

RESEARCH ARTICLE

Effects of Nasal Corticosteroids on Boosts of Systemic Allergen-Specific IgE Production Induced by Nasal Allergen Exposure

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Abstract

Background

Allergen exposure via the respiratory tract and in particular via the nasal mucosa boosts systemic allergen-specific IgE production. Intranasal corticosteroids (INCS) represent a first line treatment of allergic rhinitis but their effects on this boost of allergen-specific IgE production are unclear.

Aim

Here we aimed to determine in a double-blind, placebo-controlled study whether therapeutic doses of an INCS preparation, i.e., nasal fluticasone propionate, have effects on boosts of allergen-specific IgE following nasal allergen exposure.

Methods

Subjects (n = 48) suffering from grass and birch pollen allergy were treated with daily fluticasone propionate or placebo nasal spray for four weeks. After two weeks of treatment, subjects underwent nasal provocation with either birch pollen allergen Bet v 1 or grass pollen allergen Phl p 5. Bet v 1 and Phl p 5-specific IgE, IgG₁₋₄, IgM and IgA levels were measured in serum samples obtained at the time of provocation and one, two, four, six and eight weeks thereafter.

Results

Nasal allergen provocation induced a median increase to 141.1% of serum IgE levels to allergens used for provocation but not to control allergens 4 weeks after provocation. There

and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: This study was supported by the Christian Doppler Research Association, Vienna, Austria, and by the following research grants from commercial funders: Biomay, Vienna, Austria, and Thermofisher/Phadia, Uppsala, Sweden. Trial Medication was provided by GlaxoSmithKline. Friedrich Horak has received funding from GSK and collaborated with them on a conduct clinical trial: "A Randomized, Double-blind, Placebo Controlled, Incomplete Block, 3 Way Cross Over Study in Subjects With Allergic Rhinitis to Assess the Effect of Intranasal Repeat Doses of SB-705498 When Administered Alone or in Conjunction With Intranasal Fluticasone Propionate on the Symptoms of Rhinitis in the Vienna Allergen Challenge Chamber." Verena Niederberger has received research grants from the Austrian Science Fund (FWF), Allergy Therapeutics, UK, and Biomay, Vienna, Austria, has received honoraria for speaking at meetings from Thermofisher and has consulted Biomay, Vienna, Austria. Christian Lupinek has received honoraria from Thermofisher. Friedrich Horak has received research grants from Allergy Therapeutics, Calistoga, GSK, Meiji Seika, MSD, Oxagen, Schering Plough, and Stallergenes. Rudolf Valenta has received research grants from the Austrian Science Fund (FWF), from Biomay AG, Vienna, and Thermofisher, Uppsala, Sweden. He serves as a consultant for Biomay AG, Vienna, Austria, and Thermofisher, Uppsala, Sweden. All other authors have no conflicts of interest to declare. There are no further patents, products in development, or marketed products to declare. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials, as detailed online in the guide for authors.

were no significant differences regarding the boosts of allergen-specific IgE between INCS- and placebo-treated subjects.

Conclusion

In conclusion, the application of fluticasone propionate had no significant effects on the boosts of systemic allergen-specific IgE production following nasal allergen exposure.

Trial Registration

<http://clinicaltrials.gov/NCT00755066>

Introduction

Immunoglobulin E (IgE) plays a central role in the pathogenesis of allergy and asthma. Allergen-induced cross-linking of IgE bound to the surface of mast cells and basophils via FcεRI leads to the degranulation of these cells and the release of inflammatory mediators, proteases and pro-inflammatory cytokines [1]. IgE also enhances allergen uptake and presentation to T cells by antigen presenting cells (dendritic cells, monocytes and B cells) via binding to FcεRI and the low affinity IgE receptor FcεRII (CD23) [2,3].

In addition, IgE prolongs the survival of mast cells and up-regulates the expression of its receptors (FcεRI, CD23) [4]. Furthermore, it has been demonstrated that mast cell and basophil sensitivity correlates with the levels of allergen-specific IgE antibodies [5, 6].

Several clinical studies have demonstrated that recurrent allergen contact increases the levels of allergen-specific IgE antibodies and the clinical sensitivity towards the corresponding allergens [7–12], whereas prolonged lack of allergen contact will decrease allergen-specific IgE and eventually lead to clinical unresponsiveness [13]. In this context it was shown that antigen/allergen stimulation particularly via the nasal mucosa is followed by an increase of allergen-specific IgE levels [11, 14–15].

For allergen-specific immunotherapy (SIT) it was demonstrated that the induction of allergen-specific IgG was associated with a reduction of the boosts of allergen-specific IgE production after allergen exposure, suggesting that SIT has a suppressive effect on allergen-specific IgE production [16–19].

Intranasal corticosteroids (INCS) represent a first line anti-inflammatory drug used for the treatment of allergic rhinitis but their underlying effects on the allergic immune response are not entirely clear. While the anti-inflammatory properties of corticosteroids are well studied, less is known about their impact on allergen-specific IgE levels. *In vitro* studies using cultured peripheral blood mononuclear cells (PBMC) have demonstrated that corticosteroids enhance interleukin (IL)-4-induced rises of IgE levels [20–23]. Similar observations were made in allergic patients, who exhibited a polyclonal rise of IgE antibodies in their sera after systemic treatment with prednisolone [24]. On the other hand, corticosteroids have been shown to selectively reduce rises of nasal IL-4, IL-5 and IL-13-producing cells following *in vivo* allergen exposure [25], thereby possibly being capable of down-regulating IgE production. A few studies which investigated the effects of topical corticosteroids on IgE production showed either no or a dampening effect [26–28].

In the present double-blind placebo-controlled study we used purified recombinant allergens for controlled nasal provocation in allergic subjects to investigate whether treatment with

a frequently used topical corticosteroid, i.e., nasal fluticasone propionate, impacts on systemic allergen-specific IgE levels following nasal allergen exposure.

Methods

The protocol for this trial and supporting CONSORT checklist are available as supporting information; see [S1 CONSORT Checklist](#) and [S1 Protocol](#). The study was approved by the ethical committee of the Medical University of Vienna, the ethical committee of the "Österreichischen Arbeitsgemeinschaft für klinische Pharmakologie und Therapie" and the ethical committee of the private hospital "Institut für Hypertoniker" (1090 Vienna, Kinderspitalgasse 10/15). All study participants gave written informed consent. The study has been registered at <http://clinicaltrials.gov/> under the trial number: NCT00755066. Inclusion of patients was started before registration at clinicaltrials.gov because the importance to do so was unknown to the investigators at the time. The study was however registered at the EudraCT website before inclusion of patients was initiated (Eudract-number: 2005-004274-24). Participants were included in the study between November 2005 and February 2006. The authors confirm that all ongoing and related trials for this drug/intervention are registered

Recombinant allergens

Recombinant pollen allergens (rPhl p 1, rPhl p 5, rBet v 1), were obtained from BIOMAY (Vienna, Austria). rPhl p 1 [29] and rPhl p 5 [30] represent two major timothy grass pollen allergens and rBet v 1 [31] is the major birch pollen allergen. The allergens were selected because they are recognized by the vast majority of patients sensitized to the corresponding allergen sources and are immunologically distinct from each other.

Study design

Forty eight grass and/or birch pollen allergic subjects ([Fig. 1](#), [Table 1](#)) who were sensitized to rPhl p 5 and/or rBet v 1 were enrolled in a randomized, double-blind, placebo-controlled study. Volunteers were recruited using posters to advertise the study at the Medical University of Vienna. The study design is depicted graphically in [Fig. 2](#). Study participants were randomized to receive four weeks of either fluticasone propionate (100µg daily into each nostril, corresponding to the recommended daily treatment dose of 200µg) or placebo nasal spray. After two weeks of treatment, subjects underwent nasal provocation with either rPhl p 5 or rBet v 1 on two consecutive days. A pre-treatment phase of 2 weeks before allergen exposure was chosen in order to comply with the ARIA guidelines (www.whiair.com) which suggest that the maximum efficacy of corticosteroids requires up to two weeks of treatment. Serum IgE levels to the allergen used for nasal provocation (rPhl p 5 or rBet v 1) and to a control allergen (rBet v 1 or rPhl p 5 or rPhl p 1, respectively) were the primary outcome measure and were determined in blood samples obtained on the day of nasal provocation and 1, 2, 4, 6 and 8 weeks thereafter. The whole study was performed in winter outside the birch and grass pollen season to exclude effects due to natural seasonal allergen contact.

Study subjects and study medication

All 48 volunteers who participated in the study were grass and/or birch pollen allergic as assessed by case history, standard skin prick testing with inhalant allergens and ImmunoCAP measurements (Phadia, Uppsala, Sweden). All but one of the subjects was sensitized to at least two of the three allergen molecules. Subjects' demographic and clinical data including standard test results and specific IgE levels to recombinant allergens are shown in [Table 1](#). Twenty-five

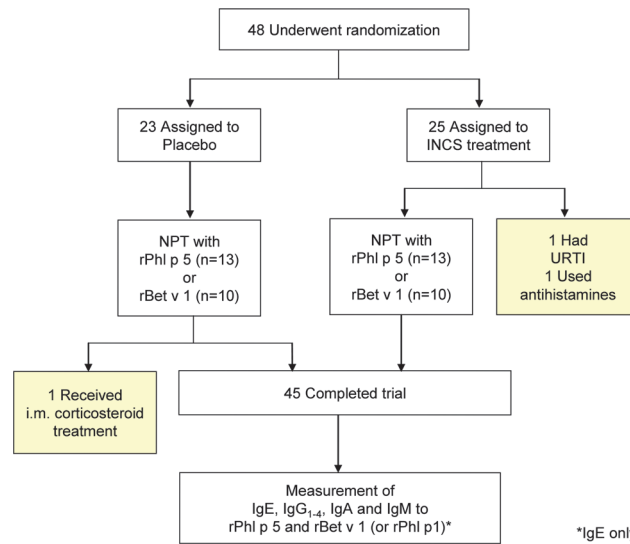


Fig 1. Distribution of study subjects. Of the 48 volunteers who were randomized, 45 completed the trial. Drop-outs are marked in yellow. INCS: intranasal corticosteroid; NPT: nasal provocation test; URTI: upper respiratory tract infection; i.m.: intra-muscular

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subjects were randomized to be treated with fluticasone propionate nasal spray and 23 subjects were assigned to placebo (Fig 1). Randomized study medication was provided in blocks of four by GlaxoSmithKline and decoding information was kept in a sealed envelope which was opened for de-blinding only in case of a systemic adverse reaction and after database closure was completed and all data were deposited with an independent statistician. The compliance of the study participants was ensured by weighing all treatment bottles after two and four weeks of treatment. All subjects had stable lung function and did not receive antihistamines or corticosteroids other than the study drug during the study. Corticosteroids were not allowed within three months and antihistamines were not allowed two weeks prior to the study. Subjects suffering from perennial allergic rhinitis and signs of airway infection during the treatment period were excluded from the study.

Nasal provocation

At the beginning of the study all study participants were asked about the severity of their nasal symptoms during the previous birch and grass pollen season. Symptoms (i.e., nasal itching, sneezing, rhinorrhea, nasal blockage) were graded as follows: 0 points: not present; 1 point: present but no discomfort; 2 points: present, some discomfort, not interfering with daily life activities; 3 points: bothersome and interfering with daily life activities or disturbing sleep [32]. The use of medication to control symptoms received 2 additional points.

Based on this score the allergen from the source which had caused more severe symptoms was chosen for nasal provocation. Twenty-six subjects were assigned to nasal provocation with rPhl p 5 and 20 subjects to nasal provocation with rBet v 1 (Fig 1). Nasal provocation with recombinant allergens was performed as previously described [11, 33]. Subjects underwent nasal application of the same allergen on two consecutive days after a two week period of treatment with either fluticasone propionate or placebo (Fig 2). Purified recombinant allergens were diluted to 20µg/ml in sterile 0.9% sodium chloride solution and administered using a metered-dose nasal pump spray delivering 40 µl of solution per spraying action. On each day of nasal provocation, two sprays of the solution were given into one nostril, resulting in a dose of 1.6µg

Table 1. Demographic and clinical data of study participants.

