

ORIGINAL ARTICLE

Experimental Allergy and Immunology

Omalizumab effectively protects against early and late allergic responses in asthma after 4 weeks

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Abstract

Background: Omalizumab is licensed for therapy in severe allergic asthma with an effect demonstrated after 8 weeks or longer treatment. As new applications for omalizumab demand precise knowledge of the onset of effects, the objective of this study was to determine the time course of the early (EAR) and late allergic reaction (LAR).

Materials and Methods: Ten patients (IgE > 300 IU/mL and < 700 IU/mL) with a significant response to allergen challenge were treated with omalizumab according to the approved dosing table. Bronchial allergen provocations (BAP) were repeated at weeks 1, 2, 4, and 8.

Results: EAR was significantly reduced after 4 weeks (Δ FEV1 28% vs 11%; $P < .001$), eNO (86 vs 53 ppb; $P < .05$) and basophil activation after 2 weeks (CD63 expression 79% vs 32%, $P < .05$) and LAR already after 1 week (Δ FEV1 26% vs 13%, $P < .05$).

Conclusion: These results demonstrate the onset of protective effects earlier than previously determined, potentially improving seasonal utilization and combination with immunotherapy.

KEYWORDS

allergen challenge, early allergic reaction, exhaled NO, late allergic reaction, Omalizumab

1 | INTRODUCTION

Moderate-to-severe allergic asthma has been effectively treated with omalizumab, a humanized anti-IgE antibody, for over 10 years. Omalizumab reduces free IgE levels, its receptors on mast cells and basophils¹ and asthma exacerbations.²

Bronchial allergen provocation (BAP) is a clinically established method to test allergic airway response to specific allergens and the effect of different drugs. First studies about the onset of protective effects of omalizumab on early and late allergic response (EAR, LAR)

after specific allergen challenges demonstrated a significant amelioration of the EAR after 4 weeks,³ although dosing scheme and route were different and not according to current recommendations. The majority of subsequent studies measured the effect on EAR and LAR after at least 8 weeks of treatment,⁴ and current recommendations of the European Medicines Agency state that patients should be treated for at least 12 to 16 weeks in order to assess clinical benefits.⁵

However, more recent studies were able to demonstrate a reduction in seasonal exacerbations after 4 weeks of treatment, suggesting a potential benefit of short, seasonal treatment approaches.^{6,7} Additionally, the combination of allergen-specific immunotherapy (SIT) for asthma after treatment with omalizumab is a promising novel therapeutic approach.⁸ Therefore, the goal of this study was to determine the time course of the onset of EAR and LAR and parameters eNO and basophil activation, as knowledge about the onset of effects on

Abbreviations: APS, Aerosol Provocation System; BAP, Bronchial allergen provocation; EAR, Early allergic response; eNO, Exhaled nitric oxide; FEV1, Forced expiratory volume in one-second; IgE, Immunoglobulin E; LAR, Late allergic response; OIT, Oral immunotherapy; PD20, Provocation dose yielding a 20% decline in FEV1; SBU, Standardized biological units; SIT, Specific immunotherapy

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allergen-induced bronchial hyperresponsiveness will be useful for treatment planning. This would strengthen these novel approaches, especially considering omalizumab currently remains the only approved anti-IgE drug.

2 | METHODS

2.1 | Study design

This study was a single-center time-course study and approved by the local ethics committee.

In initial screening visits, specific allergen sensitization was documented by percutaneous skin testing, and the investigators documented the inhaled concentration of this allergen required for a decrease in FEV1 > 20% from baseline. In a first visit, baseline (week 0) spirometry was recorded before and after inhalation of the predetermined provocative dose of allergen, and subjects received their first dose of omalizumab within 24 hours of bronchial challenge. Omalizumab was dosed by weight and IgE levels according to current dosing recommendations. It was delivered subcutaneously every 2/4 weeks (days 1, 15, 29, and 43) for 8 weeks. Study visits with BAP and serum collection occurred in weeks 1, 2, 4, and 8. eNO was measured prior to and 24 hours after allergen challenge on all study visits.

