

Effects of Treatment with Anti-immunoglobulin E Antibody Omalizumab on Airway Inflammation in Allergic Asthma

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IgE plays an important role in allergic asthma. We hypothesized that reducing IgE in the airway mucosa would reduce airway inflammation. Forty-five patients with mild to moderate persistent asthma with sputum eosinophilia of 2% or more were treated with humanized monoclonal antibody against IgE (omalizumab) ($n = 22$) or placebo ($n = 23$) for 16 weeks. Outcomes included inflammatory cells in induced sputum and bronchial biopsies, and methacholine responsiveness. Treatment with omalizumab resulted in marked reduction of serum IgE and a reduction of IgE⁺ cells in the airway mucosa. The mean percentage sputum eosinophil count decreased significantly ($p < 0.001$) from 6.6 to 1.7% in the omalizumab group, a reduction significantly ($p = 0.05$) greater than with placebo (8.5 to 7.0%). This was associated with a significant reduction in tissue eosinophils; cells positive for the high-affinity Fc receptor for IgE; CD3⁺, CD4⁺, and CD8⁺ T lymphocytes; B lymphocytes; and cells staining for interleukin-4, but not with improvement in airway hyperresponsiveness to methacholine. This study shows antiinflammatory effects of omalizumab treatment and provides clues for mechanisms whereby omalizumab reduces asthma exacerbations and other asthma outcomes in more severe asthma. The lack of effect of omalizumab on methacholine responsiveness suggests that IgE or eosinophils may not be causally linked to airway hyperresponsiveness to methacholine in mild to moderate asthma.

Keywords: eosinophils; high-affinity Fc receptor for IgE, FcεRI; interleukin-4

A significant body of evidence from epidemiologic studies, *in vivo* and *in vitro* studies in humans, and animal models has pointed to IgE as playing a key role in allergic asthma (1–4). Cross-linking, by allergen, of IgE bound to several cell types via high-affinity (FcεRI) or low-affinity (FcεRII) receptors induces cell activation and generation of a wide array of inflammatory mediators (4, 5) that have been strongly associated with mucosal inflammation, bronchial hyperresponsiveness, and symptoms of asthma. The potential importance of the airway mast cell, the key cell that uses IgE for its activation, in asthma has been highlighted by the discovery of its capacity to secrete an array of cytokines such as the two helper T cell Type 2 (Th2) cytokines interleukin (IL)-4 and IL-5, and tumor necrosis factor-α (6, 7).

Mast cells have also been closely associated with the bronchial smooth muscle layer and airway hyperresponsiveness (8, 9).

The first direct evidence that IgE is involved in allergic asthma was provided by studies of the effects of recombinant, humanized anti-IgE antibody (omalizumab) on laboratory-based allergen challenge. In these studies treatment with omalizumab reduced circulating free IgE (less than 50 ng/ml), increased the broncho-provocative dose of allergen, decreased the magnitude of the early fall in lung function, and improved methacholine hyperresponsiveness (10). Moreover, omalizumab reduced the late asthmatic response (11), which has been shown to be associated with inflammatory cell influx into the airways. Subsequent, large clinical trials have demonstrated the clinical efficacy of omalizumab on asthma exacerbation rates, use of corticosteroids, and symptoms in both adults and children (12–15).

The mechanisms underlying the clinical efficacy of omalizumab are unknown, but could involve inhibition of mast cell and basophil activation through a combination of reduced free IgE levels and IgE receptor downregulation (16, 17). Inhibition of the accumulation of inflammatory cells in the lungs has also been observed with anti-IgE in animal models of asthma (3, 18). Subsequent clinical evaluation showed that omalizumab reduced nasal eosinophilic inflammation during the pollen season in subjects with seasonal allergic rhinitis (19).

The aim of the present study was to determine whether omalizumab has antiinflammatory effects in the airways of patients with allergic asthma, which would considerably improve our understanding of the role of IgE in allergic asthma. We chose to conduct the study in subjects with mild to moderate disease in whom the well-described airway pathology of this category of asthma had not been modified by corticosteroid treatment. We hypothesized that the spectrum of antiinflammatory actions of anti-IgE treatment extends beyond IgE-bearing effector cells, such as mast cells and basophils, to include eosinophils, T lymphocytes, and Th2 cytokines. To test this hypothesis markers of inflammation were assessed in induced sputum and bronchial biopsies obtained by fiberoptic bronchoscopy before and after 16 weeks of subcutaneous treatment with omalizumab. Sputum eosinophilia, a typical feature of asthma that broadly correlates with asthma severity (20) and is associated with a greater risk of exacerbation (21), was chosen as the primary outcome variable. Secondary variables included mucosal eosinophils, mast cells, basophils, T lymphocytes, B lymphocytes, cells bearing surface IgE, and cells expressing IL-4 and IL-5 (two central Th2-type cytokines). Finally, in view of reports that the expression of FcεRI on circulating basophils is downregulated by anti-IgE treatment (17), the effect of omalizumab on the expression of high-affinity (FcεRI) and low-affinity (FcεRII, CD23) receptors for IgE on mucosal cells was studied.

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METHODS

Study Design

This 4-month, randomized, double-blind, placebo-controlled, parallel group study was conducted in five centers: University of California, San Francisco (San Francisco, CA); University of Southampton (Southampton, UK); National Jewish Medical and Research Center (Denver, CO); University of Wisconsin (Madison, WI); and Imperial College (London, UK). After a run-in period of 3 weeks, during which asthma activity and airway responsiveness were assessed to determine inclusion eligibility, subjects were randomized to 16 weeks of treatment with either omalizumab or placebo.

The dose of omalizumab was at least 0.016 mg/kg per IgE (IU/ml) every 4 weeks. Patients were treated subcutaneously with omalizumab (150–300 mg every 4 weeks or 225–375 mg every 2 weeks) on the basis of the concentration of serum total IgE and patient body weight at baseline (22). Before and after treatment, subjects underwent measurement of airway responsiveness to methacholine, sputum induction, and bronchoscopy with endobronchial biopsy, with a minimum 2-day interval between procedures. They returned for follow-up 12 weeks after completing treatment for data collection on adverse events and for determination, in serum, of free IgE levels and the presence of any anti-omalizumab antibodies.

The inclusion criteria were as follows: stable, mild to moderate asthma (defined by the criteria of the National Heart, Lung, and Blood Institute Expert Panel Report [23]) for at least 1 year; treatment with inhaled β_2 -agonists only; no acute exacerbations requiring rescue corticosteroid medication for at least 6 weeks before screening for the study; age, 18 to 50 years; total serum IgE of at least 30 to no more than 700 IU/ml; positive skin prick test for at least one common allergen (house dust mite, cockroach, dog, or cat); airway hyperresponsiveness as defined by a methacholine PC₂₀ value (provocative concentration inducing a 20% drop in FEV₁) of less than 8 mg/ml, and sputum eosinophilia of 2% or more of total nonsquamous cells.

The study was conducted in accordance with good clinical practice and the latest revisions to the Declaration of Helsinki. The protocol was approved by independent ethics committees/institutional review boards at each center, and subjects gave their written informed consent.