Characteristic	Fluticasone propionate (N = 23)	Placebo (N = 22)
Male sex—no (%)	15 (65)	10 (45)
Age—yr		
Mean	26.1±4.5	25.2±4.1
Range	20–40	19–37
No. of years with seasonal rhinitis	14.3±4.7	11.7±6.1
Specific IgE (kUA/l)		
<i>Allergen used for nasal provocation</i>		
Median	11.6	11.0
IQR	20.7	24.4
<i>Control allergen</i>		
Median	7.1	6.7
IQR	13.3	17.1
Skin prick test—wheal area (mm²)		
<i>Allergen used for nasal provocation</i>	40.3	47.2
<i>Control allergen</i>	37.6	47.1
Symptom score during previous pollen season		
<i>Allergen used for nasal provocation</i>	9.5±3.4	8.7±4.2
<i>Control allergen</i>	8.2±3.7	7.0±4.0
Total IgE (kU/l)		
Median	197	196
IQR	235.3	233.0
Sensitivity to other allergens—no. (%)		
Dust mites	10 (43)	17 (77)
Cat dander	8 (35)	11 (50)
Mold	2 (9)	3 (14)
Coexisting conditions—no. (%)		
Asthma	4 (17)	5 (23)
Conjunctivitis	23 (100)	21 (95)

*Plus-minus values are means $\pm$ SD. Wheal areas were determined by computer-aided planimetry. IQR = Interquartile range.

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of allergen per study day and in a cumulative dose of 3.2 μ g of recombinant allergen per subject. The dose used for nasal provocation was chosen because it has been used in previous studies [11] and because it is in the same range as the daily dose of allergen which a patient would be exposed to during high pollen season [34]. In fact, it has been shown that during the peak pollen season, a grass pollen allergic patient inhales approximately 5000 pollen grains per day, corresponding to 0.6 μ g of Phl p 5 allergen [34]. As Phl p 5 represents only one of 11 allergens contained in grass pollen [35], the amount of allergen used by us (i.e., 1.6 μ g of one recombinant allergen per study day) is in a dose range occurring during natural exposure.

Measurement of changes in nasal flow and nasal symptoms were recorded merely in order to ensure that nasal provocation was successful in all subjects. Therefore, these data were only a secondary endpoint of this study. Nasal flow was determined by active anterior rhinomanometry at 150 Pa measured before administration of test substances, 10 minutes after application of

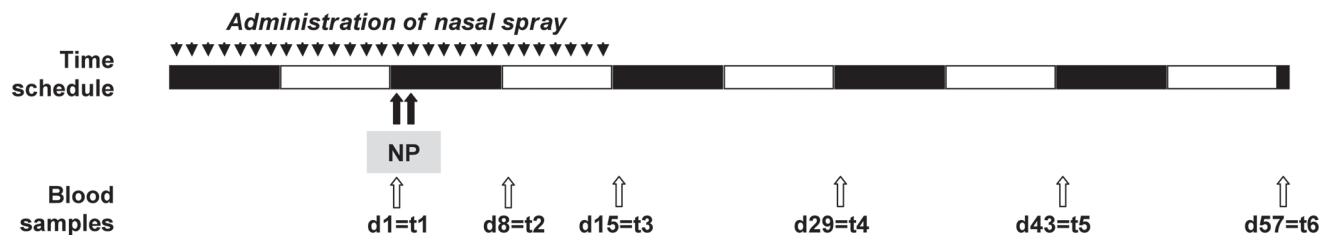


Fig 2. Study design. Patients administered nasal spray containing either nasal fluticasone propionate or placebo (black arrows) for 2 weeks before and after nasal allergen provocation (NP) performed on two consecutive days. Blood samples for measurement of allergen-specific antibodies (white arrows) were obtained at the time of the first nasal provocation (day 1 = t1) and on days 8 (t2), 15 (t3), 29 (t4), 43 (t5) and 57 (t6).

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0.9% sodium chloride, and 10 and 20 minutes after administration of test substances. Flow reduction was calculated by subtracting nasal flow after administration of test substances from nasal flow after administration of 0.9% sodium chloride solution. Subjective nasal symptoms after provocation were determined by adding scores (maximum: 12 scores) for the symptoms nasal itching, nasal congestion and nasal discharge using a 4-point scale (0: no symptoms, 1: mild symptoms, 2: moderate symptoms, 3: severe symptoms) and by counting the number of sneezes (0: no sneezes, 1: ≤ 5 sneezes, 2: 6–10 sneezes, 3: > 10 sneezes) [36, 37]. In addition, subjects recorded how long it took for their nasal symptoms to subside after nasal provocation.

Blood samples, measurement of antibodies

For the preparation of serum, blood samples were taken by puncture of the antecubital vein on the day of nasal provocation as well as 1, 2, 4, 6 and 8 weeks thereafter (Fig. 2). Serum samples were stored at -20°C until use and analyzed for IgE, IgA, IgM and IgG_{1–4} antibodies to the allergen used for nasal provocation and to an unrelated control. For subjects who had received rPhl p 5 by nasal provocation, rBet v 1 or rPhl p 1 served as control allergen, whereas for subjects who had received rBet v 1 for nasal provocation, rPhl p 5 or rPhl p 1 were used as control allergens (control allergen: Phl p 1: $n = 17$; Bet v 1: $n = 8$; Phl p 5: $n = 19$; no control allergen: $n = 1$).

Total and allergen-specific IgE levels were determined by ImmunoCAP measurements (Phadia, Uppsala, Sweden). All serum samples collected during the study were measured at the end of the study in a blinded manner. Allergen-specific IgA, IgM and IgG_{1–4} levels were measured by ELISA as described [33].

The six serum samples obtained from each subject during the course of the study were analyzed for each isotype and subclass against each of the recombinant allergens on one plate to assess accurately the time course of antibody responses. Each serum sample was tested in duplicate and mean values of the optical densities (OD) were calculated. Differences between duplicate wells were consistently $< 10\%$. Reference sera for each isotype and subclass were included on each plate to avoid plate-to-plate variations.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics, Version 20.0 (IBM Corp. Armonk, NY) and SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Sample size calculations were based on the following assumptions: It was expected that during the eight weeks period rPhl p 5/rBet v 1-specific serum IgE antibody levels would increase after challenge to a level of at least one standard deviation above baseline [11]. Our hypothesis was that this increase would be prevented by INCS by a true effect of at least 1 standard deviation at one time point. Assuming a moderate correlation of 0.4 between repeated measurements within a patient, a sample size of 19 per group (placebo, fluticasone) was calculated to result in a power of 80% to detect the

assumed difference between groups using an F-Test from a repeated measures design at a significance level of 0.05. A higher number of patients was included to account for possible drop-outs.

The changes in Phl p 5- and Bet v 1-specific IgE-levels between t1 and t2, t3, t4, t5 and t6, respectively, were assessed by linear mixed effect models. The data were log-transformed before the analyses, due to the right skewed distribution of the raw data. The differences between the IgE values at time-points t2 to t6, respectively, and the baseline value at t1 were calculated for the log-transformed data. A linear mixed model was fit to explain the differences in log-IgE values by the categorical variable time-point, treatment group (placebo vs. fluticasone) and the interaction between time-point and treatment. A random intercept for the patient was included to account for the within-patient correlation of repeated observations.

From this model, the null hypothesis of no effect of time and the null hypothesis of no treatment effect at any time point were tested by F-tests.

The effect of treatment was estimated at each time-point as the mean log-IgE difference in the fluticasone group minus the mean log-IgE difference in the placebo group. A corresponding 95% confidence interval was calculated. In addition, the average effect of treatment on the log IgE difference over all five time-points was estimated.

Following back-transformation, the estimated effects can be interpreted as median of the ratio of relative changes from t1.

The analysis was performed for Phl p 5-specific IgE levels, Bet v 1-specific IgE and combined Phl p 5- and Bet v 1-specific IgE levels.

Including the baseline (t1) value of log-IgE in the regression model was considered, to account for a possible influence of the initial amount of IgE on the change. However, for both, Phl p 5 and Bet v 1, and the combination of both allergens the effect of baseline IgE was not significant and small in magnitude and it was not included in the final models.

The calculations were done using PROC Mixed in SAS 9.4. The method due to Kenward and Roger was used to calculate degrees of freedom and standard errors.

Results

Study participants

Forty-eight birch and grass pollen allergic subjects participated in the study and were randomized to the two treatment groups ([Table 1](#); [Fig. 1](#)). Two subjects from the actively treated group were excluded from the study at the time of nasal provocation, one because he had used oral antihistamines, and the other because he had a viral upper respiratory tract infection. Another subject from the placebo group was excluded after nasal provocation because he had received intramuscular corticosteroids for a medical problem that was unrelated to the study ([Fig. 1](#)).

Demographic characteristics of placebo- and INCS-treated subjects are displayed in [Table 1](#). The two groups were comparable regarding demographic parameters, allergic history, symptoms as well as total and specific IgE levels to the allergens used for nasal provocation and the control allergens.

Allergen-specific systemic IgE levels are boosted by nasal allergen exposure

After two weeks of treatment with 200µg fluticasone propionate or placebo nasal spray, 26 subjects were exposed to Phl p 5 and 20 subjects to Bet v 1 by nasal provocation ([Fig. 2](#)). Symptoms and flow reduction after nasal provocation were recorded to ensure that the provocation procedure was performed successfully in all patients but these results were not a primary outcome of

this study. Nasal provocation was followed by an increase in the symptom scores in all 43 patients. There was however no relevant difference between those subjects who had been pre-treated with steroid and placebo nasal spray regarding their nasal symptoms after the first nasal provocation (median placebo: 7 scores, median steroid: 6 scores, n.s.) and second nasal provocation (median placebo: 6 scores, median steroid: 6 scores, n.s.). Also, we observed a decrease in nasal flow in 21 of the 22 subjects in the placebo group (median decrease: day 1: 223 ml/s, $p < 0.001$, day 2: 217 ml/s, $p < 0.001$) and in 19 of the 21 subjects in the steroid treated group (median decrease: day 1: 185 ml/s, $p < 0.001$, day 2: 167 ml/s, $p < 0.001$). There was a trend towards lower flow reduction in the INCS group which was however not statistically significant. Subjects recovered significantly more quickly from their nasal symptoms after nasal provocation if they had been pre-treated with nasal steroids (median time to recovery: placebo: 165 minutes, steroids: 60 minutes, p -value: 0.019).

Systemic allergen-specific IgE-levels were the primary endpoint of this study. Two, 4, 6 and 8 weeks after nasal provocation we observed a substantial and statistically significant ($p < 0.001$) rise of allergen-specific IgE levels to the allergen used for nasal provocation but not to the control allergen (average of the median increases for Phl p 5 and Bet v 1 at t4: 141.1%, Fig. 3). The maximum rise of specific IgE was measured four weeks after provocation. This rise was followed by a plateau/slight decline of specific serum IgE levels until week 8 (Fig. 3). No relevant changes of IgE levels to the control allergens were observed (Fig. 3). There was no statistically significant difference between the placebo- and nasal steroid-treated group regarding the development of allergen-specific IgE (Fig. 3, Table 2). In more detail, the ratio of IgE increase in the fluticasone versus placebo treated patients was almost 1 at all time-points, indicating no difference, in both the group exposed to nasal Phl p 5, Bet v 1, and a combination of the two groups (Table 2, column: "Ratio of relative changes from t1"). A calculation of confidence intervals revealed that the risk of overlooking a beneficial average blunting effect of fluticasone on IgE increases of more than 14% was less than 2.5% (Table 2). Furthermore, no relevant correlations were found between the baseline IgE-levels at t1 and the magnitudes of the relative changes in IgE-levels in the subsequent observation period (data not shown).

