

Which Allergen Extract for Grass Pollen Immunotherapy? an in Vitro Study

F. Marcucci,¹ L. Sensi,¹ G. Di Cara,¹ C. Incorvaia,²
P. Puccinelli,³ S. Scurati,³ and F. Frati³

¹Institute of Pediatrics, Department of Medical and Surgical Specialty and Public Health, Perugia, Italy

²Allergy/Pulmonary Rehabilitation, ICP Hospital, Milan, Italy

³Medical and Scientific Department, Stallergenes, Milan, Italy

Background: Grass pollen is a major cause of allergy throughout the world. The only treatment targeting the causes and not only the symptoms of allergy is specific immunotherapy (IT). A number of controlled trials demonstrated the efficacy of IT in grass pollen allergic subjects, most using extracts of multiple grasses but some using extracts of a single grass. The optimal grass extract for IT has not yet been established. **Methods:** This study is aimed at investigating the IgE-binding pattern in sera from IT-naïve patients from central Italy with allergic rhinitis and/or asthma caused by grass pollen. A 5-grass extract was used (containing *Dactylis glomerata*, *Poa pratensis*, *Lolium perenne*, *Antoxanthum odoratum* and *Phleum pratense*) and compared to *Phleum pratense* alone, which is the most frequently used single grass extract, by the RAST-inhibition technique. **Results:** The 5-grass extract showed, by RAST-inhibition, a significantly higher binding compared to the *Phleum pratense* extract for *Antoxanthum odoratum* and *Poa pratensis*, while the two extracts for immunotherapy showed similar binding affinity for *Phleum pratense* and the non-*Pooideae* grass, *Cynodon dactylon*. **Conclusions:** The use of a mixed-grass pollen extract seems to be the optimal choice when applying specific IT in grass pollen-allergic subjects from the Mediterranean area.

Keywords Grass pollen, Allergy, IgE profile, RAST-inhibition, Specific immunotherapy.

INTRODUCTION

The common term “grasses” refers to the botanical family of *Gramineae* and in particular to the subfamily of *Pooideae*. This includes the species most

commonly responsible for allergic sensitisation in Europe, such as *Anthoxanthum odoratum*, *Dactylis glomerata*, *Festuca elatior*, *Holcus lanatus*, *Lolium perenne*, *Phleum pratense* and *Poa pratensis*. Another species causing respiratory allergy, though only in southern regions, is *Cynodon dactylon*.

Grass pollen is a major source of respiratory allergy throughout the world (D'Amato et al., 2007; Erbas et al., 2007; Freye, 2008). The various species have variable importance in different geographical areas. In Europe, *Dactylis glomerata*, *Poa pratensis*, *Lolium perenne* and *Anthoxanthum odoratum* are homogeneously distributed, while *Phleum pratense* is the dominant grass in Northern regions and in the United Kingdom, and *Cynodon dactylon* is found only in Southern regions.

Allergy to grass pollen can be treated by symptomatic drugs, but only specific immunotherapy is able to act on the cause of allergy and to modify the natural history of the disease (Bousquet et al., 1998). This treatment was introduced in 1911 (Noon, 1911) and received the first scientific demonstration of efficacy by a controlled trial in 1954 (Frankland et al., 1954). Both these studies dealt with grass pollen allergy and the treatment was definitely established as effective in meta-analyses, concerning traditional, subcutaneous immunotherapy (SCIT) (Abramson et al., 2003; Calderon et al., 2007) as well as sublingual immunotherapy (SLIT) (Wilson et al., 2005; Penagos et al., 2008).

Most immunotherapy studies were performed using extracts containing multiple grasses, but there have been trials using only *Dactylis glomerata*, *Phleum pratense*, or *Lolium perenne*, on the basis that they have all the relevant grass allergens. However, to date there are no immunological data demonstrating that extracts of single grasses and multiple grasses are equivalent in terms of IgE-binding capacity in sera from grass allergic patients. This study is aimed at investigating the IgE-binding pattern in sera from immunotherapy-naïve patients with allergic rhinitis and/or asthma caused by grass pollen. A 5-grass extract compared to *Phleum pratense* was used, as this is the most frequently used single grass extract, for the RAST-inhibition technique.

