

Allergen-mediated modulation of CD23 expression is interferon- γ and interleukin-10 dependent in allergic and non-allergic individuals

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Summary

Background CD23 plays an important role in IgE regulation. The modulation of CD23 expression during specific immunotherapy (SIT) has been described previously. In the present study, we investigated in detail the effects of complete birch pollen allergen extract (BPA) on CD23 expression of peripheral blood mononuclear cells (PBMCs) *in vitro*.

Methods PBMCs from 14 birch pollen-allergic (bp-allergic) patients and eight non-bp-allergic controls were stimulated with IL-4 and increasing doses of BPA. CD23 expression on monocytes and B cells was measured by flow cytometry; sCD23 release and the levels of IFN- γ and IL-10 secretion were determined by ELISA. To analyse the mechanisms on CD23 expression in more detail, neutralizing anti-IFN- γ and anti-IL-10 antibodies were added to IL-4 and BPA-stimulated cultures.

Results IL-4 induced CD23 expression on B cells and on monocytes and sCD23 release in the bp-allergic and non-bp-allergic groups. The addition of BPA to IL-4-stimulated PBMC decreased CD23 expression significantly and dose-dependently on B cells in both groups. CD23 expression on monocytes was also decreased in both groups after the addition of BPA, but higher doses were required in the non-bp-allergic population. IL-4-induced sCD23 release was also significantly decreased after the addition of BPA. IFN- γ and IL-10 were induced by BPA in both the bp-allergic and non-bp-allergic groups. The addition of neutralizing anti-IFN- γ antibodies increased CD23 expression on B cells, which were stimulated with IL-4 and BPA, but had no effect on monocytes, whereas the addition of anti-IL-10 antibodies increased CD23 expression on monocytes but not on B cells.

Conclusion These data indicate that early immunological effects like down-regulation of CD23 on B cells and monocytes, which are observed during SIT are dose dependent, mediated by IFN- γ and IL-10 and seem not to depend *per se* on the sensitization state of an individual.

Keywords birch pollen allergen, CD23 expression, IFN- γ , IL-10, sCD23, SIT

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Introduction

Atopic diseases are associated with a number of distinctive immunological features, e.g. elevated total IgE and allergen-specific IgE levels, and also a Th2-dominated cytokine profile. CD23/Fc ϵ RII is a type II membrane glycoprotein, which is expressed on a variety of cells, mainly lymphocytes and monocytes. IL-4 is a potent inducer of CD23 expression on B lymphocytes and monocytes, whereas IFN- γ has been shown to inhibit IL-4-induced CD23 expression on B cells [1]. By contrast, the effect of IFN- γ on CD23 expression on monocytes remains controversial [2]. In addition, CD23 exists in a soluble form (sCD23) after autoproteolytic cleavage from the membrane [3]. Besides binding IgE molecules, CD23 has other physiological ligands such as CD21 [4], CD11b and CD11c [5], which are responsible for the numerous immuno-

logical functions of CD23. The effects of CD23 on IgE production are complex and diverse. An increasing effect of sCD23 on IgE production has been described by Wheeler et al. [3] and Mayer et al. [6]. They demonstrated that inhibitors of the metalloproteinase, which catalyses the cleavage of CD23 and thus decreases the sCD23 level, inhibit selectively IgE production. CD21–CD23 interaction has been shown to increase IL-4-induced IgE production [4]. Recently it was demonstrated that an antagonistic anti-CD23 antibody inhibits IL-4/CD40-induced IgE production by inhibiting ϵ -germline transcription [7].

Inhibitory effects through CD23 have been described after IgE binding or IgE–immune complex binding to surface CD23 as a negative feedback regulation in IgE production [8]. In mice, most data have shown inhibitory effects of CD23 on IgE synthesis [9, 10], indicating profound differences in IgE regulation between humans and mice.

There is increasing evidence that atopic patients differ from non-atopic individuals regarding surface expression of CD23 and release of sCD23, but these findings are controversial.

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Higher levels of sCD23 and surface expression of CD23 in birch pollen-allergic (bp-allergic) compared with non-atopic individuals have been described [11–14].

