

# Combination treatment with omalizumab and rush immunotherapy for ragweed-induced allergic rhinitis: Inhibition of IgE-facilitated allergen binding

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**Background:** The combination of anti-IgE (omalizumab) therapy with ragweed injection immunotherapy for seasonal allergic rhinitis results in a significant reduction in systemic side effects and enhanced efficacy compared with immunotherapy alone. One proposed mechanism of immunotherapy is to induce regulatory antibodies that inhibit facilitated antigen presentation.

**Objectives:** We sought to determine whether the combination protocol has a cumulative effect on inhibition of facilitated antigen presentation both during and after discontinuation of treatment.

**Methods:** Ragweed allergen immunotherapy with and without omalizumab therapy was tested in a 4-arm, double-blind, placebo-controlled study. Flow cytometry was used to detect serum inhibitory activity for IgE-facilitated CD23-dependent allergen binding to B cells as a surrogate marker for facilitated antigen presentation. Serum ragweed-specific IgG4 was measured by means of ELISA.

**Results:** Immunotherapy alone resulted in partial inhibition of allergen-IgE binding after 5 to 19 weeks of treatment compared with baseline ( $P < .01$ ). Complete inhibition of allergen-specific IgE binding was observed in both treatment groups receiving omalizumab ( $P < .001$ ). Allergen-specific IgG4 levels were only increased after immunotherapy ( $P < .05$ ), both in the presence and absence of anti-IgE treatment. Combined treatment resulted

in the induction of long-lasting inhibitory antibody function for up to 42 weeks compared with either treatment alone.

**Conclusion:** Ragweed immunotherapy induced serum regulatory antibodies that partially blocked binding of allergen-IgE complexes to B cells. Additional treatment with anti-IgE, by directly blocking IgE binding to CD23, completely inhibited allergen-IgE binding.

**Clinical implications:** The combination of ragweed immunotherapy and anti-IgE resulted in prolonged inhibition of allergen-IgE binding compared with either treatment alone, events that might contribute to enhanced efficacy. (J Allergy Clin Immunol 2007;120:688-95.)

**Key words:** Allergy, immunotherapy, omalizumab, anti-IgE, IgE-facilitated allergen binding, ragweed

Allergen immunotherapy is the only known antigen-specific immunomodulatory treatment for seasonal allergic rhinitis.<sup>1</sup> Immunotherapy reduces immediate allergen-induced symptoms and concentrations of inflammatory mediators in nasal lavage fluid, including histamine and prostaglandin D<sub>2</sub>.<sup>2</sup> Successful treatment is associated with blunting of seasonal increases in allergen-specific IgE levels, together with increasing levels of allergen-specific IgG, particularly IgG4.<sup>3-5</sup> Other proposed mechanisms of immunotherapy include immune deviation<sup>6</sup> and the induction of regulatory T cells producing suppressive cytokines, such as IL-10 and TGF- $\beta$ .<sup>4,7</sup>

T-cell activation is dependent on the processing and presentation of allergens by antigen-presenting cells (APCs). In allergy an additional mechanism of antigen processing exists in which allergen-specific IgE bound to allergen can be internalized and presented to T cells through low- or high-affinity IgE receptors expressed on APCs. This process occurs preferentially at low allergen concentrations and results in a 10- to 100-fold reduction in threshold levels required to trigger T-cell activation *in vivo*.<sup>8</sup> A number of studies have demonstrated that sera obtained from subjects receiving allergen immunotherapy can inhibit IgE-facilitated antigen presentation to allergen-specific clones or, more simply, inhibit binding of allergen-IgE complexes to B cells (IgE-facilitated allergen binding [FAB] assay).<sup>9-12</sup> This activity has been copurified with IgG<sup>11</sup> and, more recently, with IgG4.<sup>5</sup> Therefore, IgG4 can suppress not only allergen-induced IgE-dependent

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#### Abbreviations used

APC: Antigen-presenting cell  
FAB: Facilitated allergen binding  
FITC: Fluorescein isothiocyanate

histamine release by basophils<sup>13</sup> but also allergen-specific T-cell responses *in vitro* by inhibiting the binding of allergen-IgE complexes to APCs.<sup>14</sup>

Therapy with anti-IgE, using the humanized monoclonal anti-IgE antibody omalizumab, acts to reduce circulating levels of free IgE, inhibits early- and late-phase responses to allergen, and decreases tissue eosinophils, lymphocytes, and IL-4<sup>+</sup> cells.<sup>15-19</sup> Clinically, treatment with anti-IgE is well tolerated and can reduce the requirements for inhaled corticosteroids and protects against disease exacerbation.<sup>20-22</sup> A combination of anti-IgE therapy and allergen immunotherapy might offer advantages that neither method provides separately.<sup>23</sup> A recent double-blind, placebo-controlled trial of anti-IgE therapy with rush immunotherapy has investigated this possibility in the context of ragweed-induced allergic rhinitis.<sup>24</sup> Results demonstrate that patients receiving anti-IgE and immunotherapy had reduced seasonal severity scores and had fewer adverse events compared with those receiving immunotherapy alone.

