

The effect of anti-IgE treatment on in vitro leukotriene release in children with seasonal allergic rhinitis

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Background: Binding of allergens with IgE to the IgE receptors on mast cells and basophils results in the release of inflammatory mediators as sulfidoleukotrienes (SLTs), triggering allergic cascades that result in allergic symptoms, such as asthma and rhinitis.

Objective: We sought to investigate whether anti-IgE (Omalizumab), a humanized monoclonal anti-IgE antibody, in addition to specific immunotherapy (SIT) affects the leukotriene pathway.

Methods: Ninety-two children (age range, 6-17 years) with sensitization to birch and grass pollens and with seasonal allergic rhinitis were included in a phase III, placebo-controlled, multicenter clinical study. All subjects were randomized to one of 4 treatment groups. Two groups subcutaneously received birch SIT and 2 groups received grass SIT for at least 14 weeks before the start of the birch pollen season. After 12 weeks of SIT titration, placebo or anti-IgE was added for 24 weeks. The primary clinical efficacy variable was symptom load (ie, the sum of daily symptom severity score and rescue medication score during pollen season). Blood samples taken at baseline and at the end of study treatment after the grass pollen season were used for separation of leukocytes in this substudy. After in vitro stimulation of the blood cells with grass and birch pollen allergens, SLT release (LTC₄, LTD₄, and LTE₄) was quantified by using the ELISA technique.

Results: Before the study treatment, SLT release to birch and grass pollen exposure did not differ significantly among the 4 groups. Under treatment with anti-IgE + SIT-grass (n = 23), a lower symptom load occurred during the pollen season compared to placebo + SIT-grass (n = 24, *P* = .012). The same applied to both groups receiving birch SIT (n = 23 and n = 22, respectively; *P* = .03). At the end of treatment, the combination of anti-IgE plus grass SIT, as well as anti-IgE plus birch SIT,

resulted in significantly lower SLT release after stimulation with the corresponding allergen (416 ng/L [5th-95th percentile, 1-1168] and 207 ng/L [1-860 ng/L], respectively) compared with placebo plus SIT (2490 ng/L [384-6587 ng/L], *P* = .001; 2489 ng/L [1-5670 ng/L], *P* = .001). In addition, treatment with anti-IgE was also followed by significantly lower SLT releases to the allergens unrelated to SIT (grass SIT: 300 ng/L [1-2432 ng/L] in response to birch allergen; birch SIT: 1478 ng/L [1-4593 ng/L] in response to grass pollen) in comparison with placebo (grass SIT: 1850 ng/L [1-5499 ng/L], *P* = .001; birch SIT: 2792 ng/L [154-5839 ng/L], *P* = .04).

Conclusion: Anti-IgE therapy reduces leukotriene release of peripheral leukocytes stimulated with allergen in children with allergic rhinitis undergoing allergen immunotherapy independent of the type of SIT allergen used. (*J Allergy Clin Immunol* 2002;110:728-35.)

Key words: Allergy, anti-IgE, Omalizumab, children, sensitization, rhinoconjunctivitis, specific immunotherapy, leukotrienes, Cellular Allergen Stimulation Test-ELISA, therapy

The allergic response in sensitized individuals is mediated by IgE-dependent mechanisms.¹ After sensitization of susceptible individuals and the synthesis of allergen-specific IgE, atopic individuals respond to environmental allergens with a type I hypersensitivity reaction. Allergens interact with basophil- or mast cell-bound specific IgE and trigger cross-linking of IgE receptors. This cross-linking provokes a cascade of events with cell degranulation and the release of inflammatory mediators, such as histamine or tryptase,^{2,3} as well as the synthesis and release of prostaglandins,⁴ leukotrienes, and cytokines.^{5,6} Taken together, these mediators orchestrate the early inflammatory response with mucosa edema and smooth muscle contraction, clinically resulting in allergic diseases such as asthma and rhinitis.

Increased understanding of the pathology for allergic diseases has led to new therapies to target specific molecular networks, including antibodies against the T_H2 cytokine IL-5, soluble IL-4 receptors, and recombinant T_H1 cytokines, such as IL-12.⁷⁻⁹

Recently, a recombinant, humanized, monoclonal IgG1 anti-IgE antibody has been developed. Anti-IgE (Omalizumab, formerly rhu-Mab-E25) recognizes the specific Fc portion of circulating IgE that binds to the high-affinity IgE receptor FcεRI.¹⁰ Although it binds to free IgE, it does not

