

For reprint orders, please contact: reprints@futuremedicine.com



Long-term effect of monophosphoryl lipid A adjuvanted specific immunotherapy in patients with grass pollen allergy

Stefan Zielen^{*1}, Jennis Gabrielpillai¹, Eva Herrmann², Johannes Schulze¹, Ralf Schubert¹ & Martin Rosewich¹

¹Department for Children & Adolescents, Division of Allergology, Pulmonology & Cystic fibrosis, Children's Hospital, Goethe University, Theodor Stern Kai 7, 60590 Frankfurt, Germany

²Department of Biostatistics, Goethe University, Frankfurt am Main, Germany

*Author for correspondence: Tel.: +49 69 6301 5732; Fax: +49 69 6301 6061; Stefan.zielen@kgu.de

Background: Ultra-short course pollen immunotherapy adjuvanted with monophosphoryl lipid A (MPL) is attractive to conventional allergen-specific immunotherapy (AIT). Long term efficacy of MPL-AIT has not been evaluated. **Methods:** 68 patients (age 16.75 ± 5.3 years) with allergic rhinitis to grass pollen were investigated. Group 1: 21 controls; Group 2: 19 after complete AIT, and Group 3: 28 with AIT and treatment cessation: 4 years range 3–6 years ago. **Results:** The clinical symptoms (running nose, sneezing, conjunctivitis and the weekly overall score) were significantly reduced in patients group 2 and 3 compared with controls without AIT $p < 0.0001$. T-regulatory cells and TH1/TH2 cytokine pattern did not differ between patient groups. **Conclusion:** The patients in our trial with grass pollen allergy exhibited significant and long-lasting improvements after MPL-AIT, however larger trials are needed to support this finding.

First draft submitted: 9 January 2018; Accepted for publication: 12 March 2018; Published online: 22 March 2018

Keywords: allergen immunotherapy • allergic rhinitis • grass pollen allergy • long-term effects • monophosphoryl-A adjuvanted (MPL)

Introduction

IgE-mediated diseases, such as allergic rhinoconjunctivitis (AR) and asthma represent a serious health problem throughout the world. The lifetime prevalence of AR is approximately 20%. It often has an impact on social life, school performance and labor productivity of patients [1]. While pharmacological treatment such as antihistamines or topical applied steroids are alleviating symptoms of AR, allergen immunotherapy (AIT) can modify the underlying immunological mechanism of allergic diseases [2,3]. Development of tolerance induced by AIT can be described by persistence of clinical improvement and altered allergen-specific T- and B-cell response [4,5]. The prevailing immunological consensus is that T-regulatory (Treg) cells are induced by AIT [6]. The Treg-cell response is characterized by an abolished allergen-induced specific T-cell proliferation and suppressed T helper (TH)1- and TH2-type cytokine secretion; Treg cells potently suppress specific IgE production while simultaneously inducing non-inflammatory IgG4 and IgA isotype antibodies [6,7]. Since the introduction of AIT in 1911, quality and standardization of allergen extracts have improved substantially: preferably, standardized allergen extracts should be used either unmodified or modified with well-defined biological activity [8,9]. Modified extracts (allergoids) are available with less reactive B-cell epitopes that have a reduced IgE binding, while T-cell epitopes and immunogenicity should be preserved [10]. In Europe, mainly semi-depot extracts are used in the application of AIT [11]. In this case, the allergen or allergoid is physically bound to a carrier, such as aluminum hydroxide or tyrosine. Still conventional AIT consists of more than 40 injections over three years. Alternatively, ultra-short course allergy vaccines have been developed consisting of four injections of allergoids adsorbed to L-tyrosine and monophosphoryl lipid A (MPL) adjuvant offering distinct clinical advantages over conventional AIT [12]. MPL is an adjuvant derived from the lipopolysaccharide of *Salmonella minnesota* R 595. MPL is predominantly increasing the number of TH1-cells [12–14]. Clinical experience with pollen allergoids adjuvanted with MPL has been extensive, with more than 5000 subjects treated in clinical and post-marketing studies and approximately 260,000 patients receiving the drug until

now [15–18]. However, little is known about the long-term effect of ultra-short course allergy vaccination so far. This is indeed the case for many different AIT extracts and regimes. Only few long-term studies are available, since the famous study of Durham *et al.* [19] who reported a prolonged clinical remission and a disease-modifying effect of AIT up to 4 years after cessation of treatment.

In this cross-sectional study, we compared treatment success after ultra-short allergy vaccination against grass in three patients groups, one directly after 3 years treatment, one after cessation of treatment 3–6 years later, and that of an untreated control group.