Rises of allergen-specific antibody isotypes other than IgE induced by nasal provocation were only moderate

Rises of allergen-specific antibody isotypes/subclasses other than IgE were observed for Phl p 5-specific IgG₄ levels in both placebo- and INCS-treated subjects who had received rPhl p 5 intranasally ($p < 0.001$, difference between placebo and INCS-treatment: n.s., Fig. 4). However, the statistical significance of this result should be regarded with caution because of the small effect size and of the high number of secondary analyses that were performed. Also a small induction of Phl p 5-specific IgA levels was noted in the placebo group after nasal exposure to Phl p 5 (Fig. 4, n.s.). IgG₄ levels to Bet v 1 and IgG₁₋₃, IgA and IgM levels were very low in most subjects before provocation and did not change substantially during the weeks after provocation (Fig. 5).

Discussion

Increases of allergen-specific IgE production in allergic patients after nasal or respiratory allergen contact may have unfavourable effects for several reasons. In fact it has been demonstrated that higher levels of allergen-specific IgE caused increased clinical sensitivity to the culprit allergens [7–12]. Furthermore, increases of IgE levels have been shown to prolong the survival of and to up-regulate the expression of Fcε receptors on mast cells, basophils and antigen presenting cells [5]. The latter leads to enhanced degranulation and release of inflammatory mediators,

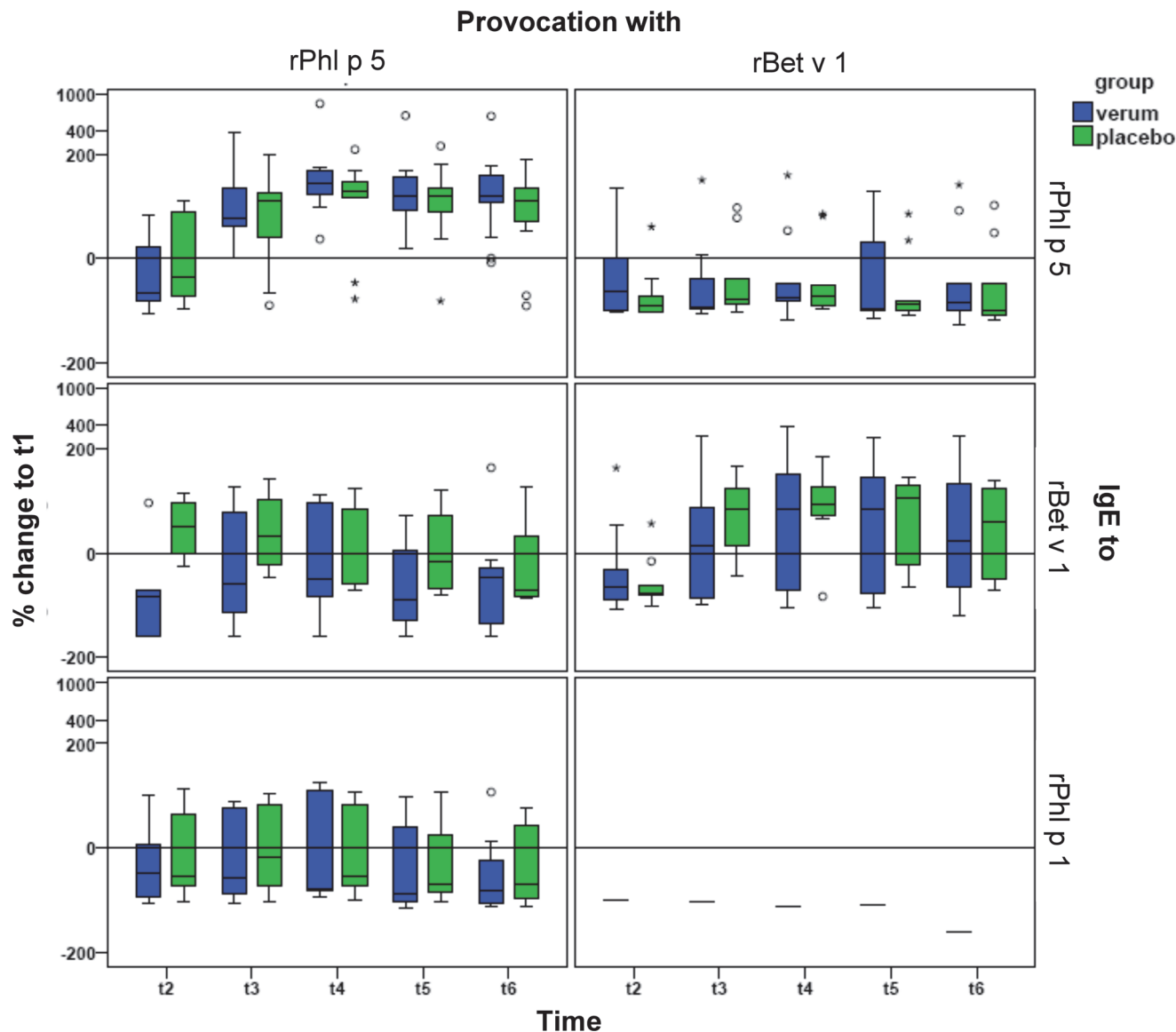


Fig 3. Development of allergen-specific IgE levels. Relative changes of IgE-levels (y-axes) to Phl p 5, Bet v 1 and Phl p 1 (top to bottom: right labels of each chart) compared to t1 (day 1) are shown for all visits (t2: day 8; t3: day 15; t4: day 29; t5: day 43; t6: day 57, x-axes). Results for the steroid-treated group are shown in blue, the placebo-group in green. Results from participants challenged with Phl p 5 are depicted in the left column, those for the Bet v 1-challenged group on the right side. Outliers that lie between 1.5 and 3 times the interquartile range below the first or above the third quartile are shown as open circles ("o"), those that lie beyond 3 times the interquartile range are depicted by asterisks ("*"). There were no significant differences between the fluticasone and placebo groups at any time point.

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proteases and pro-inflammatory cytokines from mast cells and basophils [38] and to increased IgE-facilitated antigen presentation to T cells and thus to their activation [2, 3].

Therapies such as SIT which can reduce allergen-specific IgE production offer the advantage that they modulate the course of allergic disease in addition to the reduction of allergic symptoms [16–19]. In fact, it has been demonstrated that SIT can prevent the transition from mild (i.e., rhinitis) to severe (i.e., asthma) manifestations [39] and that SIT has long-term effects even after discontinuation [40].

Table 2. Effect of fluticasone versus placebo on the median relative change in allergen-specific IgE levels.

Allergen	Time-point	Ratio of relative changes from t1	95% CI low	95% CI up
Phl p 5	t2	0.899	0.654	1.236
	t3	1.064	0.775	1.459
	t4	1.220	0.890	1.674
	t5	1.091	0.796	1.497
	t6	1.189	0.865	1.634
	average	1.086	0.822	1.436
Bet v 1	t2	1.039	0.710	1.521
	t3	0.948	0.649	1.385
	t4	1.040	0.712	1.518
	t5	1.080	0.739	1.577
	t6	1.050	0.719	1.533
	average	1.030	0.727	1.460
Combined	t2	0.964	0.758	1.227
	t3	1.013	0.798	1.286
	t4	1.142	0.899	1.449
	t5	1.090	0.858	1.384
	t6	1.127	0.887	1.432
	average	1.065	0.861	1.317

The ratio of relative changes from t1 is the relative change in the fluticasone group divided by the relative change in the placebo group. A ratio of 1 corresponds to no differential effect between fluticasone and placebo, a ratio below 1 corresponds to a beneficial blunting effect of fluticasone on allergen-induced IgE levels. 95% CI low and 95% CI up indicate the lower or upper bound of a 95% confidence interval, respectively.

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The effects of other frequently used forms of anti-allergic treatment on allergen-specific IgE antibody production have not yet been studied in defined experimental model systems. INCS are a first line of treatment for seasonal and perennial allergic rhinitis [41] as well as for other chronic inflammatory diseases of the nose and paranasal sinuses (e.g., nasal polyps, chronic sinusitis) [42]. They seem to provide excellent control of local inflammatory conditions and are therefore widely used in adult as well as paediatric patients [43, 44]. In asthmatic patients, it has been shown that early and sustained treatment with corticosteroids may even prevent some of the irreversible airway dysfunction [45–46]. Since steroids have mainly an effect on chronic and late phase allergic inflammation but not on acute IgE-mediated mast cell degranulation, it was not unexpected that we did not find statistically significant differences between the symptoms and nasal patency in the placebo-treated and steroid-treated subjects, which was measured shortly after nasal provocation. Steroid-treated subjects recovered significantly more quickly after provocation, indicating an effect of nasal steroids on the late phase response. Furthermore, there was a trend towards less severe reduction of nasal flow after provocation in patients treated with INCS. It may thus be speculated that while the beneficial effect of steroid treatment was not measurable immediately after an acute single provocation, it should be much more obvious during prolonged allergen contact.

It has been shown earlier in an experimental model of allergic sensitization in rhesus monkeys and in clinical studies in ragweed allergic patients that the application of steroids may prevent boosts of IgE production following allergen contact [27, 47].

We therefore felt that it is important to investigate in a well-controlled clinical study utilizing defined and clinically relevant allergen molecules, whether a frequently used INCS, i.e.,

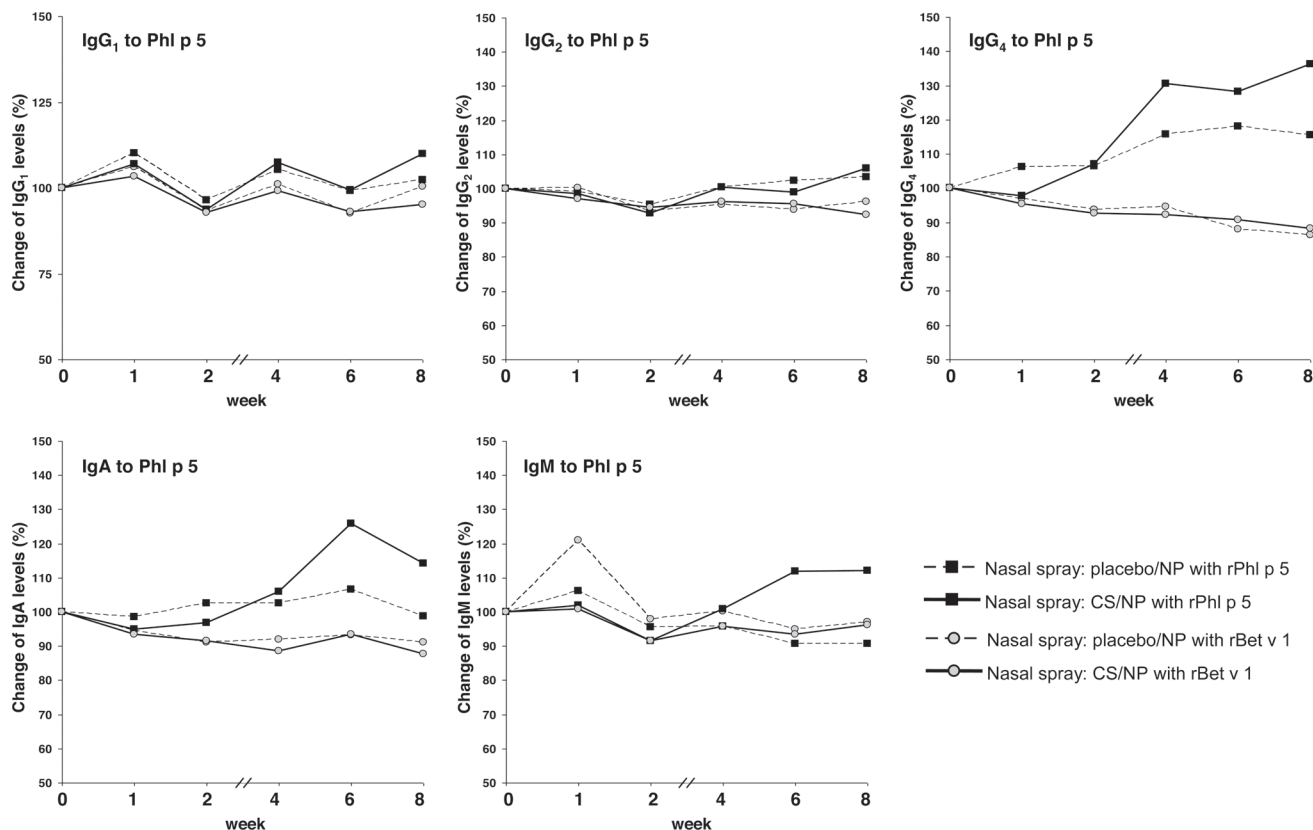


Fig 4. Development of Phl p 5-specific antibody responses. Percentage changes of Phl p 5-specific IgG₁, IgG₂, IgG₄, IgA and IgM antibody levels (y-axes) at the day of the first nasal provocation (0) and thereafter (x-axes). Uninterrupted lines: Patients with steroid spray; dotted lines: Patients with placebo spray; black squares: provocation with rPhl p 5; grey dots: provocation with rBet v 1.