Here we validated the IgE-FAB assay using ragweed allergen. Furthermore, we aimed to test serum samples obtained from subjects treated with a combination of anti-IgE and ragweed immunotherapy, anti-IgE or immunotherapy alone, or placebo in the IgE-FAB assay. We hypothesized that we would detect synergy between anti-IgE therapy and inhibitory IgG activity by means of ragweed immunotherapy in inhibiting allergen-IgE binding to B cells.

## METHODS

### Patients

Patients (21-51 years of age) with a minimum 2-year history of ragweed-induced allergic rhinitis and no recent immunotherapy were selected (Table I). Subject groups showed similar baseline characteristics, except for total IgE, which was lower in the omalizumab-only group compared with the immunotherapy-only group ( $P < .05$ ). Patients were required to have a positive skin prick test result to short ragweed extract (ALK-Abelló, Round Rock, Tex), as defined by a wheal 3 mm greater in diameter than that elicited by the saline control and a baseline serum IgE level of greater than 10 and less than 700 IU/mL. The protocol was reviewed and approved by the National Institute of Allergy and Infectious Diseases Allergy and Asthma Data and Safety Monitoring Board and the relevant institutional review boards. Additional serologic analyses were approved by the Royal Brompton Hospital Ethical Committee.

### Clinical study

A randomized, 4-arm, double-blind, placebo-controlled study was previously carried out and described in detail elsewhere.<sup>24</sup> Briefly,

4 groups included patients treated with immunotherapy combined with omalizumab, immunotherapy alone, omalizumab alone, and a placebo group. Omalizumab (Xolair; Novartis Pharmaceuticals Corp, East Hanover NJ; Genentech Inc, South San Francisco, Calif; Tanox, Inc, Houston, Tex) was administered during a 9-week pretreatment phase (–9 to 0 weeks) and during the 12-week period of immunotherapy (0 to 12 weeks). Immunotherapy with aqueous short ragweed extract (ALK-Abelló; Greer Laboratories, Lenoir, NC) was administered by using a rush regimen, as previously described. Serum samples were collected before omalizumab treatment (week –9), before the start of immunotherapy (week 0), and at weeks 5, 9, 13, and 19. Long-term follow-up samples were collected at study weeks 31, 43, and 55 (corresponding to weeks 18, 30, and 42 after treatment). For this study, randomly selected serum samples from the Creighton University study site were tested in a blinded manner at the National Heart and Lung Institute, Imperial College London.

### Specific antibody measurement

Total serum free IgE levels were measured by Novartis Pharmaceuticals (Basel, Switzerland) by using solid-phase ELISAs with a fluorometric technique and human serum as standard. Ragweed-specific IgG4 was measured by means of ELISA (coating allergen concentration, 5 µg/mL; 1:100 dilution of sera; pooled IgG4 from subjects receiving standard immunotherapy; detection with biotinylated anti-human IgG4 mAb [BD PharMingen, San Diego, Calif]).

### B-cell culture

The EBV-transformed B-cell line (a kind gift from ALK-Abelló, Hørsholm, Denmark)<sup>25</sup> was grown in RPMI 1640 (Invitrogen, Paisley, United Kingdom) supplemented with 10% heat-inactivated FCS (PAA laboratories, Yeovil, United Kingdom), 1% (vol/vol) L-glutamine, and 1% (vol/vol) penicillin/streptomycin (Invitrogen) at 37°C, 5% CO<sub>2</sub>, and 95% relative humidity. Cell density was maintained at less than  $0.5 \times 10^6$  cells/mL.

