Airway inflammation in asthma : from concept to the clinic

Rensen, E.L.J. van


Chapter
7

Anti-IgE-induced reduction in airway responses to inhaled allergen is paralleled by decreased eosinophilia in bronchial biopsies and sputum in patients with asthma

Elizabeth L.J. van Rensen, Christine E. Evertse, W. Annemarie A.M. van Schadewijk, Gareth Ayre, Thais Mauad, Pieter. S. Hiemstra, Peter J. Sterk, Klaus F. Rabe

Submitted for publication

Abstract

Background Anti-IgE, omalizumab, has been shown to inhibit the allergen response in patients with asthma. This has not been directly related to changes in inflammation.
Objective We hypothesised that anti-IgE exerts its effects by reducing airway inflammation. To that end, the effect of anti-IgE on allergen-induced inflammation in bronchial biopsies in 25 patients with asthma was investigated in a randomised, double blind, placebo-controlled study.
Methods Allergen challenge followed by a bronchoscopy at 24h was performed at baseline and after 12 weeks of treatment with subcutaneous anti-IgE or placebo. PC_{20} methacholine and induced sputum was performed at baseline, 8 and 12 weeks of treatment. Changes in the early and late responses to allergen, PC_{20}, inflammatory cells in biopsies and sputum were compared between anti-IgE and placebo.
Results Both the early and late asthmatic response were suppressed to 15.3% and 4.7% following anti-IgE as compared with placebo ($p<0.002$). This was paralleled by a decrease in eosinophil counts in sputum (from 4% to 0.5%) and post-allergen biopsies (from 15 to 2 cells/0.1mm^2) ($p<0.03$). Furthermore, biopsy IgE+ cells were significantly reduced between both groups, whereas high-affinity IgE receptor and CD4+ cells were decreased within the anti-IgE group. There were no significant differences for PC_{20} methacholine
Conclusion The response to inhaled allergen in asthma is diminished by anti-IgE, which is paralleled by a reduction in eosinophilic inflammation in bronchial mucosa and sputum and a decline in bronchial IgE positive cell counts post-allergen without changing PC_{20} methacholine. This suggests that the benefits of anti-IgE in asthma may be explained by a decrease in eosinophilic inflammation and IgE bearing cells.

Introduction

Thirty years after it was first identified, there is substantial evidence that immunoglobulin E (IgE) plays a key role in allergic asthma (1). In a population-based study, Burrows *et al.* were the first to show a strong association between serum IgE levels and self-reported asthma (2). Furthermore, high levels of circulating IgE have been shown to correlate with the risk of emergency room admissions in patients with asthma (3). IgE induces mediator release of mast cells and basophils via binding to high-affinity receptors, and thereby leads to activation of immune responses (4). Taken together, this makes immunomodulation of IgE an interesting strategy for new therapeutic interventions in asthma (5).

A recombinant, humanized, monoclonal antibody directed against IgE has been developed. This antibody decreases the levels of circulating IgE by binding to the constant region of the IgE molecule, which prevents free IgE from interacting with high- and low-affinity IgE receptors (FcϵRI and FcϵRII) (6). Anti-IgE has been found to decrease IgE levels and downregulate FcϵRI expression on basophils and dendritic cells in blood (7;8).

The first clinical studies with intravenous anti-IgE have shown that both the early (EAR) and late (LAR) asthmatic response to inhaled allergen are attenuated in patients with asthma (9;10). Subsequent large phase 3 trials, involving both pediatric and adult patients with moderate to severe asthma, have demonstrated the clinical beneficial effect of subcutaneous anti-IgE treatment by improving asthma control (11-13). A recent double-blind, placebo-controlled study confirmed the effectiveness of anti-IgE treatment in inadequately controlled severe persistent asthma by showing a reduction in exacerbation rate and emergency room visits and an improvement in quality of life and morning peak flow (14).

The anti-inflammatory activity of anti-IgE was established in a biopsy study of mild asthmatic patients (15). Following 16 weeks treatment with anti-IgE significant reductions in IgE positive cells and eosinophils in the bronchial mucosa were found (15). It remains unclear whether the inhibition of the allergen response by anti-IgE treatment can be explained by a reduced airway inflammation.

The aim of the present study was to determine whether treatment with anti-IgE decreases the early and late responses to inhaled allergen and whether this is associated with a reduced allergen-induced airway inflammation in bronchial biopsies. Furthermore, the effect of anti-IgE on peak flow, airway hyperresponsiveness and inflammatory cells in sputum was investigated in patients with asthma in a randomized, double blind, placebo-controlled study.

Table 1. Patient characteristics

characteristic	anti-IgE (n=12)	placebo (n=13)
age (years)	20.5 (18-24)	21 (19-29)
female sex, n (%)	12 (100)	10 (77)
smoking history, n (%)		
never	11 (92)	12 (92)
ex-smoker	1 (8)	1 (8)
duration of asthma (years)	10.5 (1-19)	9.0 (4-22)
total IgE (IU/ml)	154 (51-674)	321 (35-593)
FEV_1 (%pred)	96.0 (82-115)	88.8 (72-114)
PC_{20} methacholine (mg/ml)*	0.48 (1.61)	1.02 (1.93)

Data are presented as median (range); * g-mean (SD in dd);
There were no significant differences between the groups.