METHODS

Study Design and Population

This is a non-interventional, open-label study performed on the sera of immunotherapy-naïve patients with allergic rhinitis and/or asthma to grass pollens. The objective of the study is to assess the IgE binding pattern with the 5-grass extract compared to the *Phleum pratense* extract in serum samples. The samples were obtained from a population of grass pollen allergy sufferers from central Italy who were eligible for specific immunotherapy. Study participants had to fulfill all the following inclusion criteria: male or female subjects aged between 7 and 14 years; seasonal allergic respiratory

disease (rhinitis and/or asthma), defined according to ARIA (Bousquet et al., 2001) and GINA (Bousquet et al., 2007) guidelines, with at least 2 years of symptoms; a positive skin prick test, i.e., a wheal at least 5 mm in diameter (Dreborg, 1989), with a 5-grass mix and with a *P. pratense* extract from Stallergenes (Antony, France).

Subjects meeting at least one of the following conditions were excluded from the trial: administration of subcutaneous or sublingual immunotherapy in the 3 years prior to the start of the present study; treatment with systemic steroids, antihistamines or immunosuppressors; severe psychological disorders or systemic disease; contra-indications for immunotherapy listed by the Position Paper on allergen immunotherapy (Bousquet et al., 1998). Given that all study procedures were part of the participants' standard diagnosis and care prior to potential initiation of specific immunotherapy, approval by an independent ethics committee was not required. All patients or their legal tutors gave a written consent for publication of the data.

Before beginning the study, PS software (<http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize>) was used to define the sample size, and calculate the minimum number of subjects needed. The calculation was based on a coefficient of variability of below 15% in the UNICAP100 RAST-inhibition Phadia protocol (as determined in our laboratory) and mean inhibition rates for all the allergens tested – approx. 0.35 for the *Phleum* extract (p_1) and 0.71 for the 5-grass mixture (p_2). The results gave 30 as the minimum number of subjects needed to detect a statistically significant difference (with an alpha risk of 5% and a power of 80%) between the two extract preparations.

The study featured a single visit (visit 1), where inclusion/exclusion criteria were evaluated in order to decide whether the blood sample was to be included in the study. During this visit, the patient and his/her parents or tutor were informed on the study's goals and procedures and written signed informed consent and a record of the patient's medical history were obtained. There was a clinical examination and the study inclusion/exclusion criteria were evaluated. Skin prick tests (see below) were performed on the enrolled patients and a 10 ml venous blood sample was taken for the required 5 ml of serum (the UNICAP RAST-inhibition protocol used required a total of 4.5 ml of serum). Serum samples were prepared immediately and stored at -20°C until the time of the assay.

Allergen Preparations and Skin Prick Tests

Allergen preparations at 300 Index of reactivity (IR) concentration of *Anthoxanthum odoratum* (G1), *Cynodon dactylon* (G2), *Dactylis glomerata* (G3) *Lolium perenne* (G5) *Phleum pratense* (G6), and *Poa pratensis* (G8) for skin prick tests were obtained from Stallergenes (Antony, France) and were used according to the manufacturer's instructions. A negative control (with glycerol-saline

solution) and a positive control (histamine hydrochloride 10 mg/ml) were also performed. The prick test was carried out on the forearm, with the skin being lanced with drops of the respective allergens and control solutions. After 20 minutes, wheal diameters were traced accurately onto clear plastic adhesive tape and subsequently quantified. A skin test was considered as positive when the allergen wheal diameter was greater than 5 mm and/or 50% of the positive control's wheal diameter (Dreborg, 1989).