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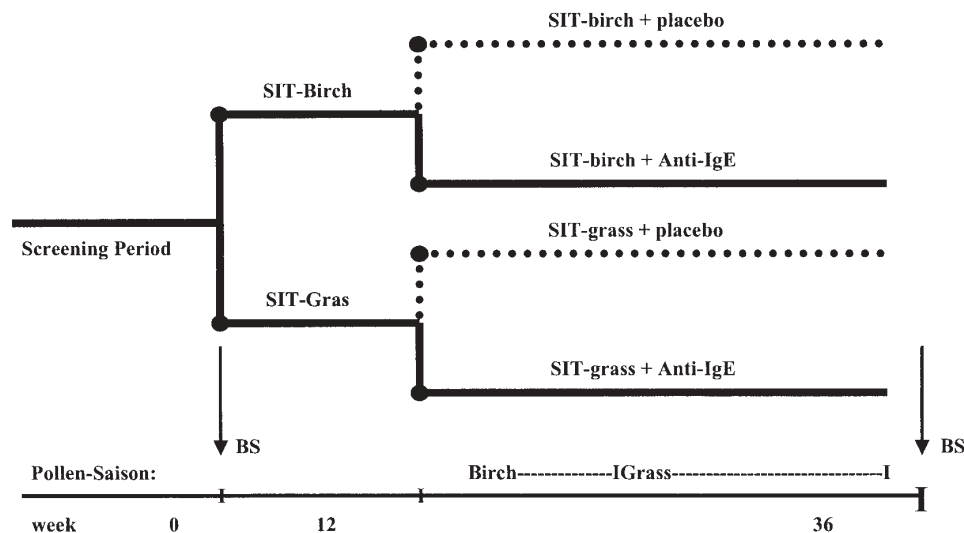


FIG 1. Study design. BS, Time point of blood sampling.

Abbreviations used

PE: Parameter estimate
SIT: Specific immunotherapy
SLT: Sulfidoleukotriene

interact with other immunoglobulins, such as IgA or IgG, or to cell-bound IgE and does not provoke histamine release from basophils or mast cells. Anti-IgE treatment decreases serum IgE levels in a dose-dependent manner, causes a downregulation of FcεRI receptors on basophils,¹¹ and inhibits both the early¹² and the late phase¹³ of the allergic response. Because the clinical response is caused by mediator release,¹⁴ the question arises of whether the effect of anti-IgE treatment can be quantified by measuring the SLT release. The aim of this study was to investigate the effect of anti-IgE in addition to specific immunotherapy (SIT) in children with seasonal allergic rhinitis on in vitro release of sulfidoleukotrienes (SLTs).

METHODS

Population

During a phase III, placebo-controlled clinical study, a total population of 225 children and adolescents in 17 centers in Germany were enrolled to investigate the effect of anti-IgE in patients with seasonal allergic rhinitis and a clinical history of birch and grass pollen-induced seasonal allergic rhinoconjunctivitis within the previous 2 years or longer.¹⁵ Of these 225 children, we were able to collect 2 corresponding blood samples from 92 subjects (mean age, 12.2 years; age range, 6.3–17.6 years; 61 [66.3%] boys and 31 [33.7%] girls) from 5 centers. The clinical study results of the whole study population were published earlier.¹⁵ The presence of IgE specific for birch and grass pollen was confirmed in all subjects (≥ 0.7 U/L, CAP class ≥ 2). Subjects who reported complaints of perennial allergic rhinitis were excluded, as were those with total serum IgE levels outside the range 30 to 1300 IU/mL and body weight of greater than 100 kg for reasons of dosing.

Study design

All subjects were randomized to one of 4 treatment groups. Two groups received birch SIT, and 2 groups received grass SIT for at

least 14 weeks before the start of the birch pollen season. After 12 weeks of SIT titration, placebo or anti-IgE was added for 24 weeks (Fig 1).

Daily symptom severity scores (eyes and nose) and rescue medication use (scored) were assessed during the pollen seasons, whereby the symptom severity score was the mean of the daily symptom severity score, and the rescue medication score was the mean of the daily rescue medication score during the pollen seasons.

The pollen seasons for birch and grass in Germany are sequential, well defined, and run from February to July, depending on the location of the center. Pollen counts were assessed systematically in all centers, and the start and end of the pollen seasons were defined as a threshold of greater than 10 grains/m³ per 24 hours for 3 consecutive days for birch and greater than 5 grains/m³ per 24 hours for grass. The efficacy analysis was based on diary information collected only during the defined pollen seasons (on average 84 days in this study).

The venous blood samples were obtained by means of cubital venipuncture before and after treatment (autumn 1999 and summer 2000). All blood samples were stored at room temperature and transported to the laboratory within 24 hours. There the preparation of the blood samples was performed immediately.

