

Omalizumab (Xolair) in children with seasonal allergic rhinitis: Leukotriene release as a potential *in vitro* parameter to monitor therapeutic effects

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To investigate the effect of omalizumab, a humanized monoclonal antibody, in addition to specific immunotherapy (SIT) on *in vitro* sulfidoleukotriene release (SLT) (A) before, (B) directly after, and (C) 1 yr after treatment with omalizumab. Children and adolescents (6.3–17.6 yr) with sensitization to birch and grass pollens and suffering from seasonal allergic rhinitis were included in a Phase III, placebo-controlled, multicenter clinical study. Within the four-arm study, patients were randomly chosen to receive SIT for either birch or grass pollen and either subcutaneous omalizumab or placebo for 24 wk during the pollen season. Thereafter, omalizumab or placebo treatment ended, but SIT therapy continued. Blood samples were collected from 92 (A, B) and 78 children (C), respectively. Leukocytes were isolated and stimulated with grass and birch pollen allergens. In the supernatants, SLT (LTC₄, LTD₄, LTE₄) were measured using ELISA [cellular allergen stimulation test, DPC-Biermann, Germany]. At the end of treatment the combination of omalizumab + SIT-grass [median SLT-release: 2125 (before) and 416 ng/ml (after omalizumab treatment); $p < 0.001$] as well as omalizumab + SIT-birch [1404 and 207 ng/ml; $p < 0.001$] resulted in significantly lower SLT release after stimulation with the corresponding allergen compared to placebo + SIT-grass [2231 and 2490 ng/ml] or placebo + SIT-birch [1324 and 2489 ng/ml]. One year after omalizumab or placebo treatment, there was no significant difference in SLT release between the 4 groups (omalizumab + SIT-grass: 2855; SIT-grass + placebo: 2543; omalizumab + SIT-birch: 2417; SIT-birch + placebo: 2573 ng/ml). These results strongly suggest that the observed effects of decreased SLT release after omalizumab treatment were attributable to the treatment with omalizumab, rather than to SIT therapy.

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An increased understanding of pathophysiologic mechanisms underlying the development of allergic disorders has resulted in several new therapeutic strategies. One of the new treatment modalities is omalizumab (Xolair[®]; formerly

rhu-MAb-E25, Novartis Pharma GmbH, Nürnberg, Germany), a recently developed recombinant, humanized monoclonal IgG1 anti-immunoglobulin-E (IgE) antibody. Omalizumab recognizes the specific Fc portion of

circulating IgE that binds to the high and low affinity IgE receptor [FcεRI and FcεRII or cluster of differentiation (CD)23], but does not interact with or to cell-bound IgE or other immunoglobulins and does not provoke mediator release from basophils or mast cells (1). To date, several clinical studies have demonstrated the safety and efficacy of omalizumab for the treatment of atopic disorders. For individuals with allergic asthma, omalizumab significantly weakens both the early- and late-phase response to inhaled allergens. Furthermore, omalizumab reduces the frequency of asthma exacerbations, improves symptom scores, and reduces the requirements for steroid medication (2–4). In patients with seasonal allergic rhinitis, omalizumab reduces rescue medication and improves quality of life (5, 6). Recently, we conducted a randomized double-blind placebo-controlled trial to assess the efficacy and safety of subcutaneously administered omalizumab or placebo, combined with specific immunotherapy (SIT), in children and adolescents with seasonal allergic rhinitis in both birch- and grass-pollen seasons. We observed that the combined therapy reduced symptom load, a combined score for daily medication use and daily eye(-) and nose symptoms over the entire pollen season by 48% (SIT-grass; $p < 0.011$) and 35% (SIT-birch; $p < 0.01$) over SIT alone. Therefore, we concluded that omalizumab therapy conferred a protective effect regardless of allergen type (6).

In addition to the clinical findings, we were able to identify a significant reduction in the *in vitro* leukotriene release in response to allergens in patients treated with SIT + omalizumab as compared with patients treated with SIT + placebo (7). However, the question remains, if the observed effects are related to omalizumab alone or to the combined therapy of omalizumab and SIT. Based only on our study design, a definitive clarification of this issue is difficult because we included no pure placebo group. Nevertheless, we hypothesized that a convergence of the sulfidoleukotriene (SLT) concentration between the two groups 1 yr after the last omalizumab injection would be suggestive of an anti-IgE effect on SLT release. Therefore, we re-evaluated our study population 1 yr after the last omalizumab injection with ongoing immunotherapy.