Methods

Patients

68 patients (45 male, 23 female, 7 to 28 years old, mean age 16.75 ± 5.3 years) with AR to grass pollen were enrolled in the study. The patients were selected from the outpatient Department of Pediatric Allergy, Pneumology and Cystic Fibrosis in the children's hospital of the Goethe-University in Frankfurt/Main, Germany.

The criteria for inclusion were as follows: signed informed consent; participants >6 and ≤ 28 years of age who had a clinical history of AR and/or allergic asthma grade 1, according to GINA guidelines, during the grass pollen season; elevated serum allergen-specific IgE (RAST $\geq 2/\geq 0.71$ kU/ml) to grass pollen; and no significantly abnormal findings on physical examination.

Reasons for exclusion included the following: forced expiratory volume in 1 s (FEV1) less than 65% of the predicted value prior to enrollment, concomitant therapy with inhaled corticosteroids (ICS) of at least 3 months in duration; the use of systemic steroids; and the regular use of antihistamines. Further patients with serious underlying conditions were excluded from the study.

Human guidelines of good clinical practice, the German Drug Act and the declaration of Helsinki (1964) and Edinburgh (2000) were followed in the conduct of the trial. The local ethics committee approved the study. All parents and all patients older than 14 years of age provided written informed consent. The trial was registered at ClinicalTrials.gov Identifier: NCT01830673.

Study design

This trial was a non-randomized prospective cross-sectional case–control study that encompassed two study visits and a diary phase during the peak pollen season. At visit one, a complete medical history, including concomitant diseases and medications, a physical examination, measurement of exhaled nitric oxide (eNO) and spirometry were performed. Then blood samples were obtained (see parameters below). Visit 2 occurred 4 weeks after the end of the grass the pollen season to collect the diary data.

Allergen specific immunotherapy

All patients except the controls had received four injections consisting of tyrosine-adsorbed *Phleum pratense* allergoid and enhanced with the adjuvant monophosphoryl lipid A (MPL) (Pollinex[®] quattro, Bencard, Munich, Germany, subsidiary of Allergy therapeutics, Worthing, UK). One cycle of AIT comprised four injections with increasing allergen units 300, 800, 2000 and 2000, respectively.

Symptom diary

During the grass pollen season, the patients from the area of Frankfurt/M, Germany, were asked to record their symptoms and medication use during the peak pollen season (for 1 week in May and 1 week in June 2012). The following five nasal and conjunctival symptoms (runny nose, blocked nose, itchy nose, sneezing and itchy eyes) and four asthma symptoms (shortness of breath, wheezing, chest tightness and coughing) were recorded in a diary. Intensity of every item was rated from 'no symptoms' [0] to 'severe symptoms' [3]. Further, the use of the following medications was recorded and weighted as follows: disodium cromoglicate [1], antihistamines [1], beta agonists [1], leukotriene antagonists [4], topical steroids [3], and systemic steroids [6] as recently described [38]. Scores of each symptom were summed up for one week and the mean value was determined for a weekly overall score (WOS). The WOS of both weeks were summed.

Lung function tests

Spirometry was performed with the MasterScreen[®] body plethysmograph by VIASYS Healthcare GmbH (Hoechst, Germany).

Measurement of exhaled nitric oxide

The exhaled NO (eNO) measurements were performed using the NIOX[®] (Aerocrine, Solna, Sweden), according to the ATS guidelines.

Blood sampling & laboratory allergy

The total IgE, specific IgE, and specific IgG/IgG4 responses to *P. pratense* were analyzed using a two-sided chemiluminescent assay (Immulite 2,000, Siemens AG Healthcare, Erlangen, Germany); 18 ml of serum drawn with a Sarstedt Monovette[®]). The analysis was performed according to the manufacturer's instructions.

Staining of lymphocytes

Whole blood samples (9 ml EDTA EDTA Monovette) were stained for differentiation for TH1 and TH2 cells by flow cytometry. For TH1 cells, the chemokine receptor CXCR3 was stained by a CD183 APC antibody, to differentiate TH2 cells, the chemokine receptor CCR4, was stained by a CD194 antibody [19].

For staining of Treg cells (CD4 + CD25 + FoxP3 positive cells) an intracellular staining procedure was used. Isolated peripheral blood mononuclear cells were surface-stained with anti-CD4-fluorescein isothiocyanate and anti-CD25-phycoerythrin monoclonal antibodies. After fixation and permeabilization, intracellular staining was accomplished applying anti-human FoxP3 allophycocyanin (APC) antibody (Natutec, Frankfurt am Main, Germany). A flow cytometer (Becton Dickinson, CA, USA) analyzed 10,000 events (on each sample). Data analysis was performed with CellQuest software (Becton Dickinson). All laboratory tests began within 2 h after the blood samples were drawn.