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nasal fluticasone propionate, has effects on boosts of IgE production induced by nasal allergen contact.

Although there is considerable evidence for local IgE production in the nasal mucosa [48], we found that even a two week pre-treatment before allergen provocation, followed by a two week treatment after provocation with fluticasone propionate compared to placebo had no significant effects on the boosts of allergen-specific IgE production. Similar results were obtained for two different pollen allergens, the highly immunogenic major grass pollen allergen Phl p 5 and the less immunogenic major birch pollen allergen, Bet v 1 [49].

There are at least two explanations for the discrepancy between our study and earlier studies describing a reduction of allergen-specific IgE levels upon administration of corticosteroids. In the rhesus monkey study [47] triamcinolone acetonide was given systemically and, in the ragweed trial, steroids (i.e., beclomethasone propionate, triamcinolone acetonide) with a high bioavailability of more than 40% and a systemic immunosuppressive effect were used, whereas the bioavailability of nasal fluticasone propionate used in our study is less than 1% [50].

In a recent study we found that boosts of allergen-specific IgE production can only be obtained with intact folded allergen which is capable of interacting with the B cell receptor but not by unfolded allergen derivatives which contain the allergen-specific T cell epitopes but cannot bind to the B cell receptor [33]. The failure of steroids to suppress allergen-specific IgE production may therefore be explained by their inability to influence secondary IgE production in memory B cells and plasma cells.

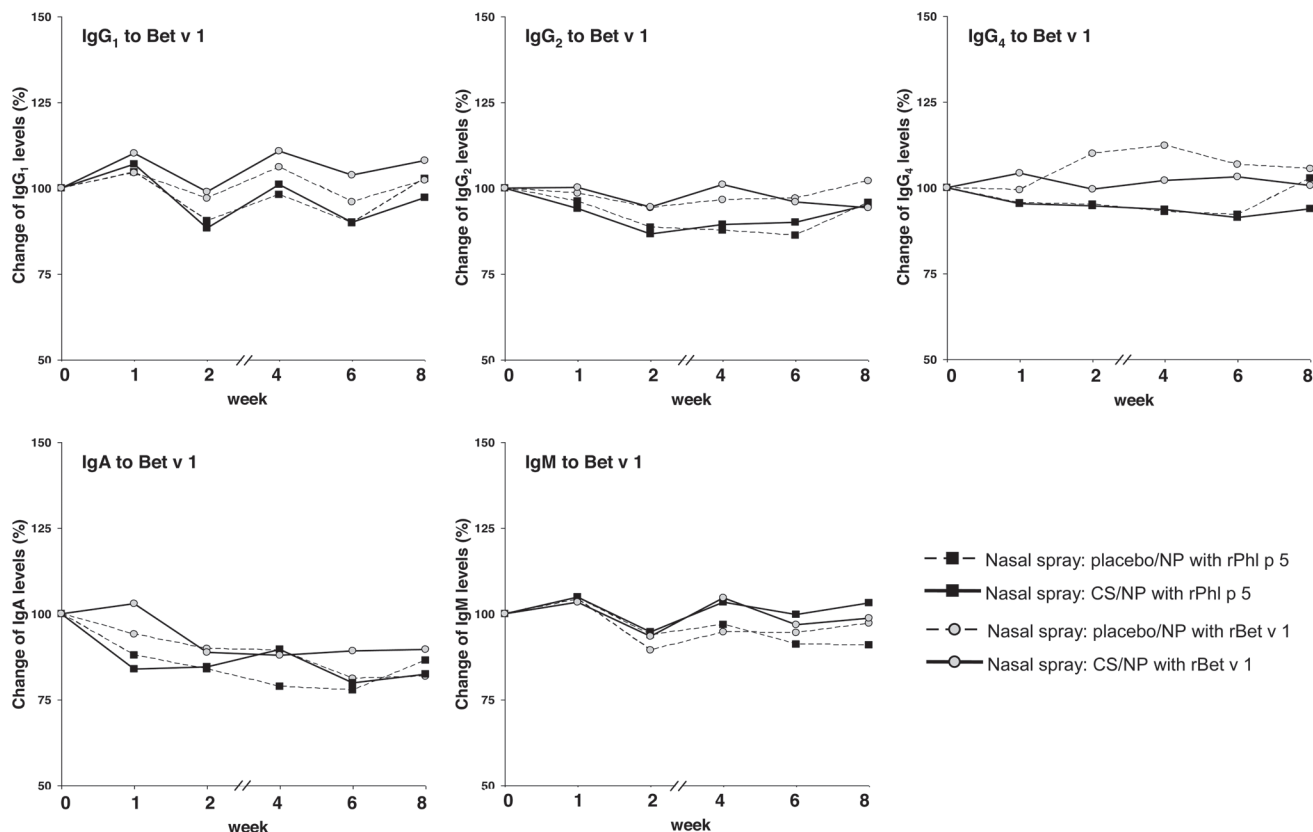


Fig 5. Development of Bet v 1-specific antibody responses. Percentage changes of Bet v 1-specific IgG₁, IgG₂, IgG₄, IgA and IgM antibody levels (y-axes) at the day of the first nasal provocation (0) and thereafter (x-axes). Uninterrupted lines: Patients with steroid spray; dotted lines: Patients with placebo spray; black squares: provocation with rPhl p 5; grey dots: provocation with rBet v 1.

doi:10.1371/journal.pone.0114991.g005

In summary, we found that intranasal application of 200 µg of fluticasone propionate over 4 weeks, a regimen which corresponds to the recommended treatment for allergic patients, had no blunting effect on the boost of systemic allergen-specific IgE production following nasal provocation with 1.6 µg of Phl p 5 or Bet v 1, two major pollen allergens, administered on two consecutive days, and did not affect the production of allergen-specific IgE antibodies. Thus, other effects of intranasal corticosteroids, like a reduction of Th2-cytokine producing cells [25] as well as decreased eosinophil and mast cell numbers [51] may account for the beneficial clinical effect of intranasal corticosteroids.

One limitation of our study is that nasal provocation exposed patients to relatively high doses of allergen, corresponding to a day's worth of allergen exposure, in a short time. We cannot exclude that INCS affect systemic IgE levels differently in patients who encounter lower doses of allergens over a more prolonged period of time. Therefore, it will certainly be interesting to study the effects of INCS on allergen-specific IgE production and subsequent allergen sensitivity in large groups of patients exposed to allergen in a pollen chamber or under conditions of natural allergen exposure. Furthermore, it will be interesting to study local allergen-specific antibody production in nasal secretions of allergic patients after allergen contact. However, the results from our trial indicate that unlike SIT, INCS cannot down-modulate allergen-specific IgE production.

Supporting Information

S1 CONSORT Checklist.
(DOC)

S1 Protocol. Study protocol submitted to the Ethics committee.
(DOC)

S1 Study Protocol Amendment. Amendment 1 and Amendment 2 to the study protocol, submitted to the Ethics committee during the study.
(DOC)

S2 Study Protocol Amendment. Amendment 1 and Amendment 2 to the study protocol, submitted to the Ethics committee during the study.
(DOC)

S1 Table.
(PDF)

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Author Contributions

Conceived and designed the experiments: CE FH SS RV VN PL PZ. Performed the experiments: CE CL FH RV VN PL PZ. Analyzed the data: CE CL FH SS RV VN RR PL PZ. Contributed reagents/materials/analysis tools: CL FH SS RV RR. Wrote the paper: CE CL FH SS RV VN RR.

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S2_Study_Protocol_Amendment.doc
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CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4-5
	2b	Specific objectives or hypotheses	5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	n.a.
Participants	4a	Eligibility criteria for participants	7
	4b	Settings and locations where the data were collected	6
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	7-8
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	10, 11
	7b	When applicable, explanation of any interim analyses and stopping guidelines	n.a.
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	7
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	7
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	7
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	7
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	7

		assessing outcomes) and how	
Statistical methods	11b	If relevant, description of the similarity of interventions	
	12a	Statistical methods used to compare groups for primary and secondary outcomes	10, 11
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	Figure 2
	14b	Why the trial ended or was stopped	n.a.
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Figure 1
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Figure 3, Figure 4, 5
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	16, 17
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	16, 17
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	17
Other information			
Registration	23	Registration number and name of trial registry	6
Protocol	24	Where the full trial protocol can be accessed, if available	Is attached
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	18

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming; for those and for up to date references relevant to this checklist, see www.consort-statement.org.

The effect of intranasal corticosteroids on the immune response following nasal allergen challenge in patients suffering from seasonal allergic rhinitis

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Abbreviations

APC	antigen presenting cells
AR	allergic rhinitis
CRF	case record form
INCS	intranasal corticosteroids
mAb	monoclonal antibody
NPT	nasal provocation test(ing)
SPT	skin prick test(ing)

1 Introduction

1.1 Background

Allergic rhinitis (AR) is a common manifestation of Type I allergy, an IgE-mediated hypersensitivity disease affecting more than 25% of the population (Wüthrich, 1995). Diagnosis is based upon case history, *in vivo* (skin tests, provocation tests) and *in vitro* (specific IgE) tests. Therapeutic options of allergic rhinitis comprise allergen avoidance, immunotherapy and symptomatic treatment with local or systemic antihistamines, local or systemic corticosteroids, cromones, decongestants, and antileukotrienes (Members of the workshop, 2004).

In seasonal AR annual fluctuations in detectable specific serum IgE levels are long known, reaching a maximum after the pollen season with subsequent reduction until exposure reoccurs (Sadan, 1969; Yunginger 1973). An allergen exposure-induced IgE boost has also been observed after nasal provocation (Naclerio, 1997) but not after skin prick testing (SPT). Furthermore, it has been shown that the rise of allergen-specific serum IgE results in an increase in cutaneous sensitivity and that skin test results reflect immediate type respiratory sensitivity (Niederberger, 2001; Niederberger, unpublished observations).

Conceivable immunological mechanisms leading to the rise of allergen specific IgE after allergen contact via the respiratory mucous membranes are, on the one hand, the IgE epitope-dependent activation of allergen specific B-memory cells and subsequent IgE production in the nasal mucosa or, on the other hand, allergen uptake and processing by mucosal antigen presenting cells (APC) which then migrate to the draining lymph nodes where activation of B-cells and IgE production occur.

Nasal provocation testing (NPT) is a useful tool to study the reaction of the nasal mucosa to inhalants under standardized conditions and a standard diagnostic procedure in clinical routine. It allows to discriminate between clinically irrelevant sensitization and true allergy. In rare cases, NPT is the only means available to provide evidence for allergic sensitization because local IgE production in nasal mucosa without systemically elevated IgE levels can account for AR (Huggins KG,

1975). Active anterior rhinomanometry represents a sensitive method to assess objective clinical parameters of nasal obstruction (nasal flow, nasal resistance and flow decrease) (Bachmann, 1982; Clement, 1984; Riechelmann, 2002).

