

Inhibition of allergen-IgE binding to B cells by IgG antibodies after grass pollen immunotherapy

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Background: Among atopic individuals, levels of allergen-specific IgG antibodies have been inversely associated with the degree of allergen sensitization. Additionally, allergen-specific IgG antibodies are markedly increased by allergen injection immunotherapy. These observations have led to proposals that allergen-specific IgG antibodies might have protective properties in atopic individuals.

Objective: We hypothesized that after grass pollen immunotherapy, these antibodies disrupt IgE-dependent allergen processing by antigen-presenting cells.

Methods: We have developed a novel flow cytometric assay based on detection of allergen-IgE binding to the low-affinity IgE receptor on B cells to examine the blocking effects of sera collected from 18 patients who participated in a double-blind, controlled trial of grass pollen immunotherapy for 1 year.

Results: In all 10 patients who received active therapy, there was induction of activity that inhibited allergen-IgE binding to B cells ($P = .02$, vs placebo subjects), as well as subsequent allergen presentation to T cells. This activity copurified with IgG and was allergen specific, because sera taken from patients treated with grass pollen immunotherapy but who were also birch pollen sensitive did not inhibit IgE-birch pollen allergen binding to B cells. **Conclusion:** We conclude that allergen-specific IgG antibodies induced by immunotherapy can disrupt formation of allergen-IgE complexes that bind to antigen-presenting cells and facilitate allergen presentation. (*J Allergy Clin Immunol* 2003;112:915-22.)

Key words: Antigen presentation, IgG, CD23, immunotherapy, B cell

Atopic disease is characterized by inappropriate production of allergen-specific IgE, which is dependent on the cytokines IL-4 and IL-13, thought to be produced primarily by allergen-specific T_H2 cells. T-cell activation is, in turn, dependent on processing and presentation of aeroallergens by antigen-presenting cells (APCs), including B cells, dendritic cells, and monocytes/macrophages.

Abbreviations used:

APC:	Antigen-presenting cell
CD:	Cluster of differentiation
EBV:	Epstein-Barr virus
FcεRI:	High-affinity IgE receptor
FcεRII:	Low-affinity IgE receptor
FITC:	Fluorescein isothiocyanate
HLA:	Human leukocyte antigen
IT:	Immunotherapy
mAb:	Monoclonal antibody
Phl p:	<i>Phleum pratense</i>
RAST:	Radioallergosorbent test
SU:	Standard quality unit(s)

Positive feedback exists within the allergic response at many levels. For example, IL-4 produced by activated mast cells,¹ basophils,² T_H2 cells,³ and eosinophils⁴ can induce differentiation and expansion of T_H2 cells from uncommitted precursors.⁵ Additionally, allergen-specific IgE bound to aeroallergens can be taken up and presented to T cells via low-affinity IgE receptors (cluster of differentiation [CD] 23, FcεRII) expressed on B cells⁶ and other APCs or via high-affinity IgE receptors (FcεRI) expressed on monocytes⁷ and dendritic cells.⁸ As a result of these latter mechanisms, the threshold levels of aeroallergen exposure required to trigger T-cell activation in vivo might be lowered.

Immunotherapy (IT) is an effective treatment for atopic disease, in particular for severe, seasonal, allergic rhinitis.⁹⁻¹¹ Among the immunologic changes described in association with IT are reductions in mucosal recruitment of inflammatory cells,¹² reduced basophil reactivity to allergen,¹³ moderate reductions in IgE,¹⁴ deviation of T_H2 responses to allergen in favor of T_H1 cytokines,¹⁵⁻¹⁸ induction of IL-10-producing regulatory T cells,^{19,20} and increases in allergen-specific IgG antibodies, particularly of the IgG₄ isotype.^{17,21} The possible role of allergen-specific IgG as a “blocking antibody” in IT and protection from atopic disease in general have been the subjects of much attention. In an observational study, reduced reactivity to cat allergens was observed in children in whom there was concomitant natural exposure to high levels of cat allergen and IgG production (including IgG₄).²² Furthermore, in cross-sectional studies based on small numbers of patients who had received IT, IgG was reported to