The study protocol was approved by the ethics committee of all participating hospitals, and written consent was obtained from the parents of patients.

Immunotherapy

The birch and grass SIT therapy (ALK-Scherax/Abello, Berlin, Germany) was given subcutaneously. The birch pollen extract contained 12.3 µg of major allergen (Bet v 1) per 100,000 SQ units. The grass pollen extract was a mix of 6 grasses and *Secale* species, containing the following: *Phleum pratense*, 20.2 µg of major allergen/100,000 SQ units; *Avena eliator*, 12.0 µg of major allergen/100,000 SQ units; *Dactylis glomerata*, 12.0 µg of major allergen/100,000 SQ units; *Lolium perenne*, 12.5 µg of major allergen/100,000 SQ units; *Poa pratense* 15.2 µg of major allergen/100,000 SQ units; *Festuca pratense*, 18.6 µg of major allergen/100,000 SQ units; and *Secale cereale*, 10.2 µg of major allergen/100,000 SQ units. This provided an average of 14.4 µg of major allergen/100,000 SQ units. For both the birch and grass pollens, 12 SIT injections at 1-week intervals were given during the build-up phase before the birch season, followed by 5 maintenance injections at 4-week intervals. The mean cumulative dose of birch SIT allergen extracts administered before the birch season was

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TABLE I. Clinical data of symptom scores and medication use during the birch and grass pollen season

	Group 1: grass SIT + anti-IgE	Group 2: grass SIT + placebo	P value	Group 3: birch SIT + anti-IgE	Group 4: birch SIT + placebo	P value
No.	23	24	NS	23	22	NS
Daily medication use	0.09 (0-0.25)	0.41 (0-1.51)	.01	0.13 (0-0.94)	0.65 (0-1.26)	NS
Daily eye symptoms	0.14 (0-0.5)	0.35 (0.01-0.77)	.007	0.36 (0.02-0.94)	0.58 (0.01-0.91)	NS
Daily nose symptoms	0.39 (0.02-0.8)	0.47 (0.1-0.98)	NS	0.52 (0.07-1.02)	0.62 (0.02-1.26)	.036
Mean symptom load	0.37 (0.03-0.78)	0.8 (0.12-2.16)	.012	0.54 (0.12-1.19)	0.93 (0.12-2.01)	.03

Values are given as medians (5th and 95th percentiles). Differences between groups were tested by using the Wilcoxon 2-sample test.

NS, Not significant.

394,300 SQ units, and a mean cumulative dose of 478,000 SQ units was administered for grass SIT before the grass season.

Anti-IgE therapy

After 12 weeks of SIT titration, placebo or anti-IgE was added for 24 weeks (Fig 1). Anti-IgE was administered subcutaneously on the basis of body weight and serum total IgE levels, providing a dose of at least 0.016 mg/kg per international unit per milliliter of IgE every 4 weeks.

SLT determination in cell supernatants

Peripheral blood SLT release in response to the different stimuli was measured by using the Cellular Allergen Stimulation Test–ELISA method (Bühlmann Laboratory AG, Schönenbuch, Switzerland).¹⁶ In brief, whole blood (3 mL) was subjected to dextran sedimentation for 90 minutes at room temperature. After centrifugation at room temperature (130g for 15 minutes), the leukocyte pellet was resuspended in a stimulation buffer containing 20 ng/mL IL-3. IL-3 enhances the mediator release without inducing its synthesis.¹⁷ Thereafter, aliquots of 200 μ L of the cell suspension were added in duplicate to 50 μ L of stimulation buffer as a negative control. Ionomycin (Calcium-Ionophor; Sigma, Taufkirchen, Germany) and activating monoclonal anti-IgE receptor antibodies (Le 27, 0.5 μ g/mL; Bühlmann Laboratory AG, Schönenbuch, Germany) different from therapeutic Omalizumab were administered for positive control determination and to allergens (6 grass MIX, GX1; common birch T3; Bühlmann Laboratory AG) in concentrations of 2 ng/mL for cell stimulation. This allergen dose was chosen after preliminary experiments, in which the optimal dose-response relationship was determined (data not shown).

After an incubation period of 40 minutes at room temperature, the samples were centrifuged, and then the supernatants were frozen at -20°C until analysis of SLT levels with a murine anti-SLT mAb-based ELISA. The anti-SLT mAb possesses the same affinity for LTC₄ and its 2 metabolites, LTD₄ and LTE₄, but does not cross-react with LTB₄, arachidonic acid, or other compounds with L-cysteine or with glutamine side chains. The value of the negative control was subtracted from the stimulated samples beforehand, and all results were expressed in nanograms per liter.