Until now, crude allergen extracts which not only contain the disease-eliciting molecules but also other allergenic and non-allergenic compounds are used for SPT, NPT, and *in vitro* tests to diagnose allergy in daily clinical practice. However, during the last decade, purified natural or recombinant allergen molecules representing the epitope complexity of the natural allergen have been obtained for most allergen sources (Valenta, 1999). To date, several recombinant allergens are already commercially available for routine *in vitro* use and complement allergen extracts for allergy diagnosis.

Among these recombinant allergens, rPhl p 5 and rBet v 1 represent the recombinant counterparts of natural Phl p 5, a major grass pollen allergen, and natural Bet v 1, the major birch pollen allergen. In addition to their *in vitro* use, both allergens have been used for *in vivo* testing, i.e. skin testing, nasal, conjunctival, and even bronchial provocation testing, in numerous studies (Godnic-Cvar, 1997; Heiss, 1999; Arquint, 1999; Van Hage-Hamsten, 2002; Niederberger, 2001; Arzneimittelbeiratsgutachten AG700). Thereby, it has been shown that they exhibit the same immunological properties as their natural counterparts without giving rise to increased safety concerns (Van Hage-Hamsten, 2004).

In parallel, hypoallergenic allergen derivatives have been engineered as candidates for immunotherapy, the only causative treatment of allergy, in order to limit side effects while conserving structures encountering for its protective effects.

For Bet v 1, a hypoallergenic derivative was obtained by dividing the molecule into two halves. These two fragments F1 (amino acids 1-74) and F2 (amino acids 75-160) were extensively evaluated in *in vitro* and *in vivo* experiments and recently entered immunotherapy studies. They have proved to show a marked reduction in IgE binding capacity without losing T-cell stimulating properties and in clinical studies skin test reactivity to the fragments was more than 100-fold reduced as compared to native Bet v 1 (Vrtala, 1997; Van Hage-Hamsten 2002; Niederberger, 2004).

Intranasal corticosteroids (INCS) are regarded as a highly effective, first line treatment for patients suffering from AR with moderate to severe and/or persistent symptoms. They should be given regularly and, in severe cases, therapy may be started before the beginning of the pollen season (Van Cauwenberge, 2000). INCS

downregulate allergic inflammation in the nasal mucosa via a broad range of effects on transcription factors and cytokine production (e.g. IL-4, 5, 6, 8, 13, ICAM-1, VCAM-1) in numerous cells (epithelial cells, lymphocytes, eosinophils, basophils, mast cells, langerhans cells) and reduction of inflammatory cell infiltration. However, although a connection between chronic inflammation and symptoms has been established in allergic rhinitis, it is not clear how the anti-inflammatory effects of INCS are associated with their ability to reduce early-phase symptoms (Mygind, 2001). Side effects of INCS are mild and confined to the nasal mucosa in almost all cases (Passalacqua, 2000).

Mometasone furoate is considered the most potent INCS presently on the market. At the same time intranasal mometasone has an extremely low rate of systemic side effects. In studies on the pharmacodynamic properties of oral and/or intranasal mometasone furoate no suppression of the hypothalamic-pituitary-adrenal axis function was found. Its systemic absorption after intranasal administration is extremely low with a bioavailability of $\leq 0.1\%$. It undergoes extensive hepatic metabolism, so that systemic activity from the swallowed portion is negligible. In AR, the recommended therapeutic dose of aqueous mometasone furoate is 200ug administered once daily as an intranasal spray (100ug to each nostril). The dose may be doubled if control of symptoms is insufficient. Improvement of nasal symptoms starts after several hours and reaches its maximum after 7 to 14 days of continuous treatment. In clinical trials with mometasone furoate, the overall incidence of adverse events was similar to placebo. Observed side effects were usually mild to moderate in severity and of short duration. The only side effect which was observed more frequently with mometasone than with placebo was mild epistaxis. There is no association of adverse tissue changes (e.g. atrophy) with the use of the drug (Onrust, 1998; Szefer, 2001).

Previous studies (Welsh, 1987; Naclerio RM, 1993; Pullerits T 1997) investigating the effect of intranasal corticosteroids on the humoral immune response to seasonal allergen exposure have yielded controversial results.

Welsh and colleagues categorized 120 patients allergic to ragweed pollen into four groups based on type of treatment (placebo, cromolyn, flunisolide, or beclomethasone) and compared ragweed specific serum IgE levels pre- and postseasonally. All intranasal topical treatments failed to reduce the seasonal increase of ragweed-specific IgE antibody levels. In contrast, Naclerio *et al.* (1993)

found that treatment with INCS decreased the rise of ragweed-specific IgE in two smaller trials comparing either beclomethasone dipropionate or triamcinolone acetonide with placebo. Similar results were reported by Pullerits in 1997 in 20 birch pollen allergic patients.

In only one study, nasal provocation was used to address this question in a systematic and controlled approach (Naclerio, 1997). Patients were treated with beclomethasone dipropionate for seven days before and throughout 3 consecutive days of intranasal challenges with ragweed pollen extract. No effect of INCS on the humoral immune response was found.

However, all previous clinical studies were performed with allergen extracts containing a number of different allergens in vague amounts. To date, the availability of recombinant allergens allows the application of defined allergen amounts and to evaluate allergen-specific immune responses following allergen exposure.

1.2 Rationale of the study

Previous studies on the effect of INCS on the humoral IgE response after allergen exposure yielded conflicting results. In this study we aim to investigate the effect of INCS on allergen exposure-induced IgE rises by the means of controlled NPT with pure recombinant allergens. NPT mimics natural allergen exposure but allows to standardize its conditions (e.g. amount, period). The use of recombinant allergens allows to investigate IgE responses specifically to the disease-eliciting antigens.

In a second part of the study, the immunological mechanism underlying the allergen induced boost of IgE production will be investigated. The use of non-IgE reactive fragments of the major birch pollen allergen Bet v 1 (Vrtala, 1997) vs. the complete IgE-reactive molecule will allow us to determine whether the boost of IgE production depends on IgE-mediated mechanisms or purely T-cell dependent mechanisms.

2 Study objectives

2.1 Primary objective

To evaluate the effect of mometasone furoate vs. placebo applied as nasal spray on the increase of allergen specific serum IgE levels following nasal exposure to

recombinant allergens. Nasal sprays will be applied for four weeks starting two weeks before nasal provocation and serum IgE levels will be followed during eight weeks after nasal provocation.

2.2 Secondary objectives

- To assess the allergen-specificity of the increased serum IgE levels after allergen exposure.
- To evaluate if allergen-specific serum IgE levels are reflected in allergen-specific skin prick test reactivity *in vivo* measured immediately before and six weeks after nasal provocation.
- To evaluate if allergen-specific serum IgE levels are reflected in allergen-specific effector cell activation *in vitro* measured by histamine release and CD203c assays immediately before and six weeks after nasal provocation.
- To determine whether the two recombinant halves of the major birch pollen allergen Bet v 1 which have shown a marked reduction in IgE binding capacity without losing T-cell stimulating properties are able to induce the increase of allergen-specific serum IgE levels following nasal exposure.
- To determine the effect of INCS on nasal flow at NPT.

3 Overall design and plan of the study

3.1 Overview

In total, 90 patients will be included in the study on a voluntary basis. 30 mainly grass pollen and 30 mainly birch pollen allergic patients sensitized to rPhl p 5 and rBet v 1 will enter a single-center, randomized, placebo-controlled, double-blind study. In parallel, another 30 birch pollen allergic patients will be evaluated in an observational study.

Screening. During a first screening visit patients will be orally informed about the study protocol and will be asked to read and sign the consent form. History will be obtained, and anterior rhinoscopy, FEV1 measurement, SPT with recombinant allergens, and blood sampling (40 ml) will be performed to determine inclusion and exclusion criteria. A part of the serum sample will be directly sent to the laboratory for

analysis of total IgE and specific IgE to rPhl p 5 and rBet v 1 and the rest will be stored at -20°C . In addition, in female patients, pregnancy will be excluded with a standard urine pregnancy test, which will be repeated monthly.

Inclusion. Patients suitable according to the inclusion and exclusion criteria will be enrolled in the study and a study number will be allotted. Patients in the double-blind, placebo-controlled part of the study will be invited to the inclusion visit.

During this visit they will receive the study medication (nasal sprays containing mometasone furoate or placebo) which will be provided by Aesca Ges.m.b.H., Traiskirchen, in an encoded manner. They will be instructed in the correct use of nasal sprays. Nasal sprays will be administered during four weeks, namely 14 days prior to and 14 days after the NPT. Patients in the observational part of the study will not receive a nasal spray. They will not have to attend this visit.

The first day of nasal spray application will be referred to as *day 1* of the study.

Nasal provocation 1. The third visit will take place two weeks after the beginning of administration of nasal sprays (mometasone or placebo) (*day 15*). Any adverse events will be recorded. A blood sample (40 ml serum, 20 ml heparinized blood) will be obtained. Heparinized blood will be analyzed regarding CD203c expression and basophil histamine release and sera will be stored at -20°C for future determination of baseline parameters. SPT with recombinant Phl p 5, Bet v 1 and its fragments and a first NPT with either rBet v 1 or rPhl p 5 will be performed ("nasal priming").

Selection of the allergen will be based on symptom severity as reported by the patient during seasonal pollen exposure. Patients will be monitored for at least 60 minutes after nasal allergen challenge. Patients in the observational part of the study, who will not receive a nasal spray, will be challenged with the recombinant fragments F1 and F2 of Bet v 1.

Nasal provocation 2. One day after the first nasal challenge (*day 16*) NPT will be repeated with the same allergen to induce the increase of serum IgE levels. Again, patients will be monitored for at least 60 minutes after nasal allergen exposure.

Observation period. Blood samples (40ml) will be collected on *days 23* (visit 5), *30* (visit 6), and *44* (visit 7). On *day 58* (visit 8), 60 ml blood (40 ml serum and 20 ml heparinized blood) will be taken and SPT with recombinant allergens will be repeated.

End of study. On *day 72* (visit 9), a final blood sample (40 ml) will be obtained and the study will be completed.

The study will be conducted between November and February, i.e. outside the birch and grass pollen season, to avoid interference by natural pollen exposure.

3.2 Endpoints

3.2.1 Primary endpoint

- change of rPhl p 5 or rBet v 1 (according to the allergen used for NPT) specific serum IgE antibody levels during 8 weeks following NPT.

3.2.2 Secondary endpoints

- change of rBet v 1 or rPhl p 5 (according to the allergen not used for NPT) specific serum IgE antibody levels during 8 weeks following NPT.
- change of mean wheal diameter (skin prick test reactivity) to rPhl p 5 and rBet v 1 6 weeks after NPT compared to baseline immediately before NPT.
- change of effector cell reactivity to rBet v 1 and rPhl p 5 in histamine release and CD203c assays 6 weeks after NPT compared to baseline immediately before NPT.
- reduction of nasal flow at NPT.

3.3 Sample size considerations

It is expected that during the eight weeks period rPhl p 5/rBet v 1 specific serum IgE antibody levels will increase after challenge up to a level of about one standard deviation above baseline (Niederberger, submitted). This increase will be prevented by INCS and will not occur in the group challenged by the fragments. Sample size is estimated based on a target significance level of 5% and a power of 80%. Applying a two factor ANOVA design with four time points and three groups, overall 19 patients per group are necessary.

4 Study population

4.1 Number of subjects

A total of 90 volunteers with a history of seasonal AR to grass and/or birch pollen will be included in the study.

4.2 Patient recruitment

Patients will be recruited by postings in the area of the Vienna Medical University (see Appendix 2) and by telephone contact of patients who have previously consented to being notified about clinical studies.