We and others have described the modulation of CD23 during the course of immunotherapy [15–18] with a reduced expression of CD23 on B cells after 1 week of rush venom immunotherapy. As expected, the stimulation of peripheral blood mononuclear cells (PBMCs) with IL-4 induced CD23 expression on monocytes and B cells. Furthermore, the IL-4-induced expression of CD23 and the release of sCD23 was decreased after the addition of allergen (birch pollen allergen extract (BPA)). Surprisingly, this was observed not only in bp-allergic but also in non-bp-allergic individuals. In order to investigate responsible mechanisms for this observation, we detected the release of the cytokines IFN- γ and IL-10 in the presence of allergen in both groups. Increased IL-10 and IFN- γ secretion occurred not only in bp-allergic but also in non-bp-allergic donors, supporting the observation of decreased CD23 expression in the presence of allergen in both groups. Our data indicate that early immunological effects, which are observed during SIT, do not seem to depend on the IgE sensitization state of an individual.

Materials and methods

Patients

Fourteen adult patients from the Department of Dermatology and Allergy (Charité, Berlin, Germany) suffering from seasonal allergic rhinoconjunctivitis were included into this study. The patients had been diagnosed as being sensitized to birch pollen by the skin prick test (ALK, Hamburg, Germany) and detection of specific IgE to birch pollen (CAP-class ≥ 2 , Pharmacia, Uppsala, Sweden). Eight non-allergic, non-birch-pollen-sensitized healthy individuals were included as the control group.

Blood samples, cell isolation and cell culture

After approval by the local ethics committee and informed consent, heparinized blood was obtained from the patients and controls. PBMCs were separated from heparinized whole blood by Ficoll density gradient centrifugation and washed repeatedly in phosphate-buffered saline (PBS) before being used for further analysis. Cell cultures were performed in flat-bottomed 24-well plates in RPMI 1640 medium supplemented with 10% fetal calf serum, glutamine (2 mM), penicillin (100 U/mL) and streptomycin (100 μ g/mL). The cells were incubated at 37°C in a water-saturated atmosphere containing 5% CO₂.

Reagents

IL-4 was from R&D (Minneapolis, MN, USA) and used at 5 ng/mL. BPA (10 000 SQ Bet v 1 = 1.23 μ g/mL) was a kind gift from ALK-Scherax (Hamburg, Germany) and was used at 10 000, 1000 and 100 SQ/mL diluted in RPMI 1640. Neutralizing anti-IL-10 and anti-IFN- γ were from R&D and were used at a final concentration of 5 μ g/mL. The isotype control antibody was from Sigma (Dreieich, Germany) and used at the same concentration to exclude non-specific effects.

Flow cytometry

1×10^6 cells were resuspended in 100 μ L staining buffer (2% BSA in PBS; pH 7.4; 0.1% sodium azide), incubated on ice with 10 μ L anti-CD19 FITC (Biosource, Ratingen, Germany) and with 10 μ L of anti-CD23 PE (Biosource) for 30 min. The cells were then washed and fixed in PBS with 2% paraformaldehyde. Antibodies with the appropriate isotype (IgG1-FITC and IgG2a-PE supplied by Immunotech, Krefeld, Germany) were used as negative controls.

The tests were carried out using a Coulter XL flow cytometer and Becton Dickinson FACScalibur and evaluated using Coulter System 2 and Cellquest software. Monocytes were identified according to their characteristics in granulatio and size in a forward-side scatter dotblot and stained with anti-CD14 antibodies. B cells were identified by anti-CD19 antibodies. The percentage of double-positive cells after deduction of the isotype control values and the mean fluorescence intensity (MFI) of the PE-conjugated antibody were determined.

Cytokine ELISA

sCD23, IFN- γ and IL-10 were determined in supernatants of 1×10^6 PBMC/mL cultured for 72 h according to the manufacturer's instruction (R&D Systems).

Proliferation assay

For studies of B cell proliferation, the WST-1 proliferation assay was used (Roche Applied Science, Germany). Briefly, 10^5 cells were cultured for 72 h with IL-4 (5 ng/mL) in the absence or presence of BPA (1000–10 000 SQ/mL). In the last 2 h, 10 μ L of the WST-1 reagent was added to each well. Cellular enzymatic activity was determined by measuring optical densities with a photometer at 450 nm. All experiments were performed in triplicate and data expressed in arithmetic means.

Statistics

The Wilcoxon test for non-parametrical, paired data using SPSS software was carried out to analyse the statistical significance of within-group changes. Between-group changes were analysed by the Mann–Whitney *U*-test for non-parametrical, unpaired data.

