

Effect of Omalizumab on Adenosine 5'-Monophosphate Responsiveness in Subjects with Allergic Asthma

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Key Words

Omalizumab · Anti-immunoglobulin E · Immunoglobulin E · Allergic asthma · Adenosine 5'-monophosphate · Methacholine

Abstract

Background: The objective of this study was to evaluate the effects of omalizumab on bronchoconstriction induced by methacholine and adenosine 5'-monophosphate (AMP). **Methods:** Thirty-four subjects with mild to moderate allergic asthma were randomized to receive placebo (n = 16) or omalizumab (n = 18) subcutaneously during 12 weeks. Airway responsiveness to AMP was measured at baseline and after 4 and 12 weeks of treatment, whereas the response to methacholine was measured at baseline and after 12 weeks of treatment. **Results:** After 4 weeks of treatment, the increase in AMP PC₂₀ (provocative concentration required to produce a 20% fall in FEV₁) was significantly greater in the omalizumab group than in the placebo group, the mean difference in the change between the groups being 1.52 doubling concentrations (95% CI, 0.25–2.79, p = 0.02). Compared with baseline, the mean AMP PC₂₀ values at 12 weeks were increased by 1.91 doubling concentrations with omalizumab (p < 0.001) and 1.01 doubling con-

centrations with placebo (p = 0.16), but changes were not significantly different between the treatment groups. Changes in methacholine PC₂₀ values were not significantly different between the omalizumab and placebo groups. **Conclusions:** In subjects with allergic asthma, omalizumab reduces the response to AMP without decreasing the response to methacholine. These findings are consistent with the conclusion that the contribution of IgE to the development of AMP bronchoconstriction is more important than their role in the induction of methacholine hyperresponsiveness.

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Introduction

Airway inflammation plays a central role in the pathogenesis of asthma and is associated with an increase in airway responsiveness to a number of nonsensitizing bronchoconstrictive stimuli. Although methacholine and histamine have become gold standards as bronchial provocants to quantify airway hyperresponsiveness, adenosine 5'-monophosphate (AMP) has been introduced more recently as a bronchoconstrictive stimulus. Whereas histamine and methacholine act by a direct effect on the airway smooth muscle, AMP-induced bronchoconstriction

tion occurs predominantly indirectly by stimulation of adenosine A_{2B} receptors on 'primed' mast cells, causing mast cell degranulation and the release of histamine and leukotrienes with subsequent smooth muscle contraction [1, 2]. Although methacholine and AMP responsiveness are significantly related [3, 4], some reports have indicated that increased responsiveness to AMP is not necessarily accompanied by bronchoconstriction in response to methacholine [5–7]. Therefore, it has been reported that they might reflect different components of airway dysfunction [5, 6, 8].

A population-based study showed a close and highly significant relationship between serum IgE levels and the prevalence of asthma [9]. Since then, others have confirmed the view that the majority of asthma occurs in association with allergy [10, 11]. Central to the mechanisms of allergic inflammation is the release of a number of pro-inflammatory mediators after allergen binding to IgE on the surface of effector cells [12]. An emerging concept of immunotherapy, therefore, is to inhibit IgE responses through the use of anti-IgE antibodies. Omalizumab (XolairTM; formerly rhuMAb-E25), a recombinant humanized monoclonal anti-IgE antibody, is the first anti-IgE agent with clinical benefits in the treatment of allergic asthma, when used as an intravenously administered formulation [13] and, more recently, as a subcutaneous injection given every 2–4 weeks in patients with moderate to severe allergic asthma [14–19] and in subjects with concomitant allergic asthma and allergic rhinitis [20]. Omalizumab works by forming complexes with circulating free IgE. It binds to the receptor-binding portion of IgE (Cε3) and because the epitope that is recognized by omalizumab encompasses binding regions for both high-affinity and low-affinity IgE receptors, the monoclonal antibody eliminates the ability of IgE to bind to both types of receptors [21]. In addition to inhibiting binding of IgE to mast cells and basophils, omalizumab also markedly downregulates the expression of high-affinity receptors on basophils [22], mast cells [23] and dendritic cells [24].

Three clinical studies have examined the effects of omalizumab on the airway responsiveness to inhaled methacholine among patients with mild allergic asthma [25–27]. In two studies, there was a modest (no statistically significant) improvement of airway responsiveness to this direct bronchoconstrictor agent after treatment with intravenous omalizumab compared to placebo [25, 26]. The third study showed that subcutaneous omalizumab had no significant effect on methacholine responsiveness in subjects with mild to moderate allergic asthma

[27]. In these studies, however, the effect of omalizumab on methacholine responsiveness was not a primary outcome.