4.3 Eligibility criteria

4.3.1 Inclusion criteria

- male or female 18-50 years of age
- moderate to severe allergic rhinitis to grass and/or birch pollen for at least two seasons according to history
- sensitization to rPhl p 5 and rBet v 1 as demonstrated by UniCAP within a range from 3.0-80 kUA/l and a positive (wheal diameter $\geq$ 3 mm larger than the negative control and half of the histamine control) SPT with rPhl p 5 and rBet v 1 at 20 μ g/ml
- willingness to comply with the study protocol
- written informed consent

4.3.2 Exclusion criteria

- perennial allergic rhinitis
- history of asthma necessitating treatment
- FEV1 <70% of predicted value
- abnormalities at auscultation of heart or lungs
- history of anaphylaxis
- severe atopic dermatitis
- total serum IgE >2000 kU/l

- previous or ongoing immunotherapy (subcutaneous or sublingual) to grass or birch pollen
- nasal provocation testing during the previous six month
- known allergy/intolerance to mometasone furoate or loratadine
- known allergy/intolerance to cellulose, glycerol, polysorbate, benzalkonium chloride, cornstarch, lactose
- contraindications for the use of INCS:
 - o acute or chronic infections of the upper respiratory tract
 - o surgery of the nose during the previous year
 - o hypersensitivity to components of the drug
- contraindications for NPT
 - o acute rhinosinusitis
 - o acute allergic reaction of the immediate type at other organs
- nasal polyposis or significant nasal anatomical deformities
- vasomotor rhinitis
- autoimmune disease, chronic or acute infectious disease, malignancy
- severe psychological disorder
- treatment with systemic or topical (intranasal, inhaled, external) corticosteroids from 3 month prior to the study
- treatment with other immunosuppressant drugs from 6 month prior to the study
- treatment with antihistamines or disodium cromoglycate from 2 weeks prior to the study
- treatment with intranasal adrenergic drugs from 3 days prior to the study
- treatment with systemic adrenergic drugs
- treatment with psychopharmacological drugs from 2 weeks prior to the study
- cardiovascular or pulmonary disease
- contraindication for adrenaline
- participation in any other clinical trial within the previous 3 month
- pregnant, lactating or sexually active women with childbearing potential who are not using a medically accepted birth control method
- a mental condition rendering the subject unable to understand the nature, scope and possible consequences of the study, and/or evidence of an uncooperative attitude
- known alcohol or drug addiction or abuse

- unlikely to be able to complete the study

4.4 Subject identification and de-personalization

All subjects enrolled must be identifiable throughout the study. The investigator will maintain a personal list of subject names and numbers to enable records to be found at a later date.

Subject numbers will be allotted to patients suitable for the study according to the inclusion and exclusion criteria. Numbers 1-30 will be used for (primarily) grass pollen allergic patients who will be exposed to rPhl p 5 at NPT and numbers 31-60 for (primarily) birch pollen allergic patients who will be exposed to rBet v 1 at NPT. Patients in the observational part of the study will receive numbers 61-90.

4.5 Subject withdrawal

The subjects will be advised in the Informed Consent Forms that they have the right to withdraw from the study at any time without prejudice. The subject may also be withdrawn from the study at the investigator's discretion at any time. The date of the withdrawal, the person who initiated withdrawal and the primary reason for withdrawal (e.g. adverse events, withdrawal of consent, pregnancy, violation of the treatment plan) will be recorded in the CRF.

5 Study procedures

5.1 Case history

Patients will be asked for their medical history including demographic data and concomitant medication, their allergic symptoms including intensity and duration and their allergic medication history. History will be considered suggestive of seasonal AR if rhinorrhea, nasal congestion, nasal itching or sneezing occur during birch and/or grass flowering season, i.e. from March to July in the study area.

5.1.1 Symptom score

The following symptom score will grade severity of AR. Patients will be asked for nasal itch, sneezing, rhinorrhea, and nasal blockage during the previous pollen

season when symptoms were worst and to grade each symptom by applying the following scale: 0, not present; 1, mild – present, no discomfort; 2, moderate – present, some discomfort, not interfering with daily life activities; 3, severe – bothersome and interfering with daily life activities or disturbing sleep. The use of medication to control symptoms will account for 2 points. AR will be considered moderate/severe if a sum score of 4 or higher is obtained.

5.2 Physical examination

5.2.1 Auscultation

Auscultation of the heart and lungs will be performed at the screening visit and only patients with normal findings will be included in the study.

5.2.2 Anterior rhinoscopy

Anterior rhinoscopy will be performed once during the screening visit to exclude anatomical variances (e.g. septal deviation) or pathological changes (e.g. nasal polyps) interfering with accurate rhinomanometry. It will be performed by using a speculum with the patient seated with the head slightly back.

5.3 FEV1 measurement

FEV1 measurements will be performed during the first visit and only patients with a FEV1 of at least 70% of the predicted value will be included in the study.

5.4 Skin prick test

5.4.1 Method

SPT is a rapid and accurate way of identifying the causative allergens in atopic individuals. Allergens are inserted into the dermis by gently pricking the skin through a drop of an allergen-containing solution with a sterile lancet. In previously sensitized individuals, IgE molecules on the surface of dermal mast cells are bridged and degranulation of the mast cells occurs. Preformed granules containing histamine are released, resulting in the appearance of a small, itchy swelling and a reddening of the skin within 15 minutes (wheal and flare-reaction). Therefore, SPT identifies the atopic

state by demonstrating tissue bound IgE and represents the *in vivo* counterpart of serum specific antibody tests.

5.4.2 Procedure

SPT will be performed on the volar forearms of the subjects. 20 µl aliquots of each test solution will be applied at a distance of at least 2 cm between each individual application point. Sterile prick lancets, which will be discarded after each application to avoid possible cross-contamination, will be used to gently prick the skin through the drops. After being pricked, the patient will keep his forearm in horizontal position. After 20 minutes, allergen solutions will be wiped off and margins of the wheals will be traced with a ball point pen. Transparent tape will be bonded on the irritated skin in order to transfer the outline of the wheal to the tape. The taped copies are then fixed to a SPT form with each tape being added to the corresponding allergen solution.

The surface of the wheals will be calculated by digital planimetry. For inclusion, wheals of at least 3 mm in diameter larger than the negative control and at least half of the histamine control will be regarded as positive reactions.

5.4.3 Test solutions

Two recombinant allergens, the fragments F1 and F2 (mix), and 2 control solutions will be tested as described (Vrtala, 1997; Heiss, 1999; Niederberger, 2001). The recombinant allergens rBet v 1 and rPhl p 5 will be diluted in 0.9% sodium chloride solution at 1, 5, 10, and 20 µg/ml. The recombinant fragments of Bet v 1 F1 and F2 will be diluted at 0.5, 2.5, 5, and 10 µg/ml and mixed in equal amounts which will correspond to the dilutions containing the whole rBet v 1 molecule (equimolarity). Solutions for SPT will be aliquoted on a per patient and test basis before the beginning of the study. Aliquots will be stored at –20°C and thawed about to use. As controls sterile 0.9% NaCl solution (negative control) and histamine hydrochloride 10 mg/ml (positive control) will be used (Allergopharma Joachim Ganzer KG, Reinbek, Germany). They will be stored according to the manufacturer's instruction.

5.4.4 SPT in study conduct

SPT will be performed 3 times during the study (screening visit, nasal provocation visit 1 (day 15), and visit 8 (day 58)). At the screening visit a total of 5 prick tests (rBet

v 1, rPhl p 5, and mix F1/F2 at 20 µg/ml, 2 controls) will be performed. At the nasal provocation visit and visit 8 a total of 27 prick tests (rBet v 1, rPhl p 5, mix F1/F2 at 1, 5, 10, and 20 µg/ml and the positive control in duplets, negative control in singlet) will be performed.

Blood samples will be obtained before SPT.

5.4.5 Safety precautions

As a safety precaution, patients will be monitored for 30 minutes after testing. Although anaphylactic events are very unlikely but always have to be considered possible, a complete emergency kit will be available immediately in case of anaphylactic reactions.

5.5 Blood sampling

Blood samples will be taken by puncture of the antecubital vein before skin testing. Maximum 60 ml per visit will be taken for the preparation of serum, basophil histamine release assays, and CD203c assays. Precisely, 40 ml blood will be obtained at visits 1, 5, 6, 7, and 9 for the preparation of serum which will be stored at –20°C until use, and 60 ml (40 ml for the preparation of serum and 20 ml heparinized blood for immediate analysis in histamine release and CD203c assays) at visits 3 and 8.

5.5.1 Measurement of total IgE

Measurement of total IgE will be performed by CAP-FEIA measurements (Pharmacia, Uppsala, Sweden), representing a standard procedure in routine diagnostics.

5.5.2 Measurement of specific IgE

Allergen specific antibodies in the sera will be measured by UniCAP-FEIA (Pharmacia, Uppsala, Sweden) at the beginning of the study to check inclusion criteria and at the end of the study in all collected serum samples in one assay to eliminate interassay variation. Results will be indicated in kUA/l.

5.5.3 Dot Blot

To exclude IgE reactivity to the recombinant fragments F1 and F2 of Bet v 1 in the group of patients being challenged with the fragments their sera will be analysed by IgE-dot blot. For this purpose, the fragments F1 and F2 will be dotted onto nitrocellulose strips. Strips will be incubated with the sera to probe the nitrocellulose-bound proteins and bound IgE antibodies will be detected by ¹²⁵I-labeled anti-human IgE and subsequent autoradiography (Vrtala, 1997).

5.5.4 CD203c and histamine release assay

In parallel to the SPTs at the nasal provocation 1 and visit 8 (day 58) heparinized peripheral blood will be obtained for CD203c and histamine release assays. Both assays allow the demonstration of IgE-dependent effector cell activation (Hauswirth, 2002).

For CD203c assays blood aliquots will be incubated with serial dilutions of recombinant allergens (from 10⁻⁵ to 1 µg/ml), anti-IgE antibody (1 µg/ml) or PBS. Cells will be washed and incubated with phycoerythrin-labeled CD203c mAb to stain cells presenting the CD203c molecule on their surface as a marker for IgE-dependent cell activation. Cells will be analyzed by flow cytometry.

For histamine release assays basophils will be enriched by dextran sedimentation and exposed to recombinant allergens or anti-IgE mAb (Valenta, 1993). After incubation, cells will be centrifuged and supernatants will be analysed for histamine content by using a commercial radioimmunoassay (Immunotech).

5.6 Pregnancy testing

In female patients, pregnancy will be excluded with a standard urine pregnancy test.

5.7 Nasal provocation and rhinomanometry

5.7.1 Method

The purpose of rhinomanometry is to measure objectively the impact of the allergen provocation on the nasal discharge. Nasal resistance is calculated by measuring the flow through the nose as well as the pressure of force required to cause the airflow.

Active anterior rhinomanometry offers a very accurate method to objectively assess clinical parameters (nasal flow, nasal resistance and nasal flow increase) of nasal obstruction. Nasal flow and nasal resistance are observed at pressure levels of 75, 150 and 300 Pa. Statistical evaluation focuses on the sum of the right and left nasal airflow (ml/s) at a pressure difference of 150 Pa (inspiration).

In active anterior rhinomanometry, the patient – wearing an airtight face mask over the mouth and nose and having the mouth closed – breathes through one nostril to evaluate the nasal flow while a sensor in the other nostril is used to measure the difference in prenasal and choanal pressure. The system used is connected to a computer. Transducer signals of transnasal airflow and pressure are amplified, digitised and shown as curve on the screen allowing on-screen control, immediate printing and saving of data.

The evaluation of the reports focuses on the sum of nasal flow (ml/s) at 150 Pa. For statistical evaluation the change of this summarised flow from the baseline is of special interest.

5.7.2 Procedure

Patients will be allowed to adapt to room climate for 15 minutes before establishing baseline levels of nasal parameters without any interaction.

Then, ten minutes after applying the diluent (0.9% sodium chloride solution) to the nostril showing the higher nasal flow at baseline a second measurement will be obtained in order to rule out any non-specific hyperreactivity to the diluent *per se* (negative control and reference).

Finally, nasal provocation will be performed by applying 2 puffs of the aqueous allergen solution into the same nostril using a metered-dose nasal pump spray delivering 15µl of solution per spraying action. Patients will be asked to hold their breath during application of the allergen solution to prevent inhalation. Active anterior rhinomanometry will be performed 10 and 20 minutes after provocation.