Subjects

Of 144 screened subjects, 45 subjects (24 females) aged 19 to 48 years were randomized (1:1) into the study (Table 1). Ninety-nine subjects were excluded because they had failed to meet one or more of the

inclusion criteria, the most common reason being insufficient sputum eosinophils (2% of total inflammatory cells) and serum IgE concentration exceeding the top of the therapeutic range (700 IU/ml). An exception was made for one subject whose IgE levels were 808 IU/ml. Thirty subjects were classified as having mild asthma with an FEV₁ greater than 80% predicted; 15 had an FEV₁ less than 80% predicted and were, therefore, classified as having moderately severe asthma. The majority of the subjects had never taken inhaled corticosteroids; 6.7% had taken inhaled corticosteroids within the past year, but of these subjects all had been off this treatment for at least 6 weeks before enrollment.

A computer-generated randomization scheme was used to provide balanced blocks of numbers for each of the two treatment groups within each center.

Methacholine Challenge

Airway responsiveness was measured by methacholine challenge, using a standardized dosimeter method and according to a standard operating procedure from the University of California, San Francisco, the details of which have been previously published (24). Briefly, subjects inhaled increasing concentrations of methacholine, ranging from 0.03 to 32 mg/ml, delivered via a dosimeter. Five breaths of each concentration were inhaled from functional residual capacity to total lung capacity, and FEV₁ was measured three times at 3 minutes, and the best of three values was recorded. Challenge proceeded until the FEV₁ fell to 20% or more of the postdiluent value.

Sputum Induction

Sputum induction, processing, and analysis were conducted according to a standardized protocol in all centers (11). Sputum cytology was preliminarily analyzed at individual centers and study eligibility was based on these data. All slides were subsequently reanalyzed at a single center (University of California, San Francisco) and these data were used for analysis of treatment efficacy.

Bronchoscopy and Bronchial Biopsy

Bronchoscopy was conducted according to a standardized protocol for all sites (25). Lung sampling was randomized, with the second bronchoscopy always conducted in the contralateral lung. Six bronchial biopsies were taken from subcarinae in the lower lobes and processed into glycol methacrylate resin, as previously described (26). Biopsy samples were analyzed by immunohistochemistry at the University of Southampton, using a streptavidin–biotin peroxidase detection system as previously published (26). Samples were accepted for further analysis if there was

TABLE 1. BASELINE CHARACTERISTICS OF THE TWO TREATMENT GROUPS

Characteristic	Omalizumab (n = 22)	Placebo (n = 23)
Age, yr		
Median	26	26
Range	19–44	20–48
Female sex, n (%)	16 (73)	8 (35)
Total IgE, IU/ml		
Median	155.5	141
Range	27–808	38–500
Duration of asthma, yr		
Median	14	20
Range	2–40	2–40
FEV ₁ , percentage of predicted value at screening		
Mean (SD)	84 (9.4)	86 (13.6)
Range	67–103	65–112
FEV ₁ , L		
Mean (SD)	3.04 (0.45)	3.35 (0.68)
Range	2.25–3.99	1.70–4.86
Methacholine PC ₂₀ , mg/ml		
Geometric mean	1.01	0.54
Range	0.11–8.50	0.05–3.65
Smoking history, n (%)		
Never	18 (82)	20 (87)
Ex-smoker	4 (18)	3 (13)

Definition of abbreviations: PC₂₀ = concentration inducing a 20% drop in FEV₁.

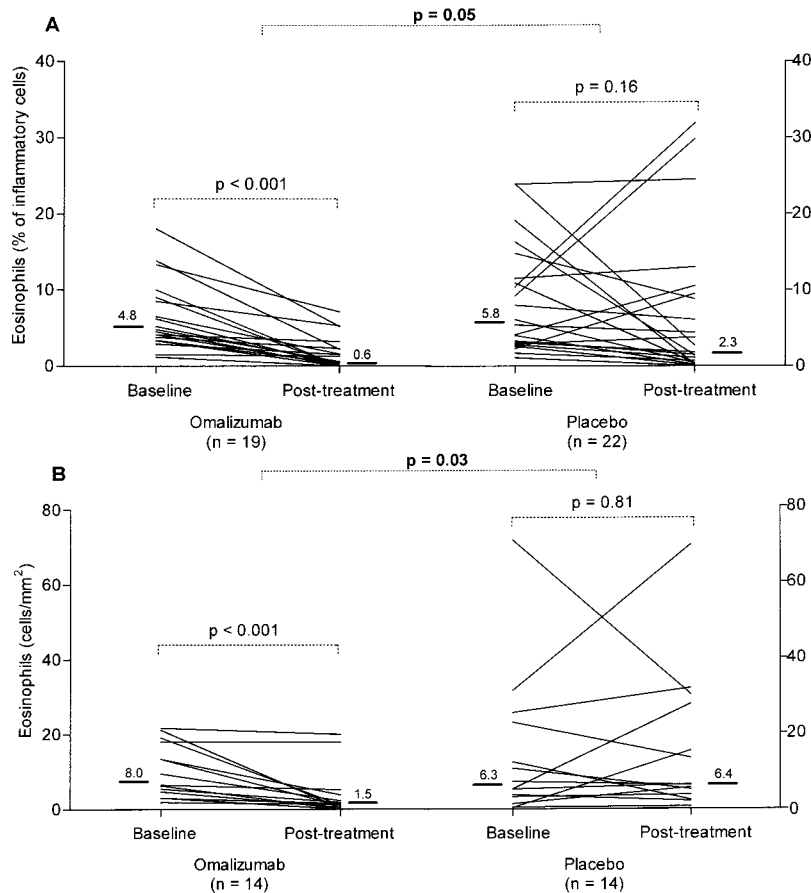


Figure 1. Effect of 16 weeks of treatment with either omalizumab or placebo on airway eosinophil counts. (A) Percentages of eosinophils in induced sputum; (B) eosinophil counts in the bronchial submucosa. Horizontal bars represent medians. *p* Values for within-group changes from baseline were calculated by Wilcoxon signed rank test. *p* Values in **boldface** are for between-group difference for change from baseline (percentage of eosinophils in induced sputum, calculated by analysis of covariance; eosinophil counts in submucosal bronchial biopsies, calculated by Wilcoxon rank sum test).

at least 0.5 mm² of analyzable area of tissue section, excluding smooth muscle and blood clots, without evidence of crushing. In addition, samples were assessed for epithelial staining if intact, well-orientated epithelium was present. Two-micrometer-thick glycol methacrylate sections were immunostained for the following: mast cells (AA1 antibody to identify tryptase-positive cells; DakoCytomation, Cambridge, UK), basophils (BB1 antibody; a gift from A. F. Walls, Southampton, UK),

eosinophils (EG2 antibody directed at eosinophil cationic protein; Pharmacia, Milton Keynes, UK), CD3⁺ T lymphocyte subset (UCHT1 for total T cells; DakoCytomation, Ely, UK), CD4⁺ T lymphocyte subset (BD Biosciences, Abingdon, UK), and CD8⁺ T lymphocyte subset (DakoCytomation). For IL-4 associated with inflammatory cells two antibodies were used: 3H4 and 4D9 (ImmunoKontakt, Abingdon, UK). The 3H4 antibody results in a cell surface, ring-staining pattern. In