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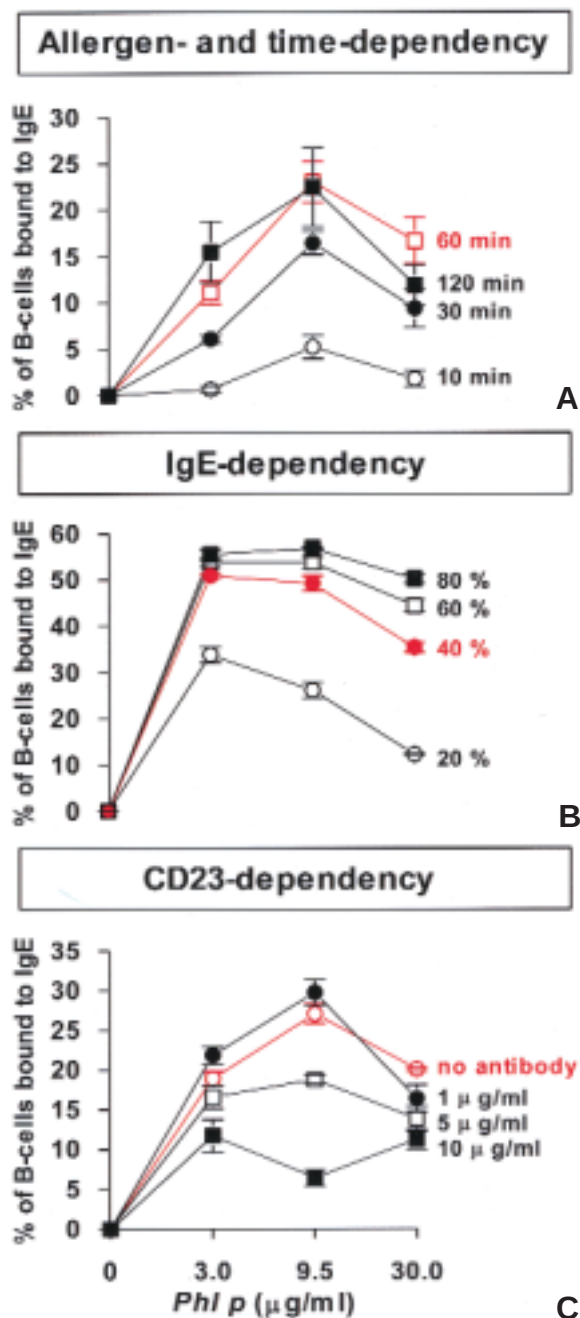


FIG 1. Characterization of allergen-IgE binding to EBV-transformed B cells. Binding is allergen dose and time dependent (the length of incubation of B cells with allergen-IgE complexes is given in minutes) (A), dependent on the concentration of IgE-containing serum (80% is equivalent to 630 SU/mL Phl p-specific IgE; 60% = 476 SU/mL; 40% = 316 SU/mL; 20% = 158 SU/mL) (B) and mediated via CD23 (concentrations of anti-CD23 antibody are given in μg/mL; C).

inhibit basophil histamine release and IgE-facilitated presentation of allergen by B cells to T-cell clones.²³ Although induction of allergen-specific IgG is a usual feature of successful IT, it has been claimed that the levels of IgG induction do not always correlate with clinical

protection, especially against bee and wasp stings.^{24,25} Nevertheless, serum concentrations of allergen-specific IgG might represent too crude a measure of activity.²⁶

We hypothesized that allergen-specific IgG does indeed have blocking activity and that manifestation of this as inhibition of IgE-facilitated allergen presentation is a key effector mechanism in conventional IT. We developed a novel flow cytometric assay, which measures interdependent binding of allergen and IgE to CD23 on B cells. In a placebo-controlled trial, we tested the ability of serum obtained before and after IT to inhibit this IgE-facilitated allergen binding to B-cells.

METHODS

Study subjects

Forty patients with a history of severe, seasonal, allergic rhinitis that was not controlled by antiallergic drugs and a positive skin-test reaction (weal > 5 mm) to *Phleum pratense* (Phl p; Aquagen, ALK-Abelló, Hørsholm, Denmark) were randomized to treatment with IT or placebo injections for 1 year. From a randomly selected subgroup of 10 patients (7 men, 3 women; mean age, 36 years; range, 18 to 58) on active treatment and 8 patients (2 men, 6 women; mean age, 38 years; range, 26 to 51) on placebo treatment, serum samples were taken before treatment and after 1 year of treatment during the peak pollen season. The groups were matched for size of the immediate skin response to intradermal allergen injections (Phl p, 10 BU in 0.03 mL) with a mean weal diameter IT group and 22.7 mm (range, 10.3 to 40.0 mm) in the placebo group. Phl p-specific IgG was measured in serum samples by using an automated fluoroenzyme assay (uniCAP 100, Pharmacia Diagnostics, Uppsala, Sweden). Phl p-specific IgE was measured with the Magic Lite Assay (ALK-Abelló).²⁷

Grass pollen IT

The study was approved by the Ethics Committee of the Royal Brompton Hospital, and all subjects gave written, informed consent. The details of the IT protocol have been published elsewhere.²⁸ After a 6-week up dosing phase, patients received 100,000 standard quality units (SU) of Phl p (Alutard SQ, ALK-Abelló) containing 20 μg Phl p 5 monthly for 12 months.