2.2 | Study population

Ten patients with mild, well-controlled allergic asthma (FEV1 ≥ 65%, no exacerbation in the last 4 weeks, no corticosteroids (inhaled and systemic) 3 months prior, no active smoking), confirmed by positive skin prick testing and positive methacholine challenge, between the age of 18 and 65 years were included in this study. Serum IgE had to be > 300 and < 700 IU/mL, and they had to give their informed consent to be included.

2.3 | Specific allergen challenge

BAP to determine the allergen concentration, which leads to a 20% decrease of FEV1 from baseline (PD20FEV1), was performed once in the initial screening visit as described before.⁹ In short, specific allergens (mite, birch, grass, Allergopharma KG, Reinbeck, Germany) were resolved at 5000 SBU/mL (standardized biological units) and aerosolized utilizing the Aerosol Provocation System (APS, VIASYS Healthcare, Hochberg, Germany) in incremental steps from 10 SBU/mL to maximally 310 SBU.⁹ FEV1 was measured 8 minutes after inhalation and PD20FEV1 calculated utilizing logarithmic interpolation. For single-dose BAP at all study visits, the calculated concentration was inhaled and FEV1 determined after 10, 15, 30 and 60 minutes, and every hour for a total of 8 hours.

2.4 | eNO measurement

eNO values were measured before and 24 hours after BAP utilizing a chemiluminescence analyzer (NIOX MINO, Aerocrine, Sweden).

Exhalation flow was kept at a rate of 50 mL/s, and eNO was determined at a plateau.

2.5 | Basophil activation test

Cellular antigen stimulation test (CAST; Buhlmann Laboratories, Allschwil, Switzerland) was used to analyze basophil activation. Briefly, leukocytes were isolated and stimulated with allergen mix of the relevant allergens (mite, grass, birch) or positive controls. Afterward cells were triple-labeled with anti-CD123-PE, anti-CD63-FITC, and anti-HLA-DR-Per-CP monoclonal antibodies (Becton Dickinson, San Jose, Calif., USA) and analyzed by flow cytometry (FACSCalibur, Becton Dickinson). (Appendix S1)

2.6 | Statistical analyses

Values are presented as median with range; significance was tested utilizing Mann-Whitney testing for comparison of single time points. *P*-values refer to comparisons with baseline values. Statistical test was performed and graphs created with Graph Prism 6.

3 | RESULTS

3.1 | Patient collective

Ten patients (6 male, 4 female) included in this study had a median age of 24.5 years (range 20–36 years), IgE levels of 397 IU/mL (336–667 IU/mL), eNO values of 58.2 ppb (29.3–150 ppb), PD20FEV1 concentrations of 32.3 SBU (6.42–114.88 SBU) and baseline FEV1 values of 105.8% (91.2%–121.6%).

3.2 | Bronchial allergen challenge

Utilizing the maximal decrease measured for every patient within the first hour of the BAP to determine the EAR, median decrease in FEV1 at baseline (week 0) was 31.1% (25.5%–58.5%). After 4 weeks, the decrease in lung function was reduced to approximately half the baseline values (14.4% [0%–35.5%], *P* < .001). A further decrease after 8 weeks (11.5% [–4.7%–36.3%], *P* < .001) was not significantly different. Seven patients exhibited a LAR (maximal FEV1 decrease 3–8 hours after BAP), and significant results for the improvement of the LAR were observed after 1 week (FEV1 decrease at baseline 25.7% [14.3%–40.2%], after 1 week 13% [–0.3%–19.5%], *P* < .05). This decrease was greater after 2 weeks (6.9% [5.3%–33.3%], *P* < .05) and 4 weeks (1.2% [–9.9%–8.2%], *P* < .001) and sustained after 8 weeks (3.6% [–4.7%–13.8%], *P* < .001) (Figure 1).