Results

Expression of CD23 on peripheral B cells and monocytes

Initially, a time course analysis (12, 24, 48 and 72 h) of IL-4-induced CD23 expression revealed a peak expression at 72 h (data not shown). Therefore, this time point was used for further analysis. To investigate the effect of BPA on IL-4-induced expression of CD23 (Fc ϵ -RII) on peripheral B cells and monocytes, the cells were stained 72 h after blood sampling either unstimulated, stimulated with IL-4 or after the addition of IL-4 and 1000 SQ/mL, respectively 10 000 SQ/mL BPA.

To exclude an artificial Th1 response by BPA by LPS contamination, a Limulus assay was performed, resulting in

an lipopolysaccharide (LPS) amount of 128 ng/mL at a concentration of 10 000 SQ/mL BPA (kindly provided by Dr Kamzy, Aventis, Germany). Since LPS was shown to enhance proliferation [19], the functional relevance of BPA on the proliferation of PBMCs of atopic and non-atopic patients was determined. The data show that the proliferation remained unchanged by BPA (1000–10 000 SQ/mL, $n = 5$, data not shown).

CD23 expression on B cells is downregulated by BPA

The baseline expression of CD23 was moderate on B lymphocytes in bp-allergic patients, which was significantly upregulated after stimulation with IL-4 for 72 h from 9% to 56.3% (Fig. 1a). Also, the mean fluorescence intensity (MFI) was significantly up-regulated after IL-4 stimulation (unstimulated: 0.38; IL-4-stimulated: 1.7; data not shown).

As indicated in Fig. 1a, a dose-dependent significant decrease of IL-4-induced CD23 expression on B cells regarding both the percentage of positive cells (1000 SQ BPA: 29.5%; 10 000 SQ BPA: 23.1%; Fig. 1a) and the MFI (1000 SQ/mL BPA: 0.58; 10 000 SQ/mL BPA: 0.49; data not shown) was observed after the addition of birch pollen allergen.

Regarding baseline CD23 expression on unstimulated B cells 72 h after isolation, no significant differences between bp-allergic and non-bp-allergic individuals were found (bp-allergic: 9%; non-bp-allergic: 5.9%; Figs 1a and b). However, non-bp-allergic individuals showed slightly lower baseline CD23 expression levels in accordance with data describing differences between atopic patients and non-atopic individuals with higher CD23 levels in atopic patients. A significant increase of CD23 expression was also apparent in non-bp-allergic individuals after stimulation with IL-4 (Fig. 1b); however, this increase was not as pronounced as in bp-allergic patients (bp-allergic patients: 56.3%, vs. non-bp-allergic individuals: 49.7%). This difference between the groups did not reach statistical significance. Interestingly, non-bp-allergic individuals also showed a significant and dose-dependent

decrease of CD23 expression after the addition of BPA (1000 SQ BPA: 30.6%; 10 000 SQ BPA: 24.8%; Fig. 1b). The MFI of CD23 expression showed the same pattern (unstimulated: 0.19, IL-4-stimulated: 1.73, 1000 SQ/mL BPA: 0.82; 10 000 SQ/mL BPA: 0.68; data not shown).

CD23 expression on monocytes is down-regulated by BPA

Since relevant expression of CD23 occurs not only on B cells but also on monocytes, we analysed these cells regarding CD23 expression as well. Nearly no baseline expression of CD23 on unstimulated cells from bp-allergic patients (0.02%; Fig. 2a) and non-bp-allergic individuals (0%; Fig. 2b) was seen on monocytes. As observed in B cells, also a significant increase of CD23 expression (28.3%) in the bp-allergic group after stimulation with IL-4 for 72 h (Fig. 2a) was seen. MFI also showed a significant increase (unstimulated: 1.3; IL-4-stimulated: 4.85; 1000 SQ BPA: 2.95, 10 000 SQ BPA: 2.01; data not shown). Similar to the findings on B cells, CD23 expression on monocytes from bp-allergic patients was highly significantly decreased after the addition of BPA in a dose-dependent manner (1000 SQ/mL BPA: 10.5%; 10 000 SQ/mL BPA: 9%; Fig. 2a).