Methods

Two-hundred and twenty-five children and adolescents in 17 centers in Germany were enrolled to investigate the effect of omalizumab in patients with seasonal allergic rhinitis and a

clinical history of birch and grass pollen-induced seasonal allergic rhinoconjunctivitis within the previous 2 yr or longer (6). Of these 225 children, blood samples were collected from 92 (before and after administration of omalizumab or placebo) and 78 children (1 yr later), respectively. All blood samples were taken outside the pollen-season. The first blood sample was collected between September and October, about 2 wk (± 2 wk) prior to the start of SIT and 14 wk (± 2 wk) prior to the first omalizumab injection. The second and third blood samples were collected during July and August of the two consecutive years, which was between 4–6 wk and 12–14 months after the last omalizumab or placebo injection. With respect to SIT administration, the blood samples were collected at the end of a regulatory 4-wk interval (range 4–5.5 wk after the last SIT injection).

The inclusion criteria were reported elsewhere (6). In brief, we recruited children with (1) allergic rhinitis and a clinical history of birch and grass pollen induced seasonal allergic rhinoconjunctivitis within the previous 2 yr or longer (2), a presence of IgE specific for birch and grass pollen (≥ 0.7 U/l; CAP class ≥ 2), (3) no history of complaints of perennial allergic rhinitis or asthma, (4) total IgE levels within the range 30–1300 IU/ml and (5) bodyweight < 100 kg (Table 1). Signed informed consent was obtained from all subjects and/or their guardians. In our study, subjects were supplied with certain permitted rescue medications for use as needed according to a pre-defined schedule: topical levocabastine (eyedrops and nasal spray) and inhaled salbutamol (step 1), cetirizine (step 2) and prednisolone (step 3). Topical steroids, leukotriene antagonists and cromones were not permitted.

The study design was approved by the ethics committees of the participating centers. Within the four treatment arms, each subject was started on subcutaneous SIT-birch or SIT-grass, and omalizumab or placebo was given before and maintained during the anticipated pollen seasons (SIT-birch or SIT-grass; ALK-Scherax/Abello, Berlin, Germany). Omalizumab was administered subcutaneously, based on bodyweight and serum total IgE, providing a dose of at least 0.016 mg/kg per IU/ml of IgE every 4 wk. Peripheral blood SLT release in response to the different stimuli was measured by the cellular allergen stimulation test (CAST)-ELISA method (Bühlmann Laboratory AG, Schönenbuch, Switzerland) (8). The following stimuli were added: stimulation buffer as negative control, ionomycin (Calcium-Ionophor; Sigma, Taufkirchen,

Table 1. Main characteristics of our study population

	Group 1 (SIT-grass + anti-IgE)	Group 2 (SIT-grass + placebo)	p-value	Group 3 (SIT-birch + anti-IgE)	Group 4 (SIT-birch + placebo)	p-value
Number	23 (20)	24 (20)	n.s.	23	22	n.s.
Age (mean, 5–95)	12.3 (6.7–17.1)	12.0 (6.9–15.0)	n.s.	12.1 (8.0–15.9)	12.4 (7.3–17.3)	n.s.
Symptom load mean	0.37 (0.03–0.78)	0.8 (0.12–2.16)	0.012	0.54 (0.12–1.19)	0.93 (0.12–2.01)	0.03
IgE (IU/l; mean, range)	482 (87–1030)	426 (52–941)	n.s.	397 (45–1000)	428 (75–998)	n.s.
<i>Before therapy</i>						
SLT release:						
Background	269 (150–430)	244 (124–457)	n.s.	293 (160–1244)	303 (191–2274)	n.s.
Positive control	4333 (1774–7388)	3866 (2011–8916)	n.s.	3714 (1709–6467)	3506 (1246)	n.s.
<i>After omalizumab or placebo</i>						
SLT release:						
Background	357 (249–568)	344 (173–1761)	n.s.	396 (192–741)	382 (238–1663)	n.s.
Positive control	8664 (3203–13096)	9790 (6646–11991)	n.s.	9840 (6177–11673)	9031 (6022–12146)	n.s.
<i>one year after omali-zumab placebo</i>						
SLT release:						
Background	331 (144–994)	373 (101–571)	n.s.	297 (118–985)	401 (156–1941)	n.s.
Positive control	3496 (1476–5925)	3714 (1103–7375)	n.s.	2751 (1147–6472)	3981 (1259–5801)	n.s.

Group 1 received SIT-grass + omalizumab; group 2, SIT-grass + placebo; group 3, SIT-birch + omalizumab; and group 4, SIT-birch + placebo.

Number of participants after 1 and 2 yr (in parenthesis), mean age, clinical symptom scores during the first year, baseline IgE as well as SLT release (ng/l; median and the 5th and 95th percentile; negative control and positive control) before, direct after and 1 yr after anti-IgE therapy are plotted. No significant differences were observed between the four groups with respect to the listed variables (Kruskal–Wallis test).

