Immunoglobulin E and G Antibody Profiles to Grass Pollen Allergens During a Short Course of Sublingual Immunotherapy

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Abstract
Background: Various studies have shown the clinical efficacy of sublingual immunotherapy in grass pollen-induced rhinoconjunctivitis. However, even short-term treatment with grass extracts might cause sensitizations to formerly unrecognized antigens.
Objective: To determine whether the antibody profiles are changing in patients receiving a defined grass pollen extract prior to and during the grass pollen season.
Methods: A randomized, double blind, placebo-controlled, multicenter phase II/III trial was started prior to the commencement of the grass pollen season. Patients with grass pollen allergy were randomly allocated to four groups, and received daily a standardized tablet at different doses. Treatment was started 8 weeks prior to the beginning of the pollen season and stopped at the end of the season. Blood samples were taken at the beginning of the study, at the beginning and the end of the pollen season, and one year after commencement of the study.
Results: At the beginning of the study, all patients tested positive for the major grass pollen allergens, but negative to the minor antigens. In all patients, the degree of antibody reactivity rose considerably after starting active treatment and fell back to the initial values within one year. Immunoglobulin (Ig) E antibodies to the minor antigens remained negative, independent of treatment and seasonal exposure. In contrast to IgE, specific IgG antibodies to all allergens tested revealed no specific trend.
Conclusions: Immunotherapy with grass allergen tablets was accompanied by an increase in grass-specific IgE antibodies, which further increased during pollen exposure, followed by a post-treatment drop in patient- and disease-specific antibodies. During this short course of treatment, no patient developed any additional sensitizations.

Key words: Grass pollen allergy. Antibody profile. IgE. IgG. Sublingual immunotherapy. Tablet.

Resumen
Antecedentes: Varios estudios han demostrado la eficacia clínica de la inmunoterapia sublingual en el tratamiento de la rinoconjuntivitis inducida por el polen de gramíneas. Sin embargo, incluye el tratamiento de corta duración con extractos de gramíneas puede causar sensibilizaciones a antígenos previamente no reconocidos.
Objetivo: Estudiar posibles cambios en los perfiles de anticuerpos en los pacientes que reciben un extracto de polen de gramíneas definido antes y durante la estación de polinización.
Métodos: Antes de empezar la estación de polinización de las gramíneas, se inició un estudio multicéntrico de fase II/III, aleatorio, a doble ciego y controlado con placebo. Los pacientes con alergia al polen de gramíneas se asignaron de manera aleatoria a cuatro grupos distintos, y se les administró un comprimido diario estandarizado con distintas dosis. El tratamiento se inició ocho semanas antes del inicio de la estación de polinización y dejó de administrarse al finalizar dicha estación. Se extrajeron muestras de sangre al principio del estudio, al principio y al final de la estación de polinización, y al año de haberse iniciado el estudio.
Resultados: Al inicio del estudio, todos los pacientes mostraban una reacción positiva a los alérgenos mayoritarios del polen de gramíneas, y negativa a los antígenos menores. En todos los pacientes, el grado de reactividad de los anticuerpos aumentó considerablemente tras iniciar el tratamiento activo y bajó a los valores iniciales en el plazo de un año. La IgE frente a los antígenos menores siguió siendo negativa, independientemente del tratamiento y de la exposición estacional. La IgG específica frente a todos los alérgenos probados no reveló ninguna tendencia específica.
Introduction

Allergy to grass pollen is one of the most common inhalant allergies leading to impaired quality of life and increased expenditure in the health care system [1]. The grass allergen tablet investigated in this clinical trial was developed to make specific immunotherapy (SIT) available to a large number of patients suffering from immunoglobulin (Ig)E-mediated allergy to grass pollen [2]. Our aim was to determine whether natural allergen exposure and/or this short term-treatment using a purified, but not patient-adjusted vaccine, would initiate a specific immune response and/or cause additional unwanted sensitizations.