3.3 | Exhaled NO

eNO values measured before BAP were significantly reduced after 4 weeks of omalizumab therapy (58.2 ppb [29.3–172.2 ppb] vs 33.2 ppb [11.7–85.9 ppb], *P* < .001). For eNO values determined

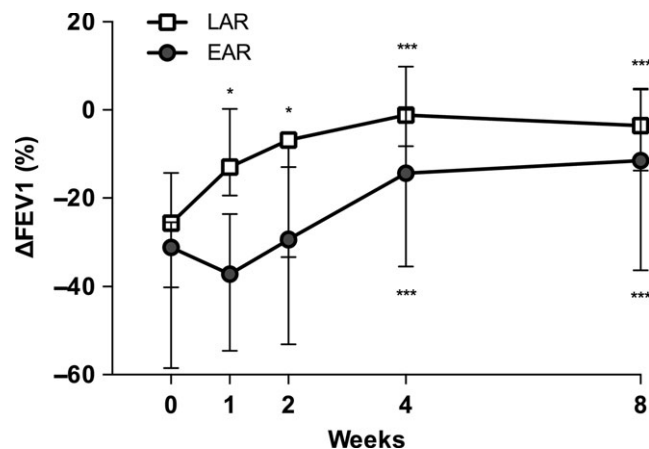


FIGURE 1 Early and late allergic response (EAR, LAR) after bronchial allergen provocation (BAP): LAR is significantly improved after 1 week of omalizumab treatment (Δ FEV1 [baseline FEV1-maximum decreased FEV1] median 25.7% [range 14.3%-40.2%] vs 13% [-0.3%-19.5%], $P < .05$, $n = 7$), EAR after 4 wks of treatment (Δ FEV1 31.1% [25.5%-58.5%] vs 14.4% [0%-35.5%], $P < .001$, $n = 10$). Neither LAR nor EAR improved further after 4 wks

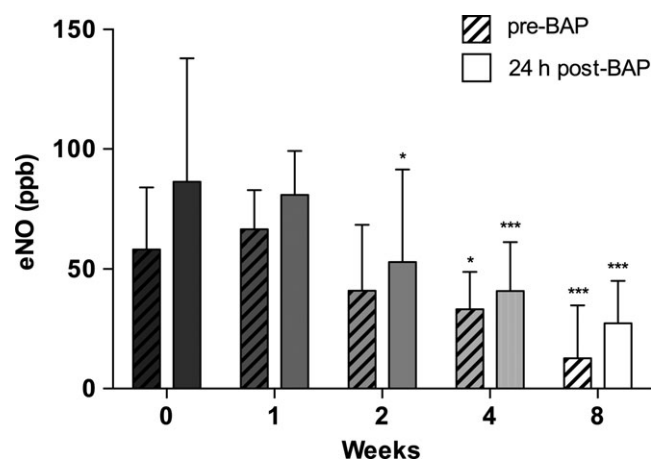


FIGURE 2 eNO values before and 24 h after BAP: eNO values 24 h after ABP are significantly decreased after 2 wks of omalizumab treatment (86.4 ppb [65.3-199.8 ppb] vs 52.9 ppb [31.3-161.3 ppb], $P < .05$), whereas eNO values measured before ABP are significantly decreased after 4 weeks (58.2 ppb [29.3-172.2 ppb] vs 33.2 ppb [11.7-85.9 ppb], $P < .001$)

24 hours after allergen challenge, the reduction was significant at 2 weeks (86.4 ppb [65.3-199.8 ppb] vs 52.9 ppb [31.3-161.3 ppb], $P < .05$) (Figure 2).