### IgE-FAB assay

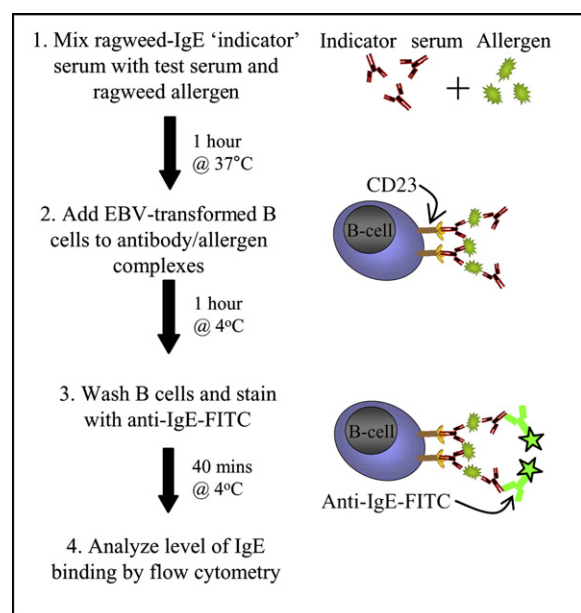
Indicator serum from a subject with ragweed pollen allergy containing high concentrations of ragweed-specific IgE (RAST >100 IU/mL; PlasmaLab, Everett, Wash) was incubated with crude short ragweed allergen extract (Greer laboratories, Lenoir, NC) for 1 hour at 37°C. EBV-transformed B cells ( $2 \times 10^5$ ) were incubated together with the allergen-IgE complexes for 1 hour at 4°C (Fig 1). Cells were washed, and allergen-IgE complexes bound to B cells were detected by means of flow cytometry (FACS Calibur; Becton Dickinson, Mountain View, Calif) by using a polyclonal human anti-IgE fluorescein isothiocyanate (FITC)-labeled antibody (DakoCytomation, Cambridge, United Kingdom). Equal volumes of indicator serum and patients' sera were incubated with 0, 0.1, and 1 µg/mL crude short ragweed allergen extract to assess the potential inhibitory activity of serum samples obtained from the clinical study or from ragweed immunotherapy donors. Results from these studies are expressed as "relative B-cell binding," where binding observed by indicator serum alone is normalized to 100% and changes in binding induced by the addition of patients' serum to the indicator is related to this value. Some experiments used biotinylated allergen to detect allergen-IgE complex binding, where B cells were stained with streptavidin-phycoerythrin (BD Biosciences) and FITC-labeled anti-IgE and analyzed as previously. In blocking experiments EBV-transformed B cells were preincubated with anti-CD23 antibody (DakoCytomation) or an isotype control for 1 hour at 37°C before being added to allergen-IgE complexes. In an additional experiment, crude ragweed allergen extract was replaced by the major ragweed allergen Amb a 1 (a kind gift from Dynavax, Berkeley, Calif) at a concentration of 0.03 µg/mL.

**TABLE I.** Subject characterization

Treatment group	OM/IT	OM/placebo	IT/placebo	Placebo/placebo
No. of subjects	7	11	9	9
Age (y)	39 (29-43)	44 (41-48)	32 (29-41)	41 (38-46)
M/F	4/3	0/11	4/5	4/5
Total IgE (IU/mL)	55 (37-132)	26 (16-41)	112 (78-187)	44 (24-65)
Ragweed skin test (mm)				
Wheal response	8 (6-11)	8 (7-10)	9 (6-11)	9 (6-9)
Erythema response	28 (17-38)	34 (25-40)	28 (18-49)	27 (22-36)

Data are shown as medians (lower quartile-upper quartile).

OM, Omalizumab; IT, immunotherapy.