It has long been known that the density of IgE receptors on effector cells is correlated with free serum IgE levels [28]. Thus, by removing free IgE from the circulation, omalizumab downregulates IgE receptor expression on basophils and, potentially, mast cells [22]. This downregulation of IgE receptors reduces the potential for basophil/mast cell activation. We hypothesized that, if bronchoconstriction induced by AMP depends, at least in part, on the state of activation of airway mast cells [1, 2], then omalizumab may provide greater bronchoprotection against the mast cell stimulus AMP than against the direct methacholine stimulus. To test this hypothesis, we performed a 12-week, double-blind, placebo-controlled, parallel group study comparing the effect of omalizumab on airway responsiveness to the two stimuli in subjects with allergic asthma. The primary outcome of the study was the provocative concentration of agonist required to produce a 20% fall in FEV₁ (PC₂₀) for AMP level. Secondary outcomes of the study were PC₂₀ for methacholine and FEV₁.

Subjects and Methods

Subjects

Male or female allergic asthmatics aged 18–65 years were eligible if they met the following inclusion criteria: mild to moderate persistent allergic asthma [29]; positive immediate responses (wheal diameter ≥ 3 mm) on skin prick testing to house dust mite allergens (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), dog, or cat; baseline FEV₁ ≥ 80% of the predicted value and FEV₁/FVC% ≥ 70%; total serum IgE ≥ 30 to ≤ 700 IU/ml and body weight ≤ 150 kg to allow optimal dosing of omalizumab; treatment with inhaled short-acting β₂-agonists alone or with inhaled corticosteroids (ICS) in doses equivalent to 200–1,000 µg of beclomethasone dipropionate per day for ≥ 6 months prior to randomization; no specific immunotherapy within the past 3 months, and bronchoconstriction in response to both AMP and methacholine at screening. All patients were to be exposed to at least one of the perennial allergens to which they had a positive skin prick test result for the duration of the study. Asthma had to be stable, with no significant change in regular medication for ≥ 3 months prior to the screening visit.

All asthmatic patients had mild to moderate bronchial asthma and none were receiving any regular medication for asthma other than ICS and/or occasionally short-acting inhaled β₂-agonists. All subjects were nonsmokers, and none had chronic bronchitis, emphysema, allergic bronchopulmonary aspergillosis or respiratory tract infections during the 4 weeks before the study. Females of childbearing potential had to use reliable contraceptives, and pregnant or lactating women were excluded from participation. No relevant concomitant diseases were present.

The study protocol was approved by the Medical Ethics Committee at each participating institution and the National Regulatory Agency (case No. 01-0530). Written informed consent was obtained from each subject prior to participation.

Study Design

This study was a randomized, double-blinded, placebo-controlled, parallel-group trial conducted in four centers in Spain. After a 5- to 9-day baseline period, patients were randomized to receive either omalizumab or placebo administered subcutaneously every 2 or 4 weeks over a 12-week period. The dose and dosing intervals were based on the subject's body weight and baseline serum IgE. This ensured for each patient a dose of at least 0.016 mg/kg per international unit per milliliter of IgE every 4 weeks. Subjects thus received 150–300 mg every 4 weeks or 225, 300 or 375 mg every 2 weeks. Trial personnel administered medication in a blinded fashion, which was maintained at each center by using a non-trial-related individual to prepare trial medication or placebo injections before administration.

Pulmonary function and airway responsiveness to AMP were measured at baseline and after 4 and 12 weeks of treatment, whereas airway responsiveness to methacholine was measured at baseline and after 12 weeks of treatment. In 20 subjects (12 receiving omalizumab and 8 receiving placebo), airway responsiveness to both bronchoconstrictor agents was also determined 12 weeks after discontinuation of treatment. Concentration-response studies with either methacholine or AMP were performed on two different days (at least 4 but no more than 9 days apart). In all subjects the AMP challenge test was performed first.

The dose of intranasal or ICS (if used) was maintained unchanged during the study. Salbutamol MDI, oral antihistamines (except astemizole) and intranasal antihistamines were used on an 'as-needed' basis to control pulmonary or nasal symptoms, respectively. No other medications were allowed to be used during the study. Subjects were asked not to take ICS for 12 h, albuterol for at least 6 h, oral antihistamines for at least 72 h and intranasal antihistamines for at least 24 h before each challenge.

Details of adverse events were collected every 4 weeks during the study on a form that recorded all events, irrespective of suspected relationships to the study drug and of mild, moderate or serious severity. Hematology evaluations (hemoglobin, hematocrit, white blood cell count and differential, red blood cell, and platelet counts) were conducted at baseline and at the end of 12 weeks of double-blind treatment.