Statistical analysis

All analyses were performed by using the Statistical Analysis System software (SAS version 6.12). Differences between the groups were tested by using the Wilcoxon 2-sample procedure. The differences before and after treatment were analyzed by using the Wilcoxon 1-sample procedure.

To investigate the potential influence of anti-IgE treatment on SLT release, we performed multiple linear regression analysis and defined the SLT production to the specific allergen stimulation (grass or birch) after treatment as the outcome variable. First, we considered baseline SLT production to each allergen, the type of SIT, and treatment with anti-IgE or placebo as the explanatory vari-

ables. In an extended model, we took the leukocyte counts and the numbers of basophils and eosinophils into account as the explanatory variables. SLT concentrations have been log transformed for analysis. The model equation is as follows:

$$\text{LOG [SLT after therapy]} = \text{INTERCEPT} + \beta [\text{SLT release before therapy}] + \beta \text{SIT} + \beta \text{Immun},$$

where Immun is defined as anti-IgE therapy or placebo and SIT as specific immunotherapy.

RESULTS

Characteristics of patients and clinical data

A total of 92 children (61 boys and 31 girls) participated in the study. The distribution of the clinical data of the analyzed population is presented in Table I. There were no differences among the 4 groups regarding age, sex, and total IgE levels at baseline.

During both pollen seasons, the scores for daily medication use, daily eye symptoms, and daily nose symptoms were lower in the group receiving anti-IgE treatment compared with in the corresponding placebo group (Table I). In the 2 groups treated with grass SIT, the difference gained statistical significance for daily medication use, daily eye symptoms, and symptom load. Symptom load and nasal symptoms were significantly lower in the group treated with birch SIT and anti-IgE compared with that seen in the group treated with birch SIT and placebo (Table I).

SLT release after unspecific stimulation

At baseline, no differences were observed between the basal SLT release of unstimulated cells and results after stimulation with the unspecific stimuli, such as ionomycin and activating anti-IgE receptor antibodies (Kruskal-Wallis test, Table II). A significantly higher SLT release was observed in summer 2000 in all 4 groups in response to the unspecific stimulus ionomycin (Table III). However, there were no significant differences between the anti-IgE and placebo groups after unspecific stimulation (Table III).

SLT release after specific stimulation

The principal focus of this study involved detailed analysis of SLT release in response to the clinically relevant grass pollen and birch pollen allergens after anti-IgE versus placebo treatment. At baseline, there was no statistically significant difference in SLT release after in vitro stimulation with allergens (Table II). After grass SIT, we observed a significantly lower SLT release after

TABLE II. Age of the study population, total IgE concentration, and SLT release of unstimulated cells before and after stimulation with nonspecific and allergen-specific stimuli at baseline

	Group 1: grass SIT + anti-IgE	Group 2: grass SIT + placebo	Group 3: birch SIT + anti-IgE	Group 4: birch SIT + placebo	P value
No.	23	24	23	22	NS
Age (y)	12.3 (6.7-17.1)	12.0 (6.9-15.0)	12.1 (8.0-15.9)	12.4 (7.3-17.3)	
IgE (IU/L)	482 (87-1030)	426 (52-941)	397 (45-1000)	428 (75-998)	NS
Basal release	269 (150-430)	244 (124-457)	293 (160-1244)	303 (191-2274)	NS
Ionomycin	8664 (3203-13,096)	9790 (6646-11,991)	9840 (6177-11,673)	9031 (6022-12,146)	NS
Anti-IgE receptor antibody	4333 (1774-7388)	3866 (2011-8916)	3714 (1709-6467)	3506 (1246-7243)	NS
Grass	2125 (97-6026)	2231 (11-5336)	2271 (1-5931)	1614 (6-2793)	NS
Birch	1302 (32-4299)	1526 (1-5465)	1404 (260-1846)	1324 (80-2295)	NS

All SLT values (in nanograms per liter) of nonspecific and allergen-specific stimulated samples are expressed as values above the unstimulated background (basal release). Values are given as medians (5th and 95th percentiles). No differences among the LT values of the 4 groups were observed before therapy (Kruskal-Wallis test).
NS, Not significant.