**FIG 1.** Schematic of the IgE-FAB assay.

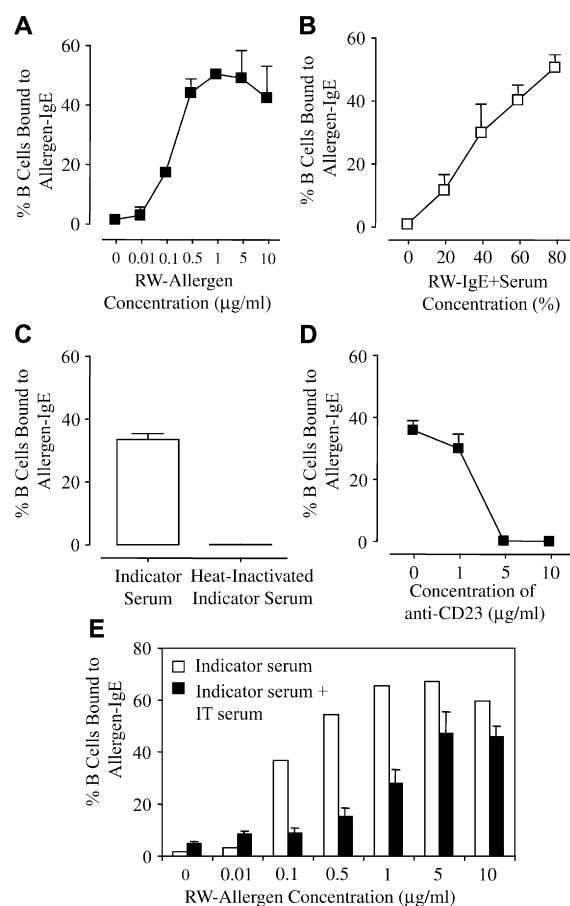
## Statistical analysis

Within-group comparisons were performed by using a 2-sided Wilcoxon matched-pairs test, and comparisons between groups used the Kruskal-Wallis test with a post test to correct for multiple comparisons or a Mann-Whitney *U* test. Differences from baseline measurements (week -9) were assessed by using the Friedman nonparametric repeated-measures test. *P* values of less than .05 were considered statistically significant.

## RESULTS

### Characterization of the ragweed-FAB assay

Initial experiments were aimed to validate the FAB assay (Fig 1) in the context of ragweed allergy. We identified sera from subjects with ragweed allergy containing high ragweed-specific IgE levels that could facilitate binding of allergen to CD23<sup>+</sup> (FcεRII) EBV-transformed B cells. Fig 2, A, shows that binding occurs in an allergen-dependent manner, and optimal binding occurs between 1 and 3 μg/mL. We next investigated the effect of diluting



**FIG 2.** Characterization of the FAB assay. **A**, Ragweed-specific IgE-containing serum (*n* = 3) and variable concentrations of ragweed allergen were preincubated for 1 hour at 37°C before addition to EBV-transformed B cells for 1 hour at 4°C. **B**, High ragweed-specific IgE-containing serum (*n* = 3) was serially diluted with assay buffer and incubated with 1 μg/mL allergen, as above. **C**, Indicator serum (*n* = 3) from patients with high ragweed-specific IgE was heat inactivated at 56°C for 30 minutes, and binding to B cells was compared with that of untreated serum in the assay, as above. **D**, B cells were pretreated with an anti-CD23 blocking antibody or isotype control antibody for 30 minutes at 4°C and used in the assay, as above. **E**, Equal volumes of immunotherapy serum were added to indicator serum and various allergen concentrations in the FAB assay. All data are shown as mean (± SE) percentages of B cells bound to allergen-IgE complexes. *RW*, Ragweed; *IT*, immunotherapy.

the ragweed-specific high-IgE “indicator” serum in the assay. Results show that binding of allergen-IgE complexes to B cells has a linear relationship to the amount of indicator serum used in the assay (Fig 2, B). Heating sera to a temperature of 56°C selectively destroys the ability of IgE to bind to FcεR but does not affect FcR binding of other antibody classes.<sup>26</sup> Results shown in Fig 2, C, show that heat-inactivated indicator sera can no longer support facilitated antigen binding. Preincubating B cells with a blocking antibody against CD23 completely inhibited allergen-IgE complex binding to B cells at antibody concentrations of 5 μg/mL or greater (Fig 2, D). We next tested the effects of adding sera obtained from subjects who had received ragweed immunotherapy for at least 1.5 years in the assay. Results shown in Fig 2, E, reveal that addition of immunotherapy sera could inhibit allergen-IgE binding, optimally at low allergen concentrations (0.1–1 μg/mL).