Materials and Methods

Forty subjects (27 men and 13 women, all Caucasian) from Graz, Innsbruck and Salzburg were enrolled in a phase II/III drug trial guided by the Parexel GmbH Institute of Clinical Pharmacology in Berlin, Germany [3]. The principal inclusion criteria were as follows: age 18-65 years, a 2–year or more clinical history of significant grass pollen-allergy-induced seasonal allergic rhinoconjunctivitis; specific IgE antibody to *Phleum pratense*; positive skin prick test to *P pratense* (ALK Prick SQ; ALK-Abelló A/S, Hørsholm, Denmark); and forced expiratory volume in 1 second greater than 70% of the predicted value. This multicenter study was performed in several European countries, but only patients from Austrian centers were included in this evaluation, since pollen number, quality, and exposure time are similar in these 3 Austrian cities. The study was approved by the individual local ethics committees.

Active treatment involved grass allergen tablets (GRAZAX, ALK-Abelló A/S), which are orodispersible tablets containing grass allergen extract of standardized quality from *P pratense*. The patients received either 2 500, or 25 000, or 75 000 standardized quality tablet (SQ-T) or placebo; 100 000 SQ-T corresponds to 20 µg of the major allergen Phl p 5. These extracts were tested for activity of Phl p 5 and 6. No data are given by the company regarding Phl p1, 7 and 12, but their presence is indisputable in an extract of *P pratense*. Individuals with persistent symptoms of allergic rhinitis were permitted to

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**Figure 1.** Trial diagram showing randomization, treatment and follow-up periods, as well as the time of season and days when blood was drawn to determine immunological parameters. Short arrows indicate the points at which immunological parameters were analyzed; SQ-T, standardized quality tablet.
receive additional single-blind rescue medication (Loratadine, Schering Plough, Kenilworth, New Jersey, USA) or placebo. Daily treatment was begun 8 weeks prior to the start of the grass pollen season and continued during the season. All patients were followed carefully for adverse side effects, symptoms, and consumption of rescue medication.

Patients’ sera were investigated for total IgE as well as grass pollen specific antibodies prior to the study, prior to the season (ie end of treatment), and 1 year after initiation of the study (figure 1). Total IgE, specific IgE- and IgG-antibodies to two major (rPhl p 1, rPhl p 5) as well as two minor cross-reactive grass pollen allergens (rPhl p 7, rPhl p 12) [4] were determined using the ImmunoCAP system (Phadia, Uppsala, Sweden). These grass pollen specific marker allergens are employed as diagnostic gate keepers, since patients who are sensitized against the major grass pollen allergens rPhl p 1 and rPhl p 5 are considered best suited for grass pollen-specific immunotherapy whereas patients who are also positive in the rPhl p 7/rPhl p 12 test appear less suitable for that treatment [7]. The ImmunoCAP system was chosen, since it was the first to be cleared by the Food and Drug Administration for the quantitative measurement of specific IgE and is as well evaluated for both the detection of antigen-specific IgG antibodies and the use of recombinant allergens. Quality criteria such as detection limits, coefficient of variation, and other performance characteristics are well defined.

**Results**

All patients who were randomized completed the trial and were included in the analysis. Patient characteristics were similar between the treatment groups at baseline, and compliance was high in all treatment groups.

Total IgE antibodies, as compared to the beginning of the study, rose quite considerably upon treatment (mean value from 105 to 138 kU/L). The peak during the pollen season was followed by a normalization after the season (153 as compared to 98 kU/L) (Table 1).

All patients in all treatment groups had low titers of specific IgE antibodies to the major grass pollen antigen rPhl p 1 and almost all to rPhl p 5 at the beginning of the study (figure 2). These titers remained stable in the grass pollen placebo group to a great extent until the season started and then rose considerably. In the active treatment group, specific rPhl p 1 and rPhl p 5 increased in a dose-dependent fashion within the first weeks of treatment and then soared during the pollen season. One year after initiation of the study – and nearly 8 months after the end of the pollen season – these IgE titers had returned to the initial counts (figure 2). In contrast to these IgE antibodies specific to the major grass allergens, IgEs to the minor allergens rPhl p7 and rPhl p 12 remained negative in all patients throughout the treatment and observation period (figure 2).