TABLE III. SLT release in response to grass pollen and birch pollen allergen after therapy with SIT and anti-IgE or placebo

	Group 1: grass SIT + anti-IgE	Group 2: grass SIT + placebo	P value	Group 3: birch SIT + anti-IgE	Group 4: birch SIT + placebo	P value
No.	23	24	NS	23	22	NS
Basal release	357 (249-568)	344 (173-1761)	NS	396 (192-741)	382 (238-1663)	NS
Ionomycin	11,918 (1-14,566)	11,695 (19-15,270)	NS	12,172 (9040-15,262)	12,072 (6173-16,144)	NS
Anti-IgE receptor antibody	4665 (2509-8127)	3513 (1198-7379)	NS	4732 (2987-9841)	4403 (1152-6862)	NS
Grass	416 (1-1168)	2490 (384-6587)	.001	1478 (1-4593)	2792 (154-5839)	.04
Birch	300 (1-2432)	1850 (1-5499)	.001	207 (1-860)	2489 (1-5670)	.001

All SLT values (in nanograms per liter; median [5th and 95th percentiles]) of nonspecific and allergen-specific stimulated samples are expressed as values above the unstimulated background (basal release). The differences between the anti-IgE and placebo groups after therapy were tested (Wilcoxon 2-sample test), and *P* values were reported.

stimulation with grass allergen in the anti-IgE group compared with that seen in the placebo group ($P = .001$, Table III). Consistent with these results, we recorded, after treatment with birch SIT, a lower SLT release in response to birch allergen in the anti-IgE group compared with that seen in the placebo group ($P = .001$, Table III). We stimulated the blood cells in the birch and grass SIT groups additionally with the allergen not related to SIT. Compared with the group treated with grass SIT plus anti-IgE, we found a lower SLT release in response to birch allergen than in the placebo group ($P = .001$, Table III). In the group treated with birch SIT plus anti-IgE, the SLT release after stimulation with grass allergen was significantly lower than that in the placebo group ($P = .04$, Table III).

To describe individual changes during therapy, we examined the difference between release at baseline and release after therapy (Figs 2 and 3). In the grass SIT plus anti-IgE group, the mean SLT release in response to grass allergen was lower after treatment (416 ng/L) compared with at baseline (2125 ng/L; Wilcoxon 1-sample procedure, $P = .0001$), whereas the release in the grass SIT plus placebo group was on a similar level at baseline and after treatment (2231 and 2490 ng/L, respectively; $P > .05$; Fig 2). According to these results, birch allergen-stimulated SLT release in the birch SIT groups was lower after anti-IgE treatment (207 ng/L) than at baseline (1404 ng/L, $P =$

.0001), whereas no significant change in the placebo group was found (1324 and 2489 ng/L, respectively; $P > .05$; Fig 3). Also, for the allergen not related to the SIT, lower SLT releases were found after anti-IgE (grass SIT: birch allergen, 300 ng/L; birch SIT: grass allergen, 1478 ng/L) compared with baseline values (1302 ng/L [$P = .0001$] and 2271 ng/L [$P = .01$], respectively; Figs 2 and 3).

Multiple linear regression models

Using multiple linear regression, we investigated the potential influence of anti-IgE treatment on SLT release after therapy. SLT production to the specific allergen stimulation (grass or birch) after treatment was defined as the outcome variable. Baseline SLT production to each allergen, type of SIT, and treatment with anti-IgE or placebo were considered as the explanatory variables.

In the groups treated with grass SIT, a significant effect of the anti-IgE treatment (parameter estimate [PE] = -3.0 , $P = .0001$) and the baseline SLT release (PE = 0.46 , $P = .0001$), but not of the immunotherapy (PE = -0.31 , $P = .64$), on the SLT release in response to grass pollen allergen was seen. In the groups treated with birch SIT, we observed a significant effect of the anti-IgE treatment (PE = -3.87 , $P = .0001$) and the baseline SLT release (PE = 0.33 , $P = .0006$), but not of the immunotherapy (PE = -0.15 , $P = .77$), on the SLT release in response to birch pollen allergen. In an extended model we also took the