Stimulation of peripheral blood lymphocytes

Peripheral blood lymphocytes were separated by Ficoll centrifugation. Then 1×10^6 cells per well were simulated for 24 h either with culture medium (negative control), grass pollen antigen or a combination of CD3/CD28 antibodies for maximum cytokine production as described [20].

Cytometric bead array

The concentrations of six cytokines were determined in cell supernatants after stimulation of whole blood for 24 h using the BD[™] cytometric bead array (CBA) Flex Set System (BD Bioscience-PharMingen, CA, USA) to measure the IL-2, IL-12/23, IL-5, IL-10, TNF- α and IFN- γ levels as described previously [21]. The lower detection limits of the cytokines were IL-2, 1.0 pg/ml; IL-12/23, 1.6 pg/ml; IL-5, 1.1 pg/ml; IL-10 1.6 pg/ml TNF- α , 0.7 pg/ml, and IFN- γ , 1.8 pg/ml.

End points & statistical analysis

In this non-randomized prospective cross-sectional case-control study, no hypothesis was formulated, and the data were analyzed descriptively. Due to the exploratory design of the trial, there was no formal estimation of sample size accounting for type I error rate, power, standard deviation, and effect size. The sample size of 20 patients per treatment group was considered sufficient to guarantee a probability of 95%, that changes in immunological parameters like specific IgG and IgG4 between groups could be detected as it was possible in a previous trial [22].

Primary outcome measures: induction of Treg cells by flow cytometry.

Secondary outcome Measures: determination of Th1 and Th2 cells by flow cytometry, inflammatory cytokine production of lymphocytes and reduction of symptom scores (reduction in nasal, conjunctival and bronchial symptoms and the weekly overall score).

The data were statistically analyzed and graphed with GraphPad Prism software, version 5.01 (GraphPad Software Inc., CA, USA, Bias for Windows version 10.08 epsilon-Verlag, Darmstadt, Germany). The parameters were tested for normal and log-normal distributions using the Kolmogorov-Smirnov test. For comparison between groups, the one-way analysis of variance (ANOVA) was used. A probability of $p < 0.05$ was considered statistically significant.

Results

Patient characteristics

We were able to enroll 68 patients with AR sensitized to grass pollen. Patients were divided into three groups according to their AIT history:

- Group 1: Controls without previous AIT.

Table 1. Patient characteristics at baseline.

	Group 1 Controls without AIT	Group 2 Post AIT	Group 3 Cessation of AIT 3–6 years
Number (n)	21	19	28
Age (years)	22.7 (12–28)	12.1 (7–24)	16.6 (10–22)
Males	8	17	20
FVC (L/%)	94.0 (80.6–134.6)	95.2 (72.5–115)	101.4 (86.7–154.1)
FEV1 (L/%)	105.6 (77.2–142.1)	102.3 (81.1–133.8)	108.8 (81.3–169.6)
FEV1/FVC (%)	87.0 (77.9–98.5)	88.7 (76.1–98.3)	83.3 (76.5–100)
eNO (ppB)	33.1 (5.5–75.4)	18.1 (2.8–33.7)	11.6 (1.8–73.2)
sIgE grass (KU/L)	82.0 (5–100)	86.1 (28.8–100)	18.3 (0.1–100)
sIgE rye (KU/L)	63.2 (0.9–100)	63.8 (15.1–100)	7.6 (0.1–76)

Median and range are shown.

Table 2. Grass and Rye specific IgG and IgG4 levels in all groups.

	Group 1 Controls without AIT	Group 2 Post AIT	Group 3 Cessation of AIT 3–6 years
IgG grass (μ/l)	6925 (200–137000)	42100 (7580–109000)	11600 (4070–39100)
IgG4 grass (μg/l)	230 (200–1189)	5267 (408–22802)	508 (200–39665)
IgG rye (μ/l)	5725 (2000–126000)	20800 (10500–72300)	7510 (3340–115000)
IgG4 rye (μg/l)	200 (200–702)	2548 (200–15159)	325 (200–14141)

Median and range are shown; group 2 levels were significantly elevated about group 1 and 3, $p < 0.001$. Group 3 had significantly higher levels than group 1, $p < 0.01$.

- Group 2: Patients directly after AIT (after completion the 2nd or 3rd year of treatment).
- Group 3: patients with cessation of AIT for at least 3 years.

The patient groups were not well balanced for age, eNO, and specific IgE, as summarized in Table 1. This was in part due to the selection criteria since controls had to be adolescents or young adults according to the ethic vote. As expected untreated patients had higher eNO levels than patients treated with AIT.

Specific IgE levels of group 3, patients after cessation of AIT 3–6 years, had significantly lower specific IgE levels than group 1 and 2. This led to an investigation of historical IgE levels in patients group 3 before AIT was initiated.