As seen in bp-allergic patients, CD23 expression was negligible on unstimulated monocytes of non-bp-allergic individuals and was significantly increased after stimulation with IL-4 (20.9%, Fig. 2b). In contrast to the bp-allergic population the non-bp-allergic population showed only a slight and non-significant decrease after the addition of 1.000 SQ BPA (18.9%). However, a significant decrease after the addition of higher amounts of BPA was observed (10.7%; Fig. 2b). The differences between the bp-allergic and non-bp-allergic groups did not reach statistical significance regarding CD23 expression on monocytes.

Furthermore, addition of polymyxin B ($c = 0.1$ – $50 \mu\text{g/mL}$) in the presence of BPA shows that CD23 expression was not altered and that stimulation with heat-inactivated BPA resulted in a partial loss of CD23 down-regulation, indicating

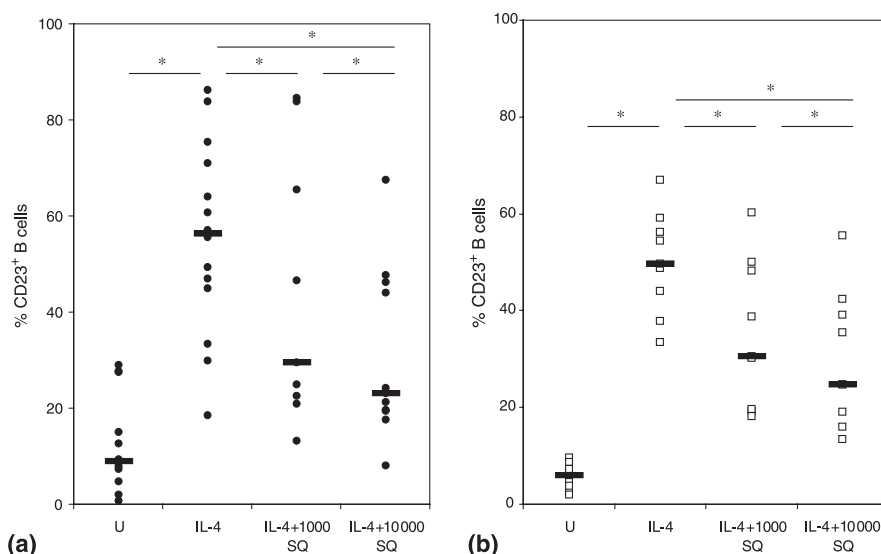


Fig. 1. Percentage of CD23-positive B cells in (a) atopic and (b) non-atopic individuals. Effect of IL-4 stimulation and addition of 1000 SQ, 10 000 SQ birch pollen extract. U: unstimulated cells. The dots represent the level of a single individual, and the bar represents the median. * $P < 0.02$.

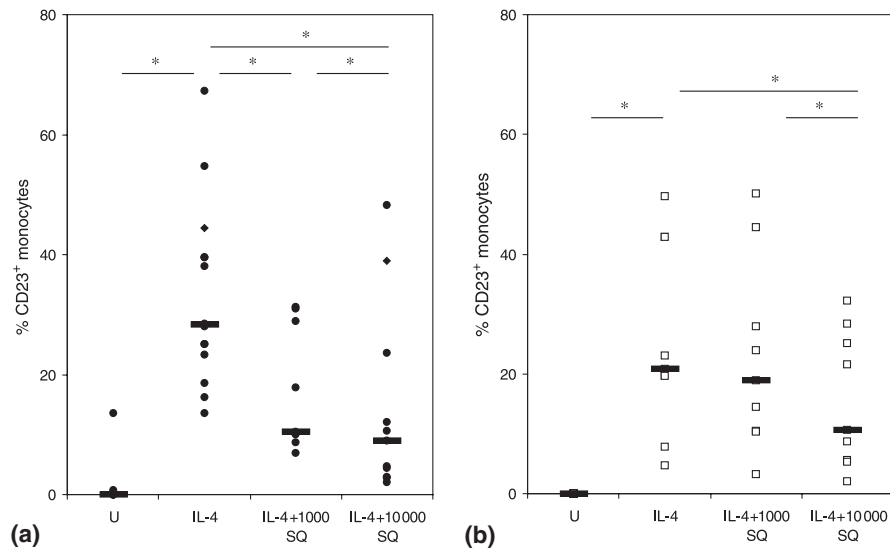


Fig. 2. Percentage of CD23-positive monocytes in (a) atopic and (b) non-atopic individuals. Effect of IL-4 stimulation and addition of 1000 SQ, 10 000 SQ birch pollen extract. U: unstimulated cells. The dots represent the level of a single individual, and the bar represents the median. * $P < 0.05$.

that a direct protein effect by BPA rather than an LPS-dependent effect is responsible for the observed CD23 down-regulation by BPA.