Pulmonary Function

Spirometry was performed with calibrated spirometers, according to standardized guidelines [30]. Baseline FEV₁ and FVC were measured until three reproducible recordings differing <5% were obtained. Maneuvers were accepted as technically satisfactory if the back-extrapolated volume was <150 ml or 5% FVC, and if the expiratory time was at least 6 s. Highest values were used for analysis. Reference values were those of the European Community for Coal and Steel [31].

Inhalation Challenge Tests

Inhalation provocation tests were performed using the method described by Chai et al. [32] and according to standardized guidelines [33]. Methacholine (Provocholine™ Methapharm, Brantford, Canada) and AMP (Sigma Chemical Co., St. Louis, Mo.,

USA) were dissolved freshly in 0.9% saline solution to produce a doubling concentration range of 0.095–25 mg/ml for methacholine and from 0.39 to 400 mg/ml for AMP. Solutions of AMP were passed through bacterial-retentive filters with 0.22 µm porosity. Each solution was administered from a jet nebulizer attached to a breath-activated dosimeter (model MB3; Mefar, Brescia, Italy) at a nebulization time of 1 s with a pause time of 6 s. The nebulizer delivers particles with an aerodynamic mass median diameter of 3.5–4.0 µm at an output of 10 µl per breath. Subjects inhaled the aerosolized methacholine and AMP solutions in five inhalations from functional residual capacity to total lung capacity through a mouthpiece with the subject's nose clipped. Normal saline solution was inhaled initially, followed by five breaths of doubling concentrations of methacholine or AMP at 2- to 3-min intervals. Due to the effect of a deep inspiration on subsequent airway tone [34], single measurements of FEV₁ were made 60–90 s after the inhalation of each concentration, unless the forced expiratory maneuver was judged to be technically unsatisfactory. The test was interrupted when a ≥20% decrease in FEV₁ from the postsaline solution inhalation value was recorded or when the highest concentration was reached. The PC₂₀ was calculated using an algebraic formula [35].

Data Analysis

An intention-to-treat approach was followed in the analysis of efficacy data. All patients with a baseline and at least one postrandomization measurement were included in the analysis according to the group to which they were randomized. The safety population comprised all patients who received at least one dose of omalizumab or placebo. The primary outcome of the study was the PC₂₀ for the AMP level. Secondary outcomes of the study were PC₂₀ for methacholine and FEV₁.

To detect a between-treatment difference in PC₂₀ AMP of 1.5 doubling concentrations in the intent-to-treat population, with an SD of 0.5 (logarithmic value) and at 80% power (0.05 level of significance), it was calculated that a minimum sample size of 16 evaluable patients per treatment group was required in the present trial.

Data were analyzed with standard statistical software packages (SPSS, version 10.0 for Windows; SPSS; Chicago, Ill., USA, and InStat for Windows, version 3.00; GraphPad Software; San Diego, Calif., USA). All PC₂₀ and serum IgE values were log-transformed before analysis and presented as geometric means with 95% confidence intervals (CIs). All other numerical variables are reported as arithmetic means with 95% CI. Changes in PC₂₀ values were also expressed [33] in terms of doubling concentrations of methacholine or AMP calculated as $\Delta \log PC_{20} / \log 2$. Comparisons of the baseline characteristics of the two groups were performed by unpaired Student's *t* tests for continuous data and by Fisher's exact tests for categorical data. Comparisons of treatment effects of placebo and omalizumab on PC₂₀ AMP, PC₂₀ methacholine, and FEV₁ were made using two-factor repeated-measures analysis of variance to analyze the effect of the two independent variables, treatment and time, on the outcome variables described previously. Correlations between variables were calculated with Pearson correlation coefficient. All comparisons were two-tailed, and *p* values less than 0.05 were considered significant.

Table 1. Baseline characteristics of the two treatment groups

	Omalizumab	Placebo	p
Number	18	16	
Age, years	32 (27–37)	30 (25–35)	0.69
Sex			0.49
Male	7	9	
Female	11	7	
ICS treatment, yes/no	16/2	14/2	0.98
ICS dose (beclomethasone equivalent), µg/day	412 (278–545)	465 (320–630)	0.51
INCS treatment, yes/no	8/10	5/11	0.50
INCS dose (beclomethasone equivalent), µg/day	144 (55–233)	138 (50–238)	0.91
IgE level ^a , IU/ml	171.4 (109.6–267.3)	227.0 (147.2–350.0)	0.35
FEV ₁ , % predicted	100.0 (92.3–107.3)	101.4 (94.9–107.9)	0.74
FVC, % predicted	106.2 (98.7–113.7)	108.8 (101.5–116.1)	0.61
FEV ₁ /FVC, %	79.9 (75.7–84.1)	80.1 (77.0–83.3)	0.94
PC ₂₀ ^a , mg/ml			
Methacholine	1.27 (0.82–1.98)	2.13 (1.48–3.07)	0.54
AMP	14.32 (9.72–21.12)	31.20 (18.59–52.37)	0.37

Beclomethasone dipropionate (equivalent dose of ICS and intranasal corticosteroid) was calculated on the basis of fluticasone propionate being twice as potent as beclomethasone dipropionate or budesonide, so that the equivalent fluticasone propionate dose was multiplied 2-fold. Data are presented as number or mean (95% CI) unless otherwise indicated. INSC = Intranasal corticosteroid.