### Analysis of inhibitory properties of sera obtained from the clinical study with the FAB assay

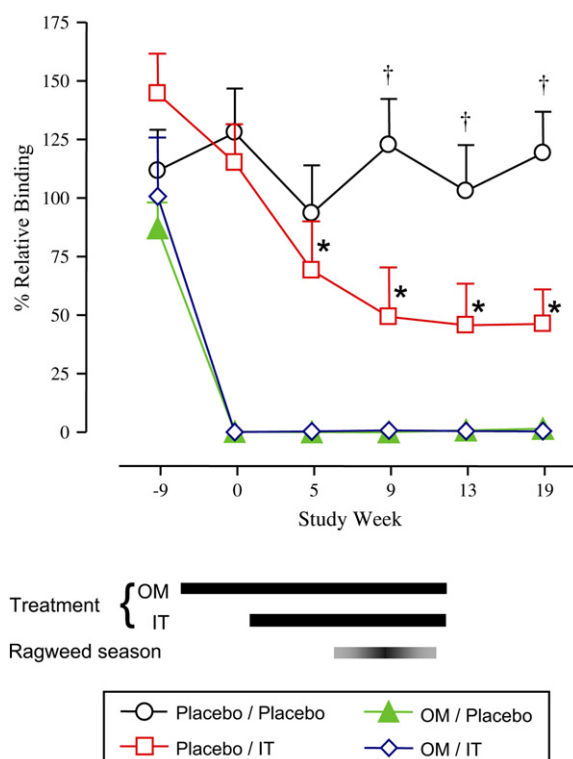
We investigated the inhibitory properties of sera obtained from the clinical study on allergen-IgE binding by using the validated ragweed-FAB assay (Fig 3). Sera obtained before treatment showed similar binding among treatment groups. In the group receiving immunotherapy only, there was a significant decrease in binding observed after 5 weeks of treatment (baseline,  $144 \pm 17$  [mean  $\pm$  SE]; week 5,  $69 \pm 21$ ;  $P < .01$ ), this was maintained until all treatment ceased at week 12 ( $P < .001$ ) and for a further 7 weeks after short-term withdrawal ( $P < .001$ ). In the placebo group no changes in binding were observed from baseline measurements. Statistically significant differences between the immunotherapy group and the placebo group were observed from study weeks 9 to 19 ( $P < .05$ ). In subjects treated with omalizumab, with or without immunotherapy, complete inhibition of binding occurred during the treatment phase (study weeks 0 to 12) and after the withdrawal of omalizumab up to week 19 ( $P < .001$ ).

### Inhibition of FAB by using crude ragweed extract or the major ragweed allergen Amb a 1

We compared inhibition of FAB at selected time points during the clinical study using either whole ragweed extract or the major allergen Amb a 1. Using either form of allergen, we observed decreases in binding in the immunotherapy-only group but not in the placebo group (Fig 4). Addition of sera from groups treated with omalizumab showed complete inhibition of binding of IgE to both ragweed extract and Amb a 1 in the FAB assay.

### Detection of FAB with biotinylated allergen

It is possible that omalizumab present in serum samples could interfere with binding of the FITC-labeled antibody used to detect allergen-IgE binding on B cells in the FAB assay. To address this possibility, an alternative method of detecting IgE-allergen complexes was used with biotinylated allergen to detect binding to B cells. A significant



**FIG 3.** Assessment of serum inhibitory properties during the study. Inhibitory activity of serum was tested in the FAB assay, where binding of indicator serum alone was normalized to 100%. Data are shown as means  $\pm$  SE. Differences between baseline measurements and other time points were assessed by using the Friedman nonparametric repeated-measures test (\* $P < .05$ ). Differences between placebo and immunotherapy groups were assessed by using a Mann-Whitney U test († $P < .05$ ). IT, Immunotherapy; OM, omalizumab.

reduction in binding was observed at the end of the study (week 19) compared with baseline samples (week -9) in the immunotherapy-only group ( $P < .05$ ), and no changes were detected in the placebo group (Table II). Again, the highest inhibition of allergen-IgE binding to B cells was detected in groups that received omalizumab, independent of additional immunotherapy treatment (Table II).

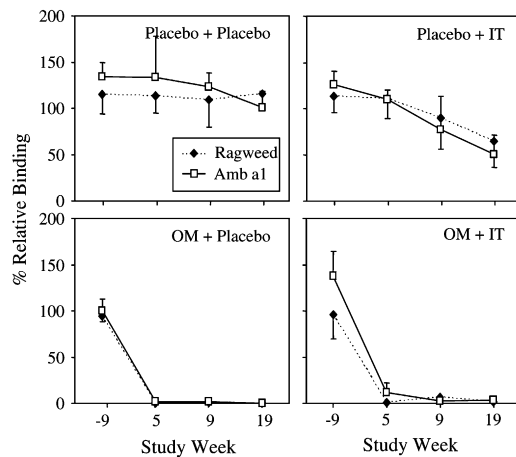
### Allergen-specific IgG4

We measured allergen-specific IgG4 levels by means of ELISA in all 4 treatment groups. Sera from the group that received immunotherapy alone (Fig 5) had significantly higher levels of IgG4 at study week 9 ( $P < .01$ ). These levels were maintained for at least 6 weeks after the withdrawal of treatment (study week 19,  $P < .01$ ). Increases in IgG4 levels were observed in the group receiving immunotherapy in combination with omalizumab from study weeks 13 to 19 ( $P < .05$ ). Groups not receiving immunotherapy did not show increases in allergen-specific IgG4 levels.