IL-4-induced sCD23 release is decreased by BPA

CD23 is released from the cell surface by proteolytic cleavage and we asked next whether the modulation of CD23 expression was accompanied by changes of sCD23 release. As expected, sCD23 was significantly enhanced after stimulation with IL-4 for 72 h in bp-allergic and non-bp-allergic individuals. The addition of 1000 SQ/mL BPA caused a significant decrease of sCD23 release in both groups (Fig. 3). There was no significant difference between the bp-allergic and non-bp-allergic groups, although the increase after IL-4 stimulation was slightly higher (bp-allergic: 158 pg/mL vs. non-bp-allergic: 174 pg/mL) and the BPA-induced decrease was slightly lower (bp-allergic: 45 pg/mL vs. non-bp-allergic: 83 pg/mL) in the non-bp-allergic group.

IFN- γ is increased by BPA independent of the sensitization state

Since it has been shown that IFN- γ production is increased during SIT [20–22] and that IFN- γ is capable of down-modulating CD23 expression [1], we next analysed the production of this cytokine in our model. The data show that the release of IFN- γ in the supernatants of unstimulated cells was higher in the non-bp-allergic group than in the bp-allergic patients, but due to a large interindividual variability this difference did not reach statistical significance (levels ranging from: bp-allergics: undetectable to 44 pg/mL, non-bp-allergic: undetectable to 144 pg/mL). However, in the presence of 1000 SQ/mL BPA alone, IFN- γ production of unstimulated PBMC was not induced in any group (data not shown). As expected, the addition of IL-4 to the culture wells did not alter IFN- γ release in either of the groups (Fig. 4).

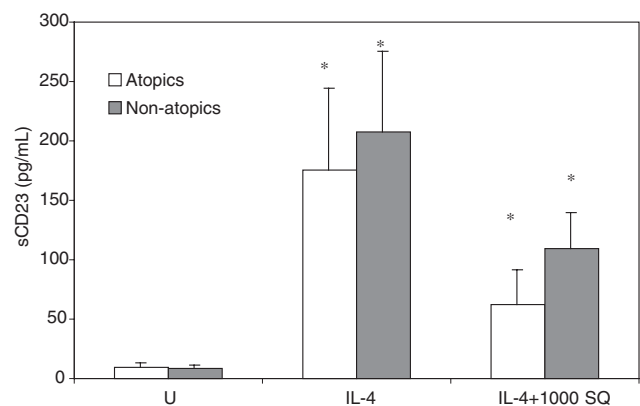


Fig. 3. sCD23 release in atopic and non-atopic individuals. PBMCs were cultured unstimulated (U), with IL-4 or with IL-4 and 1000 SQ birch pollen extract for 72 h, and sCD23 levels in supernatants were measured by ELISA. Columns represent the means $\pm$ SEM of duplicate cultures. * $P < 0.05$ in statistical analysis of within-group changes by the Wilcoxon test.

Addition of 1000 SQ/mL BPA induced a significant increase in IFN- γ secretion in both groups (Fig. 4).

BPA increases IL-10 release significantly in bp-allergic and non-bp-allergic patients

Like IFN- γ , it has been shown previously that IL-10 production is up-regulated during SIT [23]. Therefore, we also analysed IL-10 production in stimulated PBMCs from bp-allergic and non-bp-allergic donors in the presence of BPA. The baseline production of IL-10 in unstimulated cells was low and reached a median of 8.3 pg/mL in bp-allergic patients and 13.2 pg/mL in non-bp-allergic individuals (Fig. 5). Addition of 1000 SQ/mL BPA to unstimulated PBMCs did not induce IL-10 production (data not shown). IL-4 did not increase IL-10 secretion in the bp-allergic group, but a