In 11 of 28 patients of group 3 historical levels were recorded. Interestingly, the specific IgE levels to grass and rye were significantly higher before AIT than during follow up: sIgE grass (KU/L) before 100 (0.7–100) vs 18.3 vs (0.1–100); $p < 0.05$ and for sIgE rye (KU/L) 100 (0.3–100) vs 7.6 (0.1–76); $p < 0.01$.

Baseline lung function (forced vital capacity [FVC], forced expiratory volume in one second [FEV1], FEV1/FVC) was within the normal range ($>70\%$ pred.) and showed no significant differences between the groups. There were in total 16 polysensitized patients, six in group 1, three in group 2 and seven in group 3.

Symptom diary

The diary return rate in of patients in both observation periods May and June were 63.2% (43/68). Nasal and eye symptoms were significantly reduced in group 2 and group 3 (Figure 1A and Figure 1B). In addition, the WOS in group 1 was 10.46 ± 7.79 and significantly reduced in group 2 5.61 ± 4.67 , and group 3 5.68 ± 3.73 ; $p < 0.001$ (Figure 1C).

Specific IgG & IgG4 to grass & rye

As a surrogate marker for a recent AIT, IgG and IgG4 levels were found to be significantly increased in group 2 vs group 1 and group 3; $p < 0.001$ (Table 2). In addition, levels of group 3 were significantly higher than in controls $p < 0.01$.

Evaluation of TH1/TH2 cells & T-regulatory cells

The TH1/TH2 cells comparison median (range) of the groups showed no significant differences (TH 1 Group 1:16.7% [9.3–28], group 2: 10.6% [4.4–18.6] and group 3: 16.7% [6.4–32.4]; n.s) and (TH 2 Group 1:15.5% [63–41.8], group 2: 13.4% [4.4–28.7] and group 3: 13.8 [7.2–26.7]; n.s).

