

Original article

Eosinophils in bronchial mucosa of asthmatics after allergen challenge: effect of anti-IgE treatment

Background: Anti-IgE, omalizumab, inhibits the allergen response in patients with asthma. This has not been directly related to changes in inflammatory conditions. We hypothesized 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 randomized, double-blind, placebo-controlled study.

Methods: Allergen challenge followed by a bronchoscopy at 24 h was performed at baseline and after 12 weeks of treatment with anti-IgE or placebo. Provocative concentration that causes a 20% fall in forced expiratory volume in 1 s (PC₂₀) methacholine and induced sputum was performed at baseline, 8 and 12 weeks of treatment. Changes in the early and late responses to allergen, PC₂₀, inflammatory cells in biopsies and sputum were assessed.

Results: Both the early and late asthmatic responses were suppressed to 15.3% and 4.7% following anti-IgE treatment as compared with placebo ($P < 0.002$). This was paralleled by a decrease in eosinophil counts in sputum (4–0.5%) and postallergen biopsies (15–2 cells/0.1 mm²) ($P < 0.03$). Furthermore, biopsy IgE+ cells were significantly reduced between both the groups, whereas high-affinity IgE receptor and CD4+ cells were decreased within the anti-IgE group. There were no significant differences for PC₂₀ methacholine.

Conclusion: The response to inhaled allergen in asthma is diminished by anti-IgE, which in bronchial mucosa is paralleled by a reduction in eosinophils and a decline in IgE-bearing cells postallergen without changing PC₂₀ methacholine. This suggests that the benefits of anti-IgE in asthma may be explained by a decrease in eosinophilic inflammation and IgE-bearing cells.

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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. (2) were the first to show a strong association between serum IgE levels and self-reported asthma. 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 down regulate FcεRI expression

on basophils and dendritic cells (DC) in blood (7, 8), and increase markers of apoptosis in circulating eosinophils and decrease Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF), interleukin-2 (IL-2) and IL-13 expression in circulating T lymphocytes (9).

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 (10, 11). Subsequent large phase-3 trials, involving both paediatric and adult patients with moderate to severe asthma, have demonstrated the clinically beneficial effect of anti-IgE treatment by improving asthma control (12–14). A 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 (15).

The anti-inflammatory activity of anti-IgE was established in a biopsy study concerning mild asthmatic

patients (16). Following 16 weeks treatment with anti-IgE, significant reductions in IgE positive cells and eosinophils in the bronchial mucosa were found (16). 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 this study is 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 hyper-responsiveness and inflammatory cells in sputum was investigated in patients with asthma in a randomized, double-blind, placebo-controlled study.

Methods

Subjects

Twenty-five nonsmoking 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 β_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 in 1 s (FEV_1) was $>70\%$ predicted (17) and all subjects were hyper-responsive to inhaled methacholine [provocative concentration causing a 20% fall in FEV_1 (PC_{20}) <4 mg/ml] (18). The fall in FEV_1 during the 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. This 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 h was performed at baseline and at 12 weeks.

Table 1. Patient characteristics

Characteristic	Anti-IgE ($n = 12$)	Placebo ($n = 13$)
Age (years)	20.5 (18–24)	21 (19–29)
Female gender, 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). There were no significant differences between the groups.

*g-mean (SD in DD).

Treatment

The dose (150–375 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 at least 0.016 mg/kg per IgE (IU/ml) (19). 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 prebronchodilator peak flow measurements were recorded. Mean Peak Exploratory Flow (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 hyper-responsiveness

Patients were not allowed to take any short-acting β_2 -agonists for at least 8 h prior to spirometry. A standardized methacholine challenge was applied to determine airway hyper-responsiveness (18). To determine the PC_{20} methacholine, patients inhaled increasing doses of methacholine for 2 min until a fall of at least 20% in FEV_1 had been reached.

Sputum induction and processing

Prior to induction, each subject inhaled 200 μ g salbutamol. Sputum was induced by inhalation of NaCl 4.5% during 3×5 min intervals, according to a recommended protocol validated in our laboratory (20, 21). Sputum samples were processed according to the whole sample method. Differential cell counts were expressed as a percentage of 250 nonsquamous cells (21).