Methods

Subjects

Twenty-five non-smoking asthmatic volunteers (18-29 years) participated in the study [Table 1]. All patients had a history of episodic chest tightness and wheezing and were only using short-acting $ß_2$-agonists on demand. All were atopic to house dust mite (HDM) and were having a total serum IgE between 30 and 700 IU/ml. The baseline forced expiratory volume (FEV_1) was > 70% predicted (16) and all subjects were hyperresponsive to inhaled methacholine (provocative concentration causing a 20% fall in FEV_1 (PC_{20}) <4 mg/ml) (17). The fall in FEV_1 during the late asthmatic response (LAR) following inhaled allergen was at least 15%. All patients were clinically stable and had no respiratory chest infection 2 weeks prior to the study. The study was approved by the medical ethics committee of the Leiden University Medical Center and all volunteers gave a written informed consent.

Design

This study had a randomized, placebo-controlled, parallel, double-blind design. Anti-IgE or placebo was administered for 12 weeks every 2 or 4 weeks. At baseline, after 8 and 12 weeks of treatment, PC_{20} methacholine was determined and sputum induced. Allergen challenge followed by a bronchoscopy at 24 hours was performed at baseline and 12 weeks.

Treatment

The dose (150 mg to 375 mg) and frequency (every 2 or 4 weeks) of treatment was determined by weight and baseline total serum IgE level of each patient and had to be al least 0.016 mg/kg per IgE (IU/ml) (18). A research nurse who was not involved in any other measurement of the study administered the subcutaneous anti-IgE or placebo.

Diary cards

Patients kept diary cards from 2 weeks prior to and during the 12 weeks of the study. Morning and evening pre-bronchodilator peak flow measurements were recorded. Mean PEF values of the 2 weeks prior to baseline measurements of the study and the mean PEF values of the 2 weeks prior to the 12 week-measurements of the study were used in the analysis.

Spirometry and Airway hyperresponsiveness

Patients were not allowed to take any short-acting ß2-agonists for at least 8 hours prior to spirometry. A standardized methacholine challenge was applied to determine airway hyperresponsiveness (17). To determine the PC_{20} methacholine, patients inhaled increasing doses of methacholine for 2 minutes until a fall of at least 20% in FEV_1 had been reached.

Sputum induction and processing

Prior to induction, each subject inhaled 200 µg salbutamol. Sputum was induced by inhalation of NaCl 4.5% during 3x5 minutes intervals, according to a recommended protocol validated in our laboratory (19;20).
Sputum samples were processed according to the whole sample method. Differential cell counts were expressed as a percentage of 250 non-squamous cells (20).

Allergen challenge

Allergen challenges were performed according to a standardized protocol (17;21). Purified aqueous allergen extract of *Dermatophagoides pteronyssinus* (SQ 503; Vivodiagnost, ALK, Benelux), with 0.5% phenol as a preservative, was diluted ranging from 2,000-15.63 BU/ml. PC_{20} allergen was predicted from PC_{20} methacholine and skin-test sensitivity, derived from a multi-dose skin prick test, according to Cockcroft's method (21). Starting three concentrations below the predicted PC_{20} allergen, 3 ml of consecutive doubling concentrations of allergen were aerosolized for 2 minutes using a DeVilbiss 646 nebulizer (output 0.13 ml/min). The response to allergen was determined by measuring FEV_1 in duplicate 10 min after each inhalation of allergen. After reaching a fall of at least 20%, FEV_1 measurements were repeated 10, 20, 30, 40, 50, 60, 90, and 120 minutes and then hourly until 7 hours after the last inhalation. In the analysis, EAR (from 0-3 hours post-allergen) and LAR (from 3 to 7 hours post-allergen) were defined as the maximum % fall in FEV_1 from baseline and as the area under the time-response curve (AUC) (17). Patients received exactly the same allergen dose at the end of the study as they inhaled during the baseline allergen challenge.

Bronchoscopy and immunohistochemistry

Fiberoptic bronchoscopy was performed according to a standardized and validated protocol (22). Six biopsy specimens were taken at (sub)segmental level from either the right lung (first bronchoscopy; right lower lobe and/or the middle lobe) or the left lung (second bronchoscopy; lingula and left lower lobe). Two biopsies were immediately frozen and stored at –80°C.

The remaining 4 biopsies were fixed for 24 hrs in buffered formalin and paraffin embedded. Three µm thick, HE stained slides were used for checking biopsies quality (size, crushing, epithelial and mucosal representation) and the two technically best biopsies were selected for immonuhistochemistry. Slides were immunostained for IgE, high and low affinity IgE receptor (FcϵRI and FcϵRII), eosinophils (EG2), mast cells (AA1), neutrophil elastase (NE), macrophages (CD68), and T lymphocytes CD3, CD4, and CD8. In short, the sections were incubated with an optimal dilution of the primary antibodies in 1% BSA/PBS at room temperature for 60 min. As a secondary antibody, the horseradish peroxidase conjugated anti-mouse or anti-rabbit EnVision system (DAKO, Glostrup, Denmark) was used, with NovaRED (Vector, Burlingame, CA) as the chromagen. The sections were counterstained with Mayer's hematoxylin (Klinipath, Duiven, the Netherlands). For negative controls, the first antibody was omitted from this procedure. All biopsies were coded and sections analysed in a blinded fashion, using a fully automated image analysis system (23). Images were digitized using a three-chip colour camera (433.10^3 pixels, 660x496µm^2, 3x256 grey values) (KS-400 System, Kotron/Zeiss). The whole available area of lamina propria was determined by manually delineating the basement membrane. Lamina propria was defined by the widest possible 125 µm deep zone beneath the basement membrane of at least 86,000 µm^2. The automated counting of the number of positively staining cells consisted of the following steps: level off background noise, normalize staining intensity, delete noise, fuse stained fragment, delineate stained clusters, determine cell counting by an algorithm. This method has been shown to be fully reproducible and to have good agreement with interactive cell counting (23). Data were expressed as cells/0.1mm^2.