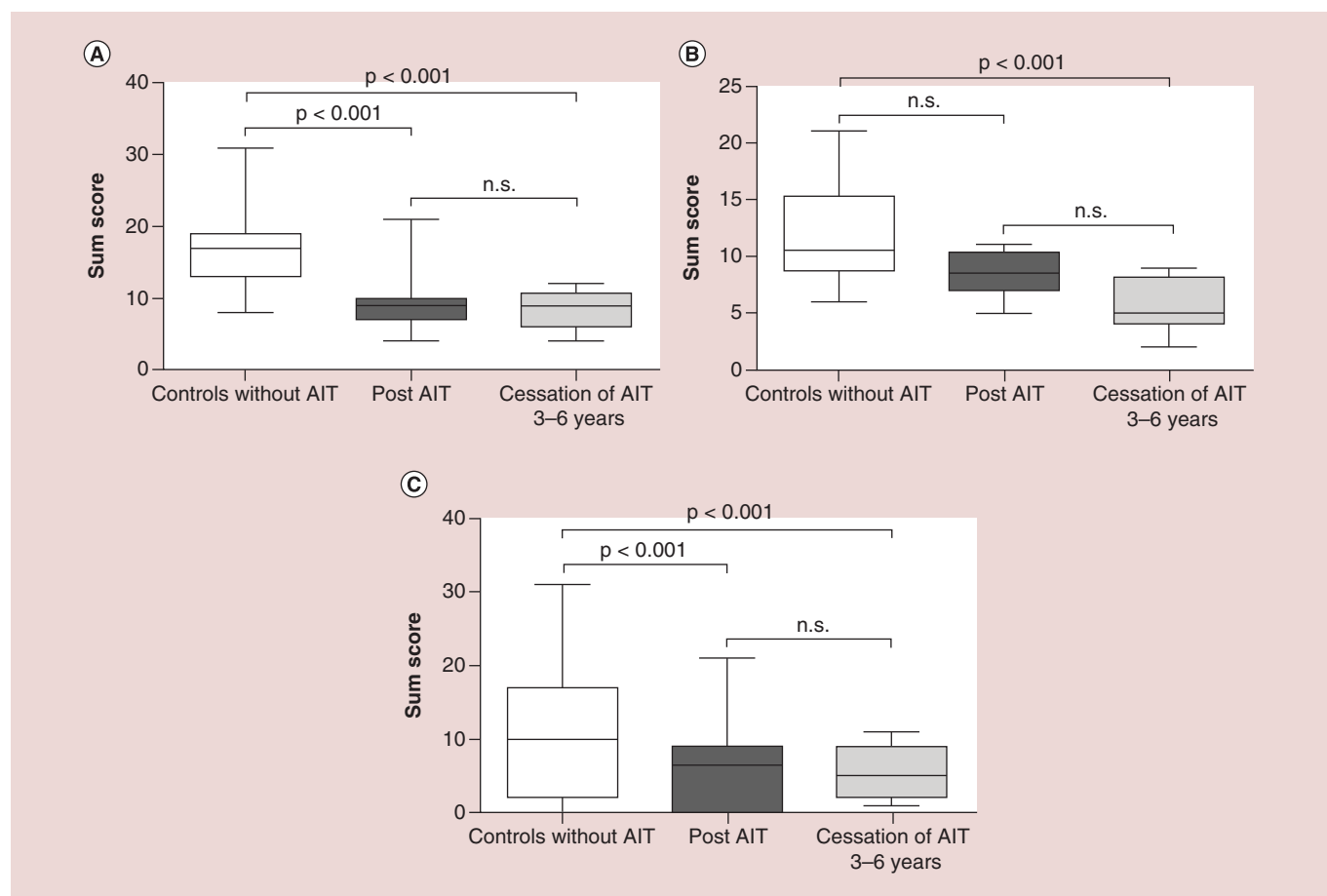


Figure 1. (A) Nasal symptom score during the grass pollen season. (B) Eye symptoms during the grass pollen season. (C) Weekly overall score (WOS) during the grass pollen season.

In addition, the distribution of Treg cells was similar between the groups (Group 1: $3.2 \pm 1.12\%$, group 2: $1.6 \pm 0.59\%$ and group 3: $2.95 \pm 0.72\%$, n.s.).

Stimulation of peripheral blood lymphocytes

As shown in Table 3A and B cytokine stimulation for 24 h either with grass pollen antigen or a combination of CD3/CD28 antibodies for maximum cytokine production showed no difference for the groups.

Discussion

The MPL adjuvanted ultra-short allergy vaccination is an established therapy for patients with allergic rhinitis and allergic asthma [15,16]. MPL is a well-characterized adjuvant for AIT, by inducing a strong TH1 response with relatively little associated toxicity [12,23]. Over the past 15 years, more than 750,000 injections have been performed. MPL-AIT leads to a significant clinical improvement of the symptoms and medication consumption in allergic rhinitis and allergic bronchial asthma [15–18]. Using MPL-AIT is safe and requires only four pre-seasonal injections in three consecutive years compared to conventional AIT. Thus, one of the major advantages is improved compliance in children, adolescents and even adults.

Nevertheless, many clinicians favor conventional AIT because of its traditional use and the availability of long-term data [19,24,25]. In addition, there is evidence that the cumulative amount of injected allergen is correlated with sustained treatment success. If this holds true for all types of AIT is still debatable. Only a few trials have shown clear evidence, that a greater amount of total allergen was favorable [26,27]. Other studies, comparing 3 to 5 years AIT, showed no benefits of longer treatment [28,29].

Table 3. Cytokine production after stimulation with grass antigen and CD3/28.

Cytokine production after stimulation with grass antigen	Group 1 Controls without AIT	Group 2 Post AIT	Group 3 Cessation of AIT 3–6 years
Tregs (%/CD4 cells)	3.2 (1.7–5.7)	1.6 (0.6–3.1)	2.9 (1.3–4.5)
IL-2 (pg/ml)	0 (0–24)	11 (0–66)	0 (0–21)
IL-12/23 (pg/ml)	25 (0–146)	33 (0–69)	37 (0–146)
IFN- γ (pg/ml)	0 (0–36)	0 (0–27)	0 (0–25)
IL-5 (pg/ml)	0 (0–31)	4 (0–193)	0 (0–31)
IL-10 (pg/ml)	7 (0–114)	16 (2–73)	29 (0–114)
TNF- α (pg/ml)	0 (0–108)	9 (0–93)	8 (0–108)
Cytokine production after stimulation with CD3/28	Group 1 Controls without AIT	Group 2 Post AIT	Group 3 Cessation of AIT 3–6 years
Tregs (%/CD4 cells)	3.2 (1.7–5.7)	1.6 (0.6–3.1)	2.9 (1.3–4.5)
IL-2 (pg/ml)	18 (0–24)	56 (0–2022)	17 (0–3700)
IL-12/23 (pg/ml)	138 (0–492)	91 (15–321)	138 (17–492)
IFN- γ (pg/ml)	1016 (0–2816)	967 (4–2566)	762 (0–2683)
IL-5 (pg/ml)	189 (0–966)	273 (0–928)	163 (0–660)
IL-10 (pg/ml)	112 (7–968)	93 (5–364)	112 (10–609)
TNF- α (pg/ml)	1293 (0–8975)	634 (2–4423)	576 (0–8975)

Median and range, T_{regs} were measured by cytometry, whole blood was stimulated and supernatant were measured 24 h later as described. No differences were found between groups.