Allergen challenge

Allergen challenges were performed according to a standardized protocol (18, 22). Purified aqueous allergen extract of *Dermaphagoides pteronyssinus* (SQ 503; Vivodiagnost, ALK, Benelux), with 0.5% phenol as a preservative, was diluted ranging from 2000 to 15.63 BU/ml. The 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 (22). Starting three concentrations below the predicted PC_{20} allergen, 3 ml of consecutive doubling concentrations of allergen were aerosolized for 2 min 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 min and then hourly until 7 h after the last inhalation. In the analysis, EAR (0–3 h postallergen) and LAR (3–7 h postallergen) were defined as the maximum % fall in FEV_1 from baseline and as the area under the time-response curve (AUC) (18). Patients received exactly the same allergen dose at the end of the study as they inhaled during the baseline allergen challenge.

Bronchoscopy and immunohistochemistry

Fibreoptic bronchoscopy was performed according to a standardized and validated protocol (23). 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 four biopsies were fixed for 24 h in buffered formalin and paraffin-embedded. The HE-stained slides of 3- μm thick, were used for checking biopsies quality (size, crushing, epithelial and mucosal representation) and the two biopsies that were of excellent technical quality were selected for immunohistochemistry. Slides were immunostained for IgE, high and low affinity IgE receptor (Fc ϵ RI and Fc ϵ R2), eosinophils (EG2), mast cells (AA1), neutrophil elastase (NE), macrophages (CD68) and T lymphocytes CD3, CD4 and CD8. DC-SIGN was used as a marker for myeloid DC, whereas CD83 was used as a marker for mature DC. 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, USA) as the chromagen. The sections were counterstained with Mayer's haematoxylin (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 (24). Images were digitized using a three-chip colour camera (433.10³ pixels, 660 $\times$ 496 μm^2 , 3 $\times$ 256 grey values) (KS-400 System; Kontron/Zeiss, Carl Zeiss, Göttingen, Germany). 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 (24). Data were expressed as cells/0.1 mm².

Analysis

The PC₂₀ methacholine, cell counts in sputum and biopsies were log transformed before statistical analysis. All data are presented as median (range), except for PC₂₀ methacholine that is presented as geometric mean (SD in 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 statistically significant 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 four 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).

Mean baseline FEV₁ was 96.3% predicted in anti-IgE group and 90.8% predicted in the placebo group. There were no significant changes for FEV₁ within or between groups during the study (*P* > 0.07).

Diary cards

Mean 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 mean 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) (*P* = 0.041 for the change from baseline between anti-IgE and placebo). Similar improvements were observed for evening PEF (Fig. 1).

Allergen challenge

At baseline, the maximum % fall in FEV₁ 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 reduced the EAR to a fall of 15.3% (0.0–23.8) (*P* < 0.0005). This change was significantly larger than the change in the placebo group (*P* = 0.002). The mean AUC of the EAR was also significantly reduced following anti-IgE treatment [28.0%fall* h (2.7–49.3) to 15.3%fall* h (0.0–23.8)] as compared with placebo [33.5%fall* h (14.2–41.9) to 23.2%fall* h (6.0–37.1)] (*P* = 0.002) (Fig. 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 than that 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 to 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) (Fig. 2).

Bronchial biopsies

Anti-IgE treatment markedly reduced the submucosal IgE+ cells from [median (range)] 15.8 cell/0.1 mm² (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/0.1 mm² (3.0–54.5) to 30.3 (2.5–63.0)] (*P* = 0.000 for changes from baseline between anti-IgE and placebo) (Fig. 3). Fc ϵ RI+ 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) [Fig. 3]. Most biopsy samples did not show any Fc ϵ R2+ cells and no changes following treatment were observed (*P* > 0.18).

There was a clear reduction in eosinophil counts in the anti-IgE group [15.0 cells/0.1 mm² (1.0–48.5) to 2.0 cells/0.1 mm² (0.5–3.0)] (*P* = 0.000), which was significantly different from the placebo group [14.5 cells/0.1 mm² (1.0–118.5) to 11.0 cells/0.1 mm² (1.5–77.0)] (*P* = 0.005 for the change from baseline between anti-IgE and placebo) (Fig. 4).