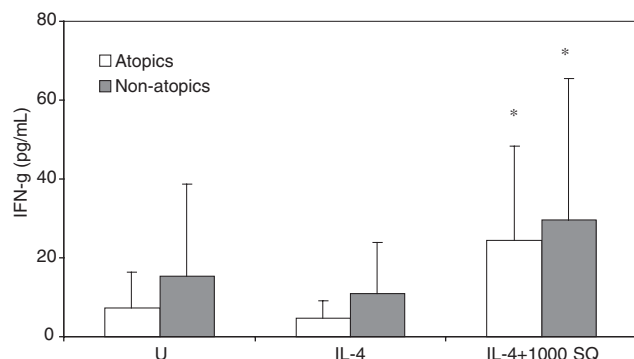


Fig. 4. IFN- γ release in atopic and non-atopic individuals. PBMCs were cultured unstimulated (U), with IL-4 or with IL-4 and 1000 SQ birch pollen extract for 72 h, and IFN- γ levels in supernatants were measured by ELISA. Columns represent the means $\pm$ SEM of duplicate cultures. * $P < 0.05$ in statistical analysis of within-group changes by the Wilcoxon test.

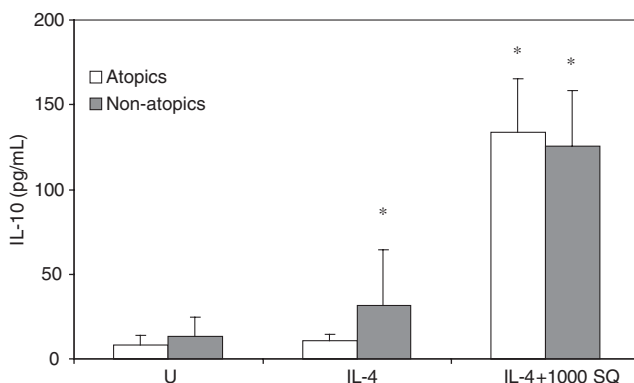


Fig. 5. IL-10 release in atopic and non-atopic individuals. PBMCs were cultured unstimulated (U), with IL-4 or with IL-4 and 1000 SQ birch pollen extract for 72 h, and IL-10 levels in supernatants were measured by ELISA. Columns represent the means $\pm$ SEM of duplicate cultures. * $P < 0.05$ in statistical analysis of within-group changes by the Wilcoxon test.

significant induction of IL-10 by IL-4 was observed in the non-bp-allergic group (Fig. 5).

The stimulation of PBMCs with IL-4 in the presence of 1000 SQ/mL BPA induced a significant increase of IL-10 production in both groups (Fig. 5). However, there was no statistical difference of IL-10 secretion between the groups.

Anti-IFN- γ diminishes BPA-dependent down-regulation of CD23 expression on B cells

In order to investigate possible mechanisms of the allergen-induced decrease of CD23 expression, we added an neutralizing anti-IFN- γ -mAb to the IL-4- and allergen-stimulated PBMC cultures. The data show that the addition of 5 μ g/mL anti-IFN- γ -mAb led to an increased expression of CD23 (Fig. 6), but this effect of anti-IFN- γ was only detectable in B cells. By contrast, the anti-IFN- γ -mAb had no effect on CD23 expression of monocytes (data not shown). However, in two of the five patients no increase but even a further decrease of CD23 expression was observed in the presence of anti-IFN- γ -mAb.