Analysis

The PC_{20} methacholine, cell counts in sputum and biopsies were log transformed before statistical analysis. All data are presented as median (range), except for PC_{20} methacholine which is presented as geometric mean (SD expressed as doubling dose). Paired t-test was applied to test for changes within groups, whereas unpaired t-test was used for changes from baseline to end between anti-IgE and placebo treatment groups. A p-value of < 0.05 was considered as statistical significance and all analyses were performed using SPSS 12.0.

Results

From one patient in the placebo group, no biopsies were obtained during the second bronchoscopy. Consequently, a total of 24 patients completed the study (anti-IgE: n=12; placebo: n=12). Three patients in the anti-IgE group and 4 patients in the placebo group did not produce sputum at one of the time points. Matched sputum samples were therefore obtained from 18 patients (anti-IgE: n=9; placebo: n=9).
Baseline FEV_1 was 96.3% predicted in anti-IgE group and 90.8% predicted in the placebo group. There were no significant changes for FEV_1 within or between groups during the study ($p>0.07$).

Diary cards

Morning PEF was significantly increased from 383.6 L/min (314.6-469.2) at baseline to 430.0 L/min (325.8-503.3) after 12 weeks of anti-IgE (p=0.038). This increase was significantly different from the change in the placebo group (398.5 L/min (342.1-613.8) to 392.1 L/min (349.3-608.5) (p=0.53 for the change within the placebo group) (p=0.041 for the change from baseline between anti-IgE and placebo groups). Similar improvements were observed for evening PEF [Figure 1].

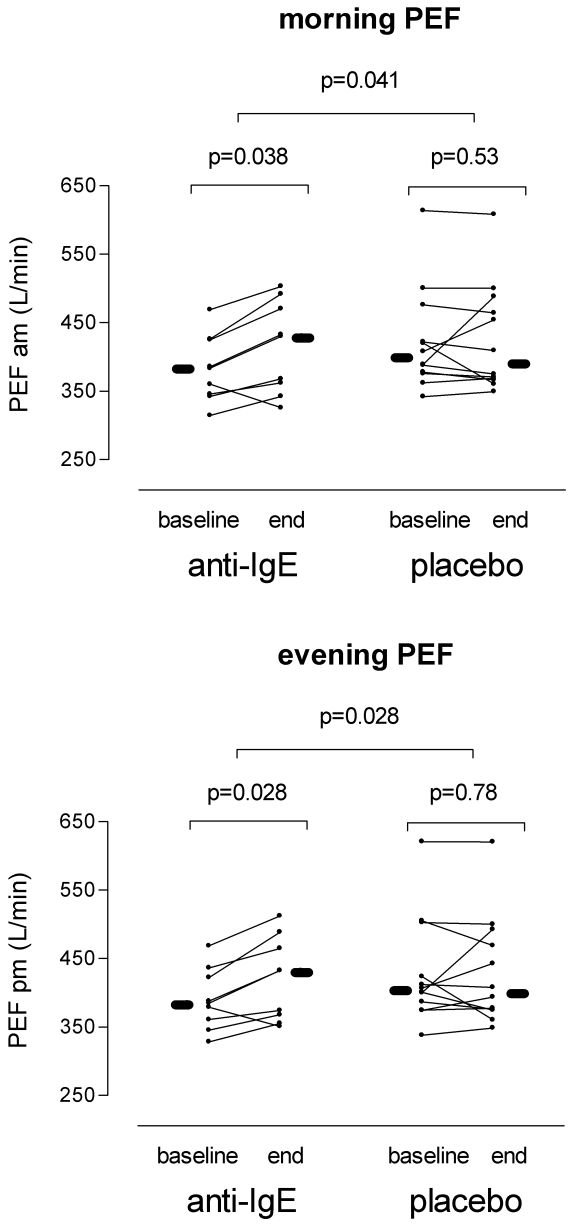

Figure 1. PEF
Individual values of morning (top panel) and evening (bottom panel) PEF at baseline and end of the study in anti-IgE and placebo treated patients.