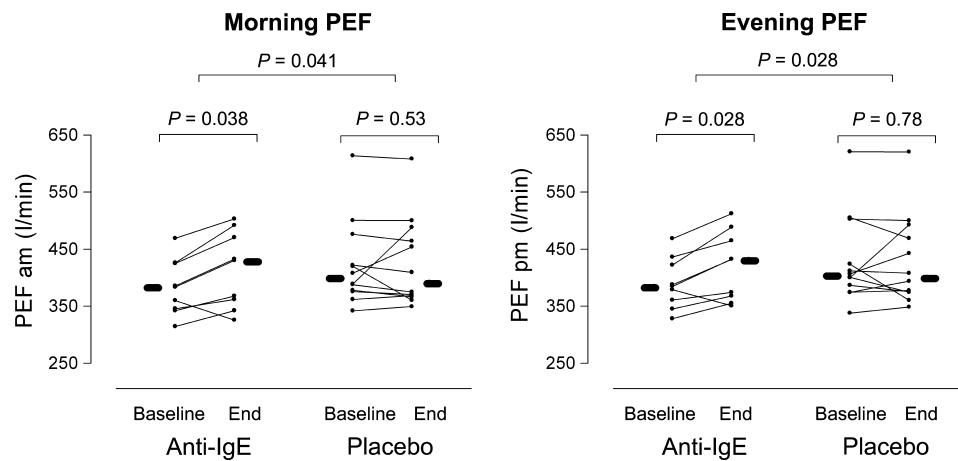


Figure 1. PEF. Individual values of morning (left panel) and evening (right panel) PEF at baseline and end of the study in anti-IgE and placebo treated patients. Horizontal bars are median.

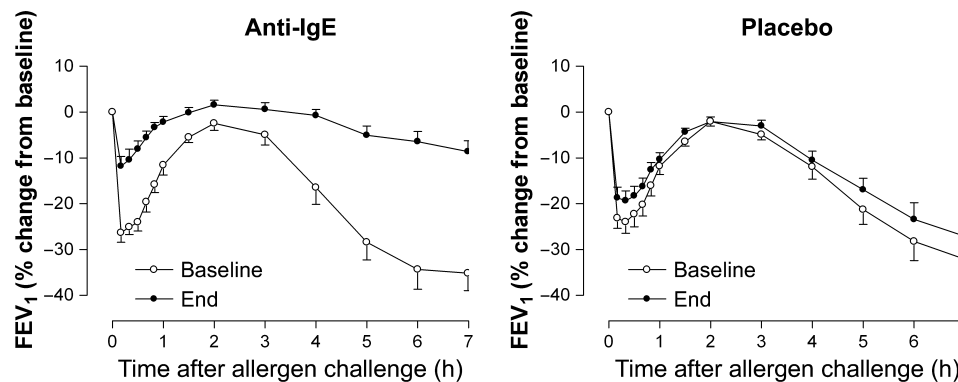


Figure 2. Allergen challenge. Airway responses to inhaled allergen between 0 and 7 h following allergen challenge for the anti-IgE group (left panel) and for the placebo group (right panel). In the anti-IgE group, EAR and LAR were significantly reduced from baseline (open circles) to end (closed circles),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