TABLE 2. MEDIAN (RANGE) CHANGE FROM BASELINE IN CELL COUNTS OF THE EPITHELIAL AND SUBMUCOSAL COMPARTMENTS OF BRONCHIAL BIOPSIES AFTER 16 WEEKS OF TREATMENT WITH OMALIZUMAB OR PLACEBO

Cell Type	Epithelium (cells/mm)		Submucosa (cells/mm ²)	
	Omalizumab (n = 10)	Placebo (n = 13)	Omalizumab (n = 14)	Placebo (n = 14)
Basophils	0 (−0.77 to 0)	0 (−0.22 to 0)	−1.02 (−8.49 to 3.89)	1.07 (−9.86 to 6.83)
Mast cells	−2.06 (−4.63 to 1.66)	−0.74 (−4.81 to 4.38)	−8.77 (−20.94 to 29.42)	3.24 (−10.76 to 31.28)
Eosinophils	−0.4 (−2.44 to 0.51)	0 (−0.79 to 9.55)	−3.95* (−20.95 to −0.10)	0.35 (−40.80 to 38.47)
T lymphocytes				
CD3 ⁺	−4.13* (−17.55 to 7.98)	3.86 (−10.02 to 33.41)	−36.96† (−258.00 to 79.54)	40.52 (−50.00 to 199.09)
CD4 ⁺	−0.53 (−1.84 to 1.60)	0.47 (−2.41 to 6.98)	−27.81† (−149.15 to 56.17)	40.90 (−28.02 to 138.89)
CD8 ⁺	−0.60 (−10.27 to 4.79)	2.86 (−6.95 to 14.14)	−8.95* (−78.31 to 32.22)	15.71 (−22.07 to 35.92)
B lymphocytes (CD20 ⁺)	−0.14 (−0.91 to 0.37)	0 (−0.79 to 0.46)	−0.83* (−20.02 to 6.53)	3.36 (−11.62 to 82.09)
FcεRI	−3.3† (−8.75 to 0)	0 (−0.41 to 7.25)	−21.26† (−48.00 to 3.13)	2.44 (−16.15 to 25.65)
FcεRII	0 (0 to 0)	0 (−0.58 to 0)	−0.76 (−5.98 to 0)	−0.73 (−5.02 to 1.55)
IL-4 (cell surface)	−1.0* (−3.64 to 0)	0 (−1.08 to 0.87)	−15.28† (−37.74 to 0.29)	0 (−20.21 to 14.67)
IL-4 (cytoplasmic)	0 (−0.14 to 0)	0 (−0.14 to 0.29)	0.47 (−7.61 to 13.96)	0.72 (−8.69 to 15.26)
IL-5	0 (0 to 0)	0 (0 to 0.45)	−0.44 (−11.06 to 6.33)	1.41 (−12.22 to 18.69)
IgE	−6.22‡ (−13.28 to −0.78)	0 (−3.15 to 10.00)	−31.33‡ (−92.45 to −6.44)	6.16 (−89.39 to 35.24)

Definition of abbreviations: FcεR = Fc receptor specific for IgE; IL = interleukin.

**p* ≤ 0.05 versus placebo (Wilcoxon test).

†*p* ≤ 0.01 versus placebo (Wilcoxon test).

‡*p* ≤ 0.001 versus placebo (Wilcoxon test).

contrast, the 4D9 antibody identifies cytoplasmic, granule-associated IL-4. Cell-associated IL-5 was identified with monoclonal antibody 7 (a gift from P. Hissey, Stevenage, UK). Cell-bound IgE was stained with an antibody from DakoCytomation. Finally, specific antibodies were used to identify FcεRI (clone 3G6; Upstate Biotechnology, Lake Placid, NY) and FcεRII (clone BU38; Binding Site, Birmingham, UK).

The epithelium and submucosa were delineated with an image analyzer and cell counting was conducted in the two compartments separately. The counts were expressed as the numbers of cells per millimeter of epithelium and square millimeter of submucosa as assessed by planimetry of the delineated areas (27).

Statistical Analysis

Efficacy was evaluated, for patients who completed the study, with matched sputum and/or biopsy samples (i.e., baseline and study end). Sample size was calculated to give sufficient power to reject a two-sided test of the null hypothesis that there is no treatment difference between omalizumab and placebo with respect to sputum eosinophil counts as the primary outcome variable (significance set at 0.05). A between-treatment difference of 2.5% eosinophils was chosen for the calculation on the basis of a previous study (11) in which mean (standard deviation) post-treatment sputum eosinophil counts were 0.56 (0.5)% and 3.1 (3.05)% in the omalizumab- and placebo-treated groups, respectively. A conservative sample size of 24 subjects per group was chosen, with the aim of having 21 subjects who completed the study. This conservative sample size also ensured appropriate power (estimated, 78.5%) to test the effects of treatment on submucosal eosinophils based on power calculation using data from Humbert and coworkers (28).

Analysis of covariance was used for between-treatment analysis of the primary outcome variable, for which a sufficient number of samples was available and for which a normal distribution could be approximated. For the secondary outcome variables nonparametric analyses were performed (Wilcoxon test for paired data). This more conservative approach was adopted to take into account the lower number of analyzable biopsies. Explorative analyses, using the Spearman rank correlation, were also undertaken to determine the correlation between changes from baseline for various outcome variables.

RESULTS

Of the 45 subjects enrolled in the study, 1 subject treated with omalizumab was withdrawn because of a positive pregnancy test while another subject receiving placebo was lost to follow-up. A total of 43 subjects therefore completed the full protocol (omalizumab, $n = 21$; placebo, $n = 22$), of whom 41 had matched sputum samples (omalizumab, $n = 19$; placebo, $n = 22$). Twenty-eight subjects (omalizumab, $n = 14$; placebo, $n = 14$) had matched biopsies in which the criteria for analyzable area were met, and 23 subjects (omalizumab, $n = 10$; placebo, $n = 13$) had matched biopsies that met the criteria for epithelial analysis.

Because of a finding of thrombocytopenia associated with anti-IgE treatment in preclinical animal studies, study drug dosing was put on hold during the trial. This occurred at the end of the trial and affected a total of six randomized subjects (three in each treatment group) across all sites. The investigators, in consultation with the study sponsors, decided that only subjects who had received more than 4 weeks of treatment would undergo bronchoscopy and sputum induction after treatment and that these post-treatment investigations would occur within 2 weeks of the last injection. Of the six subjects, three had received only 4 weeks of treatment and, therefore, did not undergo either bronchoscopy or sputum induction after treatment. After completion of the study and breaking the code, these subjects were all found to have all been taking omalizumab. The other three subjects had received 8, 10, and 10 weeks of placebo treatment and underwent both bronchoscopy and sputum induction after treatment. All six subjects were included in the primary efficacy analysis according to the intent-to-treat paradigm. An analysis of data excluding these subjects (per-protocol analysis) was also

performed. The intent-to-treat and per-protocol analyses showed similar differences between treatment groups for all outcomes.

Effects on Airway Inflammation

Sputum samples that met the strict quality criteria were obtained from 21 subjects treated with omalizumab and 22 subjects treated with placebo. Among the intent-to-treat subjects, the mean percentage of eosinophils in induced sputum decreased from 6.6 to 1.7% for subjects treated with omalizumab ($p < 0.001$), but did not change significantly (from 8.5 to 7.0%) for the placebo group; the between-group difference was statistically significant when analyzed by analysis of covariance (mean absolute difference, -4.6%; $p = 0.05$) (Figure 1A).