Antibodies and reagents

Fluorescein isothiocyanate (FITC)-labeled anti-human CD14 mAb, CD19 mAb, CD20 mAb, CD23 mAb, CD25 mAb, CD3 mAb, CD40 mAb, CD80 mAb, CD86 mAb, and human leukocyte antigen (HLA)-DR mAb were obtained from R&D Systems (Abingdon, Oxon, United Kingdom). FITC-labeled anti-human IgE polyclonal antibody was obtained from Dako Ltd (Ely, Cambridgeshire, United Kingdom). FITC-labeled anti-human IgG were obtained from Cambridge Bioscience (Cambridge, United Kingdom). Whole grass pollen allergen (Phl p) biotinylated or unbiotinylated, was a kind gift from ALK-Abelló.

Measurement of allergen-IgE binding to EBV-transformed B cells and enhancement by IgE-containing sera

Epstein-Barr virus (EBV)-transformed B cells obtained from a grass-pollen-allergic subject (CD14⁻, CD19⁺, CD20⁺, CD23⁺, CD25⁻, CD3⁻, CD40⁺, CD80⁺, CD86⁺, and HLA-DR⁺) were enriched for CD23⁺ cells with anti-CD23 mAb (BD Biosciences, Oxford, United Kingdom) in conjunction with anti-IgG-coated magnetic beads (CELLlection Pan Mouse IgG kit, Dynal, Oslo, Norway). Serum from an atopic grass-pollen-allergic patient containing

high concentrations of IgE (Phl p-specific IgE, 789 SU/mL) was incubated alone or together with equal concentrations of sera from patients undergoing IT or placebo treatment for 1 hour at 37°C with serial dilutions of Phl p allergen extract (as optimized in previous experiments; Fig 1, A and B). Reproducibility of the assay was assessed by using high-IgE-containing sera from a panel of 3 separate atopic donors. In additional experiments, the atopic serum was preincubated with allergen extract for 1 hour at 37°C before sera from IT or placebo patients were added. Subsequently, EBV-transformed B cells were added at 2×10^5 /sample and incubated for another 1 hour at 4°C (as optimized by previous experiments; Fig 1, A). After incubation with sera/allergen, B cells were washed twice with PBS containing 1% bovine serum albumin (Sigma, Poole, United Kingdom) and stained on ice for 45 minutes with a polyclonal FITC-labeled anti-IgE antibody (Dako Ltd) diluted in PBS. Further experiments were performed with a mouse anti-human IgE mAb (a kind gift from ALK Abelló). Cells were then washed again, and binding of anti-IgE antibody was measured with a FACScan flow cytometer (BD Biosciences). In blocking experiments with anti-CD23 mAb, EBV-transformed B cells were incubated for 1 hour at 37°C with this antibody before being added to the IgE-containing serum preincubated with serial dilutions of allergen. For each sample, a minimum of 5000 cells were analyzed.

Detection of allergen-antibody binding to EBV B cells with biotinylated allergen

In a separate experiment, serum from an atopic, grass-pollen-allergic patient (Phl p-specific IgE, 789 SU/mL) was incubated alone or together with sera from 6 IT-treated patients for 1 hour at 37°C with 3 µg/mL biotinylated Phl p allergen extract (a kind gift from ALK-Abelló). Subsequently, EBV B cells were added. After being washed, cells were stained with streptavidin-phycoerythrin (BD Biosciences) and FITC-labeled anti-IgE antibody (Dako Ltd) and measured by a FACScan flow cytometer as before. A minimum of 5000 cells were analyzed.

IgG fractionation of sera

Sera from patients who had received grass pollen IT were fractionated over a high-affinity protein G column (HiTrap Protein G HP, Amersham Pharmacia Biotech, UK). In brief, sera were centrifuged for 10 minutes at 2000 *g*, filtered through a 0.45 µm filter, and diluted in an equal volume of binding buffer (0.02 mol/L sodium phosphate, pH 7.0). The column was washed with distilled water and equilibrated with binding buffer before the diluted serum was added. The run-through fluid was collected, and IgG bound to the column was eluted with the elution buffer (0.1 mol/L glycine-HCl, pH 2.7). All fractions were neutralized by addition of 1 mol/L Tris-HCl, pH 0.9, extensively dialyzed (Slide-A-Lyzer, Pierce, Rockford, Ill) against PBS, and concentrated (Slide-A-Lyzer Concentration Solution, Pierce) to the starting volume. Serum IgG was measured by immunoturbidimetry with a Beckman LX20 Analyzer (Beckman Coulter, Fullerton, Calif).