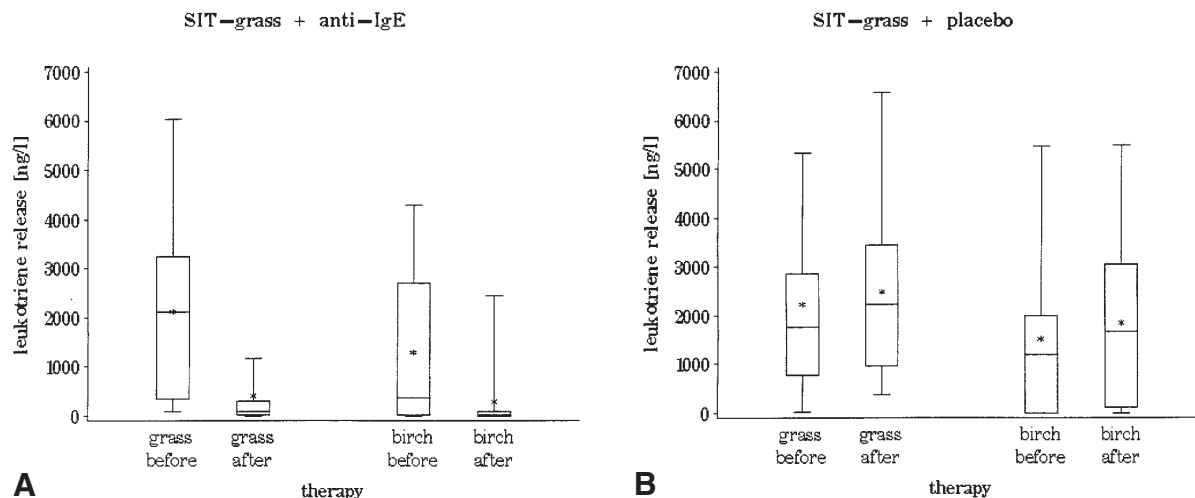


FIG 2. In vitro SLT release (in nanograms per liter) after stimulation with grass pollen allergen and birch pollen allergen in the groups treated with grass SIT plus anti-IgE (**A**) and grass SIT plus placebo (**B**) before and after therapy. The boxes include the interval between the 25th and 75th percentiles. The extremes represent the 5th and 95th percentiles. The median is shown as a horizontal line in the box and the mean as an asterisk. A statistically significant difference was observed when comparing the SLT release before and after therapy in response to grass ($P = .0001$) and birch ($P = .0002$) allergen in the grass SIT plus anti-IgE group but not in the grass SIT plus placebo group.

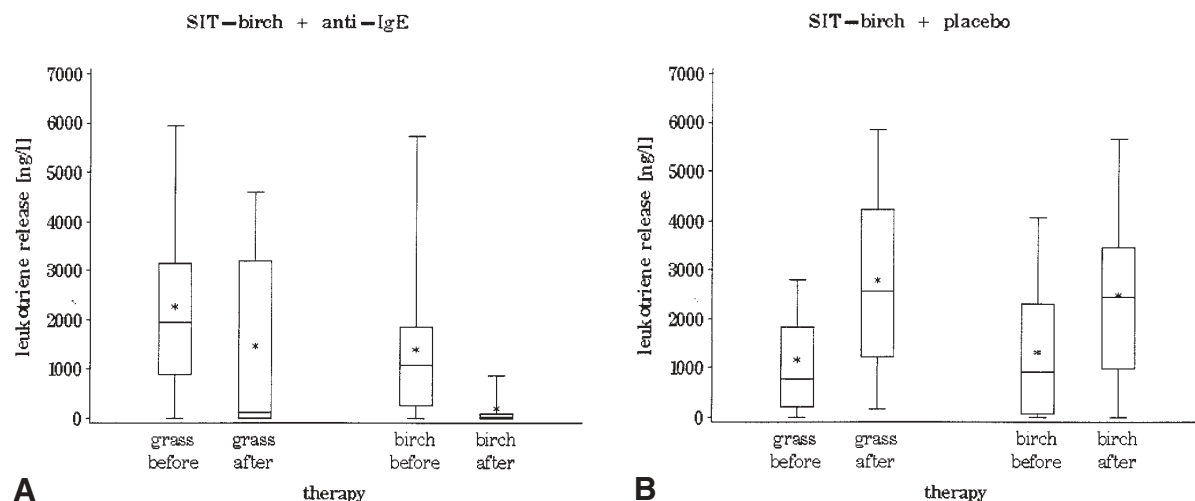


FIG 3. In vitro SLT release (in nanograms per liter) after stimulation with grass pollen allergen and birch pollen allergen in the groups treated with birch SIT plus anti-IgE (**A**) and birch SIT plus placebo (**B**) before and after therapy. The boxes include the interval between the 25th and 75th percentiles. The extremes represent the 5th and 95th percentiles. The median is shown as a horizontal line in the box and the mean as an asterisk. A statistically significant difference was observed when comparing the SLT release before and after therapy in response to grass ($P = .0011$) and birch ($P = .0001$) allergen in the birch SIT plus anti-IgE group but not in the grass SIT plus placebo group.

leukocyte counts, as well as the number of eosinophils and basophils (Table IV), as explanatory variables into account. However, we observed no significant effect of these variables on SLT release.

Correlation analysis

The in vitro SLT release in response to allergens parallels the clinical results (Table I). Additionally, we performed a correlation analysis and calculated the Spearman correlation coefficients and the P values. In the total

population we observed a weak but statistically significant positive correlation between SLT release in response to birch pollen and the use of rescue medication ($r_s = 0.27$, $P = .008$), as well as the symptom load ($r_s = 0.31$, $P = .003$). The SLT release in response to grass pollen and the use of rescue medication ($r_s = 0.18$, $P = .08$) or the symptom load ($r_s = 0.194$, $P = .06$) did not reach statistical significance. We observed no correlation between the SLT release in response to anti-IgE or ionomycin and the symptom or rescue medication scores.