Allergen-specific IgE Assays

RAST-inhibition was performed in solid phase using both Stallergens 300 IR allergens bound to paper disks with the corresponding Phadia (Uppsala, Sweden) allergens as control. The RAST-inhibition method with the standard UNICAP 100 RAST-inhibition from Phadia requires 4 data points at two-fold increasing extract dilutions in order to obtain the linear regression of the RAST-inhibition, from which the percentage of RAST-inhibition of each tested allergen is obtained. The 0% inhibition point was obtained by using serum sample and saline buffer 1:1 RAST test incubation. The 100% inhibition points for each allergen (G1, G2, G6, and G8) were measured by using the same allergen (both the Stallergens 300 IR extracts and the corresponding Phadia allergen). The two tested extracts (5-grass Mix and *Phleum pratense* 300 IR) were in liquid suspension with serum sample 1:1, 1:10, 1:100, and 1:1000. The percentage inhibition produced by the test (patient serum) sample is calculated as follows:

$$\frac{[\text{Result (0\% inhibition)} - \text{Result (inhibition by test sample)}]}{[\text{Result (0\% inhibition)} - \text{Result (100\% inhibition)}]} \times 100$$

All tests of a same patient's serum were performed in the same moment. Serum samples were thawed immediately prior to testing. For each patient, the RAST value with buffer saline solution (0% RAST-inhibition) and the respective RAST-inhibition values for *Phleum* and 5-grass Mix extracts were measured. The grass pollen extracts used to determine inhibition of specific IgE binding were (i) a 300 IR/ml 5-grass mix (*D. glomerata*, *P. pratensis*, *L. perenne*, *A. odoratum* and *P. pratense*) and (ii) a 300 IR/ml preparation of *P. pratense* pollen extract alone. All assays were performed in duplicate.

Statistical Analysis

Statistical analysis was carried out with software SPSS 13.0 for Windows (SPSS Inc., Chicago IL, USA). For each patient and each allergen, the differences in percent inhibition between the 5-grass extract and the *Phleum* extract were evaluated using a Wilcoxon test for paired data. These results were considered as "related samples", as they were produced from the same

blood sample and at essentially the same time. A non-parametric test was chosen in view of the low number of subjects studied and to minimize any error related to a possible non-normal data distribution.

RESULTS

A total of 34 patients (19 males, 15 females) were included in the study. The mean age was 10.7 years (range 7–14 years). Twenty-one patients had allergic rhinitis only and 11 had rhinitis and asthma. Regarding the individual allergen skin prick test, 31 patients were positive to *Anthoxanthum odoratum* (G1), 31 to *Cynodon dactylon* (G2), 34 to *Phleum pratense*, 30 to *Poa pratensis* (G8), 24 to *Dactylis glomerata* (G3), and 23 to *Lolium perenne* (G5). Table 1 shows the individual results for each grass. RAST-inhibition was performed only on patients with adequate specific IgE levels (at least 12.5 KU/L). i.e., on 29 patients with *Anthoxanthum*

Table 1: Results to skin prick test with each grass.

Patient no.	A. odoratum	C. dactylon	P. pratense	P. pratensis	D. glomerata	L. perenne
1	Negative	Negative	Positive	Negative	Negative	Positive
2	Positive	Positive	Positive	Positive	Positive	Positive
3	Positive	Positive	Positive	Positive	Negative	Positive
4	Positive	Positive	Positive	Positive	Positive	Negative
5	Positive	Positive	Positive	Positive	Negative	Negative
6	Positive	Positive	Positive	Positive	Positive	Positive
7	Positive	Positive	Positive	Negative	Negative	Positive
8	Positive	Positive	Positive	Positive	Positive	Negative
9	Positive	Positive	Positive	Positive	Positive	Positive
10	Positive	Positive	Positive	Positive	Positive	Positive
11	Positive	Positive	Positive	Positive	Positive	Positive
12	Positive	Negative	Positive	Positive	Positive	Negative
13	Positive	Positive	Positive	Positive	Negative	Positive
14	Positive	Positive	Positive	Positive	Positive	Positive
15	Positive	Positive	Positive	Positive	Negative	Negative
16	Positive	Positive	Positive	Positive	Positive	Positive
17	Positive	Positive	Positive	Positive	Positive	Positive
18	Negative	Positive	Positive	Positive	Positive	Negative
19	Negative	Positive	Positive	Positive	Negative	Negative
20	Positive	Positive	Positive	Positive	Positive	Positive
21	Positive	Positive	Positive	Positive	Negative	Positive
22	Positive	Positive	Positive	Positive	Positive	Positive
23	Positive	Negative	Positive	Positive	Positive	Negative
24	Positive	Positive	Positive	Positive	Positive	Positive
25	Positive	Positive	Positive	Positive	Negative	Negative
26	Positive	Positive	Positive	Positive	Positive	Positive
27	Positive	Positive	Positive	Positive	Positive	Positive
28	Positive	Positive	Positive	Positive	Positive	Positive
29	Positive	Positive	Positive	Positive	Positive	Positive
30	Positive	Positive	Positive	Positive	Positive	Positive
31	Positive	Positive	Positive	Negative	Positive	Negative
32	Positive	Positive	Positive	Negative	Negative	Negative
33	Positive	Positive	Positive	Positive	Positive	Positive
34	Positive	Positive	Positive	Positive	Positive	Positive