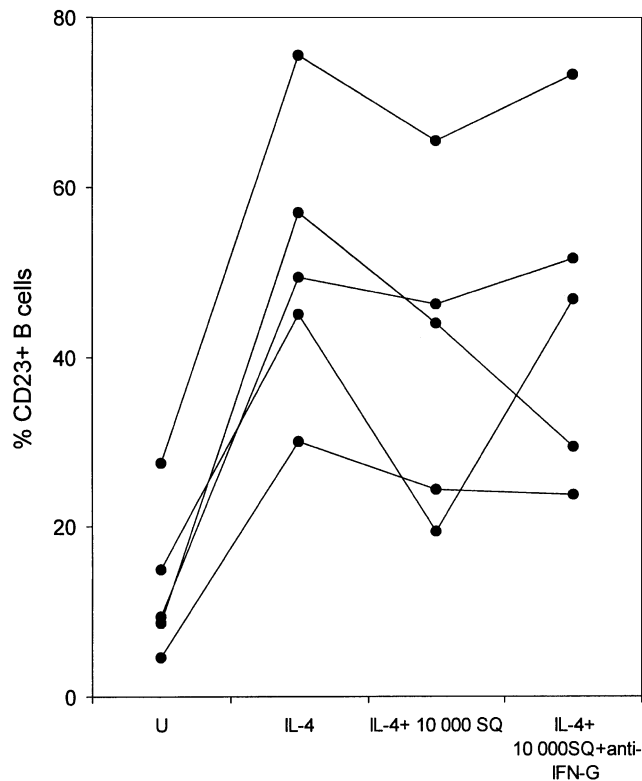


Fig. 6. Percentage of CD23-positive B cells in atopic individuals. Effect of anti-IFN- γ on IL-4 and birch pollen extract-stimulated cells. U: unstimulated cells. The dots represent the level of a single individual.

Anti-IL-10 increases allergen-reduced CD23 expression on monocytes

The influence of IL-10 on the observed BPA-induced reduction of surface CD23 was investigated using neutralizing anti-IL-10-mAb. On monocytes from bp-allergic individuals, addition of 5 μ g/mL anti-IL-10-mAb led to increased CD23 expression (Fig. 7) and diminished the effect of BPA. However, the presence of anti-IL-10-mAb did not alter the CD23 expression on B cells (data not shown).

Discussion

In the present study we analysed in detail the mechanisms of allergen-induced CD23 down-regulation, which occurs during SIT, by using an appropriate *in vitro* model. As detected previously after *in vivo* application of allergen, we show that CD23 expression is down-regulated in the presence of allergen *in vitro* and not only on B cells but also on monocytes. Surprisingly, the effect of BPA did not depend on the clinical status of the analysed individuals either regarding CD23 expression on monocytes and B cells, or on sCD23 release or secretion of IFN- γ or IL-10. Finally we demonstrate that down-regulation of CD23 by allergen is mediated by IL-10 and partly by IFN- γ .

CD23 expression occurs in both monocytes and B cells and has been shown to be induced by IL-4 [1]. This induction of CD23 on monocytes or B cells does not depend on the

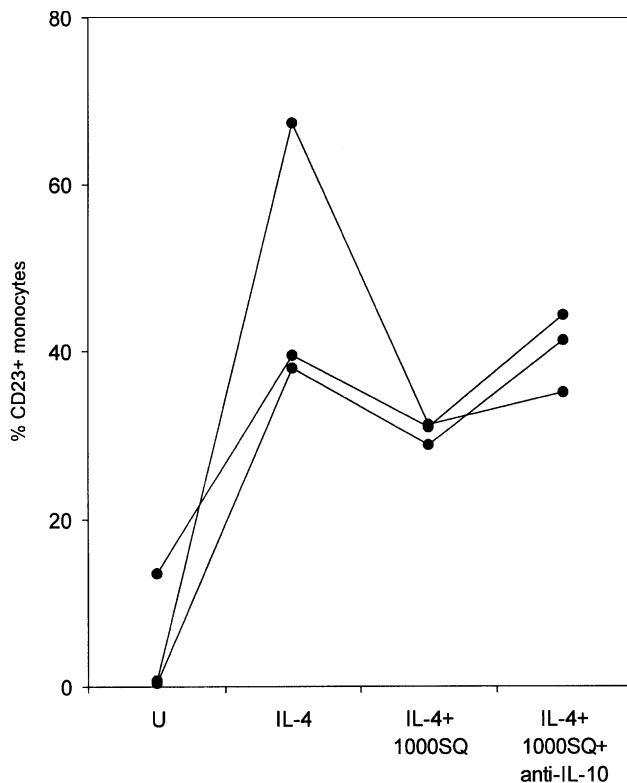


Fig. 7. Percentage of CD23-positive monocytes in atopic individuals. Effect of anti-IL-10 on IL-4 and birch pollen extract-stimulated cells. U: unstimulated cells. The dots represent the level of a single individual.

sensitization status of an individual. The CD23 promoter contains several STAT-6 binding sites [24, 25], which are responsible for CD23 induction by IL-4. IL-10, on the other hand, promotes its signals via STAT3 [26] and IFN- γ activates mainly STAT1 [27].

The difference between the effects of these inhibitory acting cytokines may be a result of different cytokine receptor expression as seen in Th1 vs. Th2 cells [28, 29]. Both cell types are known to express IL-10-R and IFN- γ -R; however, whether differing expression of receptor chains [30] or any differences regarding receptor density or responsiveness exist is not known.