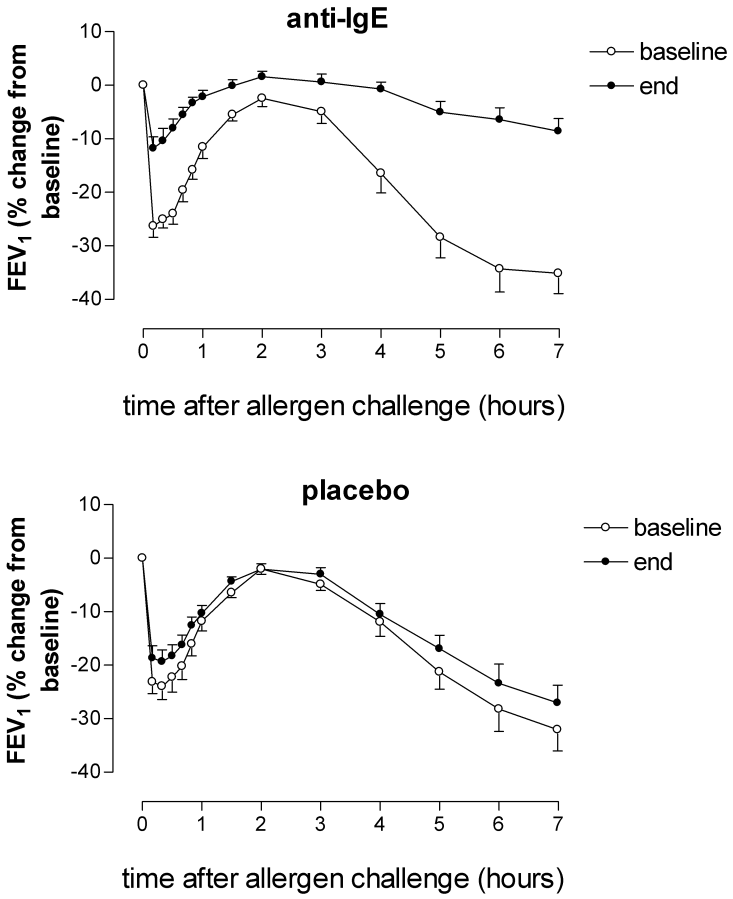

Figure 2. Allergen challenge
Airway responses to inhaled allergen between 0 to 7 hours following allergen challenge for the anti-IgE group (top panel) and for the placebo group (bottom panel). In the anti-IgE group, EAR and LAR were significantly reduced from baseline (open circles) to end (closed circles), which was also significant between the groups.

Allergen Challenge

At baseline, the maximum % fall in FEV_1 during the EAR was (median (range)) 28.9% (19.4-38.6) for the anti-IgE group and 27.0% (20.3-35.9) for placebo. Anti-IgE treatment significantly inhibited the EAR to 15.3% (0.0-23.8) (p=0.000). This change was significantly larger as the change in the placebo group (p=0.002). The AUC of the EAR was also significantly reduced following anti-IgE treatment (28.0 percentage fall per hour (%fall*h) (2.7-49.3) to 15.3 %fall*h (0.0-23.8)) as compared to placebo (33.5 %fall*h (14.2-41.9) to 23.2 %fall*h (6.0-37.1) (p=0.002) [Figure 2].

The maximum % fall during the LAR was suppressed following anti-IgE treatment from 36.1% (18.9-52.7) to 4.7% (0.0-20.6). This reduction was significantly larger as compared with placebo (31.1% (12.8-54.0) to 25.4% (14.0-48.2)) (p=0.000 for the change from baseline between anti-IgE and placebo). In addition, the reduction in AUC of the LAR was significantly larger in the anti-IgE group (95.6 %fall*h (13.9-182.9) to 9.3 %fall*h (-23.0-52.8) as compared with the placebo group (89.9 %fall*h (12.4-152.2) to 56.0 %fall*h (25.2-122.9) (p=0.000) [Figure2].

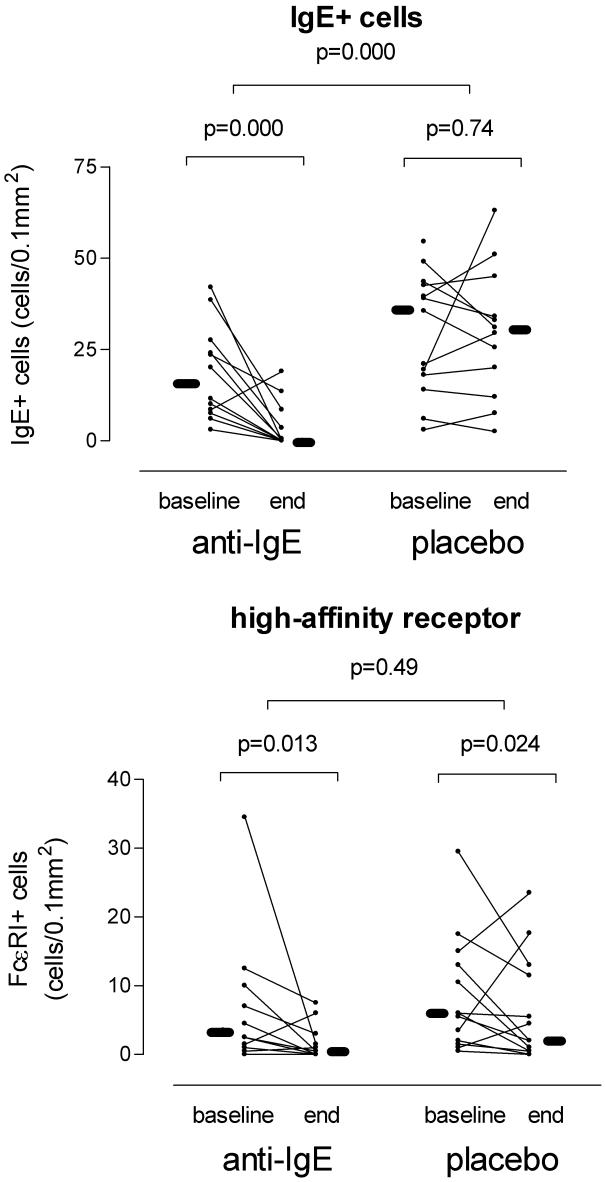

Figure 3. Biopsy IgE+ and FcϵRI+ cells
Individual values of IgE+ (top panel) and FcϵRI+ cells (bottom panel) at baseline and end of the study in anti-IgE and placebo treated patients.