3.4 | Basophil activation test

After activation with a specific allergen mix, CD63 expression on basophils measured by flow cytometry was significantly reduced after 2 weeks of treatment with omalizumab (78.5% [12.4%-98.6%] vs 31.6% [1.7%-80%], $P < .05$). Further reductions were detected at 4 and 8 weeks (17.3% [5.8%-85.7%], $P < .05$ and 7.4% [0.6%-51.4%], $P < .01$, respectively).

4 | DISCUSSION

This study demonstrates that the onset of anti-inflammatory effects as well as the effect on EAR and LAR after treatment with omalizumab is significant after 4 weeks, with some effects already occurring after 1 week. This represents the first time-course study utilizing the approved dosing and route recommendations that shows the onset much earlier than previously reported.

Airway inflammation measured by eNO and allergic IgE-sensitization measured by basophil activation were downregulated from the second week onward. Even though both parameters further decreased until 8 weeks, for FEV1 values during EAR and LAR, representing a relevant clinical correlate, no further significant improvement could be shown after 4 weeks. Therefore, even though there were no additional measurements after 12 and 16 weeks, seeing the effect reaching a plateau strengthens the point that the effect on EAR and LAR is fully effective after 4 weeks.

Whereas the mechanism underlying the LAR is not as clear, both the early decrease in FcεRI density on basophils¹ and the amount of sputum eosinophils¹⁰ have been linked to the pathophysiology and severity of the LAR. Interestingly, both basophil activation as well as eNO values decreased as early as 2 weeks after omalizumab initiation, correlating with the early improvement in LAR.

The early effects prove especially important, as recent studies show that precisely timed seasonal treatment could repress the burden of seasonal exacerbations as efficiently as year-around treatment. Omalizumab has an early clinical effect on asthma exacerbations after only 4 weeks of treatment in a large inner-city children cohort, although effects have been attributed to viral-induced exacerbations.⁶ Following up, the PROSE study confirmed that the protective effects begin within 4 to 6 weeks of the start of omalizumab treatment,⁷ in line with the results of this time-course study. However, the presented study did not measure exacerbations as clinical parameter, but provides evidence that allergen response is mitigated within weeks. In addition, the small cohort, which had mild asthma compared to the target population of Omalizumab may limit its applicability to a more diverse population.

Allergen-specific immunotherapy is another helpful tool to reduce seasonal exacerbations. The combination of SIT with omalizumab reduced seasonal asthma symptoms compared to SIT alone. It is possible that milder, allergic asthma has a more rapid response to omalizumab than more severe asthma.⁸ Nevertheless, as EAR as well as LAR is significantly reduced after 4 weeks, the combination of omalizumab with SIT before the allergen season might also be feasible for moderate-to-severe asthmatics.

In conclusion, this time-course study might improve the clinical applications of omalizumab by showing the onset of the protective effect in allergic asthma after 4 weeks of treatment.

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CONFLICT OF INTEREST

JT, MA, AL and RS have no conflict of interest to disclose. SZ received fees for lectures and advisory boards from the following companies: bene-Arzneimittel GmbH (www.bene-arzneimittel.de), Vifor Pharma Deutschland GmbH (www.viforpharma.de), Novartis AG (www.novartis.com), GlaxoSmithKline GmbH (www.glaxosmithkline.de), ALK-Abelló Arzneimittel GmbH (www.alk-abello.com), Allergy Therapeutics (www.allergytherapeutics.com), Boehringer Ingelheim (www.boehringer-ingenheim.de), Allergopharma GmbH (www.allergopharma.de) and Biotest (www.biotest.com). MR received fees for lectures from the following companies: Novartis AG (www.novartis.com), GlaxoSmithKline (www.glaxosmithkline.de), Allergopharma GmbH (www.allergopharma.de) and Allergy Therapeutics (www.allergytherapeutics.com). JS received fees for lectures from Novartis AG (www.novartis.com). IB is an employee of Novartis AG. This does not alter the authors' adherence to Allergy policies on sharing data and materials.