The results of immunohistochemical analysis of bronchial biopsies are shown separately for the epithelial and submucosal compartments in Table 2. Fourteen subjects in each group had adequate paired biopsies for immunohistochemical analysis of the submucosa. The other biopsies did not meet the strict quality control criteria imposed by the protocol. Further biopsies from seven subjects could not be included for analysis of the epithelium either, because there was insufficient intact epithelium or

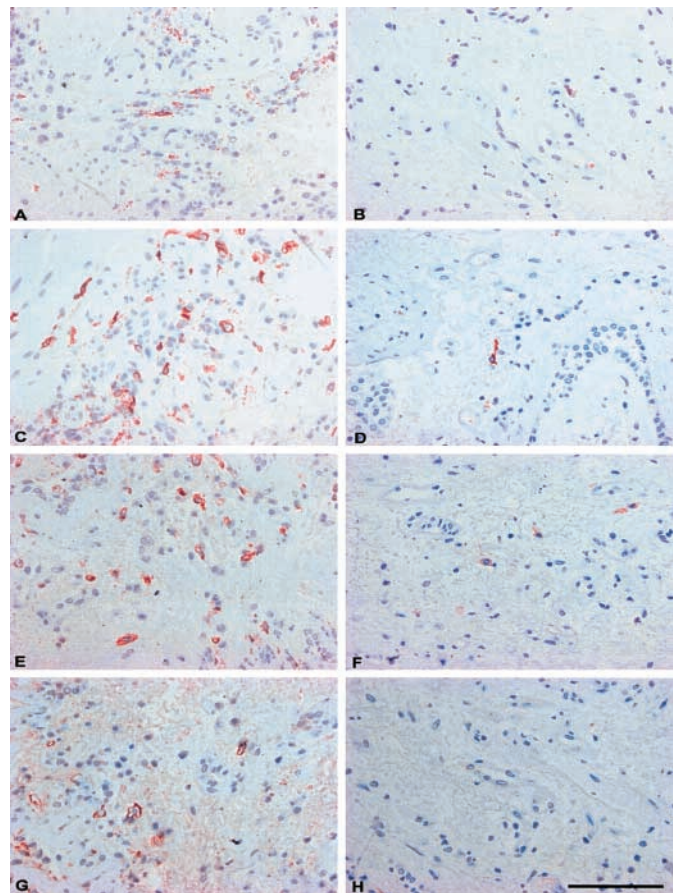


Figure 2. Immunohistochemical staining of bronchial biopsies before (left) and after (right) 16 weeks of treatment with omalizumab. Representative sections from one subject show pre- and post-treatment eosinophil staining with antibody against eosinophil cationic protein (ECP) (A and B), cell surface immunoglobulin E (IgE) (C and D), high-affinity IgE receptor (E and F) and interleukin-4 (IL-4) (G and H). Note the cytoplasmic granule-associated staining of ECP and the cell surface, ring-staining pattern obtained with antibodies for IgE, the IgE receptor, and IL-4. The latter is identified with antibody 3H4. Scale bar: 100 μ m.

the epithelium was cut tangentially. Thus, epithelial analysis could be conducted in biopsies from 10 subjects treated with omalizumab and 13 subjects treated with placebo.

The significant decrease in sputum eosinophils in the omalizumab-treated group was mirrored by a significant decrease in the numbers of eosinophils in both the epithelial and submucosal compartments. In the submucosa, the marked reduction in median (range) eosinophil counts from 8.0 (1.9 to 21.7) cells/mm² to 1.5 (0 to 20) cells/mm² ($p < 0.001$) was significantly ($p = 0.03$) different from the nonsignificant change from 6.3 (0.3 to 71) cells/mm² to 6.4 (0.7 to 69.8) cells/mm² observed in the placebo group (Figures 1B, 2A, and 2B). Significant between-group changes were also seen for IgE⁺ cells in the epithelium and submucosa ($p = 0.001$ and $p < 0.001$, respectively) (Figures 2C, 2D, and 3A); FcεRI⁺ cells in the epithelium and submucosa ($p = 0.01$ and $p < 0.001$, respectively) (Figures 2E, 2F, and 3B); cells displaying a ring-staining pattern for IL-4, using the 3H4 antibody in both the epithelium and submucosa ($p = 0.02$ and $p = 0.001$, respectively) (Figures 2G, 2H, and 4A); and numbers of epithelial and submucosal CD3⁺ T lymphocytes ($p = 0.03$ and $p = 0.01$, respectively) (Figure 5A), submucosal CD4⁺ T lymphocytes ($p = 0.005$) (Figure 5B), submucosal CD8⁺ T lymphocytes ($p = 0.05$) (Figure 5C), and submucosal B lymphocytes ($p = 0.02$) (Figure 5D). No other treatment-related effects were apparent.

There were strong and highly significant ($p < 0.001$) correlations between the reduction in biopsy (submucosal) IgE⁺ cells and FcεRI⁺ cells and between both these variables and reductions in IL-4⁺ (3H4) cells (Figure 6). There was a weaker correlation between the reduction in biopsy (submucosal) eosinophils and the reduction in cells staining for either IL-4 (3H4) or IgE

($p = 0.01$ and $p = 0.08$, respectively). There was no significant correlation between the change in eosinophils in sputum and submucosal biopsies ($p = 0.84$).

Serum free IgE Levels and Anti-omalizumab Antibodies

The six subjects affected by the clinical hold were excluded from the analysis of serum free IgE level. All omalizumab-treated patients had free IgE concentrations below 50 ng/ml (21 IU/ml) at study end, whereas no subject in the placebo group had free IgE concentrations below this level. Anti-omalizumab antibodies were not detected in any of the subjects.

Pulmonary Function

At the screening visit one patient experienced a 30.4% drop in FEV₁ with normal saline challenge and thus was not subjected to methacholine challenge. After completing treatment, and before sputum induction and bronchoscopy, the same patient had an FEV₁ measurement recorded on the case report form that was 10 times lower than previous recordings. This was, therefore, deemed a data input error and, as such, PC₂₀ and FEV₁ data from this patient were excluded from these analyses. In the omalizumab-treated group the mean $\pm$ SD baseline FEV₁ was 3.04 $\pm$ 0.45 L at baseline and 3.02 $\pm$ 0.63 L on completion of treatment ($p = 0.79$). In the placebo group the corresponding measurements were 3.35 $\pm$ 0.68 L at baseline and 3.45 $\pm$ 0.84 L on completion of treatment ($p = 0.17$). At the screening visit, the mean $\pm$ SD percentage predicted FEV₁ values were 84 $\pm$ 9.4 and 86 $\pm$ 13.6% for the omalizumab group and placebo group, respectively, and on completion of treatment were 83 $\pm$ 13.3 and 88 $\pm$ 13.9%, respectively. Similarly, there were no

