

Basophil Histamine Release Decreases during Omalizumab Therapy in Allergic Asthmatics

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Key Words

Omalizumab · Allergic asthma · Asthma treatment · Anti-IgE · Basophil histamine release

Abstract

Background: Omalizumab is an established add-on therapy efficacious in allergic diseases with additional anti-inflammatory activity in the treatment of asthma. The evaluation of responders to anti-IgE treatment is critical to maximize benefit/risk/cost ratio. The aim of the study was to monitor the efficacy of anti-IgE treatment by ex vivo basophil histamine release. **Methods:** Seventeen patients with allergic asthma were enrolled and received omalizumab at a dose of ≥ 0.016 mg/kg/IgE every 4 weeks. Histamine release from basophils was evaluated fluorometrically after dose-dependent allergen challenge at baseline and after 16 weeks of treatment. Maximal histamine release and cellular sensitivity to the allergen were calculated. Clinical measurements consisted of body plethysmography, skin prick test, β_2 -agonist usage, serum free IgE levels, peripheral eosinophils and investigator ratings of global evaluation of treatment effectiveness. **Results:** Maximal histamine release and cellular sensitivity to the allergen were significantly decreased in the omalizumab group compared to placebo. These changes were accompanied by significant changes in the clinical markers airway resistance, β -agonist usage, skin prick test

wheel area and investigator ratings of global evaluation of treatment effectiveness. **Conclusions:** Omalizumab therapy decreases basophil histamine release and cellular sensitivity with high effectivity of 95.8% (median). The decline of ex vivo basophil responses does not always parallel individual clinical improvement. Basophil-based stimulation tests should be further evaluated before being regarded as useful parameters monitoring omalizumab therapy.

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Introduction

Omalizumab is a humanized monoclonal anti-IgE antibody that binds to IgE at the same site as the high-affinity Fc ϵ RI receptor, lowering free IgE and leading to down-regulation of high-affinity IgE receptors on circulating basophils and mast cells [1, 2]. Subsequently, omalizumab treatment will attenuate the early- and late-phase responses to inhaled allergens in patients with asthma [3]. Further anti-inflammatory effects have been observed and might contribute to the clinical efficacy of omalizumab treatment [4–6].

The efficacy of omalizumab treatment has been demonstrated in large-scale clinical trials [7–9] with improvement of quality of life and decreased numbers of severe asthma exacerbations. In clinical practice these param-

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eters vary considerably and are difficult to evaluate intra-individually. Surrogate markers like FEV₁ improved in large clinical studies but do not always parallel the individual response, since the individual change can be rather small. Subsequently, there is a need for reliable parameters indicating efficacy of omalizumab therapy. Incomplete suppression of cellular mediator release has been suggested as explanation for omalizumab treatment failures. Basophil histamine release (HR) was investigated as a tool to determine treatment responses to omalizumab in comparison to other clinical parameters.

Methods

Study Design

Patients with moderate to severe allergic asthma participated in this study, being part of a randomized, double-blind, placebo-controlled, parallel-group trial. All patients, meeting the inclusion criteria described in detail elsewhere [7, 8], had a history of allergic asthma and a positive skin prick test (SPT) to at least 1 perennial allergen, that is house dust mites (*Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*), cat or dog. The study was approved by the local ethics committee and performed in accordance with the Declaration of Helsinki. All patients gave written informed consent to participate.

Omalizumab or placebo was administered subcutaneously at 2- or 4-week intervals. Doses were adjusted for body weight and total IgE at screening, facilitating omalizumab administration at a dose of at least 0.016 mg/kg/IgE every 4 weeks.

Study parameters and all tests were evaluated before the start (baseline) and after 16 weeks of treatment.

HR of Basophils

Peripheral venous blood was collected from 17 patients, 8 receiving omalizumab and 9 receiving placebo. Mixed leukocyte preparations were obtained by dextran sedimentation [10]. Cells were washed with PBS and stimulated with 100 IU/ml anti-IgE as a positive control and different concentrations (0.1, 1, 10, 100, 1,000 SQ/ml) of specific allergens (house dust mite mix, cat or dog dander; ALK, Wedel, Germany) depending on the individual allergy profile of the basophil donor.

After 60 min, cells were removed by centrifugation (800 g, 15 min, 4°C). Supernatants were analyzed fluorometrically [10]. Total histamine content was determined by lysing the cells with 2% HClO₄. Cells incubated with buffer reflected spontaneous HR (blanks <5% of the total histamine content). HR_{max} (mean ± SEM) was the highest release value expressed as a percentage of total histamine content minus blanks. HR_{sens} was defined as the inverted value of the allergen concentration initiating a 50% of maximum HR. All experiments were performed in duplicate.