In previous studies, differences of baseline CD23 expression in atopics vs. non-atopics have been described. This observation seems to depend on the kind of atopic disease (rhinitis, asthma or atopic dermatitis) and may be related to the state of systemic inflammation, but seems however not to be apparent when cells were cultured *in vitro*. After culturing PBMCs for 3 days, no significant difference between allergic and non-sensitized individuals is detectable regarding CD23 expression and sCD23 levels. Although we have not examined sCD23 release and CD23 expression directly after isolation in these patients, we could also in earlier studies detect no significant alterations of CD23 expression between wasp-allergic patients (non-atopic individuals) and birch pollen, respectively grass pollen-allergic rhinoconjunctivitis patients. By contrast, increased CD23 expression by B cells was observed after direct *ex vivo* analysis in patients suffering from atopic dermatitis [31]. Paterson et al. [32] also did not

find differences of surface expression and sCD23 release after 2 days of unstimulated culture, although they describe a larger increase in allergics than in non-sensitized individuals after stimulation with IL-4 and IL-4/CD40. Controversial data have been published by Corominas et al. [33] with no differences in CD23 or sCD23, but differing results in an earlier publication [34].

IFN- γ has been shown to regulate CD23 expression. IFN- γ inhibits IL-4-induced CD23 mRNA and sCD23 production on human B cells [2, 33, 35]. There is controversial data concerning the influence of IFN- γ on monocytes. Alderson et al. [36] described an enhancing effect of IFN- γ in combination with granulocyte macrophage-colony stimulating factor on membrane and soluble CD23, but there is also evidence of inhibitory effects [2, 35, 37]. In this study we demonstrated an inhibiting effect of IFN- γ on CD23 expression on B cells and no effect on monocytes when PBMCs were used.

In recent studies, increasing evidence of an important role of IL-10 for the efficacy of immunotherapy has been suggested. IL-10 is an anti-inflammatory cytokine. For example, it has inhibitory effects on the proliferation and cytokine response of T-lymphocytes [38]. It is produced by human TH1 as well as TH2 cells [38], monocytes and B cells [39]. IL-10 inhibits antigen-presentation of monocytes via down-regulation of HLA-II [40], ICAM-1 and B7 [41]. There seems to be a negative feedback control between IL-10 and CD23. Ligation of CD23 induces IL-10 production by monocytes [42], but CD23 expression on monocytes is downregulated by IL-10 [43, 44]. The regulation of B cell CD23 expression by IL-10 is controversial. Morinobu et al. [43] did not find a significant reduction of IL-4-induced CD23 on B cells after the addition of IL-10, which in contrast they show on monocytes. However, Ruzek et al. [45] found a partial decrease of CD23 surface expression on splenic B cells after the addition of IL-10. This is in accordance with our data, showing that IL-10 has a negative effect on CD23 expression on monocytes whereas we did not find an effect of IL-10 on CD23 expression on B cells.

It has been recently shown that IL-10-deficient mice elicit a much higher IgE production, underlining the observation that IL-10 plays an important role in the regulation of IgE [46]. It was demonstrated that IL-10-deficient mice have a higher CD4/CD8 ratio and thus IL-10 augments generation of CD8⁺ cells, which have the potential to suppress IgE production. Whether the increase of IL-10 as determined in the present study also influences the CD4/CD8 ratio has to be clarified in future studies.

The most interesting observation of the present study was that the observed effects by BPA were not restricted to the bp-allergic group. This indicates that BPA is a potent antigen with strong immunological effects regarding the induction of IFN- γ and IL-10 production, as several studies have suggested previously. Whether these effects are mediated through the activation of T cells or may also involve direct activation of monocytes or B cells through antigenic uptake or through IgG binding and consecutive Fc- γ -R activation is currently under investigation. Although we measured a small LPS contamination in the BPA extract used, we could rule out any functional relevance indicated by data from proliferation, denaturation of protein and neutralizing experiments.

Taken together, allergen-induced immunological changes are strongly dose-dependent and at least the IL-10 and IFN- γ cytokine response and in turn CD23 expression, which is observed during SIT, seem not to depend on the sensitization state of an individual. Whether this has any consequences for the therapeutical approach for allergic diseases will be determined in further studies.

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