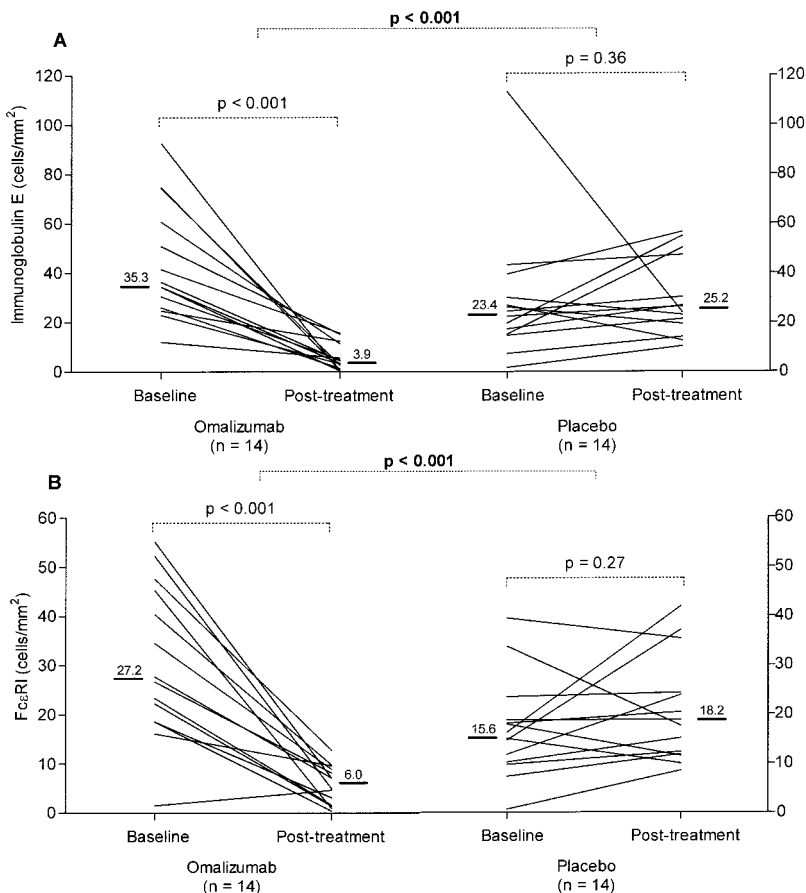


Figure 3. Effect of 16 weeks of treatment with either omalizumab or placebo on immunoglobulin E (IgE)-bearing cells and cells expressing high-affinity (FcεRI) receptors in the bronchial submucosa. (A) Cell counts positive for IgE; (B) cells expressing FcεRI receptors. Horizontal bars represent medians. *p* Values for within-group changes from baseline were calculated by Wilcoxon signed rank test; between-group difference for change from baseline (*p* value in **bold face**) was calculated by Wilcoxon rank sum test.

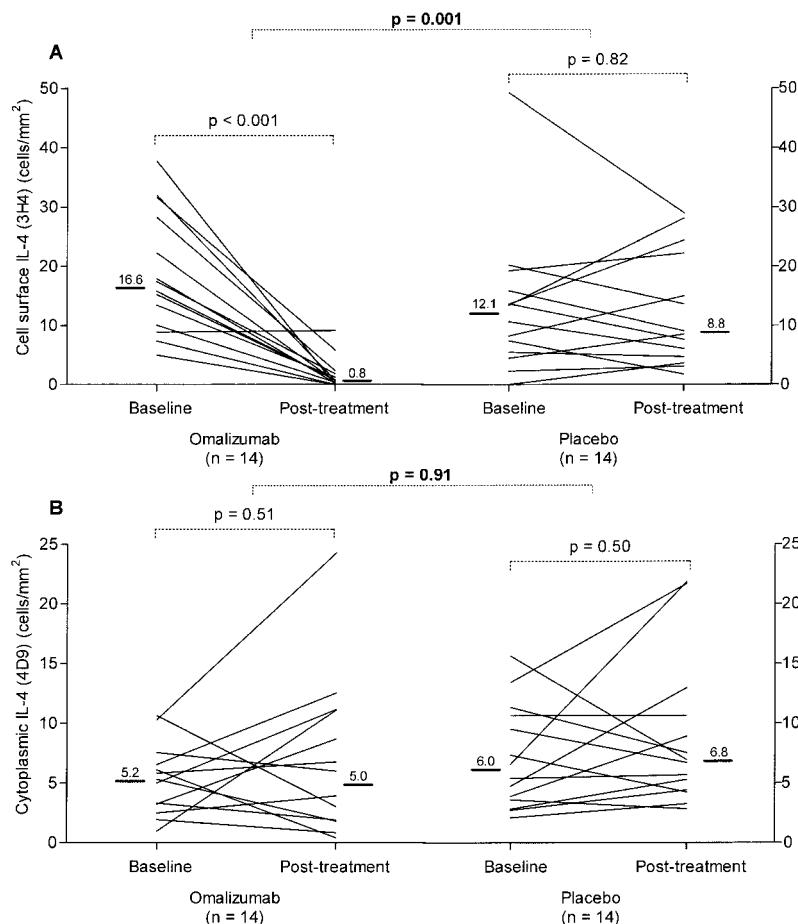


Figure 4. Counts of submucosal cells staining positively with (A) 3H4 antibody against interleukin (IL)-4, which results in a cell surface, ring-staining pattern, and (B) 4D9 antibody against IL-4, which results in cytoplasmic granule-associated staining, before and after 16 weeks of treatment with either omalizumab or placebo. Horizontal bars represent medians. *p* Values for within-group changes from baseline were calculated by Wilcoxon signed rank test; between-group difference for change from baseline (*p* value in **bold-face**) was calculated by Wilcoxon rank sum test.

significant changes in airway responsiveness ($p = 0.14$ for between-group comparison) (Figure 7). In the omalizumab group geometric mean (range) PC₂₀ methacholine changed from 1.01 (0.11 to 8.50) mg/ml at baseline to 0.73 (0.06 to 4.72) mg/ml after treatment ($p = 0.47$); corresponding values for the placebo group were 0.54 (0.05 to 3.65) mg/ml at baseline and 0.67 (0.04 to 2.23) mg/ml at study end ($p = 0.26$).

DISCUSSION

This study shows that treatment with the humanized anti-IgE antibody, omalizumab, decreases IgE in the airway mucosa of individuals with asthma as effectively as it does in the circulation. This suggests good penetration of the antibody into inflamed tissues and effective removal of IgE from both high- and low-affinity IgE receptors on inflammatory cells. The depletion of IgE from airway tissue was associated with a marked reduction in airway eosinophilia and in expression of the high-affinity IgE receptor and the central Th2 cytokine IL-4. Furthermore, treatment-related effects on CD4⁺, CD8⁺, and B lymphocyte counts were also observed, although they were not as pronounced as those of the effects on IgE, its receptors, eosinophils, and IL-4. These changes were not accompanied by measurable improvements in airway hyperresponsiveness to methacholine.

Although airway inflammation has long been considered to be central to asthma pathogenesis (29), there has been limited success to date in developing novel nonsteroidal, antiinflammatory treatments for this disease. The findings of the present study are, therefore, significant in that they show omalizumab to be the first nonsteroidal agent with major antiinflammatory activity

in the airways of individuals with allergic asthma. Indeed, this treatment led to an almost complete eradication of sputum eosinophils, which was mirrored by a similar reduction in eosinophils in bronchial biopsies, where treatment caused near depletion of eosinophils within the bronchial submucosa in all but two subjects.