### Long-term effects of omalizumab and immunotherapy treatment on allergen-IgE binding

We investigated the inhibitory properties of sera obtained at 18, 30, and 42 weeks after cessation of





**FIG 4.** Comparison of inhibitory responses with either crude ragweed allergen extract or the major ragweed allergen Amb a 1 in the FAB assay within the 4 treatment groups ( $n = 3$  per group). Data are shown as means  $\pm$  SE. IT, Immunotherapy; OM, omalizumab.

omalizumab and immunotherapy treatment (at the end of study week 12) on allergen-IgE binding to B cells in the FAB assay. Serum obtained from the placebo/placebo group had no effect on allergen-IgE binding to B cells (Fig 6). In the group receiving 12 weeks of immunotherapy treatment alone, we no longer observed inhibition of allergen-IgE binding compared with baseline measurements at either 18, 30, or 42 weeks after withdrawal of treatment. Subjects receiving omalizumab alone showed a mean  $\pm$  SE binding of  $14.0\% \pm 6.0\%$  at 18 weeks after treatment,  $54.2\% \pm 6.6\%$  at 30 weeks, and  $84.0\% \pm 6.9\%$  at 42 weeks. At 42 weeks after treatment, allergen-IgE binding was no longer significantly reduced compared with that seen in the placebo control group. Sera obtained from subjects previously receiving combined omalizumab and immunotherapy treatment had similar inhibitory activity to the omalizumab group alone at 18 weeks. In contrast, a significant difference was observed between these groups at 30 and 42 weeks after treatment ( $P < .05$  and  $P < .01$ , respectively). At all time points, differences were observed between the combined therapy group and the immunotherapy-only group (18 weeks,  $P < .001$ ; 30 weeks,  $P = .01$ ; 42 weeks,  $P = .08$ ). No significant differences in allergen-specific IgG4 levels were observed between groups at weeks 18, 30, or 42 after treatment withdrawal, although a trend for increased IgG4 levels were observed in immunotherapy-treated groups compared with baseline measurements (see Table E1 in the Online Repository at [www.jacionline.org](http://www.jacionline.org)).

## DISCUSSION

Ragweed immunotherapy was associated with increases in allergen-specific IgG4 levels and increased serum inhibitory activity, as measured by the facilitated

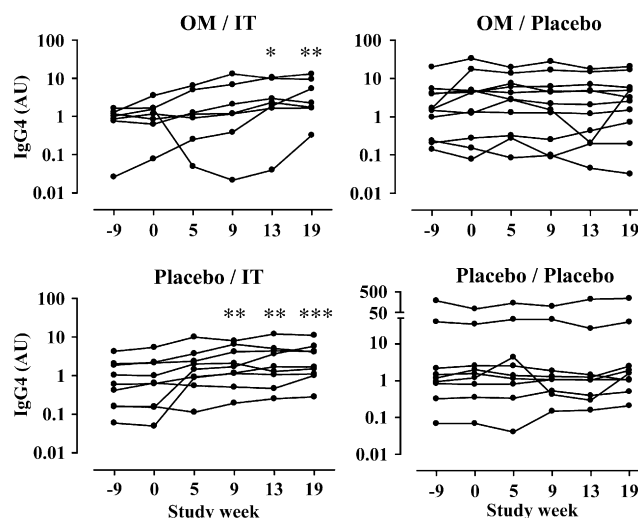
**TABLE II.** Detection of allergen-IgE complexes with biotinylated allergen

Treatment group	Study week -9	Study week 19	P value
OM/IT	111% $\pm$ 3%	34% $\pm$ 1%	<.001
OM/placebo	121% $\pm$ 13%	34% $\pm$ 1%	<.001
IT/placebo	153% $\pm$ 13%	103% $\pm$ 8%	<.05
Placebo/placebo	120% $\pm$ 14%	120% $\pm$ 9%	.98

OM, Omalizumab; IT, immunotherapy.

antigen binding assay. Treatment with omalizumab completely inhibited facilitated antigen binding but did not influence IgG4 levels. The inhibitory effects of omalizumab alone were observed for up to 30 weeks after discontinuation of treatment. Combination treatment with omalizumab and immunotherapy enhanced the serum inhibitory properties for at least 42 weeks after discontinuation compared with either treatment alone. A previous study has demonstrated that combination treatment also resulted in enhanced clinical efficacy and reduced systemic side effects.<sup>24</sup> These observations could be explained, at least in part, by the cumulative inhibitory effects of the combined therapy on allergic inflammation.