TABLE IV. Absolute numbers of leukocytes, basophils, eosinophils, monocytes, and lymphocytes

	Group 1: grass SIT + anti-IgE	Group 2: grass SIT + placebo	Group 3: birch SIT + anti-IgE	Group 4: birch SIT + placebo
Before treatment				
Total leukocyte counts	7117 (4100-8800)	7583 (5100-12,600)	7578 (6100-10,400)	7191 (5400-9100)
Basophils	88 (0-273)	145 (0-192)	106 (0-176)	71 (0-182)
Eosinophils	320 (0-780)	339 (50-1015)	431 (0-968)	321 (36-823)
Monocytes	366 (77-660)	430 (148-768)	365 (75-637)	447 (168-723)
Lymphocytes	2679 (1551-4047)	3226 (2072-5200)	3166 (1690-4928)	2907 (1674-5013)
After treatment				
Total leukocyte counts	6409 (5200-10,300)	7417 (5600-9100)	6909 (5200-9600)	7141 (4400-10,000)
Basophils	19 (0-81)	38 (0-162)	25 (0-114)	32 (0-195)
Eosinophils	250 (56-513)	371 (72-880)	289 (81-546)	450 (86-896)
Monocytes	309 (171-585)	370 (68-720)	330 (114-680)	299 (0-679)
Lymphocytes	2538 (1300-4500)	2997 (1904-4488)	2906 (1856-4446)	2772 (1376-5044)

Values are given as grams per liter $\times 1000$ (median [5th and 95th percentiles]). No differences between leukocyte values and the differential blood count among the 4 groups before and after therapy were observed (Kruskal-Wallis test).

DISCUSSION

To date, a number of clinical studies have demonstrated the safety and efficacy of anti-IgE for the treatment of allergic rhinitis^{15,18} and asthma.¹⁹⁻²¹ However, until now, no randomized controlled trials with anti-IgE have used in vitro mediator release to elucidate important pathophysiologic mechanisms. The current study was able to demonstrate that allergen-stimulated, IgE-mediated in vitro SLT release can reflect an anti-inflammatory effect of anti-IgE treatment when given in combination with SIT.

All of our patients were sensitized to birch pollen, as well as to grass pollens, and the 4 groups did not differ in regard to the history of allergic complaints at the time of blood collection or the SLT values at baseline. Only the SIT medication (grass vs birch) in relation to specific pollen exposure differed between the groups. The period of natural grass pollen exposure was later and closer to the time of posttreatment blood sampling than birch pollen exposure. In other words children with grass SIT received specific therapy for their relevant allergen closer to the time point of blood sampling than the birch SIT group. However, this might not be relevant for our findings of different SLT release in the anti-IgE and placebo groups.

The clinical symptoms were monitored over the entire pollen season. Therefore our study design allows a comparison of the effects of anti-IgE treatment on the symptom load during the pollen season and the SLT releasability at the end of the treatment period. Because we have no pure placebo group for both seasons, our data do not allow the evaluation of pure anti-IgE treatment effects. Surprisingly, after 9 months of SIT, neither placebo group differs significantly (birch: 1850 vs 2489, Table III) with regard to in vitro SLT release stimulated by grass and birch allergen (Table III and Figs 2 and 3). In contrast to this lack of SIT effects, an earlier article reported a significant reduction of allergen-specific SLT release after immunotherapy with *Dermatophagoides pteronyssinus*.²² In another study wasp venom immunotherapy resulted in a reduction in histamine and SLT release by human peripheral blood leukocytes.²³ There are different aspects

that might account for the lack of change in SLT release in the groups treated with SIT alone. First, the duration of SIT in our study was only 9 months. To date, there are no data to describe the time course of possible changes in SLT release. Clinical efficacy requires a sufficient cumulative allergen dose. Also, it is possible that changes in SLT release are dose dependent. Second, our patients were sensitized to 2 clinically relevant allergens but receiving immunotherapy for only one of these allergens. This might also account for the lack of change in SLT release in children receiving SIT alone. Third, it is possible that the single effect of SIT on allergen-induced SLT release is negligible, and the posttreatment values observed by us are related predominantly to anti-IgE treatment. However, on the face of the fact that we had no pure placebo group, this remains speculative.