Omalizumab treatment is inhaled corticosteroid sparing and is associated with reductions in asthma exacerbation rates in subjects with moderate and severe asthma in addition to positive effects on other clinical outcomes (13–15). The omalizumab-induced reductions in markers of airway inflammation described here in subjects with mild to moderate asthma provide a clue to some of the mechanisms of these beneficial clinical effects reported in more severe disease. In particular, the marked reduction in airway eosinophilia in the omalizumab-treated subgroup may explain the efficacy of omalizumab on asthma exacerbation rates. Eosinophils have long been implicated in asthma pathogenesis by way of cationic proteins and oxygen radicals that damage epithelial cells and enhance neural responses (30). They are also a source of cysteinyl leukotrienes that exert potent effects on bronchial smooth muscle, blood vessels, and mucosal glands. Airway eosinophilia is broadly related to asthma severity (20, 31) and corticosteroid treatment decreases eosinophils coincident with improvements in asthma control (27, 31–33). The mechanisms by which omalizumab decreased airway eosinophilia in this study were not specifically investigated but must involve inhibition of IgE-dependent mechanisms of eosinophil accumulation. These include allergen-induced cross-linking of IgE on the surface of effector cells, such as mast cells, causing release of eosinophil-active cytokines. One such cytokine is IL-4,

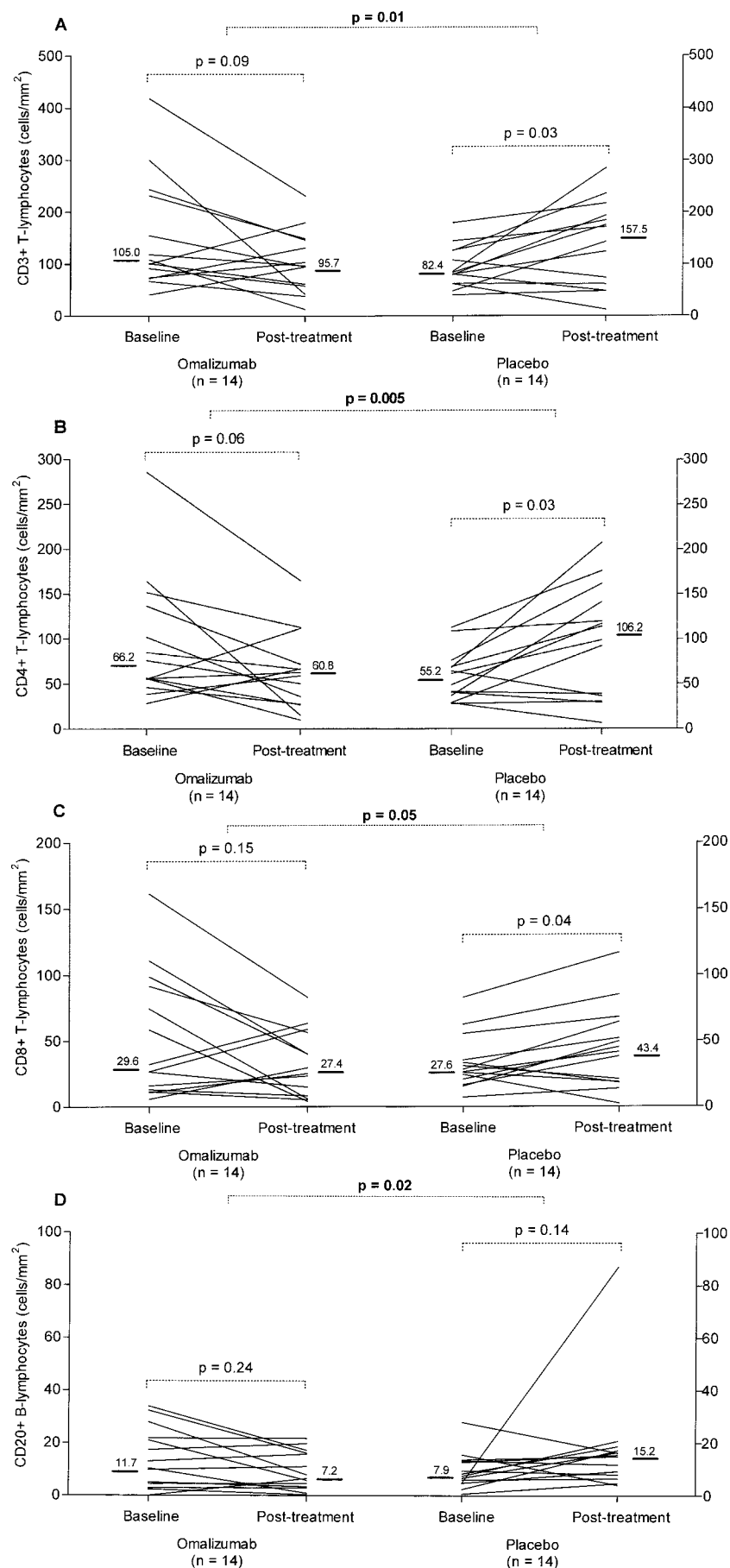


Figure 5. Submucosal counts of (A) CD3⁺ T lymphocytes, (B) CD4⁺ T lymphocytes, (C) CD8⁺ T lymphocytes, and (D) B lymphocytes (CD20⁺) before and after 16 weeks of treatment with either omalizumab or placebo. Horizontal bars represent medians. p Values for within-group changes from baseline were calculated by Wilcoxon signed rank test; between-group difference for change from baseline (p value in **boldface**) was calculated by Wilcoxon rank sum test.

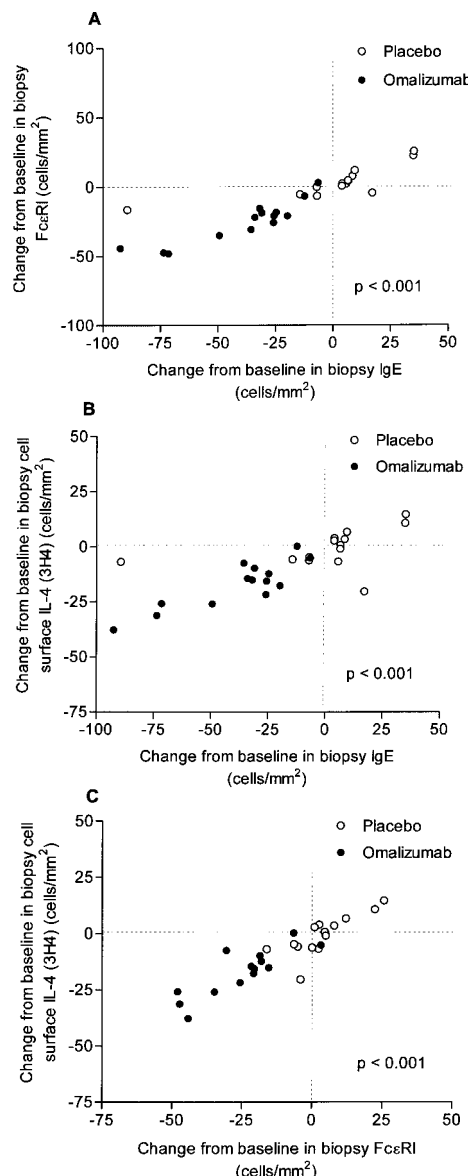


Figure 6. Correlation between change from baseline in (A) counts of immunoglobulin E (IgE)-bearing cells and cells expressing high-affinity (FcεRI) receptors, (B) cells staining positively with 3H4 antibody against interleukin (IL)-4 and counts of IgE-bearing cells, and (C) cells positive for IL-4 (3H4) and cells expressing high-affinity (FcεRI) receptors, in the bronchial submucosa after 16 weeks of treatment with either omalizumab or placebo. *p* Values from analysis of Spearman's rank correlation ($r_s = 0.92, 0.78, \text{ and } 0.89$, respectively) are all less than 0.001.