^a Geometric mean (95% CI).

Results

Sixty-eight patients were screened, of whom only 39 were eligible for randomization and received at least one dose of double-blind trial medication (omalizumab, *n* = 20; placebo, *n* = 19). Twenty-nine subjects were excluded because they had failed to meet one or more of the inclusion criteria, the most common reason being absence of bronchoconstriction in response to AMP and total serum IgE concentration >700 IU/ml. An exception was made for one subject whose IgE levels were 719 IU/ml. Three patients discontinued prematurely before the first visit after randomization: 2 patients (one in each group) declined the previous acceptance of participation, whereas 1 subject (placebo group) was excluded from the study after 3 weeks of treatment due to a serious adverse event. Additionally, 2 subjects (one in each group) were excluded from the efficacy analysis because of protocol violation. Only the data for the remaining 34 subjects who completed the study period were included in the efficacy analysis. Of those who completed the study, 18 were randomized to receive omalizumab and 16 to receive placebo. Baseline characteristics were comparable for the

two treatment groups (table 1). A total of 12 subjects in the omalizumab group and 8 in the placebo group were treated with oral antihistamines during the clinical trial (*p* = 0.49), whereas 1 and 3 subjects of each group were treated with intranasal antihistamines (*p* = 0.32). Antihistamines were, however, withheld before methacholine and AMP challenge tests.

One subject (placebo group) was excluded from the study after 6 weeks of treatment due to an asthma exacerbation and the need to use oral steroids, but both pulmonary function and AMP responsiveness at 4 weeks of treatment were determined. Thus, in the placebo group, there were evaluable data on 16 subjects for FEV₁ and AMP PC₂₀ and there were evaluable data on 15 subjects for the methacholine PC₂₀.

Lung Function

The mean change from baseline in FEV₁ over the study period is depicted in figure 1. In the omalizumab group, the FEV₁ increased from a mean (95% CI) baseline value of 3.44 liters (3.35–3.54) to 3.53 liters (3.43–3.62) after 4 weeks of treatment and to 3.51 liters (3.41–3.60) after 12 weeks of treatment (*p* = 0.39). In the placebo

group, the FEV₁ values decreased from 3.77 liters (3.66–3.88) during the baseline period to 3.62 liters (3.51–3.73) after 4 weeks of treatment and to 3.70 liters (3.58–3.81) after 12 weeks of treatment ($p = 0.18$). At week 4, the increase in FEV₁ from baseline was significantly greater in the omalizumab group than in the placebo group, the mean difference in the change between the groups being 0.23 liters (95% CI, 0.01–0.46, $p = 0.049$). At week 12, changes in FEV₁ were similar in both groups, the mean difference being 0.14 liters (–0.05 to 0.33, $p = 0.15$). When measured 12 weeks after cessation of treatment, FEV₁ values had returned to pretreatment values (data not shown).

AMP Responsiveness

Values of PC₂₀ at baseline and after 4 and 12 weeks of treatment for the two groups are shown in figure 2. The geometric mean AMP PC₂₀ value for the omalizumab group increased from 14.32 mg/ml (95% CI, 9.72–21.12) at baseline to 54.07 mg/ml (37.67–79.69, $p < 0.001$) at 4 weeks of treatment and to 53.78 mg/ml (36.48–79.28, $p < 0.001$) at 12 weeks of treatment. In this group, the PC₂₀ increased by 1.92 (1.00–2.85) and by 1.91 (1.11–2.72) doubling concentrations after 4 and 12 weeks of treatment, respectively. In the placebo group, there was an increase from 31.20 mg/ml (18.59–52.37) at baseline to 41.28 mg/ml (24.60–69.28, $p = 0.37$) after 4 weeks of treatment and to 62.73 mg/ml (37.38–105.30, $p = 0.16$) after 12 weeks of treatment. In this group, the PC₂₀ increased by 0.41 (–0.53 to 1.34) and by 1.01 (–0.43 to 2.45) doubling concentrations after 4 and 12 weeks, respectively.