The aim of our cross-sectional study was to evaluate the long-term efficacy of MPL-AIT in patients with grass pollen allergy. For the first time, our results demonstrated that MPL-AIT may deliver long-term protection in patients with grass pollen allergy. Each of the symptoms; running nose, sneezing, conjunctivitis and the weekly overall score were significantly reduced in group 3, compared to controls without treatment and similar to patients group 2.

It is important to note, that the present study was designed to investigate potential immunological effects of AIT and does not supply sufficient data for clinical treatment effects. The non-randomized design of the trial and the small sample size studied, were not expected to result in meaningful differences between the symptom scores of treated and untreated patients. In addition, it is well known, that symptom scores are profoundly influenced by placebo effects [30]. In particular, patients without treatment like group 1 tend to be less satisfied and may overestimate their symptoms [30]. This disadvantage may hold true for the comparison of patient's in group 1 versus group 2, but is less likely to affect patients of group 3, who stopped treatment more than 3 years ago. Thus, significant improvements in clinical symptoms of group 3 demonstrated the long-lasting effect of ultra-short allergy vaccination. In line with this finding, we found a decrease in sIgE levels in patients of group 3, which is a well known phenomenon after successful AIT [31,32], although this observation was retrospectively done in 11 patients only.

For conventional AIT and sublingual immunotherapy (SLIT) it is well established that AIT facilitate long-lasting treatment effects after discontinuation of treatment [19,24,25,33–36]. Jacobsen *et al.* [24] showed a protective effect of AIT on the development of allergic asthma in a 10-year follow-up study in children with grass pollen allergy. Similar to our trial, Dominicus *et al.* [34] demonstrated the long-term effects of AIT in adults after cessation of therapy. They showed persistent improvement of clinical symptoms 3 years after cessation of AIT.

Due to rapid developments in the field of AIT, there is an unmet need to objectively assess the treatment successes by biomarkers. Recently the EAACI taskforce reviewed all candidate biomarkers used in clinical trials of AIT [37]. Biomarkers were grouped into seven domains: (1) IgE (total IgE, specific IgE and sIgE/Total IgE ratio), (2) IgG-subclasses (sIgG1, sIgG4 including sIgE/IgG4 ratio), (3) serum inhibitory activity for IgE, (4) basophil activation, (5) cytokines and chemokines, (6) cellular markers (regulatory cells) and (7) *in vivo* biomarkers (including provocation tests).

As mentioned above, the present study was designed to investigate immune responses after AIT vs non-treated controls. Well known immunological parameters like IgE, IgG and IgG4 responses, number of TH2 to TH1 cells, possible increase of Treg cells, and normalizing the TH2 dominated cytokine response towards tolerance were analyzed. The results of our investigations were rather disappointing. Only, IgG and IgG4 levels significantly

increased during and after AIT, whereas all other immune parameters were not changed compared to the non-treatment group. This is difficult to explain but may be partially explained by the immunological measurements used in our trial. In keeping with recent findings of the EAACI task force, neither numbers of TH1/TH2 and Treg cells nor cytokine measurement of lymphocytes are appropriate to estimate treatment effects of clinical trials [37]. These sophisticated immunological techniques can be used to get insight into the mechanisms of AIT in mouse models and insect allergy, but are less valuable in trials of AIT in AR.

In contrast to immunological parameters, symptom and medication scores have objectively demonstrated treatment success even in small cohorts [28,34,35]. Numerous clinical trials and treatment reports have found that MPL-AIT were well-tolerated and resulted in significant reductions in symptom scores, improved provocation test results and antigen-specific IgE value, as well as increased antigen-specific IgG/IgG4 [16,18,23,38]. According to our results, this effect is long lasting and demonstrable after 3–6 years after end of MPL-AIT.

Financial & competing interests disclosure

Stefan Zielen received fees for lectures and advisory boards from the following companies: bene-Arzneimittel GmbH; Vifor Pharma Deutschland GmbH; Novartis AG; GlaxoSmithKline GmbH; ALK-Abelló Arzneimittel GmbH; Allergy Therapeutics; Boehringer Ingelheim, Lofarma GmbH, IMS Health GmbH & Co. and Biotest Pharma GmbH. Martin Rosewich received fees for lectures and advisory boards from the following companies: Chiesi, Novartis AG, GlaxoSmithKline, Allergopharma GmbH & Co. KG and Allergy Therapeutics.