which upregulates endothelial vascular cell adhesion molecule-1 (34) and, thereby, increases endothelial adhesion of eosinophils expressing the vascular cell adhesion molecule-1 ligand. Omalizumab treatment decreased immunostaining for cell-associated IL-4 in the airways, as detected with 3H4 antibody, and this decrease correlated positively and significantly with the decrease in submucosal eosinophil number. Immunostaining with 3H4 is particularly apparent in asthma and rhinitis when compared with healthy control subjects, and increases during exposure to pollen allergens in seasonal asthma (26) suggest properties of this IL-4 antibody that are relevant to atopy and asthma and its activity. Previous studies have suggested that the cell surface, ring-staining pattern seen with 3H4 might identify secreted IL-4 (6, 35).

Whether or not this antibody detects secreted IL-4 or IL-4 that is bound to the matrix surrounding the mast cell or, indeed, the mast cell itself, remains to be elucidated. Persistent IL-4 production is a characteristic of severe asthma (36), and IL-4 is resistant to corticosteroids, raising the possibility that omalizumab treatment could provide benefit in severe asthma through reductions in airway mucosal IL-4.

Previous studies have shown that treatment with omalizumab reduces expression of the high-affinity FcεRI receptor for IgE on circulating basophils (17); the current study now demonstrates a similar potent action in the airway submucosa, but also shows the selectivity of this effect because low-affinity FcεRII levels did not change. Several cell types, including mast cells, basophils, epidermal Langerhans cells, monocytes, and dendritic cells, express FcεRI, but in the current study the extent of expression on the individual cell types was not determined. The observed reduction in FcεRI expression might not only magnify the functional consequences of IgE reduction by limiting the number of IgE molecules that can bind to effector cells, but might also influence antigen presentation. IgE receptors FcεRI and FcεRII (CD23), expressed on antigen-presenting cells, are known to increase the efficiency of antigen presentation (37, 38) and IL-4 strongly enhances this effect (39, 40). Studies have shown that the expression of FcεRI is increased in the epithelium of asthmatic airways when compared with control subjects (28, 41), providing conditions for increased antigen processing. Evidence of an IgE-dependent mechanism that involves antigen presentation via FcεRII has come from studies in the mouse model of asthma (3). Mice pretreated with neutralizing antibody to FcεRII do not develop lung eosinophilia after allergen challenge (3), suggesting that eosinophilic inflammation in this model depends on IgE–FcεRII-facilitated antigen presentation to T lymphocytes. Although omalizumab did not decrease FcεRII expression in our study, the reduction in IgE levels that resulted with treatment could still disrupt the IgE-mediated mechanisms of antigen presentation in the airways and antigen-initiated cell recruitment mechanisms. In support of a role for IgE in the processes of cell recruitment in humans, administration of anti-IgE antibody to asthmatic volunteers before allergen challenge inhibits eosinophil recruitment (11). Finally, treatment with omalizumab was found to have an effect, albeit not as pronounced as for the other variables studied, on T and B lymphocytes. The dynamics of these cells within the airways over longer periods of time are unknown, but the observed differences between the two treatment arms could be related to differences in trafficking as a consequence of allergen exposure (26) and an effect of omalizumab on the stimulatory effects of allergen.

The results of this study must be seen in the context of studies targeting IL-4 and eosinophils, both of which have been viewed as central to asthma pathogenesis and both of which are shown to be markedly reduced by anti-IgE antibody in the present study. Anti-IL-5 antibody treatment has been shown not to attenuate allergen-induced late-phase responses (42) despite a marked reduction in sputum and blood eosinophils, and has also been shown not to affect asthma clinical outcomes, at least in one small clinical trial (43). Although it has been argued that anti-IL-5 antibody can only partially reduce eosinophil counts in bronchial biopsies (44), and that sufficient numbers of eosinophils persist in the airway mucosa to cause inflammatory effects, these findings have caused a lively debate about the role of eosinophils in asthma pathogenesis (45). It is noteworthy that the marked reductions in eosinophil counts seen in our study occurred in the absence of any effect on IL-5 immunostaining in the biopsies. Also relevant are data demonstrating that a treatment strategy based on reducing sputum eosinophils improves asthma control (21). Specifically, calibrating the corticosteroid dose according

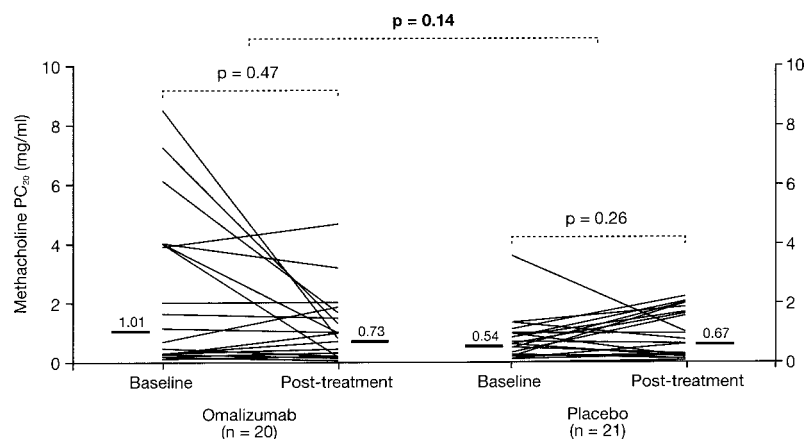


Figure 7. Effect of 16 weeks of treatment with either omalizumab or placebo on airway responsiveness (methacholine PC₂₀). Horizontal bars represent geometric means. p Values for within-group changes from baseline were calculated by Wilcoxon signed rank test; between-group difference for change from baseline (p value in **boldface**) was calculated by Wilcoxon rank sum test.

to the number of eosinophils in induced sputum as well as symptoms results in better asthma control than dosing strategies that rely on asthma symptoms alone (21). This has suggested that eosinophils do indeed have a direct role in asthma pathogenesis.

The prospect of targeting IL-4 in subjects with asthma generated considerable enthusiasm after its inhibition by soluble IL-4 receptor was shown to prevent exacerbations that resulted from acute withdrawal of inhaled corticosteroids (46, 47). However, similar to anti-IL-5 treatment, soluble IL-4 receptor was shown to have little or no efficacy in larger unpublished clinical trials. In contrast to treatments that target IL-4 and IL-5 individually, anti-IgE treatment has the potential both to inhibit IL-4 and reduce airway eosinophils. Importantly, it markedly reduces eosinophil counts both in the lumen and mucosa, which reflects action that extends beyond IL-5 and probably targets other pro-eosinophil mechanisms.