Clinical Evaluations

Free IgE. Free IgE was measured by ELISA (Novartis, Basel, Switzerland) as previously described [11]. The detection limit was 2.4 ng/ml and the lower limit of quantitation was 1 ng/ml for free IgE.

Skin Prick Test. SPT to perennial allergens (house dust mite, cat and dog dander), including positive (1 mg/ml histamine dihydrochloride) and negative control (diluent) was performed before and after 16 weeks of treatment. Wheal reactions were read after 15 min, copied to a strip of tape and analyzed using the NIH image 1.6 software (National Institutes of Health, Bethesda, Md., USA). Values were expressed as percentage of the positive control.

Eosinophil Count. Blood eosinophil levels at baseline and after 16 weeks of treatment were given as total counts and percentage of leukocytes.

Airway Resistance. Airway resistance (R_{aw}) was determined by body plethysmography (Jaeger, Würzburg, Germany).

Assessment by the Investigator. Investigator ratings of global evaluation of treatment effectiveness (IGETE) were categorized using a 5-point score: 2 = significant improvement, 1 = slight improvement, 0 = no change, -1 = slight worsening, -2 = significant worsening.

Statistical Analysis

Between-group comparisons were made using the Mann-Whitney U test. A 2-tailed p value below 0.05 was considered statistically significant. All tests compared the treatment groups by percentage changes from baseline to week 16, except IGETE given as absolute score levels reflecting clinical changes. The in-group (before and after treatment) comparisons were made using the Wilcoxon signed rank test and associations were tested by Spearman's rank correlation. p values (2-tailed) below 0.05 were considered statistically significant.

Results

Patient Characteristics

Baseline characteristics of 17 patients were similar between the groups (table 1).

HR of Basophils

HR from patients receiving omalizumab was significantly reduced (fig. 1; table 1), with changes in HR_{max} (from -28.7 to -95.8%) and HR_{sens} (from 20 to -99.8%) in nearly all patients (fig. 2). No statistically significant differences occurred in the placebo group.

Clinical Evaluations

Significant differences were demonstrated in SPT, airway resistance, use of inhaled β₂-agonists and clinical evaluation by IGETE comparing omalizumab with placebo (fig. 2; table 1). FEV₁ values failed to improve and blood eosinophils were reduced only when compared in the individuals under omalizumab therapy, but failed to decrease significantly comparing the change in the omalizumab group to placebo (fig. 2).

Table 1. Baseline characteristics and variables during therapy

Variable	Baseline		After 16 weeks of therapy	
	omalizumab	placebo	omalizumab	placebo
Subjects (female/male)	8 (4/4)	9 (3/6)		
Age, years	34 (23–49)	36 (21–61)		
IgE, IU/l	199 (58–433)	285 (45–683)		
FEV ₁ , liters	3.24 (1.47–3.95)	3.155 (2.04–4.49)	3.39 (1.5–3.92)	3.315 (2–4.61)
R _{aw} , kPa/l·s	0.46 (0.27–0.85)	0.367 (0.24–0.76)	0.33 (0.25–0.57)*, +	0.363 (0.31–0.75)
β ₂ -Agonist, puffs/day	3.1 (0–7)	1.1 (0–5)	0.15 (0–2.8)*, +	1.4 (0–5.7)
Blood eosinophils, %	5.9 (1.3–11.5)	4.2 (1.6–6.8)	3.4 (1.3–11) ⁺	3.55 (2.7–5.1)
SPT, % of histamine area	222.9 (83.8–342.8)	148.5 (105–564)	75 (56.5–238.7)**, +	153.9 (62–614.6)
Skin test results				
House dust mite	6	7		
Cat dander	6	6		
Dog dander	2	5		
Others (seasonal)	7	7		
Free IgE, ng/ml	458.5 (138–1,088)	509 (118–2,288)	12 (9–25) ⁺⁺	ND
HR _{max} , % of complete	14.6 (3.29–70.69)	14.61 (2–69.4)	4.8 (0.32–40.34)**, ++	16.792 (1.8–70.58)
HR _{sens}	833.3 (52.6–2,000)	55.6 (9.5–200)	11.1 (0–100)*, +	52.6 (5.3–83.3)
Anti-IgE release, % of complete	47.7 (3.39–65.2)	29.64 (1–43.9)	50 (34.6–68.4)	35.62 (2.2–60.3)

Data are presented as medians. Figures in parentheses are ranges, unless indicated otherwise. ND = Not detectable.