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

References

1. Bousquet J, Annesi-Maesano I, Carat F, Léger D, Rugina M, Pribil C *et al.* Characteristics of intermittent and persistent allergic rhinitis: DREAMS study group. *Clin. Exp. Allergy*. 35(6), 728–32 (2005).
2. Malling HJ, Bousquet J. Subcutaneous immunotherapy for allergic rhinoconjunctivitis, allergic asthma, and prevention of allergic diseases. *Clin. Allergy Immunol.* 21, 343–358 (2008).
3. James LK, Durham SR. Update on mechanisms of allergen injection immunotherapy. *Clin. Exp. Allergy* 38, 1074–1088 (2008).
4. Akdis CA, Blaser K. Mechanisms of allergen-specific immunotherapy. *Allergy* 55(6), 522–530 (2000).
5. Wambre E, DeLong JH, James EA, LaFond RE, Robinson D, Kwok WW. Differentiation stage determines pathologic and protective allergen-specific CD4⁺ T-cell outcomes during specific immunotherapy. *J. Allergy Clin. Immunol.* 129(2), 544–551 (2012).
6. Jutel M, Akdis M, Blaser K, Akdis CA. Mechanisms of allergen specific immunotherapy – T-cell tolerance and more. *Allergy* 61(7), 796–807 (2006).
7. Shamji MH, Durham SR. Mechanisms of allergen immunotherapy for inhaled allergens and predictive biomarkers. *J. Allergy Clin. Immunol.* 140(6), 1485–1498 (2017).
8. van Ree R, Chapman MD, Ferreira F *et al.* The CREATE project: development of certified reference materials for allergenic products and validation of methods for their quantification. *Allergy*. 63(3), 310–326 (2008).
9. Cox L, Esch RE, Corbett M, Hankin C, Nelson M, Plunkett G. Allergen immunotherapy practice in the United States: guidelines, measures, and outcomes. *Ann. Allergy Asthma Immunol.* 107(4), 289–299 (2011).
10. Akdis CA, Blaser K. Regulation of specific immune responses by chemical and structural modifications of allergens. *Int. Arch. Allergy Immunol.* 121(4), 261–269 (2000).
11. Schmitt J, Schwarz K, Stadler E, Wüstenberg EG. Allergy immunotherapy for allergic rhinitis effectively prevents asthma: Results from a large retrospective cohort study. *J. Allergy Clin. Immunol.* 136(6), 1511–1516 (2015).
12. Rosewich M, Lee D, Zielen S. Pollinex Quattro: an innovative four injections immunotherapy in allergic rhinitis. *Hum. Vaccin. Immunother.* 9(7), 1523–1523 (2013).
13. Thompson HS, Davies ML, Watts MJ *et al.* Enhanced immunogenicity of a recombinant genital warts vaccine adjuvanted with monophosphoryl lipid A. *Vaccine*. 16(20), 1993–1999 (1998).