T-cell proliferation assays and IgE-facilitated antigen presentation

T_H2 clones (AGC29, AGC32) specific for the major grass pollen allergen Phl p 5 and autologous EBV-transformed B cells used as APCs in proliferation assays were kindly supplied by Dr Peter Adler Würtzen (ALK-Abelló). Serum from a grass-pollen-allergic patient containing a high concentration of IgE was incubated alone or together with sera from patients who had received grass or birch pollen IT for 1 hour at 37°C with serial dilutions of Phl p allergen extract. Subsequently, EBV-transformed B cells (irradiated at 6000 rads) were added at 3×10^6 /mL and incubated

for 1 hour at 37°C. B cells were then washed 3 times with RPMI-1640 (Life Technologies, Paisley, United Kingdom). To measure the antigen-presenting ability of these B cells, they were added at 1×10^4 /well to the Phl p 5-specific T_H2 T-cell clones (at 2×10^4 /well) and cultured in RPMI-1640 medium supplemented with 2 mmol/L L-glutamine (Life Technologies), 100 IU/mL penicillin/streptomycin (Life Technologies), and 5% human AB serum (Sigma) for 48 hours. Proliferation was measured by adding 1 µCi of tritiated methyl thymidine (Amersham, Aylesbury, United Kingdom) for the final 12 hours of culture, followed by liquid scintillation counting. Results were expressed as the stimulation index, representing the ratio of counts per minute of allergen-stimulated T cells divided by the counts per minute of T cells cultured with medium alone (negative control).

Statistical analysis

Within-group comparisons were performed with the Wilcoxon matched-pairs signed-ranks test. Between-group comparisons were performed with the Mann-Whitney *U* test (Minitab Inc, City, Pa). *P* values <0.05 were considered statistically significant.

RESULTS

Allergen-IgE binding to B cells: effects of allergen concentration, incubation time, CD23 blockade, and serum dilution

Allergen-dependent binding of IgE to the surfaces of EBV-transformed B cells was detected as early as 10 minutes after incubation with atopic IgE-containing serum (Phl p-specific IgE, 789 SU/mL) that had been preincubated with serial dilutions of grass pollen allergen (Phl p; Fig 1, A). The binding of serum-IgE was clearly dependent on the dilution of IgE-containing serum (Fig 1, B) and concentration of the allergen. Optimal binding was detectable at a relatively low allergen concentration (3.0 µg/mL Phl p) after 60 minutes (Fig 1, A). Binding decreased with longer incubation periods and at higher allergen concentrations (30 µg/mL Phl p). Prior incubation of B cells with CD23-blocking antibodies resulted in a dose-dependent decrease of allergen-IgE binding (Fig 1, C). On the basis of these experiments, we used an incubation period of 60 minutes and 40% serum containing Phl p-specific IgE (316 SU/mL).

Sera from patients undergoing grass pollen IT disrupt allergen-IgE binding to B cells

After 1 year of IT, the actively treated group showed significantly lower symptom and medication scores compared with the placebo-treated group (Fig 2, A). Specific subcutaneous grass pollen IT for 1 year was associated with a significant increase in specific IgG antibodies (*P* = .0004; Fig 2, B) in serum. Sera were tested for their ability to block allergen-IgE binding with 3.0 µg/mL Phl p (Fig 2, C and D). In patients who had received 1 year of IT, sera collected during the pollen season significantly inhibited allergen-IgE binding to B cells when compared with sera from placebo-treated patients (*P* = .02; Fig 2, C and D). Blocking activity was observed even when post-IT sera were added after prior mixing of allergen and IgE for 1 hour at 37°C to form allergen-IgE complexes.