* $p < 0.05$ vs. placebo (change from baseline); ** $p < 0.01$ vs. placebo (change from baseline); + $p < 0.05$ vs. baseline; ++ $p < 0.01$ vs. baseline.

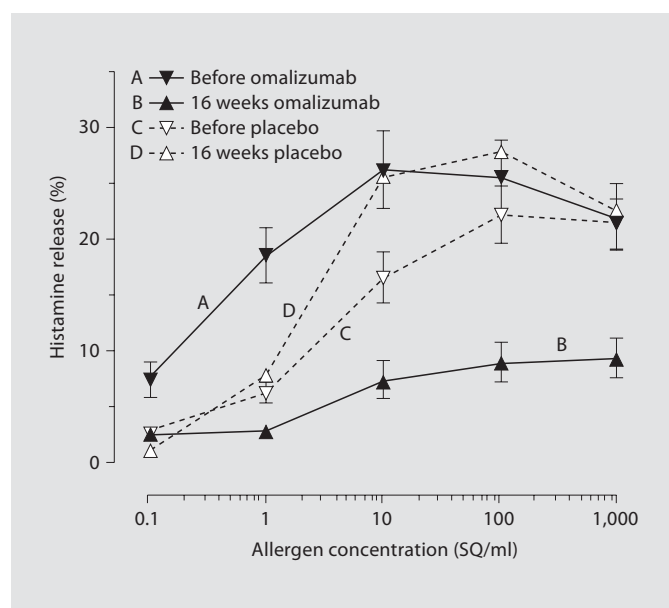


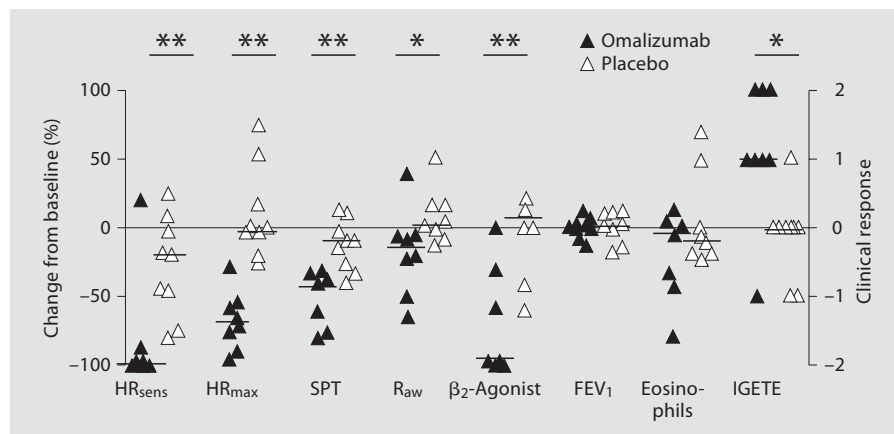
Fig. 1. Basophil HR (mean $\pm$ SEM) by individually selected perennial allergens before and after 16 weeks of therapy with omalizumab (n = 8) or placebo (n = 9).

Free IgE dropped dramatically after omalizumab treatment ($p < 0.01$; table 1). Subsequent levels were not strongly related to the clinical improvement ($r_s -0.5$; $p = 0.22$), but considerable associations occurred between changes of HR_{sens} and R_{aw} and changes of HR_{max} and SPT (fig. 3). However, only weak correlations were found between HR changes and clinical data, which did not facilitate predictions of the individual treatment outcome.

Discussion

Treatment with omalizumab leads to highly significant changes of basophil HR in all omalizumab recipients. This is reflected by decreased HR_{max} and reduced basophil sensitivity to allergens (median change: -96% compared to baseline) in all patients independently of their clinical response to omalizumab treatment. The reduction of free IgE is accompanied by significant changes in the function of basophils [12]. In addition, treatment with omalizumab leads to significant changes in airway resistance, usage of β_2 -agonists and superior global clinical evaluation, but no differences in FEV₁.

Fig. 2. Percentage change compared to baseline of laboratory and clinical variables after treatment with omalizumab or placebo. The left y-axis shows the percentage of change, the right y-axis the clinical change depicted as 5-point scale reflecting IGETE (2 = marked clinical improvement; 1 = slight clinical improvement; 0 = no change; -1 = slight clinical worsening; -2 = marked clinical worsening). * $p < 0.05$; ** $p < 0.01$.