14. Moore A, McCarthy L, Mills KH. The adjuvant combination monophosphoryl lipid A and QS21 switches T cell responses induced with a soluble recombinant HIV protein from Th2 to Th1. *Vaccine* 17(20–21), 2517–2527 (1999).
15. Drachenberg KJ, Wheeler AW, Stuebner P, Horak F. A well-tolerated grass pollen-specific allergy vaccine containing a novel adduct, monophosphoryl lipid A, reduces allergic symptoms after only four preseasonal injections. *Allergy* 56, 498–505 (2001).
16. Rosewich M, Schulze J, von Weikersthal-Drachenberg Zielen S. Ultra-short course immunotherapy in children and adolescents during a 3-yrs post-marketing surveillance study. *Pediatr. Allergy Immunol.* 21, e185–e189 (2010).
17. DuBuske LM, Frew AJ, Horak F *et al.* Ultrashort-specific immunotherapy successfully treats seasonal allergic rhinoconjunctivitis to grass pollen. *Allergy Asthma Proc.* 32, 239–247 (2011).
18. Patel P, Holdich T, Fischer von Weikersthal-Drachenberg KJ, Huber B. Efficacy of a short course of specific immunotherapy in patients with allergic rhinoconjunctivitis to ragweed pollen. *J. Allergy Clin. Immunol.* 133, 121–129 (2014).
19. Durham SR, Walker SM, Varga EM, Jacobson MR, O'Brien F, Noble W, Till SJ, Hamid QA, Nouri-Aria KT. Long-term clinical efficacy of grass-pollen immunotherapy. *N. Engl. J. Med.* 341(7), 468–475 (1999).
20. Kunicki MA, Amaya Hernandez LC, Davis KL, Bacchetta R, Roncarolo MG. Identity and diversity of human peripheral th and t regulatory cells defined by single-cell mass cytometry. *J. Immunol.* 200(1), 336–346 (2018).
21. Fußbroich D, Schubert R, Schneider P, Zielen S, Beermann C. Impact of soyasaponin I on TLR2 and TLR4 induced inflammation in the MUTZ-3-cell model. *Food Funct.* 6(3), 1001–1010 (2015).
22. Eickmeier O, Huebner M, Herrmann E *et al.* Sputum biomarker profiles in cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD) and association between pulmonary function. *Cytokine* 50(2), 152–157 (2010).
23. Rosewich M, Schulze J, Eickmeier O, Telles T, Rose MA, Schubert R, Zielen S. Tolerance induction after specific immunotherapy with pollen allergoids adjuvanted by monophosphoryl lipid A in children. *Clin. Exp. Immunol.* 160(3), 403–410 (2010).
24. Jacobsen L, Niggemann B, Dreborg S *et al.* Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma: 10-year follow-up on the PAT study. *Allergy* 62, 943–948 (2007).
25. Eng PA, Reinhold M, Gnehm HP. Long-term efficacy of pre-seasonal grass pollen immunotherapy in children. *Allergy* 57(4), 306–312 (2002).
26. Frew AJ, Powell RJ, Corrigan CJ, Durham SR. UK Immunotherapy Study Group. Efficacy and safety of specific immunotherapy with SQ allergen extract in treatment-resistant seasonal allergic rhinoconjunctivitis. *J. Allergy Clin. Immunol.* 117, 319–325 (2006).
27. Tworek D, Bochenska-Marciniak M, Kuprys-Lipinska I, Kupczyk M, Kuna P. Perennial is more effective than preseasonal subcutaneous immunotherapy in the treatment of seasonal allergic rhinoconjunctivitis. *Am. J. Rhinol. Allergy.* 27, 304–308 (2013).
28. Stelmach I, Sobocińska A, Majak P, Smejda K, Jerzyńska J, Stelmach W. Comparison of the long-term efficacy of 3- and 5-year house dust mite allergen immunotherapy. *Ann. Allergy Asthma Immunol.* 109, 274–278 (2012).
29. Tabar AI, Arroabarren E, Echechipia S, García BE, Martin S, Alvarez-Puebla MJ. Three years of specific immunotherapy may be sufficient in house dust mite respiratory allergy. *J. Allergy Clin. Immunol.* 127, 57–63 (2011).
30. Wechsler ME, Kelley JM, Boyd IO *et al.* Active albuterol or placebo, sham acupuncture, or no intervention in asthma. *N. Engl. J. Med.* 365, 119–126 (2011).
31. Gleich GJ, Zimmermann EM, Henderson LL, Yunginger JW. Effect of immunotherapy on immunoglobulin E and immunoglobulin G antibodies to ragweed antigens: a six-year prospective study. *J. Allergy Clin. Immunol.* 70, 261–271 (1982).
32. Rosewich M, Arendt S, El Moussaoui S, Schulze J, Schubert R, Zielen S. Bronchial allergen provocation: a useful method to assess the efficacy of specific immunotherapy in children. *Pediatr. Allergy Immunol.* 24(5), 434–440 (2013).
33. Cools M, Van Bever HP, Weyler JJ, Stevens WJ. Long-term effects of specific immunotherapy, administered during childhood, in asthmatic patients allergic to either house-dust mite or to both house-dust mite and grass pollen. *Allergy* 55(1), 69–73 (2000).
34. Dominicus R. 3-years' long-term effect of subcutaneous immunotherapy (SCIT) with a high-dose hypoallergenic 6-grass pollen preparation in adults. *Eur. Ann. Allergy Clin. Immunol.* 44, 135–140 (2012).
35. Zidarn M, Kosnik M, Silar M, Bajrovic N, Korosec P. Sustained effect of grass pollen subcutaneous immunotherapy on suppression of allergen-specific basophil response; a real-life, nonrandomized controlled study. *Allergy* 70, 547–555 (2015).
36. Zielen S, Devillier P, Heinrich J, Richter H, Wahn U. Sublingual immunotherapy provides long-term relief in allergic rhinitis and reduces the risk of asthma: a retrospective, real-world database analysis. *Allergy* 73(1), 165–177 (2018).
37. Shamji MH, Kappen JH, Akdis M, Jensen-Jarolim E, Knol EF *et al.* Biomarkers for monitoring clinical efficacy of allergen immunotherapy for allergic rhinoconjunctivitis and allergic asthma: an EAACI Position Paper. *Allergy* 72(8), 1156–1173 (2017).
38. Rosewich M, Girod K, Zielen S, Schubert R, Schulze J. Induction of bronchial tolerance after 1 cycle of monophosphoryl-A-adjuvanted specific immunotherapy in children with grass pollen allergies. *Allergy Asthma Immunol. Res.* 8(3), 257–263.

Reproduced with permission of copyright owner. Further reproduction prohibited
without permission.