After 4 weeks of treatment, the increase in AMP PC₂₀ was significantly greater in the omalizumab group than in the placebo group, the mean difference in the change between the groups being 1.52 doubling concentrations (95% CI, 0.25–2.79, $p = 0.02$). However, after 12 weeks of treatment, changes in AMP PC₂₀ values were not significantly different between the omalizumab and placebo groups, the mean difference being 0.90 doubling concentrations (95% CI, –0.64 to 2.44, $p = 0.24$). Following cessation of treatment PC₂₀ values returned to pretreatment values (data not shown).

Methacholine Responsiveness

Values of PC₂₀ at baseline and after 12 weeks of treatment for the two groups are shown in figure 3. The geometric mean methacholine PC₂₀ value for the omalizumab group increased from 1.27 mg/ml (95% CI, 0.82–1.98) at baseline to 2.10 mg/ml (1.35–3.27, $p = 0.11$) after

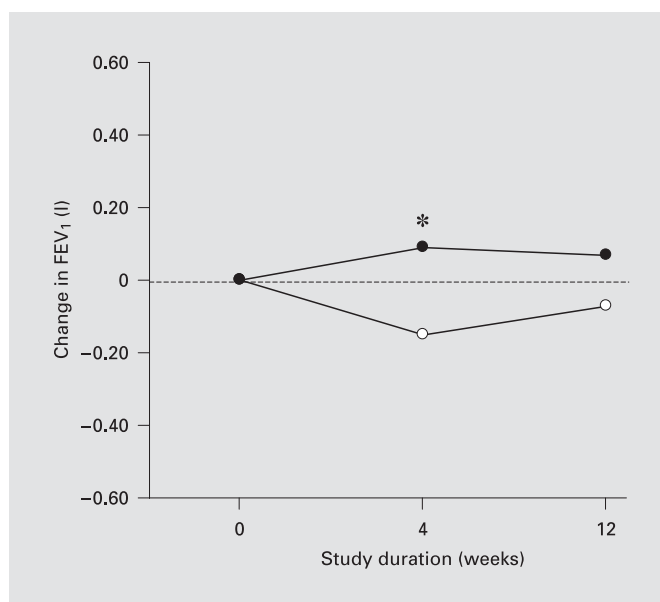


Fig. 1. FEV₁ measured at clinic visits, expressed as mean changes from baseline. ● = Omalizumab; ○ = placebo. * $p < 0.05$ compared to the placebo group.

12 weeks of treatment. The mean (95% CI) increase was 0.69 doubling concentrations (–0.20 to 1.58). In the placebo group, there was a decrease from 2.13 mg/ml (1.48–3.07) to 1.80 mg/ml (1.26–2.60, $p = 0.50$) after 12 weeks of treatment. The mean (95% CI) decrease was –0.24 doubling concentrations (–0.98 to 0.50). Changes in methacholine PC₂₀ values were not significantly different between the omalizumab and placebo groups, the mean difference being 0.96 doubling concentrations (95% CI, –0.19 to 2.12, $p = 0.11$). After cessation of omalizumab PC₂₀ methacholine values returned to pretreatment values (data not shown).

Correlations

At baseline, a significant correlation was found between methacholine and AMP PC₂₀ values ($r = 0.68$, $p < 0.001$). No significant correlation was found between changes in FEV₁ and changes in either methacholine PC₂₀ values (placebo group, $r = 0.35$, $p = 0.19$; omalizumab group, $r = 0.43$, $p = 0.07$) or AMP PC₂₀ values at week 4 (placebo group, $r = 0.42$, $p = 0.10$; omalizumab group, $r = 0.40$, $p = 0.09$) or at week 12 (placebo group, $r = 0.33$, $p = 0.21$; omalizumab group, $r = 0.23$, $p = 0.35$). Furthermore, omalizumab-induced changes in methacholine and AMP PC₂₀ values were not significantly related ($r = 0.10$, $p = 0.68$).