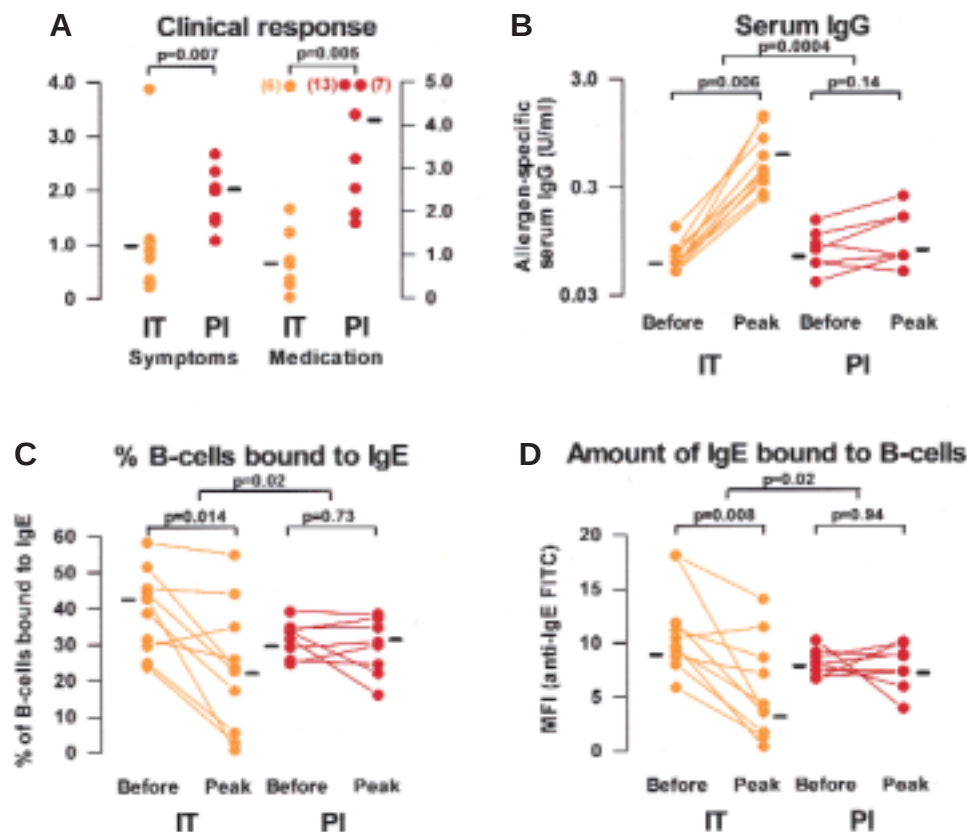


FIG 2. Grass pollen injection IT: symptom and medication scores (area under curve for whole pollen season expressed as arbitrary units $\times 10^3$) (A), concentrations of allergen-specific serum-IgG (arbitrary units U/mL) (B), and allergen-IgE binding to B cells in the presence of sera before (*Before*) and after 1-year treatment during peak pollen season (*Peak*) in patients treated with IT ($n = 10$, yellow circles, IT) or placebo ($n = 8$, red circles, PI). Results are expressed as percentage of B cells with detectable IgE on their surfaces (C) and as amount of binding of IgE to B cells (MFI, mean fluorescence intensity) (D).

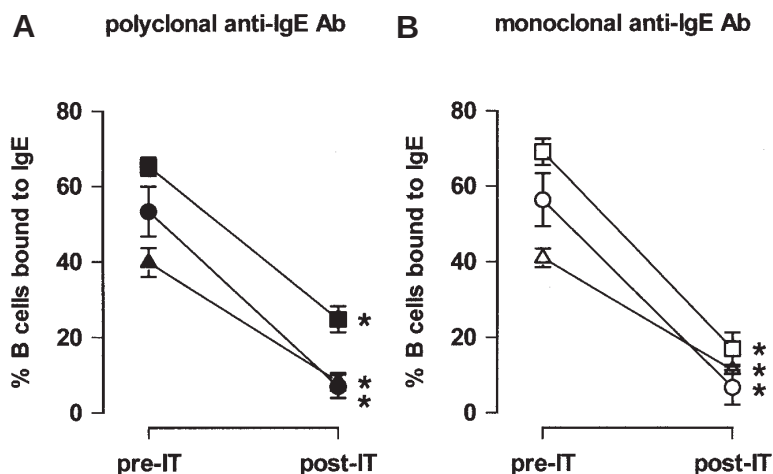


FIG 3. Detection of inhibitory activity of allergen-IgE binding to B cells by sera from IT patients with a polyclonal anti-IgE antibody is specific and reproducible with different IgE-containing sera. Three different IgE-containing sera (closed square, closed circle, and closed triangle) were incubated with 3 μ g/mL Phl p and sera from 5 patients treated with grass pollen IT. IgE-allergen complexes bound to B cells were detected with fluorescently labeled anti-IgE antibody (A) or biotinylated anti-IgE mAb followed by streptavidin-phycoerythrin staining (B). Data shown are mean values (SE mean). P values represent statistical differences of treatment effect within groups, as determined by a Student paired t test. (* $P < .01$). Data are representative of a minimum of 3 separate experiments.

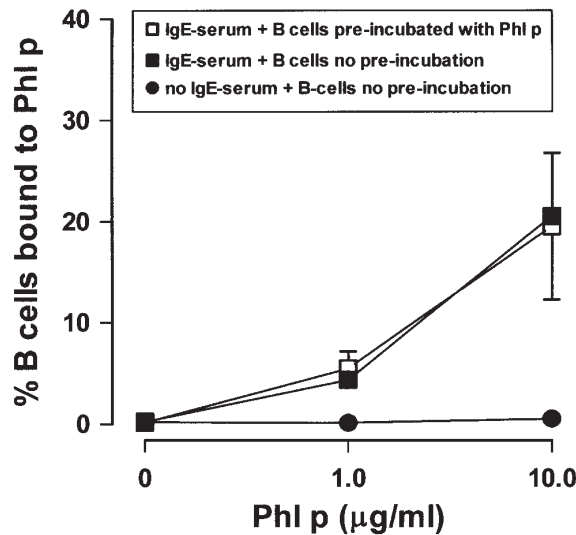


FIG 4. Binding of allergen to B cells is dependent on the presence of IgE-containing serum. Biotinylated allergen was added either alone (closed circles) or after preincubation with IgE-containing serum to untreated B cells (closed squares) or B-cells that had been pretreated for 1 hour with 100 μg/mL Phl p (open squares). Binding of allergen was measured with streptavidin-phycoerythrin and detected by flow cytometry. Data shown are mean values (SE Mean) of 3 separate experiments.