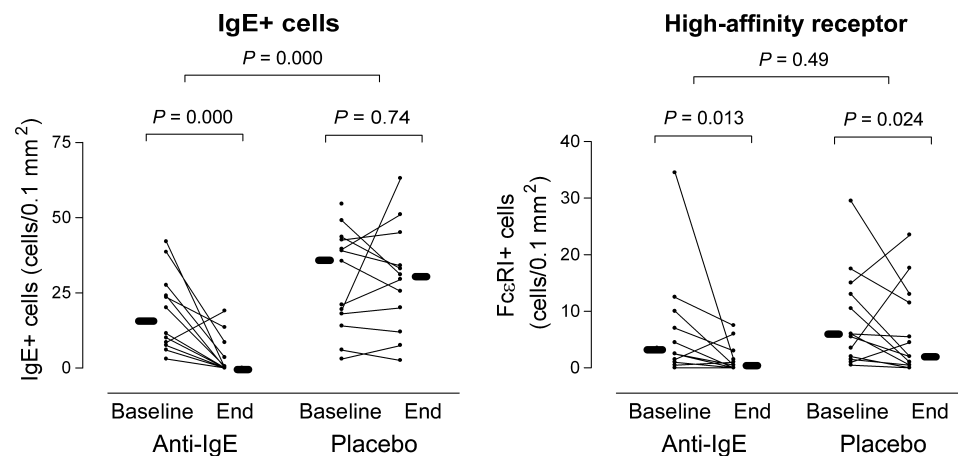


Figure 3. Biopsy IgE+ and FcεRI+ cells. Individual values of IgE+ (left panel) and FcεRI+ cells (right panel) at baseline and end of the study in anti-IgE and placebo treated patients. Horizontal bars are median.

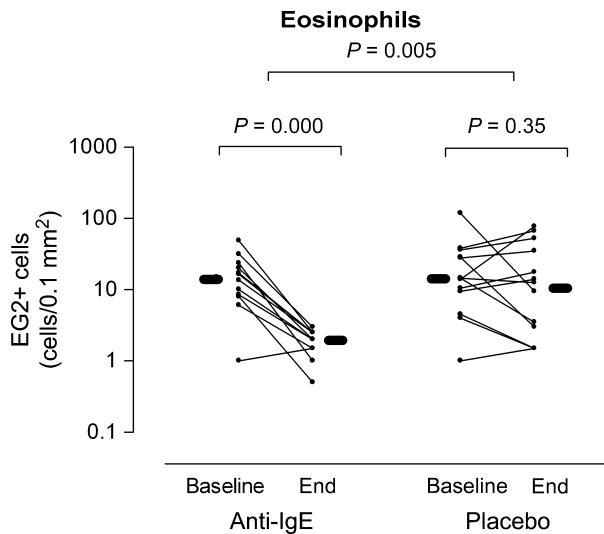


Figure 4. Biopsy eosinophils. Individual values of eosinophils at baseline and end of the study in anti-IgE and placebo treated patients. Horizontal bars are median.

There were no significant differences between anti-IgE and placebo observed for mast cells, macrophages, neutrophil elastase, B-lymphocytes and mature dendritic (CD83⁺) cells ($P > 0.09$). Myeloid dendritic (DC-SIGN⁺) cells were lower following anti-IgE treatment, but the difference with placebo treatment did not reach statistical significance ($P = 0.55$; Fig. 5). However, the change in DC-SIGN⁺ cells following anti-IgE treatment was significantly correlated with that in FcεRI⁺ cells ($r = 0.65$, $P = 0.022$) and eosinophils ($r = 0.67$; $P = 0.018$) (Fig. 6). 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 ($P > 0.14$).

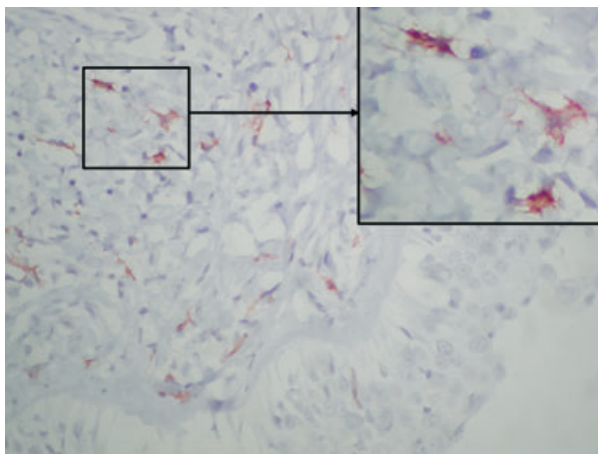


Figure 5. Immunostaining for the myeloid dendritic cell marker DC-SIGN.

Baseline myeloid DCs (DC-SIGN⁺ cells) were significantly correlated with the change in FcεRI⁺ cells ($r = 0.66$; $P = 0.02$) and the change in eosinophils ($r = 0.71$; $P = 0.009$) following anti-IgE treatment (Fig. 6).