Bronchial biopsies

Anti-IgE treatment markedly reduced the submucosal IgE+ cells from 15.8 cell/0.1mm^2 (3.0-42.0) to 0.0 (0.0-19.0) (p=0.000). This reduction was significantly larger as compared with the placebo group (35.5 cell/0.1mm^2 (3.0-54.5) to 30.3 (2.5-63.0) (p=0.000 for changes from baseline between anti-IgE and placebo) [Figure 3]. FcϵRI+ cells were also significantly reduced within the anti-IgE group (p=0.013). However this change was not significantly different from the change in the placebo group (p=0.49 for the change from baseline between anti-IgE and placebo) [Figure 3]. Most biopsy samples did not show any FcϵRII+ cells and no changes following treatment were observed (p>0.18).

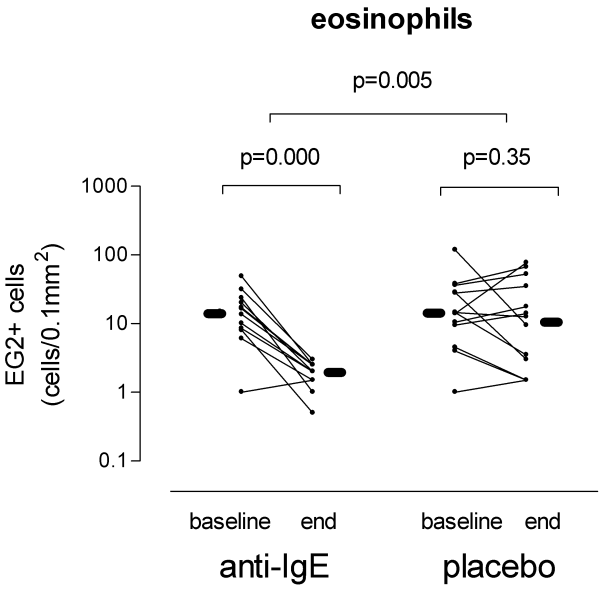

Figure 4. Biopsy eosinophils
Individual values of eosinophils at baseline and end of the study in anti-IgE and placebo treated patients.

There was a clear reduction in eosinophil counts in the anti-IgE group (15.0 cells/0.1mm^2 (1.0-48.5) to 2.0 cells/0.1mm^2 (0.5-3.0)) (p=0.000), which was significantly different from the placebo group (14.5 cells/0.1mm^2 (1.0-118.5) to 11.0 cells/0.1mm^2 (1.5-77.0) (p=0.005 for the change from baseline between anti-IgE and placebo) [Figure 4].
There were no significantly differences between anti-IgE and placebo observed for mast cells, macrophages, neutrophil elastase and B-lymphocytes (p>0.09). CD4+ T-lymphocytes were significantly reduced after anti-IgE treatment (p=0.021), however, there were no significant between-group differences for the T-lymphocytes (p>0.14).

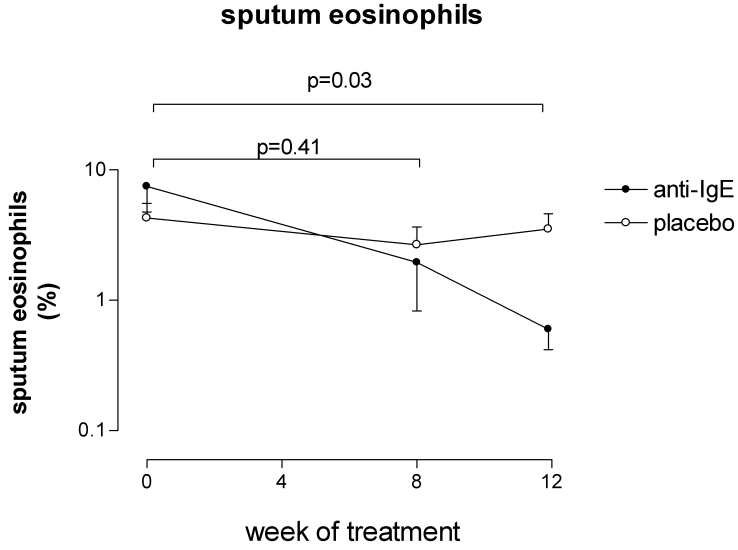

Figure 5. Sputum eosinophils
Change in sputum eosinophils from baseline, to 8 weeks and at the end of the study for anti-IgE (closed circles) and placebo (open circles).