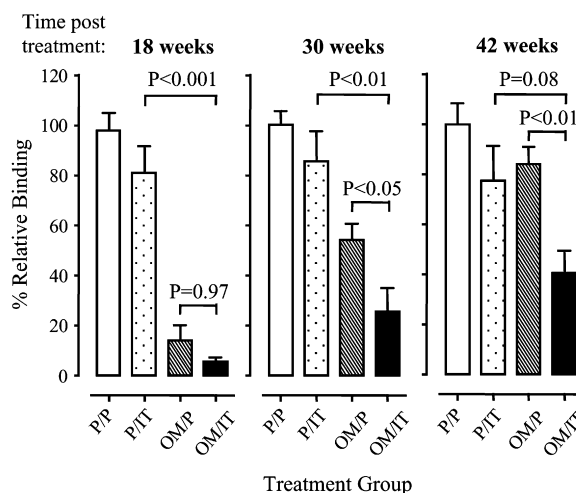
Immunotherapy acts to induce allergen-specific antibodies, which compete with IgE in the indicator sera to inhibit the formation of allergen-IgE complexes. In contrast, anti-IgE (omalizumab itself) is present in sufficient concentrations in peripheral blood during therapy to inhibit binding of allergen-IgE complexes to CD23 expressed on B cells in our assay. Therefore although ragweed immunotherapy acts to induce a population of regulatory antibodies, anti-IgE treatment has a passive action that is ultimately dependent on the presence of the drug itself. By using a different model of facilitated antigen presentation, inclusion of anti-IgE *in vitro* has previously been shown to inhibit subsequent proliferative responses of allergen-specific T-cell clones in a dose-dependent manner.<sup>27</sup> Thus although omalizumab might be effectively blocking IgE binding *per se*, we believe this is representative of *in vivo* conditions, and thus omalizumab might prevent facilitated antigen presentation and subsequent allergen-specific T-cell activation. Omalizumab has a serum elimination half-life of approximately 26 days,<sup>28</sup> and therefore should be almost completely cleared from the serum approximately 8 months after discontinuation of treatment. Interestingly, results shown here demonstrate that treatment with omalizumab alone had inhibitory effects on allergen-IgE binding up to 30 weeks after treatment withdrawal. It is possible that very small concentrations of anti-IgE antibodies are required to inhibit allergen-IgE binding to CD23. Moreover, after treatment withdrawal, combined treatment with omalizumab and immunotherapy enhanced serum inhibitory activity compared with either treatment alone. It is possible that after withdrawal of allergen immunotherapy, even in the absence of high-dose allergen stimulation, persistent low levels of high-affinity IgG4 antibodies could compete with IgE for allergen binding, thereby resulting in



**FIG 5.** Changes in allergen-specific IgG4 levels relative to pretreatment levels over the course of the study. Allergen-specific IgG4 levels were measured by means of ELISA. Individual data are shown, and differences between baseline measurements and time points were assessed by using the Friedman nonparametric repeated-measures test (\* $P < .05$ , \*\* $P < .01$ , and \*\*\* $P < .001$ ). IT, Immunotherapy; OM, omalizumab.

enhanced and persistent serum inhibitory activity with the combined treatment compared with either treatment alone. An alternative possibility is that the sustained inhibition of allergen-IgE binding to APCs (and associated inhibition of T-cell activation)<sup>29</sup> might have resulted in prolonged downregulation of  $T_H2$  lymphocyte responses, as has been observed after discontinuation of grass pollen immunotherapy.<sup>30</sup> This in turn could lead to reduced local effector cell function in the nasal mucosa<sup>31,32</sup> and decreased local specific IgE production<sup>33</sup> after withdrawal of treatment. These provide potential mechanisms by which combined omalizumab and immunotherapy treatment was observed to be more clinically effective than either treatment alone.<sup>24</sup>