Interestingly, relations not only between release magnitude (HR_{max}) and skin test reactivity, being a function of allergen-specific IgE on cutaneous mast cells, but also between allergen-driven basophil sensitivity (HR_{sens}) and airway resistance (R_{aw}), being a delicate lung function parameter, confirm the concept of omalizumab as an IgE trap scaling down mediator release and related symptoms. The presented data do not allow analyzing relative effects of reduced IgE and reductions of FcεRI on effector cells, which have elegantly been demonstrated after omalizumab treatment [12].

Despite these effects, the utility of effector cell functions, particularly basophil sensitivity, suggested as a promising tool and termed CDsens using CD63 as a basophil readout to monitor omalizumab treatment [13], have limitations due to their lack of individual predictability. In contrast to the CD63-based data [13], demonstrating close relations between CDsens and SPTsens, nasal provocation titer and relative IgE antibody concentration, we were not able to find a close association between clinical benefit and basophil parameters, particularly basophil sensitivity. These differences could be attributed to differences between CD63, resembling activation of basophils, and HR, resembling degranulation. However, fundamental changes in basophil functions after down-regulation of membrane-bound IgE and its receptor will likely lead to parallel results, regardless of the selected readout (CD63 vs. HR) or effector cell (mast cell vs. basophil) [14]. Maximal allergen-specific basophil response is clearly not appropriate to monitor omalizumab effects [13], since this readout is independent of basophil sensitivity [14] and does not parallel individual changes after anti-IgE treatment.

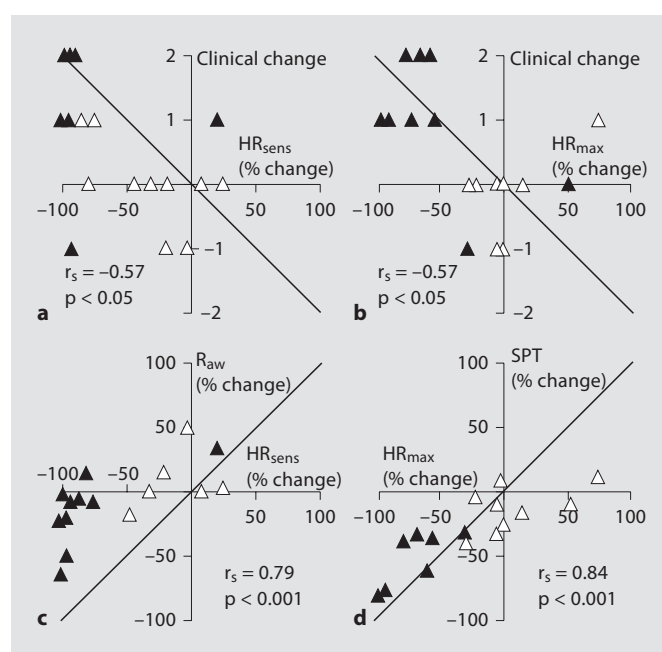


Fig. 3. Associations between clinical changes and basophil HR after omalizumab treatment. $\blacktriangle$ = Omalizumab; $\triangle$ = placebo. The diagonals show ideal concordance. **a** HR_{sens} vs. clinical change. **b** HR_{max} vs. clinical change. **c** HR_{sens} vs. R_{aw} . **d** HR_{max} vs. SPT.

It cannot be ruled out that the superior matching of the culprit allergen source responsible for maintaining the allergic inflammation in the lower airways with the allergen tested for ex vivo basophil responses could have resulted in a more close association between our experimental findings and the individual clinical outcome. However, the perennial allergen source for basophil stim-

ulation was chosen based on the individual sensitization and a corresponding case history, since allergen-specific challenge procedures for demonstrating clinical relevance were not part of the study.

Based on this small data set, detection of R_{aw} and usage of β_2 -agonists might be useful tools to evaluate interventions with omalizumab. There is still a strong need to establish robust and reliable readouts indicating clinical success, since introduced parameters like quality of life and asthma exacerbations are either not objective or difficult to determine treating individual patients.

The response rate to anti-IgE therapy has been reported to reach approximately 60% of the omalizumab-treated patients [15]. Here we could demonstrate that IgE-me-

diated hypersensitivity to allergens is decreased significantly in most patients, but only weakly associated with clinical improvement. Therefore, the role of IgE-mediated mechanisms in patients not responding to omalizumab should be critically evaluated. Provocation tests, possibly performed via surrogate mucosal routes rather than bronchial challenges, might be useful to determine the clinical contribution of present sensitizations in view of the asthma severity.

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