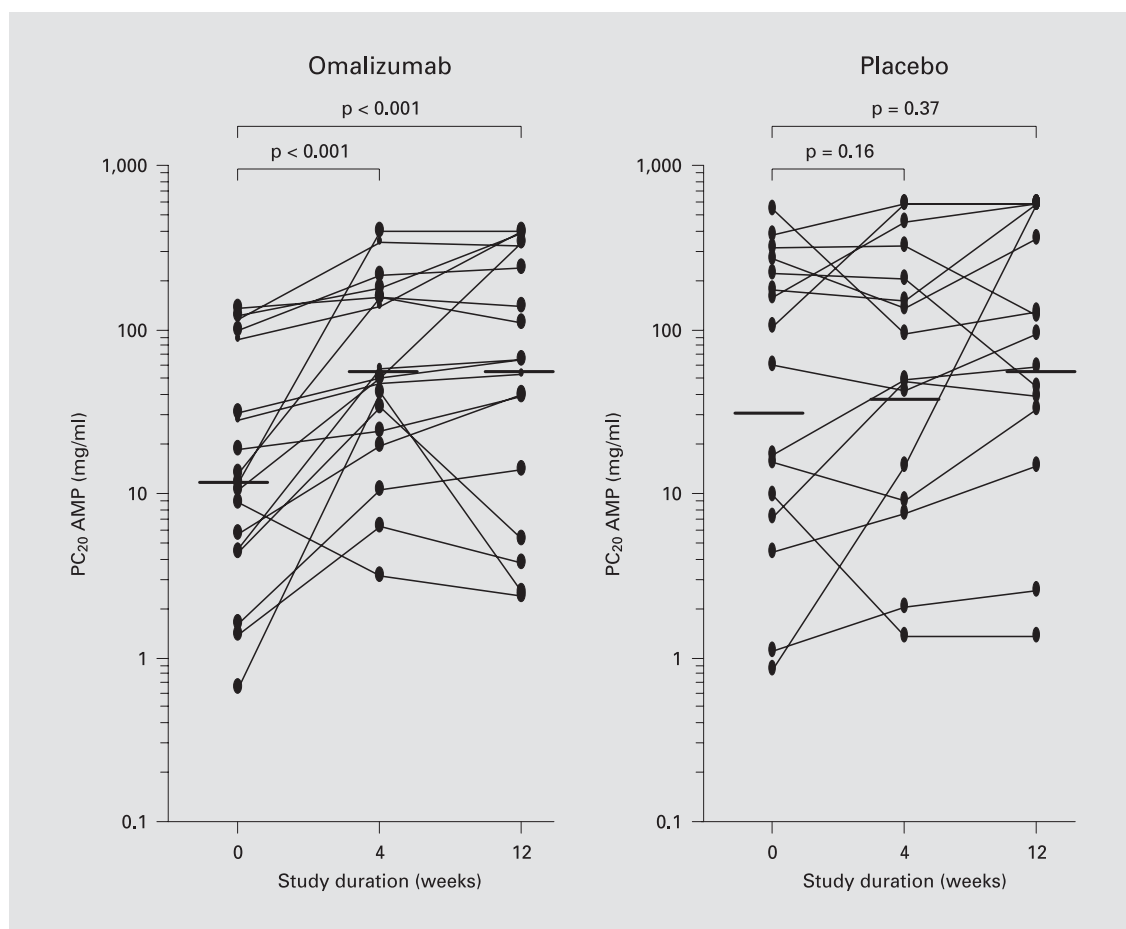


Fig. 2. Individual values for AMP PC₂₀ within the two groups at baseline and after 4 and 12 weeks of treatment are shown. Geometric means are represented by horizontal lines. After 4 weeks of treatment, the increase in PC₂₀ was significantly greater in the omalizumab group than in the placebo group ($p = 0.02$).

Safety and Tolerability

Treatment with omalizumab was well tolerated. The overall incidence of adverse events was comparable between the treatment groups. One patient on placebo experienced a serious adverse event (gastrointestinal bleeding), whereas no patient on omalizumab had serious adverse events. One additional placebo recipient had an asthma exacerbation requiring treatment with oral corticosteroids. No clinically meaningful changes in laboratory parameters were noted in any of the treatment groups. Furthermore, occurrences of platelet counts $<150 \times 10^9/l$ were not detected at baseline or during the treatment.

Discussion

The present study demonstrates that in subjects with mild to moderate allergic asthma subcutaneous treatment with omalizumab over a period of 12 weeks is associated with a significant reduction in airway responsiveness to AMP. After 4 weeks, the reduction in AMP responsiveness was significantly greater in the omalizumab group than in the placebo group. However, although the effect of omalizumab on AMP responsiveness at 12 weeks of treatment was similar to that detected at 4 weeks, the degree of attenuation was not significantly greater than placebo. In contrast to its effects on AMP, omalizumab had no effect on airway responsiveness to methacholine. These findings are the first clinical evidence of the involvement of IgE in the pathophysiology of airway con-