Previous studies have shown inhibition of IgE-mediated facilitated allergen presentation to allergen-specific T-cell clones by sera from patients after allergen-specific immunotherapy.<sup>9,11</sup> Here we used a simplified technique that detects allergen-IgE complex binding to B cells by means of flow cytometry, which is representative of subsequent T-cell responses.<sup>29</sup> Thus this assay might function as a surrogate marker for facilitated allergen presentation. We validated the assay in the context of ragweed allergen and confirmed that optimal binding occurs at low allergen concentrations.<sup>8,25</sup> The assay is dependent on the presence of IgE because heat denaturation of IgE could abrogate binding and binding occurred in a serum-dependent manner. Complete blocking of complex binding occurred in the presence of excess anti-CD23, confirming that allergen-IgE binding is solely dependent on the low-affinity receptor in this assay. We detected allergen-IgE complexes bound to EBV-transformed B cells using a fluorescently labeled polyclonal anti-human IgE antibody. It is possible that omalizumab could bind to this antibody and thus interfere with the detection of complexes, which might explain why little or no binding was



**FIG 6.** Assessment of serum inhibitory properties 18, 30, and 42 weeks after discontinuation of treatment. Inhibitory activity of the serum was tested in the FAB assay, where binding of indicator serum alone was normalized to 100%. Data are shown as means  $\pm$  SE. Differences between groups were assessed by using the Mann-Whitney  $U$  test. P, Placebo; IT, immunotherapy; OM, omalizumab.

observed after anti-IgE treatment. To address this possibility, we used an alternative detection system with biotinylated allergen and measured bound allergen-IgE complexes using fluorescently labeled streptavidin. Again, results from this assay show that omalizumab-treated subjects could inhibit biotinylated allergen-IgE binding. This suggests that detection of allergen-IgE-labeled anti-IgE antibody was not compromised by the presence of residual omalizumab.

Previous studies of allergen immunotherapy with either grass pollen or birch have shown decreases in facilitated allergen presentation after prolonged periods of treatment.

A report by van Neerven et al<sup>10</sup> shows a decrease of facilitated allergen presentation to allergen-specific T cells of more than 50% in the majority of subjects studied after 18 months of birch pollen immunotherapy. In our previous studies of grass pollen immunotherapy, we have demonstrated an average decrease of more than 75% after 2 years of treatment.<sup>5</sup> In this study subjects were treated for 12 weeks, and we observed a significant decrease in FAB of approximately 50% after 5 weeks. This suggests that changes in CD23-associated allergen-IgE binding occur early during treatment, and long-term therapy is not necessary to induce changes in this mechanism. Further results demonstrate that inhibition of FAB occurs up to 7 weeks after the withdrawal of treatment, although these effects are no longer observed after 18 weeks. Therefore the effects of 12 weeks of ragweed immunotherapy on inhibitory serum activity are relatively short lived.

Allergen-specific IgG antibodies are induced by successful immunotherapy,<sup>5,34,35</sup> but quantitative changes in specific IgG antibodies do not always correlate with clinical protection.<sup>36-38</sup> Here we demonstrate a significant increase in ragweed-specific IgG4 levels induced by treatment with immunotherapy. Treatment with omalizumab did not influence allergen-specific IgG4 levels induced by immunotherapy. Increases in ragweed-specific IgG4 levels were modest compared with other reports of immunotherapy,<sup>5</sup> although this might reflect the short treatment period (approximately 2 months) compared with other studies (1-2 years). Allergen-specific IgG4 levels do not correlate with inhibitory activity measured in the FAB assay (analysis not shown), and this finding is consistent with a previous study.<sup>5</sup> It is possible that the final inhibitory activity, as measured in the FAB assay, could be predicted by the ratio of allergen-specific inhibitory antibodies (IgG4) to IgE in the patient's serum. However, in this study it is not possible to interpret serum allergen-specific IgE results in subjects treated with omalizumab because assays are unable to distinguish between IgE contained within IgE-anti-IgE complexes, which increase because of delayed renal clearance, and free IgE, which decreases.<sup>21</sup>

In summary, ragweed immunotherapy induced serum regulatory antibodies that partially blocked binding of allergen-IgE complexes to B cells. The addition of anti-IgE to ragweed, by directly blocking IgE binding to CD23, completely inhibited allergen-IgE binding, which might have contributed to the observed enhanced efficacy with the combination therapy. Although the cost of the combination of immunotherapy with anti-IgE treatment is high, this should be considered in view of the enhanced benefit/risk ratio and the known long-term benefits of allergen immunotherapy.<sup>24</sup> Whether the prolonged inhibition of allergen-IgE binding that was seen after discontinuation of the combination compared with either treatment alone could result in a more prolonged duration of efficacy remains to be determined.

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