We validated the assay by using high-IgE-containing sera from 3 separate atopic individuals and confirmed virtually identical inhibitory activity in sera from 5 IT-treated patients (Fig 3, A). Moreover, detection of IgE with the polyclonal anti-IgE antibody was specific, because similar binding was confirmed with an anti-IgE mAb (Fig 3, B).

To assess whether allergen can bind directly to B cells, we used a biotinylated allergen extract and measured binding in the absence of IgE-containing serum (Fig 4). Binding of allergen at different concentrations was undetectable in the absence of IgE-containing serum. Moreover, preincubation of B cells with high concentrations of allergen did not influence subsequent binding of allergen-IgE complexes (Fig 4).

IgE binding corresponds to allergen binding to B cells

Detection of allergen-IgE binding was comparable when detecting binding of biotinylated allergen extract or IgE binding with a fluorescently labeled anti-IgE antibody. For example, in the presence of IgE-containing sera from atopic subjects, $28.5\% \pm 12.3\%$ (mean $\pm$ SD) of B cells bound to allergen corresponded to $23.7\% \pm 11.3\%$ of B cells with IgE on their surfaces. Moreover, in the presence of sera from 6 IT-treated patients, binding of allergen was inhibited by $76.5\% \pm 7.2\%$, similar to $77.8\% \pm 14.2\%$ inhibition of IgE binding to B cells.

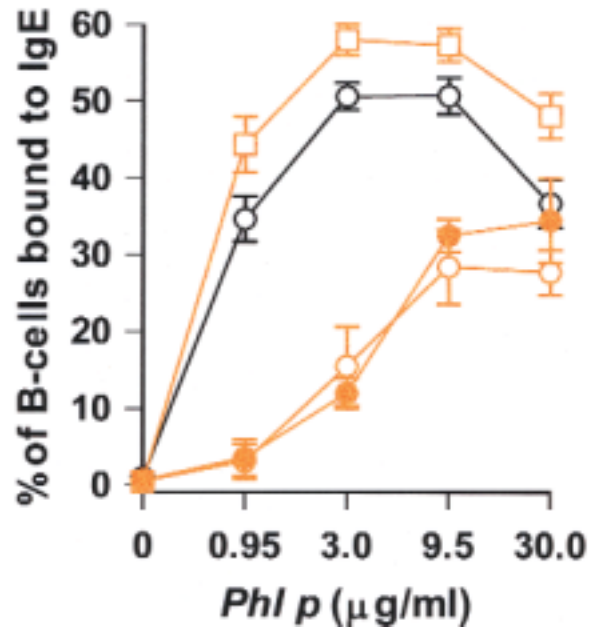


FIG 5. Inhibition of allergen-IgE binding to B cells by sera from IT patients: copurification of the inhibitory factor with serum IgG. IgE-containing serum (open black circles) and variable concentrations of grass pollen allergen (Phl p) were preincubated with whole serum (closed yellow circles), IgG-enriched fractions (open yellow circles), or IgG-depleted fractions (open yellow squares) of sera from patients treated with grass pollen IT. Data shown are median values from the sera of 5 patients.

Blocking of allergen-IgE binding to B cells by grass pollen IT sera is mediated through IgG

IgG antibodies were depleted from the sera of 5 IT-treated patients. Depletion of IgG resulted in loss of blocking activity, whereas the IgG-containing fractions exhibited a blocking activity similar to that of undepleted sera (Fig 5). To determine whether inhibition of allergen-IgE binding by IT sera was associated with an increase in IgG binding to B cells, we used an anti-IgG mAb to detect binding of allergen-IgG complexes in the presence of IgE-containing serum alone or together with sera from 6 IT-treated patients. In the presence of IgE-containing sera alone, $12.63\% \pm 4.87\%$ (mean $\pm$ SD) of B cells were bound to IgG. Addition of sera from IT-treated patients to the assay resulted in a small ($18.8\% \pm 8.98\%$) but nonsignificant ($P = 0.183$, 2-sample t test) increase in IgG binding to B cells.