Sputum

The median (range) % sputum eosinophil decreased in the anti-IgE treated group from 4.0% (0.2–28.0) at baseline, to 0.8% (0.2-10.8) after 8 weeks and to 0.5% (0.0-1.6) at the end of the study (after 12 weeks of treatment). This change was significantly different with placebo (baseline: 2.2% (0.4-10.2); 8 weeks: 1.0% (0.2-10.8); 12 weeks: 2.6% (0.4-13.8) after 12 weeks of treatment (p=0.03 for the change from baseline between anti-IgE and placebo) [Figure 5].

None of the other cell types in induced sputum showed any significant changes.

Airway hyperresponsiveness

PC_{20} methacholine was measured (geometric mean (SD expressed as doubling dose) 0.48 (1.61) at baseline, 0.94 (1.89) after 8 weeks and 1.04 (2.06) after 12 weeks in the anti-IgE group and 1.02 (1.93), 1.30 (2.16), 1.90 (2.26) in the placebo. There were no significant differences for the changes between the groups (p>0.18) [Figure 6].

Discussion

The results of this study show that anti-IgE treatment leads to a marked reduction of eosinophil counts in sputum and biopsies and IgE+ cells in biopsies. This suppression of inflammation is paralleled by a clear inhibition of both early and late response to inhaled allergen and a significant improvement in morning and evening PEF rates. However, PC_{20} methacholine was not changed by anti-IgE treatment. These findings indicate that the blunting of the allergen response by anti-IgE may be explained by a decrease in eosinophilic inflammation and IgE bearing cells. Interestingly, our results suggest that airway hyperresponsiveness to methacholine in atopic asthma is independent from IgE.

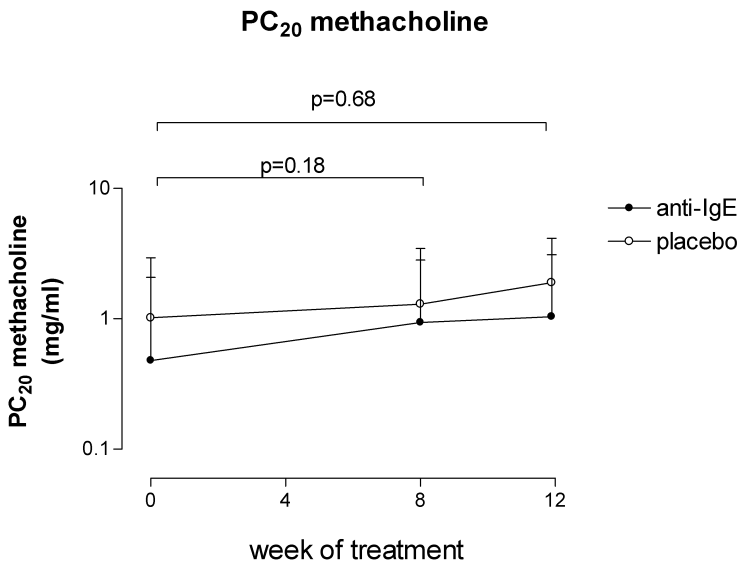

Figure 6. Airway hyperresponsiveness Change in PC_{20} methacholine from baseline, to 8 weeks and at the end of the study for anti-IgE (closed circles) and placebo (open circles).

To our knowledge, this is the first clinical study with anti-IgE demonstrating that the effects on allergen-induced airway responses are paralleled by a reduction in airway inflammation in patients with asthma. Our functional outcomes are in line with others who showed the inhibitory effect of anti-IgE treatment on early and late asthmatic response (24;25). Our results indicate that the major anti-inflammatory effects of anti-IgE are based on a marked fall in eosinophils and local IgE production in the airways. This extends the effects shown on nasal inflammation in patients with rhinitis and on airway inflammation in patients with asthma (26;27). Like Djukanovic *et al.*, we have demonstrated the anti-inflammatory effect of anti-IgE treatment in bronchial biopsies (26). In contrast with their study, we showed also a clear clinical beneficial effect of anti-IgE on morning and evening PEF and on response to inhaled allergen (26).
In the present study, airway hyperresponsiveness was not improved following anti-IgE treatment as compared with placebo. Our results are in keeping with previous studies also showing no or marginal effects on PC_{20} methacholine (24-26;28).

We do not believe that our data were influenced by measurement errors, since we used validated and reproducible methods (16;17;20;22;23). All subjects in this study were carefully selected non-smokers with stable, atopic, mild-intermittent asthma, who had not used inhaled steroids for at least 1 month prior to the study. In order to compare the allergen-induced inflammation in the bronchial biopsies, the allergen challenge at the end of study was performed in a similar manner to the baseline allergen challenge.