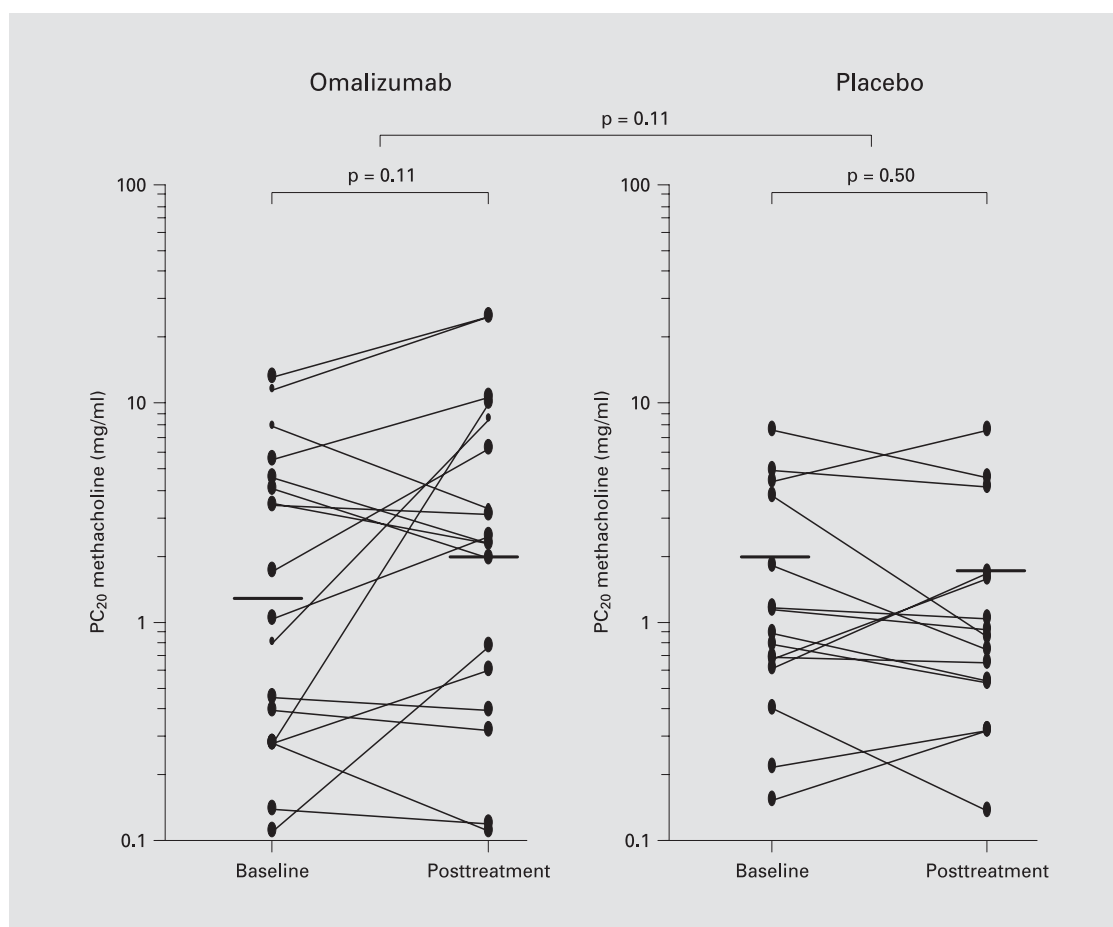


Fig. 3. Individual values for methacholine PC₂₀ within the two groups at baseline and after 12 weeks of treatment are shown. Geometric means are represented by horizontal lines. Changes in PC₂₀ were not significantly different between the placebo and omalizumab groups.

striction induced by AMP and suggest a dissociation between the mechanism that induces bronchoconstriction in response to methacholine and AMP.

To the best of our knowledge, there are no studies that have investigated the effects of treatment with omalizumab on airway responsiveness to inhaled AMP in subjects with asthma. The results of our study demonstrate that, in this group of subjects, treatment with omalizumab is associated with a significant reduction in AMP responsiveness. The protective effect of omalizumab on the response of the airway to inhaled AMP was seen within 4 weeks of treatment and after only one to two doses. Although this rapid effect might appear surprising, some studies have shown that omalizumab has a rapid clinical and biological onset of action *in vivo*. Recent findings in adults with allergic rhinitis indicated that omalizumab

caused a decrease of IgE serum levels as soon as 72 h and a reduction of the number of high-affinity IgE receptors on basophils as soon as 7–14 days after subcutaneous injections [36].

