-driven inflammation, including inflammatory bowel disease, rheumatoid arthritis and systemic lupus erythematosus [32–36]. In allergic disorders, including atopic asthma, allergic rhinitis and atopic dermatitis, tissue expression of IL-16 has been found to be prominent [22–24, 37]. Despite its association with allergic inflammation, the function of IL-16 in allergic immune responses is presently unclear and may be complex. For instance, IL-16 is a chemoattractant for CD4+ T cells and eosinophils [38], can participate in dendritic and T cell interactions [39, 40], inhibit anti-CD3-activated lymphocyte responses [19] and modulate the pattern of cytokines produced by antigen-stimulated T cells [41]. We have previously demonstrated that IL-16 abrogates the production of IL-5 and promotes IFN-γ release by antigen-specific T cells in atopic subjects, suggesting that IL-16 may exert important immunoregulatory effects on T cells in atopic disorders [41]. In this study, we further investigated the potential role of IL-16 in allergic immune responses by evaluating the effect of IL-16 on IgE production. Since IL-16 has potent effects on T cells, we conducted these experiments under conditions in which T cells were present and could respond to this cytokine. Our current results indicate that IL-16 inhibits IgE production in unfractionated PBMC stimulated with IL-4 and anti-CD40 antibody.

Despite an increasing body of evidence suggesting a regulatory role of IL-16 in cellular-mediated immune responses, little is known about the implication of IL-16 in humoral immune responses and the cellular cross-talk between B and T cells. IL-16 mRNA is expressed in abundance in lymph nodes and IL-16 immunoreactivity is prominent in human tonsils at sites of T and B cell interactions [28, 39]. Kaser et al. [39] reported that B lymphocytes themselves produce IL-16 which induces the migration of T lymphocytes in vitro. These data suggest a role

Fig. 4. a Effect of IL-16 on the expression of IFN- γ . PBMC from atopic donors ($n = 3$) were incubated with rIL-16 alone for 8 h. The expression of IFN- γ mRNA was assessed by RT-PCR. The amplified cDNA fragments have the expected lengths of 424 bp for IFN- γ and 417 bp for β -actin. Lane 1 = unstimulated cells; lane 2 = cells in the presence of IL-16. **b** Role of IFN- γ in mediating the effects of IL-16 on IgE production. Anti-CD40/IL-4-stimulated PBMC were incubated with rIL-16 in the presence or absence of anti-IFN- γ mAb or isotype control. Results are expressed as means $\pm$ SD ($n = 12$).



for B-cell-derived IL-16 as a potential regulator of B and T cell interactions in humoral immune responses, by allowing CD4 $^{+}$ T cell migration to B-cell-rich follicles in lymphoid organs. The results of the current study suggest that IL-16 may also mediate T-cell-dependent B cell responses by regulating IgE production. The potential contribution of IL-16 in B cell activation and IgE production has not been fully investigated. Previous animal studies have shown that the administration of anti-human IL-16 antibody partially decreased the upregulation of ovalbumin-specific IgE during repeated ovalbumin inhalation in sensitized mice, suggesting that IL-16 may be involved in the process of IgE production [42]. Allergen-induced airway hyperresponsiveness was also inhibited in animals treated with anti-IL-16 antibodies. These latter data

would initially appear at variance with our findings showing an inhibition of IgE production by human PBMC in vitro. However, it has been subsequently shown that the systemic administration of exogenous IL-16 inhibits allergen-induced airway hyperresponsiveness, airway eosinophilia and type 2 T helper cell (Th2) cytokine production in sensitized mice, suggesting that IL-16 may also have potent immunosuppressive effects on Th2-dominated allergic airway responses in vivo [43]. IL-16 can also inhibit inflammatory responses in nonallergic immune responses, which is consistent with the immunoregulatory role of IL-16 under some conditions [32, 44]. Furthermore, the divergent results from these studies also reveal the complexity of the potential regulatory role of endogenous and exogenous IL-16 on allergen-induced

T cell responses *in vivo* and call for further clarification. Whether endogenous levels of IL-16 reach concentrations required for the induction of immunosuppression of Th2 responses is unknown.