What is the mechanism by which anti-IgE treatment gives such a marked reduction in eosinophilic inflammation? Via cross-linking with high-affinity receptors on mast cells, IgE induces the release of preformed mediators, such as histamine and thereafter the release of newly formed mediators and cytokines such as TNF-α, IL-4 and IL-5, leading to the accumulation of eosinophils (4). Treatment with anti-IgE is likely to intervene in this mechanism. Indeed, a reduction in cell surface IL-4 following anti-IgE treatment has been demonstrated (15). A second mechanism, by which anti-IgE affects eosinophils, might be related to the inhibitory effects of anti-IgE on dendritic cell FcεRI expression (8). It has been proposed that anti-IgE can alter allergen presentation by dendritic cells (1). Anti-IgE treatment may decrease the uptake and presentation of allergens by dendritic cells, leading to a reduced T cell response, which may diminish eosinophilic airway inflammation (29).
Interestingly, we have shown that the effect of anti-IgE is much larger for the late asthmatic response than for the early response. The EAR is predominantly mediated by IgE-triggered mast cell mediator release of histamine and tryptase (30). Free IgE is markedly reduced following anti-IgE treatment, the number of mast cells was not changed in our study. Apparently, it is not the number of mast cells that is important, but it is whether they are loaded with IgE. Furthermore, resulting from the minimal amount of IgE that is probably available after treatment, cross-linking with high-affinity receptors still might occur and thereby leading to an early asthmatic response.

Airway hyperresponsiveness is an important feature of patients with asthma (31), but anti-IgE treatment did not appear to affect it. We cannot exclude that airway hyperresponsiveness might improve after longer treatment with anti-IgE, since our patients were only treated for 12 weeks. Results of therapy with anti-IL5, which also results in an abolishment of eosinophils, have shown no effect on airway hyperresponsiveness either (32). These findings confirm the dissociation of eosinophils and airway hyperresponsiveness in asthma as observed in some animal models (33). Treatment with inhaled steroids, in contrast, reduces both eosinophils and airway hyperresponsiveness (34). Possibly, inhaled steroids not only affect eosinophilic inflammation, but also affects the functional properties of airway smooth muscle, whereas the effects of anti-IgE treatment are mediated through anti-inflammatory properties alone.

Our findings may have several clinical implications. First, we have shown that PEF, allergen-induced airway responses and airway inflammation are strongly reduced after anti-IgE treatment within one study. This implies that indeed anti-IgE exerts its clinical beneficial effects by reducing airway inflammation and thereby might be an effective therapy for asthma. On the other hand, anti-IgE treatment had no effect on airway hyperresponsiveness in our study. We have previously shown that treatment aimed at reducing airway hyperresponsiveness leads to more effective control and thereby a reduction of exacerbations in patients with asthma (35). Therefore, the inability of anti-IgE treatment to normalize airway hyperresponsiveness requires further investigation.

In conclusion, treatment with anti-IgE inhibits both allergen-induced airway response and airway inflammation in patients with asthma. We have shown that the most important anti-inflammatory effect of anti-IgE is on reducing eosinophilic inflammation and IgE-bearing cells. The suggestion that airway hyperresponsiveness appears to be independent of IgE is of interest with respect to the role of anti-IgE as a new therapy for asthma.

References

1 Holgate S, Casale T, Wenzel S, Bousquet J, Deniz Y, Reisner C. The anti-inflammatory effects of omalizumab confirm the central role of IgE in allergic inflammation. J Allergy Clin Immunol 2005; 115(3):459-465.

2 Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. N Engl J Med 1989; 320(5):271-277.

3 Pollart SM, Chapman MD, Fiocco GP, Rose G, Platts-Mills TA. Epidemiology of acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits. J Allergy Clin Immunol 1989; 83(5):875-882.

4 Platts-Mills TA. The role of immunoglobulin E in allergy and asthma. Am J Respir Crit Care Med 2001; 164(8 Pt 2):S1-S5.

5 Stokes J, Casale TB. Rationale for new treatments aimed at IgE immunomodulation. Ann Allergy Asthma Immunol 2004; 93(3):212-217.

6 Presta LG, Lahr SJ, Shields RL, Porter JP, Gorman CM, Fendly BM et al. Humanization of an antibody directed against IgE. J Immunol 1993; 151(5):2623-2632.

7 MacGlashan DW, Jr., Bochner BS, Adelman DC, Jardieu PM, Togias A, McKenzie-White J et al. Down-regulation of Fc(epsilon)RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. J Immunol 1997; 158(3):1438-1445.

8 Prussin C, Griffith DT, Boesel KM, Lin H, Foster B, Casale TB. Omalizumab treatment downregulates dendritic cell FcepsilonRI expression. J Allergy Clin Immunol 2003; 112(6):1147-1154.

9 Boulet LP, Chapman KR, Cote J, Kalra S, Bhagat R, Swystun VA et al. Inhibitory effects of an anti-IgE antibody E25 on allergen-induced early asthmatic response [see comments]. Am J Respir Crit Care Med 1997; 155(6):1835-1840.

10 Fahy JV, Fleming HE, Wong HH, Liu JT, Su JQ, Reimann J et al. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. Am J Respir Crit Care Med 1997; 155(6):1828-1834.

11 Busse W, Corren J, Lanier BQ, McAlary M, Fowler-Taylor A, Cioppa GD et al. Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. J Allergy Clin Immunol 2001; 108(2):184-190.

12 Milgrom H, Fick RB, Jr., Su JQ, Reimann JD, Bush RK, Watrous ML et al. Treatment of allergic asthma with monoclonal anti-IgE antibody. rhuMAb-E25 Study Group [see comments]. N Engl J Med 1999; 341(26):1966-1973.

13 Soler M, Matz J, Townley R, Buhl R, O'Brien J, Fox H et al. The anti-IgE antibody omalizumab reduces exacerbations and steroid requirement in allergic asthmatics. Eur Respir J 2001; 18(2):254-261.