In contrast to the specific IgEs, specific IgG antibodies to all of the four antigen specificities remained at the level determined at baseline, independent of seasonal exposure or treatment group (figure 3). Remarkably, IgG levels to the four different antigens were also similarly high. Despite several months of treatment in the verum groups and despite natural exposure, none of the patients developed new sensitizations to any of the investigated grass antigen during the observation period.

The success of treatment and adverse events were carefully monitored and have been published elsewhere [3]. No patient discontinued the study because of non-compliance, adverse events or for any other study-related reason. Only mild oral pruritus was reported by almost half of actively treated subjects.

**Discussion**

Recombinant grass and tree pollen allergens may be used to improve the choice and monitoring of currently available forms of specific immunotherapy [5]. Thus, group 1 grass pollen allergens with a molecular weight of approximately 30 kD are the most frequently identified grass pollen allergens. They are expressed in all grasses, and approximately 90% of grass pollen-allergic patients show IgE antibody reactivity to group 1 allergens. Whereas Phl p 1, Phl p 2, Phl p 5 and Phl p 6 serve as specific markers for sensitization to grass pollen [6], Phl p 7 occurs as a highly cross-reactive allergen in pollen from a
variety of plant species [4]. Therefore it might be justified to use a recombinant technique to decide whether a patient may be a candidate for immunotherapy, and which antigen source would be appropriate in each individual case. Moreover, it has been speculated, that development of sensitizations against formerly negative grass pollen could occur [7].

In this study we used a battery of antibodies to determine whether treatment with a crude grass pollen extract is liable to cause new sensitizations during a short course of treatment; whether seasonal exposure causes antibody alterations in sensitized individuals; and whether short term treatment results in a selective increase in specific IgG antibodies which, as “blocking antibodies”, lead to antigen tolerance.

Treatment with grass allergen tablets was well tolerated by all subjects and all treatment groups [3]. Grass tablets containing purified grass extracts did not cause new sensitizations to the studied antibody specificities, in contrast to observations in a study with raw Fagales pollen extracts, where patients treated with crude allergen extracts developed new IgE specificities against minor allergens not recognized before treatment [8]. Our negative finding – that is positive for the patient – might be due to the fact, that although extracts were used for treatment, these were well purified extracts that were standardized for the major allergen rPhl p 5. The small number of patients treated and the short course of treatment in our study may be insufficient for definitive conclusions to be drawn. However, our observations are in accordance with a recent study demonstrating that SIT does not represent a risk factor for progression towards multiple pollen sensitization in monosensitized pollen-allergic patients [9].

Short-term treatment with this tablet appears to be safe, and no patient left the study because of adverse events. This is consistent with previously published observations regarding immunotherapy with recombinant grass pollen allergens [10,11]. Surprisingly, shortly after initiation of the study and before natural exposure was started, treatment with the relevant allergen caused a significant increase in specific antibodies in all patients, independent of whether they were treated with the grass tablet alone or with additional antihistamines. With regard to IgG antibodies, short-term grass tablet treatment does not promote the specific production of any antigen specificity. The brief course of treatment did not lead to the production of blocking antibodies. However,
we did not specifically look for IgG4 antibody concentrations as has been done in other studies [12]. The reason for this is that it was not our aim to look for criteria of efficacy of treatment, ie specific IgG4 antibodies, but rather for factors indicating new sensitizations, ie IgE and IgG antibodies that were not present before initiating the study. In addition, our studies are not absolutely comparable to similar data resulting from the identical multicenter trial regarding IgG antibody detection [3]. Whereas we tested for IgG antibodies against the well defined recombinant grass pollen antigens Phl p 1, 5, 7, and 12, respectively, in the analysis of the whole study population [3] specific IgG antibodies against a *P. pratense* extract were determined.

In summary, immunotherapy using the grass tablet appears to be safe and patient-friendly [1]. Its efficacy has been proven in phase-III studies [3]. It remains to be seen whether the long-term efficacy of this treatment is equal to that of conventional subcutaneous or sublingual treatment [13]. Besides, the mode of action is not fully elucidated. Allergen exposure stimulates initial IgE antibody response, whereas short-term treatment does not appear to exert a significant influence on the specific IgG response.

**References**


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