Germany) for positive control determination and two allergens (six grass MIX, GX1; common birch T3; Bühlmann Laboratory AG) at a concentration of 2 ng/ml for cell stimulation. The value of the negative control was subtracted from the stimulated samples beforehand and all results were expressed as ng/l. All analyses were performed using the Statistical Analysis System (sas, Version 6.12; Cary, USA). Differences between the groups were tested using the Wilcoxon Two Sample Procedure and the Kruskal–Wallis Test. The differences before and after treatment were analyzed using the Wilcoxon One Sample Procedure.

Results

Blood samples were collected from 92 (before and after administration of omalizumab or placebo; 61 boys; 31 girls) and 78 children [1 yr later; 53 boys; 25 girls; mean age 12.1 yr (range: 6.3–17.6)], respectively. In this four-arm study, Group 1 was treated with SIT-grass + omalizumab (before and after administration of anti-IgE: n = 23; 1 yr later: n = 20), Group 2 with SIT-grass + placebo (n = 24/20), Group 3 with SIT-birch + omalizumab (n = 23/21) and Group 4 with SIT-birch + placebo (n = 22/17). There were no differences between the four groups regarding sex and total IgE at baseline (Table 1).

Before and 1 yr after treatment with omalizumab/placebo and SIT, the SLT release in response to birch and grass pollen allergen was

comparable and did not significantly differ between the four groups (Figs 1 and 2). At the end of the 24 wk, SIT-grass + omalizumab and SIT-birch + omalizumab treatment resulted in significantly lower SLT release after stimulation with the corresponding allergen [416 (1–1168); 207 (1–860) ng/l] compared with SIT + placebo [2490 (384–6587) ng/l; $p = 0.001$; 2489 (1–5670) ng/l; $p = 0.001$, Wilcoxon Test] (Figs 1 and 2). No significant differences emerged between the omalizumab and the placebo groups after unspecific stimulation (Table 1).

Discussion

Therapeutic use of omalizumab in the treatment of allergic asthma and allergic rhinitis was shown in both children and adults in double-blind, randomized, placebo-controlled clinical protocols. All these studies showed that the administration of omalizumab resulted in a reduced symptom scores, a reduced requirement for medication and a reduced asthma exacerbation rate (2–6). Therefore, anti-IgE offers a new approach to interfere with the pathophysiologic changes in allergic patients in a preventive way.

Sulfolipids are potent lipid mediators derived from arachidonic acid in the 5-lipoxygenase pathway, which modulate a broad spectrum of proinflammatory activities in allergic diseases (8, 10, 11). Recently, we demonstrated that SLT release of leukocytes in response to allergens is significantly suppressed in children

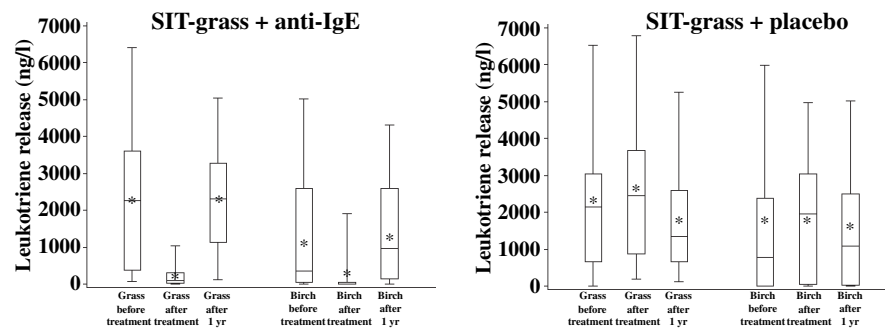


Fig. 1. *In vitro* leukotriene release (ng/l) after stimulation with grass pollen allergen and birch pollen allergen in the groups treated with SIT-grass + omalizumab (left) and SIT-grass + placebo (right) before (i), after (ii), and 1 yr (iii) after the omalizumab or placebo therapy under ongoing SIT. The boxes include the interval between the 25th and 75th percentile. The extremes represent the 5th and 95th percentiles, the median is shown as a horizontal line in the box and the mean as a star. A statistical significant difference (Wilcoxon Test) was observed when comparing the SLT release before and after therapy in response to grass and birch pollen allergen ($p < 0.001$, respectively) in the SIT-grass + anti-IgE group but not in the SIT-grass + placebo group.