Allergen-IgE binding to B cells is associated with enhanced allergen presentation to T cells, which is inhibited by sera from grass pollen IT patients

To investigate whether allergen-IgE binding to B cells translates into antigen presentation and T-cell activation, B cells preincubated with allergen, IgE-containing serum, and sera from IT patients were tested for their capacity to stimulate proliferation of an autologous Phl p

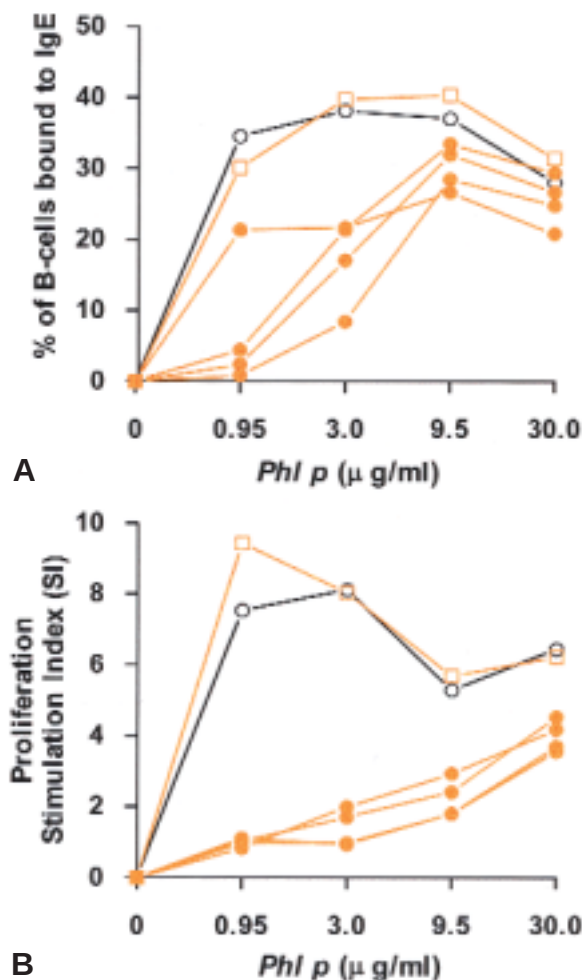


FIG 6. Allergen-IgE binding to B cells is associated with enhanced antigen presentation to allergen-specific T cells, which is inhibited by sera from IT patients: Allergen-dependent binding of IgE from IgE-containing serum in the absence (open black circles) or presence (closed yellow circles) of sera from 4 patients treated for 1 year with grass pollen IT and serum from 1 patient treated with birch pollen IT (open yellow squares). IgE-allergen complexes bound to B cells were detected with fluorescently labeled anti-IgE antibody by flow cytometry (A), or EBV-transformed B cells were irradiated and used as APCs for an autologous grass-pollen-specific T-cell clone and proliferation was measured after 24 hours by ^3H -thymidine incorporation (B). Data shown are representative of a minimum of 3 separate experiments.

5-specific $\text{T}_\text{H}2$ cell clone (AGC29). Preincubation of B cells with IgE-containing serum did indeed potentiate allergen-induced T-cell proliferation, and this was inhibited by sera from IT patients (Fig 6, A and B). Moreover, there was a correlation between the degree of allergen-IgE binding to the surface of B cells and the capacity of B cells to stimulate T-cell proliferation $r = .91$, $P = .02$). Similar results were obtained with another Phl p 5-specific $\text{T}_\text{H}2$ cell clone (AGC32; data not shown).

Inhibitory effects of sera on allergen-IgE binding to B cells are specific for the allergen used for IT

Five patients were identified who had received grass pollen IT but who were also sensitized to birch pollen allergen (weal >5 mm on skin testing with *Betula verrucosa*). Sera from these patients inhibited B-cell binding with grass pollen allergen preincubated with IgE-containing serum from a grass-pollen-allergic donor by 84% (data not shown), but not birch pollen allergen-IgE binding after preincubation with serum from a birch-pollen-allergic donor. This confirms that induction of this inhibitory activity is allergen specific, with no detectable “bystander” inhibition of IgE-facilitated presentation of an unrelated allergen to which the patients were cosensitized.

DISCUSSION

In this study, we show that binding of IgE antibodies to CD23 on the surfaces of B cells is dramatically enhanced by the presence of low allergen concentrations, consistent with formation of “polymeric” allergen-IgE complexes that bind CD23 with greater avidity than “monomeric” IgE. This allergen-IgE binding to B cells was associated with enhanced stimulation of allergen-specific T-cell clones, suggesting that binding of complexes translates functionally into enhanced antigen processing and presentation. Thus, our assay provides a valuable alternative for the investigation of IgE-facilitated allergen presentation, without the need for expensive and time-consuming T-cell cultures. IT but not placebo treatment was associated with induction of activity that inhibited allergen-IgE binding to B cells. This inhibition was due to allergen-specific IgG antibodies, because serum levels of these antibodies increased after treatment and the inhibitory activity copurified with IgG. The failure of this activity to inhibit birch pollen allergen-IgE binding to B cells confirmed that this response to IT was allergen specific.