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) (Fig. 7). None of the other cell types in induced sputum showed any significant changes.

Airway hyper-responsiveness

The PC₂₀ methacholine was measured [g-mean (SD in DD) 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 ($P > 0.18$).

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, PC₂₀ 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 hyper-responsiveness to methacholine in atopic asthma is independent of IgE.

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 EAR and LAR (10, 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

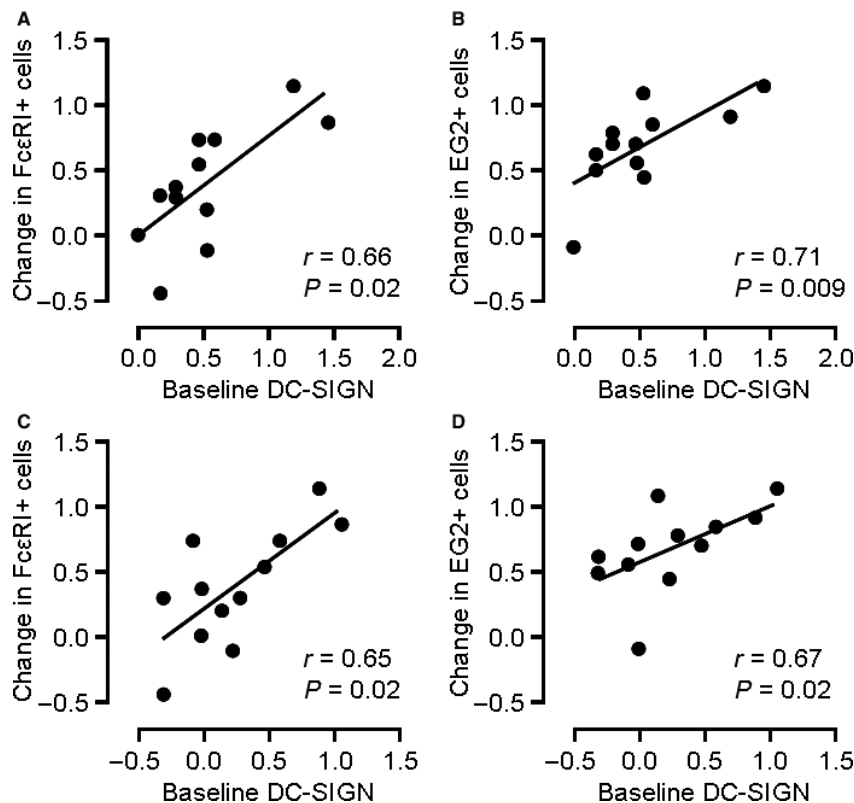


Figure 6. Correlations with DC-SIGN in the anti-IgE group. Correlation between change in FcεRI⁺ (A, C) and eosinophils (EG2; B, D), and baseline DC-SIGN (A,B) or change in DC-SIGN (C, D).

inflammation in patients with asthma (16, 26). Like Djukanovic et al. (16), we have demonstrated the anti-inflammatory effect of anti-IgE treatment in bronchial biopsies. In contrast with their study, we showed also a

clear and clinically beneficial effect of anti-IgE on morning and evening PEF and on response to inhaled allergen (16).

In this study, airway hyper-responsiveness was not improved following anti-IgE treatment as compared with placebo. Our results are in keeping with previous studies also showing absent or marginal effects on PC₂₀ methacholine (10, 16, 25, 27).

We do not believe that our data were influenced by measurement errors, as we used validated and reproducible methods (17, 18, 21, 23, 24). All subjects in this study were carefully selected nonsmokers 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