Because anti-IgE treatment influences IgE concentration and aims at a substantial decrease of mediator release by effector cells, we used in vitro SLT release to elucidate the treatment effect of anti-IgE when administered in combination with SIT. Leukotrienes are oxidation products of arachidonic acid that are synthesized in and released from basophils, mast cells, and, to a lesser extent, eosinophils. They mediate a large array of proinflammatory activities, are among the most potent bronchoconstrictors, and cause vasodilatation, increased microvascular permeability, exsudation of macromolecules, and edema, as well as neutrophilic chemotaxis and activation.^{24,25} We used the Cellular Allergen Stimulation Test for SLT detection, which is based on the quantification of the allergen-induced production of the SLTs LTC₄, LTD₄, and LTE₄, which are metabolic conversion products of LTC₄.

Our data revealed consistent results for both SIT allergens: for birch SIT and grass SIT, a much lower SLT release at the end of study treatment was seen after in vitro stimulation with grass and birch allergen in the anti-IgE compared with the placebo arms of the study. The basal SLT release of unstimulated cells was similar at both time points among all groups. The ionomycin-induced release of SLTs was significantly higher in sum-

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diseases

mer compared with the baseline values without significant differences among the 4 groups.

To study seasonal influences in parallel to the main study, we examined an external control group ($n = 9$) and measured monthly the basal SLT release and the release in response to anti-IgE receptor antibodies. In children with pollen allergy ($n = 4$) and individuals without pollen allergy ($n = 5$), we observed no systematic change in the basal release but a median increase in response to anti-IgE receptor antibodies of 66% (range, -12% to 185%) from March to May.

The cause of the latter, reflecting potentially increasing releasability during spring, is unknown. Nevertheless, the low SLT release after stimulation with allergen observed in the anti-IgE groups appears to not be a consequence of seasonal changes.

The therapeutic efficacy of anti-IgE and the decrease in SLT release after stimulation with allergens observed by us might be attributable to several pharmacologic mechanisms. In addition to the effects of neutralizing free IgE, the IgE production by B cells is diminished.²⁶ Moreover, earlier studies revealed that the density of FcεRI on basophils correlates strongly with the IgE levels in blood.²⁷ In vitro and clinical studies have described that anti-IgE treatment, which reduces plasma levels of free IgE to near zero, can also downregulate FcεRI by more than 95% after a treatment period of 5 weeks to 3 months.²⁸ Furthermore, in vitro data reported that FcεRI can be upregulated or downregulated in cultured basophils by changing the concentration of IgE up or down, respectively.²⁹ Our results revealed higher SLT release after stimulation with anti-IgE receptor antibodies in summer, with no statistical differences between the groups treated with anti-IgE (Omalizumab) and placebo. Therefore SLT release in response to an anti-IgE receptor antibody does not reflect treatment with Omalizumab. Because we have no data regarding the density of FcεRI on basophils, we cannot comment on upregulation or downregulation of IgE receptors on effector cells. To the best of our knowledge, there are no data regarding the correlation between SLT release in response to anti-IgE receptor antibodies and the IgE receptor density on basophils. We observed, on a functional level, that the remaining high-affinity FcεRI receptors are able to mediate a substantial release of SLT, even after 24 weeks of treatment with anti-IgE. This does not differ from what was seen in the placebo group. However, anti-IgE receptor needs only a small number of FcεRI receptors to induce SLT production by effector cells. We speculate that the combination of a decreased number of circulating specific IgE molecules and reduction of FcεRI receptor density on effector cells in children receiving SIT plus anti-IgE might suffice to reduce the number of receptors binding allergen-specific IgE molecules and therefore reduce SLT release after stimulation with a single allergen.

The observed effects of immunotherapy plus anti-IgE on in vitro SLT release parallels the clinical benefit for these patients. We used the symptom load, a construct combining symptom severity score and rescue medication score, to assess the clinical effects. We propose that this

score is more robust than the symptom severity score or rescue medication score alone because it assesses the benefit for patients regardless of their preference for reduce medication or clinical symptoms. The symptom load was significantly reduced in both anti-IgE groups compared with in the placebo group. Additionally, we showed a significant association between the use of rescue medication score, as well as the symptom load and in vitro SLT release, in response to birch pollen after therapy. However, the correlation coefficient was weak and only significant for some of the clinical outcomes. This might be due to the fact that our study was not designed to establish a marker for the clinical effect of anti-IgE therapy. Taken together, compared with exclusive SIT with pollen allergens, the combination of SIT and anti-IgE is associated with a significantly reduced in vitro SLT release after stimulation with allergens parallel to the reduction in symptoms and the use of rescue medication. We therefore conclude that anti-IgE therapy inhibits the SLT pathway in children with allergic rhinitis receiving immunotherapy, regardless of the type of SIT allergen.

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