Inhibition of allergen-IgE binding by IgG in our system was optimal at low allergen concentrations. It is well established that there is an optimal ratio of antibody to allergen for formation of large complexes.²⁹ At high allergen concentrations, formation of complexes is reduced because of the total number of binding sites available on antibodies: only 1 or a limited number of sites are occupied by a single allergen. In contrast, at low allergen concentrations, multiple antibodies are likely to become cross-linked by binding to a single allergen moiety. In this study, we measured total allergen-specific IgG against whole grass pollen extract to assess the global antibody response to the complete allergen as given during IT. Future studies should address the contribution of individual IgG subclasses and antibody responses to the major allergens in the extract. Although IgG antibodies are clearly involved in blocking allergen-IgE binding, they do not mediate this inhibition by binding directly to B cells, because an increase in IgG binding could not be detected on B cells in the same assay.

Induction of allergen-specific IgG antibodies is a usual feature of successful IT,^{21,30-32} in contrast to variable and modest reductions in IgE antibodies. Some investigators have reported that allergen-specific IgG₄ induced by IT can block allergen-induced, IgE-dependent histamine release by basophils,^{33,34} whereas others have suggested that IgE-allergen binding in sera of IT patients is unaltered.³⁵ Furthermore, induced IgG levels did not correlate with clinical protection.^{24,25} Other reports suggest that IT might also change antibody specificity.^{26,33,34,36} In a previous study, increases in IgG antibodies after successful grass pollen IT were accompanied by inhibition of histamine release by basophils.³⁴ This suggests that IT might induce IgG antibodies with the same specificity as IgE antibodies, resulting in direct competition for the same epitopes. Moreover, results from venom IT suggest that the change in specificity can occur soon after the start of treatment and long before a change in antibody titers could be detected.²⁶ These data complement findings from murine models of IT, where administration of high concentrations of allergen has been shown to alter antibody affinity and specificity as well as quantity.³⁷ These findings are consistent with IT acting to change the character of allergen-specific IgG, as well as inducing increases in IgG concentration.

These observations stress the importance of studying the *functional activity* of allergen-specific IgG, as a blocking antibody or otherwise, as opposed to measuring crude levels in sera. To this aim, we developed a simple flow cytometric assay that measured allergen-mediated binding of IgE to EBV-transformed B cells by using a fluorescently labeled anti-IgE antibody. We were able to show that this mechanism was mediated via CD23, dependent on allergen-specific IgE, efficient at low allergen concentrations, and detectable after only short (60 minutes) incubation. Some studies have reported raised levels of anti-IgE autoantibodies in sera from atopic subjects.^{38,39} Although binding of such antibodies would also be detectable with the polyclonal, FITC-coupled, anti-IgE antibody used in our assay, the allergen dependency of the system (ie, no IgE binding was detectable in the absence of allergen) confirms that the majority of IgE bound to B cells is complexed with allergen. Therefore, although anti-IgE autoantibodies might be present in the sera of grass-pollen-atopic patients, they do not bind in detectable amounts to B cells in our assay system. Moreover, we confirmed that binding of allergen-IgE to B cells was directly correlated with the antigen-presenting capacity of these B cells to T cells. Using this assay, we showed that allergen-specific IgG induced by grass pollen IT was able to displace IgE from allergen-IgE complexes, thereby blocking allergen-IgE binding to B cells, while itself not binding to B cells. The latter finding is in agreement with the observation that both monomeric IgG and complexes of house dust mite allergen with IgG fail to bind to the FcR for IgG on B cells (CD32).⁴⁰ Therefore, binding of allergen-IgG to B cells does not seem to play a role in allergen presentation. On the other hand, cells that express the high-affinity IgG

receptor CD64, such as monocytes, might possibly mediate antigen presentation through this mechanism.

Despite accumulating evidence that allergen-specific IgG induced by IT might have biologic activity, a number of issues remain to be addressed. For example, what is the mechanism responsible for the formation of "blocking" IgG antibodies? Do allergen-specific IgG antibodies modulate sensitivity to allergen during the natural history of hayfever?²² Can "blocking" antibodies also inhibit FcεRI-IgE-mediated antigen presentation, eg, by dendritic cells and monocytes? In addition to changes in serum immunoglobulins, immunotherapy has been associated with modulation of T-cell responses to allergen, including reduced proliferation, a shift from a T_H2-type to a T_H1-type profile of cytokine production,^{15-18,35} and more recently, with the emergence of regulatory T cells producing IL-10.^{19,41} Whether these changes are interrelated or represent separate immunologic pathways is unknown. It is possible that IL-10 might account for the observed increases in IgG, because IL-4-induced switching to IgE and IgG₄ is inhibited and potentiated, respectively, by IL-10.^{42,43} However, IL-10 alone would not account for the allergen specificity of the inhibitory activity that we observed with the post-IT sera. An alternative explanation, as suggested by Ball et al,³⁴ is that IT induces allergen-specific de novo immune responses. In summary, we have confirmed that allergen IT induces "blocking" IgG antibodies that inhibit IgE-facilitated allergen binding by B cells and presentation to T cells. Further studies are needed to assess whether this activity might predict the clinical response to treatment.

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