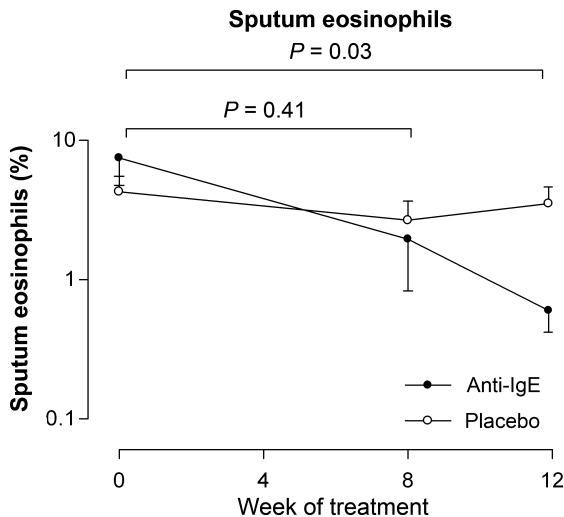


Figure 7. Sputum eosinophils. Change in sputum eosinophils from baseline to 8 weeks and at the end of the study for anti-IgE (closed circles) and placebo (open circles).

intervene in this mechanism. Indeed, a reduction in cell surface IL-4 following anti-IgE treatment has been demonstrated (16). A second mechanism, by which anti-IgE affects eosinophils, might be related to the inhibitory effects of anti-IgE on DC Fc ϵ RI expression (8). It has been proposed that anti-IgE can alter allergen presentation by DCs (1). Anti-IgE treatment may decrease the uptake and presentation of allergens by DCs, leading to a reduced T-cell response, which may diminish eosinophilic airway inflammation (28). In line with this, anti-IgE may also indirectly decrease eosinophil numbers by decreasing DC numbers. There was no effect of anti-IgE treatment on mature, CD83 positive (29) DCs, nor on myeloid DC numbers [as assessed by the myeloid DC marker DC-SIGN; (30)]. However, there was a significant correlation between changes in DC-SIGN⁺ cells, and changes in Fc ϵ RI⁺ cells and eosinophils following anti-IgE treatment. In addition, baseline DC-SIGN correlated with the effect of anti-IgE on Fc ϵ RI and IgE positive cells. These data suggest a role of DCs in the anti-inflammatory effects of anti-IgE treatment, and a local immunomodulatory effect of anti-IgE treatment.

Interestingly, our findings have shown that the effect of anti-IgE is much larger for the LAR than for the early response. The EAR is predominantly mediated by IgE-triggered mast cell mediator release of histamine and tryptase (31). 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 whether they are loaded with IgE is the essential aspect. Furthermore, resulting from the minimal amount of IgE that is probably available after treatment, cross-linking with high-affinity receptors still might occur.

Airway hyper-responsiveness is an important feature of patients with asthma (32). Anti-IgE treatment does not appear to affect this mechanism. We cannot exclude that airway hyper-responsiveness might improve after longer treatment with anti-IgE, as our patients were only treated for 12 weeks. Results from therapy with anti-IL-5, which also results in an abolishment of eosinophils, have shown no effect on airway hyper-responsiveness either (33). These findings confirm the dissociation of eosinophils and airway hyper-responsiveness in asthma as observed in animal models (34). Treatment with inhaled steroids, in contrast, reduces both eosinophils and airway hyper-responsiveness (35). Possibly, inhaled steroids not only affect eosinophilic

inflammation, but also affect 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 clinically 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 hyper-responsiveness in our study. We have previously shown that treatment aimed at reducing airway hyper-responsiveness leads to more effective control and thereby a reduction of exacerbations in patients with asthma (36). Therefore, the inability of anti-IgE treatment to normalize airway hyper-responsiveness 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 hyper-responsiveness appears to be independent of IgE, is of interest with respect to the role of anti-IgE as a new therapy for asthma.

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Conflicts of interest

This study was supported by Novartis. E. van Rensen has none to disclose; C. Evertse has none to disclose; A. van Schadewijk has none to disclose; S. van Wijngaarden has none to disclose; G. Ayre is employed by Novartis; T. Mauad has none to disclose; P.S. Hiemstra has none to disclose; P. Sterk has none to disclose; K. F. Rabe has consulting agreements with, has participated in advisory board meetings of, and received lecture fees from AstraZeneca, Boehringer, Pfizer, Novartis, AltanaPharma, MSD and GSK. The Department of Pulmonology and thus Professor Rabe, as head of the department, has received grants from AltanaPharma, Novartis, Bayer, AstraZeneca, Pfizer, MSD, Exhale Therapeutics and GSK in the years 2001–2005.

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