5.7.3 Symptom score

In addition to the objective parameters measured by rhinomanometry, patients will be asked to score the symptoms of blockage, secretion and itching by using a 4-point scale (0 = no symptoms; 1 = mild symptoms; 2 = moderate symptoms; 3 = severe

symptoms) and the number of sneezes will be counted (0 = no sneeze; 1 = 1-5 sneezes; 2 = 6-10 sneezes; 3 > 11 sneezes) to calculate a total nasal symptom score.

5.7.4 Test solutions

The recombinant allergens rPhl p 5, rBet v 1, and a mix (1:1) of the recombinant fragments will be diluted in sterile 0.9% sodium chloride solution at 50 µg/ml (25 µg/ml for each fragment), respectively, before the beginning of the study. Solutions will be aliquoted and stored at –20°C until use.

5.7.5 NPT in study conduct

Nasal provocation will be performed twice on two consecutive days in the course of the study after two weeks of treatment with either mometasone furoate or placebo.

5.7.6 Safety precautions

Typical reactions to nasal provocation comprise nasal itch, sneezing, nasal hypersecretion and nasal obstruction. Patients will be provided with handkerchiefs and, if necessary, with one tablet of 5 mg desloratadine at the investigator's discretion.

To prevent inhalation during application of the allergen solution, patients will be requested to hold their breath during application of the allergen solution. Although anaphylactic events are very unlikely at NPT but always have to be considered possible, a complete emergency kit will be available immediately in case of anaphylactic reactions.

Patients will be monitored for at least 60 minutes after allergen application.

5.8 Statistical analysis

IgE levels will be log transformed to stabilize variances and to obtain normality. Residuals of transformed variables after ANOVA will be tested by Kolmogorov-Smirnov test for deviations from normality and by Levene's test for homogeneity of variance. A two factor ANOVA with one repeated measurements factor and one between-subjects factor will be applied. Similar analyses will be performed for

secondary endpoints but as exploratory tests. Significance level for the primary endpoint is set to 5%.

6 Investigational products and study medication

6.1 Recombinant allergens, recombinant allergen fragments and control solutions

6.1.1 Recombinant Phl p 5 and Bet v 1

Recombinant Phl p 5 and recombinant Bet v 1 have been previously used for skin and provocation testing in allergic patients (Godnic-Cvar, 1997; Heiss, 1999; Arquint, 1999; Van Hage-Hamsten, 2002; Van Hage-Hamsten, 2004; Arzneimittelbeiratsgutachten AG700; Niederberger, 2001).

Recombinant proteins will be reconstituted in sterile 0.9% sodium chloride solution and dilutions of 1, 5, 10, and 20 µg/ml for SPT and 50 µg/ml for NPT will be prepared and aliquoted on a per patient and test basis.

6.1.2 Recombinant Bet v 1 fragments F1 and F2

The recombinant Bet v 1 fragments F1 (amino acids 1-74) and F2 (amino acids 75-160) have already been used for skin and nasal provocation testing (Van Hage-Hamsten, 2002; Niederberger, 2004; Arzneimittelbeiratsgutachten AG700).

Recombinant fragments were expressed in *E. coli* and purified as described (Vrtala, 1997).

6.1.3 Control solutions

Control solutions will comprise sodium chloride 0.9% as negative control and histamine 10 mg/ml as positive control. They will be obtained from Allergopharma, Reinbek, Germany, and stored according to the manufacturer's instructions.

6.2 Study medication

6.2.1 Study drug – packaging, labelling, randomization, and storage

Nasonex aquosum-Nasenspray and placebo will be supplied by AESCA Ges.m.b.H., Traiskirchen, in an encoded manner. Labels will display numbers 1-60 and be randomized in blocks of 4. Sprays will be dispensed in ascending order from numbers 1-30 for mainly grass pollen allergic patients and numbers 31 to 60 for mainly birch pollen allergic patients. To prevent shortness of the drug, nasal sprays will be available twofold for each patient. Patients will hand-in the first package and be provided with the second package at NPT 1 (halftime of the treatment period). The study drug will be stored at room temperature in safe custody of the investigator until distribution. After the treatment period nasal sprays will be returned to the investigator.

6.2.1.1 Study drug formulation and dosage

Nasonex aquosum-Nasenspray is composed of mometasone furoate, which is delivered at 50 µg per puff, and dispersible cellulose BP 65 cps (microcrystalline cellulose, carmellose-sodium), glycerol, sodium citrate dihydrate, citric acid monohydrate, polysorbate 80, benzalkonium chloride, and phenylethyl alcohol in aqueous solution.

Patients will be instructed to administer two puffs of the nasal spray in each nostril once daily (200 µg mometasone furoate per day and placebo, respectively).

6.2.1.2 Compliance

Compliance of the patients will be estimated by weighing the spray bottles at the time of return.

6.2.1.3 Safety precautions

INCS are usually well tolerated and side effects generally are mild and reversible. Patients will be asked for possible side effects at each study visit and in the meantime telephone contact to the study personnel will be possible at any time. The drug will be discontinued if intolerance occurs and examination and treatment will be provided by an otolaryngologist if necessary.

6.2.2 Concomitant medication

6.2.2.1 General

All additional medication being taken by the subjects on entry to the study or at any time during the study are regarded as concomitant medication and will be documented in the CRF. Concomitant medications should be kept to a minimum during the study but, if considered necessary, may be given if not interfering with the study protocol.

6.2.2.2 Rescue medication

Patients will be provided with one tablet of desloratadine 5 mg at the investigator's discretion if experiencing unacceptable rhinitis symptoms at NPT.

Although anaphylactic events are very unlikely in this study but always have to be considered possible, a complete emergency kit will be available immediately in case of anaphylactic reactions at SPT or NPT.

6.2.2.3 Prohibited concomitant medication

Subjects must remain off medications interfering with study procedures (SPT, NPT, treatment of anaphylactic reactions) for the duration of the study. The following concomitant treatments are not permitted during this study:

- systemic or topical corticosteroids
- other immunosuppressant drugs
- antihistamines (except desloratadine 5 mg at NPTs at the investigator's discretion) or disodium cromoglycate
- systemic or intranasal adrenergic drugs
- psychopharmacological drugs
- ACE-inhibitors or beta-blockers

If treatment with one of these drugs is considered necessary during the course of the study, subjects will be withdrawn from the study.

7 Data handling procedure

Case record forms (CRFs) will be completed for each participant. Trained personnel will check the entries and any errors or inconsistencies will be corrected immediately.

8 Adverse events

An adverse event is any event during a clinical study, including intercurrent illness or accident, which impairs the well-being of the subject; it may also take the form of an abnormal laboratory value. The term adverse event does not imply a causal relationship with the study treatment.

All subjects experiencing adverse events – whether considered associated with the use of the study treatment or not – will be monitored until symptoms subside and any abnormal laboratory value has returned to baseline, or until there is a satisfactory explanation for the changes observed, or until death, in which case a full pathologists report will be supplied. All findings must be reported on an “Adverse event” page in the case record form.

All adverse events will be reported on and documented as described below.

Adverse events are divided into the categories “serious” and “non-serious”. This determines the procedure which must be used to report/document the adverse event (see below).

8.1 Definition of serious and non-serious adverse events

A serious adverse event is:

- any event that is fatal or life-threatening
- any event that is permanently disabling
- any event that requires or prolongs hospitalization
- any event that involves cancer, congenital anomaly, or occurs as a result of overdose (application of more than the stipulated dose)

Adverse events which do not fall into these categories are defined as non-serious.

8.2 Assessment of severity of AE

Regardless of the classification of an adverse event as serious or non-serious (see above), its severity must be assessed as mild, moderate or severe, according to medical criteria alone:

mild = does not interfere with routine activities, acceptable

moderate = interferes with routine activities

severe = impossible to perform routine activities, considered as unacceptable by the physician, requires treatment, requires discontinuation of study, or has residual effect.

It should be noted that a severe adverse event needs not to be serious in nature and that a serious adverse event needs not, by definition, to be severe. Regardless of severity, all serious adverse events must be reported on as below.

8.3 Reporting/documentation of adverse events

Adverse events are collected by spontaneous reporting.

8.3.1 Reporting/documentation of serious adverse events

All serious adverse events which occur during this study, whether considered to be associated with the study medication or not, must be documented on an "Adverse event" page in the case record form.

A follow-up report including all new information obtained on the serious event must be prepared and will be collected.

The investigator will submit on request copies of all these reports to the ethics committee. Where necessary, investigators will inform the authorities.

8.3.2 Reporting/documentation of non-serious adverse events

These are to be documented on an "Adverse event" page in the case record form.

9 Ethical and legal aspects

The study will be carried out in keeping with local legal requirements and GCP.

9.1 Declaration of Helsinki

The study will be performed in accordance with the guidelines of the Declaration of Helsinki (1964), including current revisions. The use of recombinant allergens for *in vitro* and *in vivo* diagnosis follows a position paper summarizing a consensus made during a WHO Meeting held in Geneva 1993 concerning this topic (Bousquet & Valenta 1994).

9.2 Informed consent of subject

Before being admitted to the clinical study, the subject must have consented to participate after the nature, scope and possible consequences of the clinical study have been explained in a form understandable to him/her. The subject must give consent in writing. The signature of the investigator will confirm the subject's consent. The patient may withdraw the content, even without giving comments, at any time and without negative consequences for his further medical care.

9.3 Acknowledgement / approval of the study

Before the start of the study, the study protocol will be submitted to the Ethics committee of the Austrian Working Group for Clinical Pharmacology (Österreichische Arbeitsgemeinschaft für Klinische Pharmakologie), Kinderspitalgasse 10/15, A-1090 Vienna.

9.4 Insurance

All subjects participating in clinical studies are insured through Generali Versicherung AG, Landskrongasse 1-3, 1011 Wien, Tel. ++43-1-534 01-4456, (insurance number: filed subsequently) in accordance with §38 of the Austrian Medicines Act (AMG).

9.5 Confidentiality

All subjects' names will be kept secret in the investigators files. Subjects will be identified throughout documentation and evaluation by the number allotted to them during the study. The subjects will be told that all study findings will be stored and handled in strictest confidence.

10 Documentation and use of study findings

10.1 Documentation of study findings

All results collected during the study will be entered on the CRFs. All entries on the case record forms will be made legibly in black ink. If corrections are made to entries in the case record form, the words or figures will be ringed and a single stroke drawn through them. The correct value will be entered beside the old entry and date and the

correction will be initialled. Incorrect entries must not be covered with correcting fluid or obliterated, or made illegible in any way. The completed CRFs will be signed by the investigator. CRFs will be completed immediately after the final examination. The medical records upon which the CRF is based will be kept for at least 15 years.

10.2 Use of study findings

The findings of this study will be published by the investigators in a scientific journal and presented at scientific meetings. The manuscript will be circulated to all co-investigators before submission.

11 Protocol amendments

If any modifications become necessary or desirable, these will be documented in writing; major changes and changes involving patients manners require the approval of all investigators and the ethics committee.