The precise mechanisms by which IL-16 downregulates anti-CD40/IL-4-induced IgE production by PBMC are unclear at present. The inability of IL-16 to inhibit IgE production in purified B cell populations pointed to a T-cell-dependent mechanism. Consistent with this, B cells do not bear CD4, the putative receptor for IL-16. The observation that IL-16-mediated inhibition of IgE production occurs when PBMC are pretreated with IL-16 for 24 h prior to stimulation suggests an indirect effect of IL-16, possibly the release of a soluble factor by T cells. We first focused our investigation on IFN- γ , since IFN- γ is a strong inhibitor of IgE production. Moreover, we have previously shown that IL-16 enhanced IFN- γ response in allergen-stimulated PBMC [41]. Despite the induction of IFN- γ by IL-16, neutralizing anti-IFN- γ antibody did not alter the effect of IL-16 on IgE production, suggesting that the inhibitory effect of IL-16 on IgE production is unlikely to be attributed to its enhancing effect on IFN- γ production. We previously observed that PBMC from atopic donors spontaneously release IL-13 in cell culture supernatants over time. In addition, we recently showed that IL-16 inhibits the IL-13 production of allergen-stimulated PBMC from atopic donors [31]. We therefore questioned whether the IL-16-mediated effect on IgE synthesis in atopic PBMC may be related to an indirect effect on IL-13 production. Our results show that rescue doses of IL-13 did not restore the production of IgE in PBMC treated with IL-16. Since IL-16 may have biological effects on CD4-bearing monocytes, depletion experiments were conducted to investigate the potential involvement of monocytes in IL-16-mediated effects on IgE production. Our data show that IL-16 does not alter IgE production in cell preparations depleted of CD14⁺ cells, suggesting that CD14⁺ cells, presumably monocytes, may contribute to the inhibitory effect of IL-16 on IgE production. Monocytes are a source of various cytokines, chemokines and lipid mediators, some of which may have significant effects on B cell responses [45]. In line with these findings, we have previously shown that IL-16-mediated inhibition of allergen-induced cytokine production by PBMC may be partly related to an effect of IL-16 on accessory cells [31]. Interestingly, human B cell function has been shown to be regulated by interaction with soluble CD14 [46]. The biological functions of IL-16 on monocytes and dendritic cells are largely unknown. Earlier studies have shown that IL-16 modulates receptor expression and cytokine

release in monocytes and monocyte-derived dendritic cells [47, 48].

We clearly noted a distinction between atopic and nonatopic subjects regarding the effect of IL-16 on IgE production. IL-16 did not decrease the IgE that presumably resulted from *de novo* isotype switching in the non-atopic individuals. It is conceivable that atopic individuals have circulating memory B cells that will begin to develop into plasma cells and secrete IgE following T-cell-mediated stimulation. The effect of IL-16 may therefore be to diminish the interaction between memory B cells and T cells in culture, due to different chemokine and adhesion receptors rather than naïve B cells. Our depletion studies suggest that monocytes may play a role. These rapid responders may secrete mediators that are important in the early phase of IgE production, such that preformed memory B cells or plasma cells would be more sensitive to these mediators than more slowly developing cells that are undergoing isotype switching.

Cross-talk between B and T cells involves interactions through costimulatory molecules expressed on the cell surface of B and T cells. Of particular importance for IgE production is the CD40/CD40L interaction. Since no consistent IgE synthesis over baseline was obtained in IL-4-stimulated PBMC in the absence of anti-CD40 antibody, PBMC were stimulated with IL-4 in the presence of anti-CD40 antibody, which in theory overcomes the need for T cells in our model. It is therefore unlikely that CD40L function or expression is implicated in the effect induced by IL-16 on IgE production. However, we cannot exclude the possibility that IL-16 modulates the function of additional cell surface signals that operate in T and B cell interactions in this system. Further experiments would be necessary to investigate these hypotheses systematically.

In conclusion, these experiments show that IL-16 decreases IgE production by PBMC. These findings suggest that IL-16 may be an important modulator of allergic responses through its ability to downregulate IgE production.

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