To appreciate the full implications for clinical activity of the spectrum of actions of anti-IgE noted in the present study will require further studies specifically designed and powered to correlate the antiinflammatory effects, symptoms, and airway responsiveness. Although most of the subjects with asthma studied in the present study had only mild airway obstruction, they clearly had abnormal responsiveness to methacholine inhalation, which did not improve with omalizumab treatment for 16 weeks, suggesting that, at least in subjects with milder forms of the disease, there is a dissociation of antiinflammatory actions of anti-IgE and airway hyperresponsiveness. This is a surprising result because three previously published placebo-controlled studies, in which airway responsiveness was also not a primary outcome, have suggested small improvements in PC₂₀ (10, 11, 48). One showed a statistically significant but small improvement in methacholine responsiveness (average 0.9 doubling dose) after omalizumab treatment (10); the other showed a small improvement (0.8 doubling dose), which was not statistically significant (11). The third study (48) investigated the effects of omalizumab on acetylcholine responsiveness and results were reported as percentage change from baseline. The omalizumab-treated group (n = 18) had a median improvement in acetylcholine PC₂₀ of 50% (0.5 doubling dose) after 12 weeks of treatment, an effect significantly greater than in the placebo group (n = 17). Our study is the largest to date, and we might have predicted an improvement in airway responsiveness based on the large reduction in airway eosinophilia and other markers of airway inflammation. However, airway responsiveness is a complex physiological abnormality determined by a number of factors, including remodeling of the epithelium, smooth muscles, and blood vessels as well as changes in neural and contractile responses, all of which might

require longer treatment with omalizumab. It is also dependent on soluble factors present in serum. This has been shown by sensitization of bronchial rings *in vitro* with IgE-rich sera. In keeping with the observations in our study, the addition of neutralizing nonanaphylactogenic anti-IgE antibody did not reduce nonspecific hyperresponsiveness (49).

In summary, this study shows that anti-IgE treatment in mild to moderate asthma decreases IgE levels in the airway mucosa and decreases multiple markers of airway inflammation, especially eosinophils and the high-affinity receptor for IgE. Omalizumab did not improve methacholine responsiveness in this population of subjects with asthma. The antiinflammatory effects of omalizumab provide clues to the mechanism by which omalizumab reduces asthma exacerbation rates and other asthma outcomes in more severe asthma. The dissociation between the effects of omalizumab on IgE and eosinophils on the one hand and methacholine responsiveness on the other suggests that IgE and eosinophils may not mediate methacholine responsiveness in mild to moderate asthma.

Conflict of Interest Statement: R.D. has received \$2,000 as a consultant for Aventis and £1,500 for lectures sponsored by GlaxoSmithKline and has received sponsorship for basic research from AstraZeneca, Cambridge Antibody Technology, Immunex, GlaxoSmithKline, and Bayer totaling £1,545,582 and has received £349,000 from GlaxoSmithKline for participating in two multicenter studies of the antiinflammatory effects of Advair and is one of the cofounders of Synairgen and receives £15,000 per year as a consultant; S.J.W. has received £35,715 as a research grant from Merck and has received £697 from GlaxoSmithKline and £1,166 from Novartis as sponsorship to attend conferences; M.K. has been a consultant for Genentech for the years 2002–2004 at \$3,000 per year for 3 years and Merck for the years 2001–2004 at \$2,000 per year for 2 years and has been reimbursed by Genentech for the years 2002–2004 at \$8,000 per year for 2 years, Merck, Inc. for the years 2001–2004 at \$5,000 per year for 3 years, Novartis for the years 2002–2004 at \$3,000 per year for 3 years and GlaxoSmithKline for the years 2001–2003 at \$2,500 per year for 2 years and was the principal investigator on a grant by Genentech from 2000 to 2003 with a total grant award of \$5,300; N.N.J. has received a lecture honorarium from Genentech and has research grants funded as part of a multicenter studies by GlaxoSmithKline, Dynavax Technologies, and Roche Pharmaceuticals; M.S. does not have a financial relationship with a commercial entity that has an interest in the subject matter of this manuscript; K.F.C. has been a member of scientific Advisory Boards for Novartis, GlaxoSmithKline, AstraZeneca, and Fujisawa and has received lecture fees from Novartis, GlaxoSmithKline, Altana, and Celgene and has been reimbursed by Novartis and Boehringer Ingelheim for attending scientific conferences and has received industry-sponsored research grants from Novartis, GlaxoSmithKline, Altana, and Celgene; W.B. is currently employed by Novartis pharmaceutical and holds Novartis stocks in 2004; A.F.-T. has been a permanent employee of Novartis Pharmaceuticals in the United States since 1984; J.M. was an employee of Novartis at the time the study was conducted and at the time of manuscript writing and submission and was subsequently employed by GlaxoSmithKline, which has an interest in respiratory inflammation; W.W.B. has received consultancy fees for the past 3 years from the following companies with a total consultancy fee for these 3 years as indicated: Bristol Myers Squibb (\$2,000), Dynavax (\$3,000), Hoffman LaRoche (\$2,000), Schering (\$3,000, 2002–2003), and Fujisawa (\$3,000) and has served on advisory boards in various capacities over the past 3 years (2001–2003) with the following reimbursements: GlaxoSmithKline (\$8,500), Aventis (\$2,000), Schering (\$4,000),

Pfizer (\$4,000, 2004), and AstraZeneca (\$2,000) and has also received honoraria for speaking or other educational activities in the past 3 years from Merck (\$7,000, 2003), GlaxoSmithKline (\$2,500, 2003), and Aventis (\$2,500, 2003) and has received industry-sponsored support for research from GlaxoSmithKline (\$750,000, 2002 and 2003), and for participation in multicenter trials: Fujisawa (\$250,000, 2002–2003), GlaxoSmithKline (\$500,000, 2001–2003), Aventis (\$200,000, 2001–2003), Hoffman LaRoche (\$120,000, 2002), Pfizer (\$100,000, 2003), Genentech/Novartis (\$100,000, 2002–2003), and Merck (\$100,000, 2003); S.T.H. chaired an international advisory group for Novartis in 2001 and served as consultant in relation to their regulatory submission of omalizumab to the EMEA in the same year (\$2,000 payment for each) and was paid by Novartis to give lectures at the AAAI and ERS meetings (total over 3 years, \$4,000), and received a grant in aid for discovery research, but in a nonrelated field of research and is also a member of the Merck research Laboratory Board of Scientific Advisors, Chairman of its Education and AIR Board, and until 2002 Chairman of the UK MSD Respiratory Advisory Board and undertakes consultancy work for Almirall Prodesfarma (Spain), Altana, and Cambridge Antibody Technology; J.V.F. has received \$308,180 from Novartis between 1999 and 2001 as a research grant for his center's participation in this study and has also received a research grant of \$138,034 from GlaxoSmithKline and between 2001 and 2003 has received \$7,000 in lecture fees and \$3,500 in consulting fees from various pharmaceutical and biotechnology companies with interests in asthma.

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