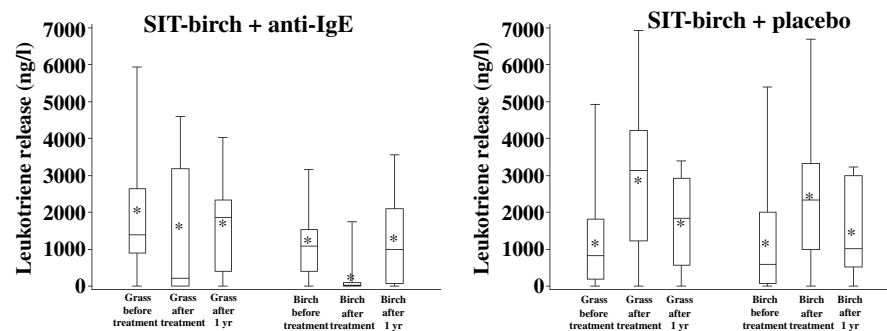


Fig. 2. *In vitro* leukotriene release (ng/l) after stimulation with grass pollen allergen and birch pollen allergen in the groups treated with SIT-birch + omalizumab (left) and SIT-birch + placebo (right) before (1), after (2), and 1 yr (3) after the omalizumab or placebo therapy under ongoing SIT. The boxes include the interval between the 25th and 75th percentile. The extremes represent the 5th and 95th percentiles, the median is shown as a horizontal line in the box and the mean as a star. A statistical significant difference (Wilcoxon Test) was observed when comparing the SLT release before and after therapy in response to grass and birch pollen allergen ($p < 0.05$ and $p < 0.001$, respectively) in the SIT-brich + anti-IgE group but not in the SIT-brich + placebo group.

treated with omalizumab + SIT (7), whereas the endogenous leukotriene B4 release in urine was unchanged (12). Here, we were able to show that SLT release in response to grass and birch pollen allergen returns to pre-treatment values 1 yr after ending omalizumab treatment, but with continued SIT.

The reason for performing the follow-up analysis was to discriminate between effects of omalizumab and immunotherapy treatment on SLT release. Because we have no pure placebo group in our study, we were only able to investigate the combined effect of omalizumab in addition to SIT on the leukotriene pathway (7). In contrast to significantly decreased SLT release in the omalizumab group directly after treatment, findings at 1 yr after the last omalizumab administration were similar in all

groups. These results strongly suggest that the observed effects of decreased SLT release after omalizumab treatment were attributable to the treatment with omalizumab, rather than to SIT therapy. However, this conclusion is hampered by the fact that an interaction between SIT and omalizumab cannot be completely excluded, i.e., the effect of omalizumab on SLT release may differ between patients with ongoing immunotherapy and patients without immunotherapy. Moreover, it would be desirable to compare the *in vitro* data with clinical parameters, i.e., symptom scores. However, this study was designed as a nested laboratory investigation to examine the SLT release in children with SIT and omalizumab or placebo. Therefore, clinical data showing a parallel decrease of SLT release and symptom scores in the anti-IgE group are only

available for the first year (6). We conclude from this data that SLT release might be particularly attractive for monitoring anti-IgE therapy.

The SLT release might also be influenced by the use of additional medications. In our study, subjects were supplied with certain permitted rescue medications for use as needed according to a pre-defined schedule: topical levocabastine (eyedrops and nasal spray) and inhaled salbutamol (step 1), cetirizine (step 2) and prednisolone (step 3). Topical steroids, leukotriene antagonists and cromones were not permitted. However, the use of medication was significantly lower in the SIT + anti-IgE groups vs. placebo. Compared with SIT-birch + anti-IgE and SIT-grass + anti-IgE groups with placebo, we observed a reduction in the median rescue medication score of 78% and 81%, respectively (6). Therefore, a bias of the decreased SLT release caused by rescue medications seems to be unlikely.

The additional effect of omalizumab on allergen immunotherapy-induced allergic reactions has important clinical implications. Very recently, a study with ragweed allergic patients confirmed our clinical findings that the addition of omalizumab to immunotherapy resulted in a significant improvement in severity scores during the pollen season (13). Moreover, this study showed that pre-treatment of patients with omalizumab for 9 wk reduced the rate of anaphylactic events during rush immunotherapy by almost 80%. It might be promising to study omalizumab pre-treatment which can lead to a safer and more effective use, and hypothetically also to longer lasting effects of allergen-specific immunotherapy for patients with allergic disorders.

To sum up, our results strongly suggest that the observed effects of decreased SLT release directly after omalizumab treatment are attributable to the treatment with omalizumab rather than to SIT therapy. Therefore, allergen-induced *in vitro* SLT release might be a promising marker for monitoring omalizumab treatment in children with allergic rhinitis.

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