AUTHOR CONTRIBUTIONS

JT contributed to the analysis of the data and drafted the manuscript. AL and MA carried out the acquisition of the data and contributed to the analysis of the data. JS and MR contributed to the study design, RS contributed to the study design and acquisition of the data. IB contributed to the study design and revised the manuscript. SZ contributed to study design, coordinated the study and revised the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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Supplemental data

Table 1: Patient characteristics

Patient	Sex (F/M)	Age (yr)	Allergen	VC (% pred.)	FEV1 (% pred.)	eNO (ppb)	PC20 Methacholin (mg/mL)	PD20 Allergen (SBU)	Total IgE (IU/mL)	Dose Omalizumab (mg)
1	M	26	Mite	88.0	103.5	81.3	2.64	30.0	463	375
2	M	22	Birch	96.6	114.4	38.6	8.0	32.89	382	300
3	F	21	Grass	90	95.4	59.1	0.40	6.5	667	375
4	M	35	Grass	92.9	112.9	30.4	6.04	42.0	410	300
5	F	20	Mite	101.9	98.8	150	0.87	6.42	381	225
6	M	36	Grass	91.6	91.2	47.8	0.08	31.7	384	300
7	M	31	Mite	97.8	105.5	29.3	1.49	38.05	336	300
8	F	27	Birch	90.1	106.1	59	8.00	14.99	658	375
9	M	21	Mite	98.6	112.3	92	3.03	97.95	475	375
10	F	23	Birch	104.4	121.6	57.4	3.13	114.88	348	225
Median		24.5		94.8	105.8	58.2	2.84	32.3	397	300
Min		20		88	91.2	29.3	0.08	6.42	336	225
Max		36		104.4	121.6	150	8	114.88	667	375

Table 2: Comparison to control group (placebo) at week 0 and week 8

	0 weeks		8 weeks	
	Placebo	Omalizumab	Placebo	Omalizumab
EAR (% Δ FEV1)	21.42 (8.88- 41.55)	31.11 (25.49-58.52)	19.6 (4.17- 46.98)	11.49 (-4.6- 36.29)
LAR (% Δ FEV1)	30.36 (29.74- 36.9)	25.7 (14.29-40.19)	23.77 (3.95-29.52)	3.57 (-4.86- 13.76)
eNO (ppb) Pre-BAP	32 (10.9-223)	58.2 (29.3-172.5)	18 (7-82.3)	12.70 (5.7- 45.7)
Change from pre-BAP eNO (%)*	46.88 (-25.6-127.5)	57.06 (-1.6-145.8)	-9.41 (-35.3- 155.9)	-47.31 (-2.7 - -82.2)

Values are shown as median (range). N for Placebo group: EAR n=12, LAR n=3, eNO Pre n=15, eNO Post n=10, Omalizumab group: n=10 for all values. *Median of individual percent change in eNO 24h after BAP relative to pre-BAP eNO at 0 weeks.

Methods

Basophil activation test

Serum was collected at each study visit. Cellular antigen stimulation test (CAST; Buehlmann Laboratories, Allschwil, Switzerland) was used to analyze the effect of omalizumab on basophil activation. Briefly, leukocytes were isolated by dextrane sedimentation from ethylenediamine tetraacetic acid (EDTA) blood. Cells were stimulated with allergen mix of the relevant allergens (mite, grass, birch) at a concentration of 20 ng/mL or anti-IgE antibody solutions as positive controls for 40 minutes. Cells without stimulation were used as negative control. Afterwards cells were triple labeled with anti-CD123-PE, anti-CD63-FITC, and anti-HLA-DR-Per-CP monoclonal antibodies (Becton Dickinson, San Jose, Calif., USA) and analyzed by flow cytometry (FACSCalibur, Becton Dickinson) equipped with an air-cooled 488-nm laser. On each sample, 150 basophils were collected and data analysis was performed with Cell Quest software (Becton Dickinson).