14 Humbert M, Beasley R, Ayres J, Slavin R, Hebert J, Bousquet J et al. Benefits of omalizumab as add-on therapy in patients with severe persistent asthma who are inadequately controlled despite best available therapy (GINA 2002 step 4 treatment): INNOVATE. Allergy 2005; 60(3):309-316.

15 Djukanovic R, Wilson SJ, Kraft M, Jarjour NN, Steel M, Chung KF et al. Effects of treatment with anti-immunoglobulin E antibody omalizumab on airway inflammation in allergic asthma. Am J Respir Crit Care Med 2004; 170(6):583-593.

16 Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society [see comments]. Eur Respir J Suppl 1993;16:5-40.

17 Sterk PJ, Fabbri LM, Quanjer PH, Cockcroft DW, O'Byrne PM, Anderson SD et al. Airway responsiveness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Report Working Party Standardization of Lung Function Tests, European

Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993 Mar;16:53-83.
18 Hochhaus G, Brookman L, Fox H, Johnson C, Matthews J, Ren S et al. Pharmacodynamics of omalizumab: implications for optimised dosing strategies and clinical efficacy in the treatment of allergic asthma. Curr Med Res Opin 2003; 19(6):491-498.
19 Fahy JV, Liu J, Wong H, Boushey HA. Cellular and biochemical analysis of induced sputum from asthmatic and from healthy subjects. Am Rev Respir Dis 1993;147(5):1126-1131.
20 in 't Veen JC, de Gouw HW, Smits HH, Sont JK, Hiemstra PS, Sterk PJ et al. Repeatability of cellular and soluble markers of inflammation in induced sputum from patients with asthma. Eur Respir J 1996;9(12):2441-2447.
21 Cockcroft DW, Murdock KY, Kirby J, Hargreave F. Prediction of airway responsiveness to allergen from skin sensitivity to allergen and airway responsiveness to histamine. Am Rev Respir Dis 1987; 135(1):264-267.
22 Sont JK, Willems LN, Evertse CE, Hooijer R, Sterk PJ, Van Krieken JH. Repeatability of measures of inflammatory cell number in bronchial biopsies in atopic asthma. Eur Respir J 1997; 10(11):2602-2608.
23 Sont JK, De Boer WI, Van Schadewijk WA, Grunberg K, Van Krieken JH, Hiemstra PS et al. Fully automated assessment of inflammatory cell counts and cytokine expression in bronchial tissue. Am J Respir Crit Care Med 2003; 167(11):1496-1503.
24 Boulet LP, Chapman KR, Cote J, Kalra S, Bhagat R, Swystun VA et al. Inhibitory effects of an anti-IgE antibody E25 on allergen-induced early asthmatic response [see comments]. Am J Respir Crit Care Med 1997; 155(6):1835-1840.
25 Fahy JV, Cockcroft DW, Boulet LP, Wong HH, Deschesnes F, Davis EE et al. Effect of aerosolized anti-IgE (E25) on airway responses to inhaled allergen in asthmatic subjects. Am J Respir Crit Care Med 1999; 160(3):1023-1027.
26 Djukanovic R, Wilson SJ, Kraft M, Jarjour NN, Steel M, Chung KF et al. Effects of treatment with anti-immunoglobulin E antibody omalizumab on airway inflammation in allergic asthma. Am J Respir Crit Care Med 2004; 170(6):583-593.
27 Plewako H, Arvidsson M, Petruson K, Oancea I, Holmberg K, Adelroth E et al. The effect of omalizumab on nasal allergic inflammation. J Allergy Clin Immunol 2002; 110(1):68-71.
28 Noga O, Hanf G, Kunkel G. Immunological and clinical changes in allergic asthmatics following treatment with omalizumab. Int Arch Allergy Immunol 2003; 131(1):46-52.
29 Lambrecht BN. Dendritic cells and the regulation of the allergic immune response. Allergy 2005; 60(3):271-282.
30 Wenzel SE, Fowler AA, III, Schwartz LB. Activation of pulmonary mast cells by bronchoalveolar allergen challenge. In vivo release of histamine and tryptase in atopic subjects with and without asthma. Am Rev Respir Dis 1988; 137(5):1002-1008.
31 National Institutes of Health, National Heart, Lung, and Blood Institute. Global initiative for asthma. Global strategy for asthma management and prevention. NHLBI/WHO. NIH Publication No. 02-3659. 2002.
32 Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'connor BJ, Walls CM et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. Lancet 2000; 356(9248):2144-2148.
33 Birrell MA, Battram CH, Wood man P, McCluskie K, Belvisi MG. Dissociation by steroids of eosinophilic inflammation from airway hyperresponsiveness in murine airways. Respir Res 2003; 4(1):3.
34 van Rensen EL, Straathof KC, Veselic-Charvat MA, Zwinderman AH, Bel EH, Sterk PJ. Effect of inhaled steroids on airway hyperresponsiveness, sputum eosinophils, and exhaled nitric oxide levels in patients with asthma. Thorax 1999; 54(5):403-408.
35 Sont JK, Willems LNA, Bel EH, van Krieken HJM, Vandenbroucke JP, Sterk PJ et al. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. Am J Respir Crit Care Med 1999;159:1043-1051.