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13 Appendices

Appendix 1 Study flow chart

Appendix 2 Posting

Study flow chart

	Screening	Inclusion	NPT 1	NPT 2	5	6	7	8	9
<i>Time (month/ after start of study medication)</i>	<i>October / November.</i>	<i>November</i>	<i>December / d15*</i>	<i>d16*</i>	<i>d23*</i>	<i>d30*</i>	<i>d44*</i>	<i>d58*</i>	<i>d72*</i>
Informed consent	x								
Eligibility	x	x							
SPT	x		x					x	
Blood sampling	x 40 ml		x 60 ml		x 40 ml	x 40 ml	x 40 ml	x 60 ml	x 40 ml
Anterior rhinoscopy	x								
FEV1	x								
INCS (hand-out/hand-in)		x	x			x			
NPT			x	x					
Check AE			x	x	x	x	x	x	x
Study completion									x

**d0 = start of study medication (nasal sprays)*

Probanden für klinische Studie gesucht

Titel der Studie:

***Immunantwort von Heuschnupfenpatienten nach nasaler
Provokation mit Allergenen unter Behandlung mit einem
kortisonhaltigen Nasenspray***

Vorraussetzungen:

- Alter: 18 – 50 Jahre
- saisonale, allergische Rhinitis („Heuschnupfen“) durch Gräser- und/oder Birkenpollen

Aufwand und Entschädigung:

- 9 Studientage (verteilt über einen Zeitraum von 3 Monaten zwischen November 2005 und Februar 2006), max. 2 Stunden/Studientag
- Aufwandsentschädigung: 200 Euro

Für nähere Information wenden Sie sich bitte an:

cornelia.egger@meduniwien.ac.at

Dr. Cornelia Egger, Institut für Pathophysiologie, Allgemeines Krankenhaus Wien
Währinger Gürtel 18-20, A-1090 Wien

AMENDMENT 1

to the protocol 03/05 (EudraCT number 2005-004274-24):

„The effect of intranasal corticosteroids on the immune response following nasal allergen challenge in patients suffering from seasonal allergic rhinitis“

Study drug – packaging, labelling, randomisation, and storage (6.2.1 of the protocol)

Rationale

The target of the study is the immune response on allergen challenge in allergic patients treated with (any) intranasal steroid vs. placebo. Initially we intended to use mometasone furoate as steroid. However, due to difficulties with the preparation of *Nasonex aquosum-Nasenspray* (mometasone propionate) and placebo blinded treatment packages early enough for conducting the study ahead of the start of the coming pollen period, *Nasonex aquosum-Nasenspray* (mometasone furoate) will be replaced by *Flixonase aquosum-Nasenspray* (fluticasone propionate), another well known nasal steroid. Fluticasone propionate and mometasone furoate applied intranasally represent equivalent drugs in terms of their safety and efficacy in the treatment of allergic rhinitis (1, 2, 3) in daily clinical practice. The study is not a company sponsored study (“Klinische Prüfung eines Arzneimittels”) but basic biomedical research on humans, according to the initial EC study application form. Therefore the target of the study not any compound stands in the focus.

Former version:

Study drug – packaging, labelling, randomization, and storage

Nasonex aquosum-Nasenspray and placebo will be supplied by AESCA Ges.m.b.H., Traiskirchen, in an encoded manner. Labels will display numbers 1-60 and be randomized in blocks of 4. Sprays will be dispensed in ascending order from numbers 1-30 for mainly grass pollen allergic patients and numbers 31 to 60 for mainly birch pollen allergic patients. To prevent shortness of the drug, nasal sprays will be available twofold for each patient. Patients will hand-in the first package and be provided with the second package at NPT 1 (halftime of the treatment period). The study drug will be stored at room temperature in safe custody of the investigator until distribution. After the treatment period nasal sprays will be returned to the investigator.

Study drug formulation and dosage

Nasonex aquosum-Nasenspray is composed of mometasone furoate, which is delivered at 50 µg per puff, and dispersible cellulose BP 65 cps (microcrystalline cellulose, carmellose-sodium), glycerol, sodium citrate dihydrate, citric acid monohydrate, polysorbate 80, benzalkonium chloride, and phenylethyl alcohol in aqueous solution.

Patients will be instructed to administer two puffs of the nasal spray in each nostril once daily (200 µg mometasone furoate per day and placebo, respectively).

New version:

Study drug – packaging, labelling, randomization, and storage

Flixonase aquosum-Nasenspray and placebo will be supplied by GlaxoSmithKline Pharma GmbH, Vienna, in an encoded manner. Labels will display numbers 1-60 and be randomized in blocks of 4. Sprays will be dispensed in ascending order from numbers 1-30 for mainly grass pollen allergic patients and numbers 31 to 60 for mainly birch pollen allergic patients. To prevent shortness of the drug, nasal sprays will be available twofold for each patient. Patients will hand-in the first package and be provided with the second package at NPT 1 (halftime of the treatment period). The study drug will be stored at room temperature in safe custody of the investigator until distribution. After the treatment period nasal sprays will be returned to the investigator.

Study drug formulation and dosage

Flixonase aquosum-Nasenspray is composed of fluticasone propionate, which is delivered at 50 µg per puff, and microcrystalline cellulose/carboxymethylcellulose (Avicel RC 591), dextrose, phenylethyl alcohol, diluted hydrochloric acid, polysorbate 80, and benzalkonium chloride in aqueous solution.

Patients will be instructed to administer two puffs of the nasal spray in each nostril once daily (200 µg fluticasone propionate per day and placebo, respectively).

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time point:		IgE to allergen used for nasal provocation (kUA/L)						IgE to control allergen (kUA/L)					
		1	2	3	4	5	6	1	2	3	4	5	6
Placebo nasal spray	nasal provocation with Phl p 5	5.51	7.15	7.37	9.98	6.90	8.16	5.50	6.49	6.21	7.27	4.83	6.05
		6.97	9.13	10.2	10.6	10.6	9.90	5.98	8.11	7.67	7.00	6.89	6.81
		7.30	8.34	11.8	12.6	11.5	11.6	26.9	31.0	45.4	39.5	39.0	40.6
		11.5	10.6	12.4	16.1	15.9	13.9	4.96	5.44	6.08	6.41	6.53	5.18
		1.18	1.06	1.61	1.90	1.76	1.85	0.68	0.62	0.79	0.59	0.59	0.64
		0.57	0.55	0.58	0.54	0.59	0.50	0.69	0.64	0.67	0.64	0.56	0.61
		38.9	37.8	58.9	84.1	94.3	72.7	18.1	17.2	17.9	19.5	18.3	14.2
		0.77	0.80	1.05	1.19	1.22	0.99	59.6	58.5	56.3	52.3	60.5	50.2
		6.95	9.27	9.05	9.64	8.04	8.38	2.96	4.05	3.25	3.09	2.75	2.42
		15.3	13.1	45.2	50.6	54.5	41.2	10.9	7.57	9.54	8.90	11.1	8.08
		37.4	28.0	29.6	31.8	31.2	29.4	21.7	16.5	15.4	15.9	15.5	14.8
		16.0	13.7	14.3	22.9	21.0	17.0	7.51	6.50	5.64	6.57	6.10	4.78
Placebo nasal spray	nasal provocation with Bet v 1	36.7	33.4	42.4	54.9	54.1	51.5	1.60	1.39	1.33	1.25	1.16	1.05
		5.38	4.59	5.14	5.93	4.96	5.19	1.34	1.06	1.11	1.26	1.05	1.11
		13.4	14.4	13.5	15.4	13.3	19.8	19.0	18.3	17.8	17.8	15.7	24.3
		44.7	36.8	53.6	50.4	44.0	42.0	12.6	10.4	10.9	9.49	8.52	9.42
		1.84	1.55	3.87	2.25	2.18	1.61	5.31	3.90	6.09	4.38	4.23	3.15
		67.5	48.7	86.4	100	100	100	40.0	28.2	28.0	31.0	27.0	28.9
		10.5	n.d.	15.7	18.9	17.9	17.2	1.94	n.d.	2.42	2.29	2.00	2.05
		0.98	0.97	0.96	0.81	0.88	0.98	15.2	16.5	14.6	12.5	12.5	14.4
		29.8	25.1	43.8	74.7	51.8	43.6	35.8	25.5	26.1	41.6	30.1	26.0
		14.9	12.9	15.6	18.2	22.8	13.8	1.65	1.18	1.32	1.51	1.95	1.05
Fluticasone nasal spray	nasal provocation with Phl p 5	34.0	28.6	37.0	49.1	40.5	35.4	0.70	0.58	0.63	0.66	0.56	0.47
		35.6	38.0	48.7	82.7	58.8	50.5	37.7	39.3	42.0	55.4	38.3	34.1
		10.8	12.5	14.3	22.2	20.5	21.1	21.6	27.6	25.9	30.4	27.2	28.4
		11.6	9.96	11.6	12.0	12.4	11.6	6.59	5.28	5.39	5.06	4.91	4.65
		11.2	9.30	53.3	100	77.5	76.5	5.62	4.60	5.17	7.60	5.34	5.33
		43.4	39.4	49.3	54.3	43.9	43.2	7.40	6.48	8.29	9.67	8.29	7.03
		21.1	24.8	23.7	29.8	25.8	n.d.	6.86	8.52	8.16	8.23	7.37	n.d.
		26.6	n.d.	49.9	46.1	37.7	47.1	7.96	n.d.	12.0	6.59	5.95	7.90
		10.7	9.72	12.2	15.6	15.7	15.3	7.75	7.31	7.13	6.58	6.16	6.50
		10.6	7.61	11.4	18.2	13.9	14.1	7.89	5.63	6.11	6.64	5.15	5.22
		14.4	9.85	15.4	24.0	18.5	19.0	17.1	11.5	11.8	14.2	10.5	10.9
		45.9	45.6	73.1	100	100	98.3	27.0	26.4	31.7	34.1	28.9	27.2
Fluticasone nasal spray	nasal provocation with Bet v 1	2.39	2.09	3.97	5.26	4.68	4.17	0.35	0.35	0.35	0.35	0.35	0.35
		54.1	45.0	64.1	99.0	94.2	84.8	0.86	0.62	0.59	0.74	0.64	0.68
		26.0	20.8	22.7	29.9	27.9	25.9	22.1	16.3	16.8	17.7	16.5	16.1
		5.89	5.74	5.89	5.09	4.96	5.28	100	100	96.2	85.2	79.1	82.9
		19.7	13.4	14.7	13.7	13.5	10.9	0.60	0.43	0.42	0.38	0.39	0.35
		5.15	4.77	10.7	18.2	14.7	13.2	2.07	1.49	1.57	1.86	1.49	1.35
		0.84	0.60	0.95	1.08	1.13	0.86	3.82	2.92	2.61	2.25	2.29	1.82
		1.85	3.79	7.29	8.83	7.03	7.35	3.68	5.85	6.68	7.28	5.65	6.18
		6.34	6.76	6.49	7.58	9.11	6.42	4.68	5.48	4.70	4.99	6.17	4.43
		12.7	11.5	10.1	11.5	10.9	11.0	5.17	4.87	3.96	4.28	3.79	4.27
		5.56	4.98	4.54	4.87	5.86	6.89	8.00	7.27	6.85	7.57	8.21	9.72

time points:

- 1 immediately before nasal provocation
- 2 1 week after nasal provocation
- 3 2 weeks after provocation
- 4 4 weeks after provocation
- 5 6 weeks after provocation
- 6 8 weeks after provocation

AMENDMENT 2

to the protocol 03/05 (EudraCT number 2005-004274-24):

„The effect of intranasal corticosteroids on the immune response following nasal allergen challenge in patients suffering from seasonal allergic rhinitis“

Inclusion criteria (4.3.1 of the protocol)

Rationale

The primary objective of the study is to evaluate the effect of mometasone furoate vs. placebo applied as nasal spray on the increase of allergen specific serum IgE levels following nasal exposure to recombinant allergens. As a secondary objective we intended also to assess the allergen-specificity of the increased serum IgE levels after allergen exposure by including only double-sensitized (sensitization to rPhl p 5 *and* rBet v 1) subjects in the study. However, due to difficulties with the recruitment of enough eligible subjects according to the sample size considerations and in order not to compromise the primary endpoint of the study, subjects sensitized to one allergen only (rPhl p 5 *or* rBet v1) will be also included in the study and specific IgE levels will be accepted from 0.7 kUA/l (RAST class 2) as demonstrated by UniCAP.

Former version:

Inclusion criteria

- sensitization to rPhl p 5 and rBet v 1 as demonstrated by UniCAP within a range from 3.0-80 kUA/l and a positive (wheal diameter $\geq$ 3 mm larger than the negative control and half of the histamine control) SPT with rPhl p 5 and rBet v 1 at 20 µg/ml

New version:

Inclusion criteria

- sensitization to rPhl p 5 and/or rBet v 1 as demonstrated by UniCAP within a range from 0.7-80 kUA/l and a positive (wheal diameter $\geq$ 3 mm larger than the negative control and half of the histamine control) SPT with rPhl p 5 and/or rBet v 1 at 20 µg/ml