After 12 weeks, treatment with omalizumab was associated with a significant within-group attenuation in airway responsiveness to AMP. However, although the magnitude of the reduction in AMP responsiveness (1.91 doubling concentrations) was similar to that detected at 4 weeks (1.92 doubling concentrations), the degree of attenuation at 12 weeks was not significantly greater than placebo. This was probably the consequence of the high placebo response. At 12 weeks of treatment, patients receiving placebo achieved substantial improvement (1 doubling concentration) in AMP responsiveness and this high placebo response reduced the magnitude of the

differences between placebo and omalizumab. The reason for the improvement in the placebo group is not clear. One possibility is that it reflects the placebo effect of administering parenteral therapy for a disease traditionally treated with inhaled medication. This explanation is supported by data from some phase III clinical studies that have identified substantial improvements in many variables in the group treated with subcutaneous placebo [15,16]. Additionally, most patients enrolled in this study were already receiving ICS and, therefore, a second explanation for the high placebo response in the present study might be improved compliance with ICS treatment. Results of the other secondary efficacy variables were apparently inconsistent with this last explanation, because neither methacholine PC₂₀ nor baseline FEV₁ values increased as a result of placebo treatment. However, it has been demonstrated that the bronchial response to AMP improves to a larger extent after therapy with ICS than the response to methacholine [37–39]. In addition, our subjects were well controlled and had normal pulmonary function at baseline, so that improvement in FEV₁ would have been hard to demonstrate. We are, therefore, confident that the marked placebo effect is probably the expression of an improved compliance with ICS treatment resulting from the frequent assessment by a physician.

On the other hand, omalizumab therapy appears to have minimal, if any effect on airway responsiveness to methacholine. This confirms and extends the results of other trials investigating the effect of omalizumab administered by intravenous or subcutaneous injection on methacholine responsiveness in subjects with mild to moderate asthma not treated with ICS [25–27]. By contrast, Noga et al. [40] studied the effects of subcutaneous omalizumab on the airway responsiveness to inhaled acetylcholine in patients with moderate to severe allergic asthma treated with ICS. The results from this study demonstrated that treatment with omalizumab for 16 weeks was associated with a significant reduction in acetylcholine responsiveness compared with placebo. There are several possible explanations for this apparent contradiction. In the study by Noga et al. [40] the bronchoconstrictor agent was acetylcholine, whereas the bronchoconstrictor used in this study was methacholine. Furthermore, subjects in the study by Noga et al. [40] were studied at baseline and after 16 weeks of treatment, whereas our subjects were tested at baseline and after 12 weeks of treatment. It is possible that the treatment period was too short to achieve a significant improvement. Other reasons for such discrepancies might be related to differences in patients' characteristics, to diversity in the disease activ-

ity in the subjects studied, or to important differences in the statistical analysis.

Although the results of some prospective studies suggest that the development of airway hyperresponsiveness to methacholine and IgE levels are linked [41], it has been difficult to prove the contribution of IgE to the development of methacholine hyperresponsiveness. Our results suggest that, at least for the response to this direct bronchoconstrictor, this contribution is not causal. It has long been appreciated, however, that the various stimuli used to assess bronchial responsiveness act through different mechanisms and reflect different components of asthma pathogenesis [5, 6, 8]. Consistent with this notion, in the present study we have shown that reduction in responsiveness to AMP is not necessarily accompanied by reduction in responsiveness to methacholine. Our results also demonstrate that PC₂₀ AMP improved in response to treatment with omalizumab to a greater extent than did PC₂₀ methacholine (1.91 vs. 0.69 doubling concentrations, respectively). Furthermore, our results suggest that in subjects with allergic asthma, changes in airway responsiveness to methacholine and AMP after treatment with omalizumab are not correlated. This different effect could be caused by the fact that the two bronchoconstrictors are identifying different alterations in the lower airways. The response to AMP may reflect acute inflammatory changes in the bronchial mucosa, whereas methacholine responsiveness might predominantly be dependent on structural changes of the airways [42].

Three months after cessation of omalizumab, FEV₁ and PC₂₀ values had returned to pretreatment values. This suggests that 12 weeks of treatment with omalizumab does not result in any permanent reduction in methacholine or AMP responsiveness in asthma.

The present results have clinical implications. Our observation that airway hyperresponsiveness to indirect bronchoconstrictor agents may diminish after treatment with omalizumab even in patients treated with ICS is important because triggers that frequently induce bronchial obstruction in the real world, such as exercise and allergens, act by an indirect mechanism. In the present study, however, we deliberately selected a substantial proportion of subjects treated with ICS. The reason to do so was largely based on the argument that omalizumab will be administered to subjects concomitantly treated with ICS. It could be argued, however, that the effects of omalizumab on airway responsiveness might be different in subjects not treated concomitantly with ICS, and therefore it would be of interest to repeat this type of study in steroid-naïve subjects with asthma.

In summary, in this study of patients with mild to moderate persistent allergic asthma, we have shown that omalizumab reduces the response to inhaled AMP without decreasing the response to methacholine. These findings are consistent with the conclusion that the contribution of IgE to the development of bronchoconstriction in response to AMP is more important than their role in the induction of methacholine hyperresponsiveness.

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