Table 2: Mean $\pm$ standard error (range) % RAST inhibition by a 5-grass mix and a *Phleum* extract for 4 grass pollen allergens in the sera of 32 immunotherapy-naïve, paediatric patients with allergic rhinitis and/or asthma.

Allergen	% RAST inhibition by the 5-grass mix	% RAST inhibition by the <i>Phleum pratense</i> extract	Statistical difference between the 2 sets of results
G1 <i>Anthoxanthum odoratum</i>	89.4 $\pm$ 1.06 % (80.7–99.5)	79.5 $\pm$ 1.65 % (63.4–96)	$p < 0.0001$
G8 <i>Poa pratensis</i>	82.7 $\pm$ 1.43 % (64.0–99.3)	68.2 $\pm$ 3.43 % (24.4–90)	$p < 0.0001$
G6 <i>Phleum pratense</i>	87.3 $\pm$ 1.09 % (73.3–99)	81.6 $\pm$ 1.24 % (68–95.4)	$p = \text{NS}$ (0.231)
G2 <i>Cynodon dactylon</i>	60.0 $\pm$ 4.07 % (10.2–85)	57.3 $\pm$ 5.09 % (2.3–95)	$p = \text{NS}$ (0.097)

odoratum (G1), 29 with *Cynodon dactylon* (G2), 24 with *Phleum pratense* (G6), and 24 with *Poa pratensis* (G8). RAST-inhibition was not performed with *Dactylis glomerata* (G3), nor with *Lolium perenne* (G5) because only a few patients (12 and 11, respectively) had high specific IgE levels (data not reported).

For each positive grass pollen, each subject was evaluated with the RAST-inhibition method in order to test the IgE binding of *Phleum* and 5-grass mix extracts. RAST-inhibition performed by Stallergenes 300 IR and the corresponding Phadia allergens showed similar results, thus only the former were used for statistical analysis. Table 2 shows the results of RAST inhibition by the 5-grass Mix and the *Phleum pratense* extract against the single 4 grass pollen allergens. The 300 IR 5-grass Mix extract (containing G1, G3, G5, G6, and G8) showed a significantly higher binding affinity compared with the 300 IR *Phleum pratense* extract for *Anthoxanthum odoratum* and *Poa pratensis*, while the two extracts for immunotherapy showed similar binding affinity for *Cynodon dactylon* and *Phleum pratense*. Both extracts showed a reduced binding affinity against the non-*Pooidea* grass *Cynodon dactylon*. High levels of RAST-inhibition were detected in 29/29 patients with the 5-grass mix and in 21/29 with *Phleum pratense* extract using *Anthoxanthum odoratum*, in 20/24 patients with a 5-grass mix and in 14/24 with *Phleum pratense* using *Poa pratensis* extract. This is illustrated in Figure 1.

DISCUSSION

In people sensitized to grass pollen, the allergic symptoms of rhinitis and asthma are caused by the contact between the allergens contained in such pollen plants and the specific IgE antibodies they produce. To date, more than 150 allergens from 52 grass species are known and classified in 13 groups according to similar physicochemical and immunologic properties

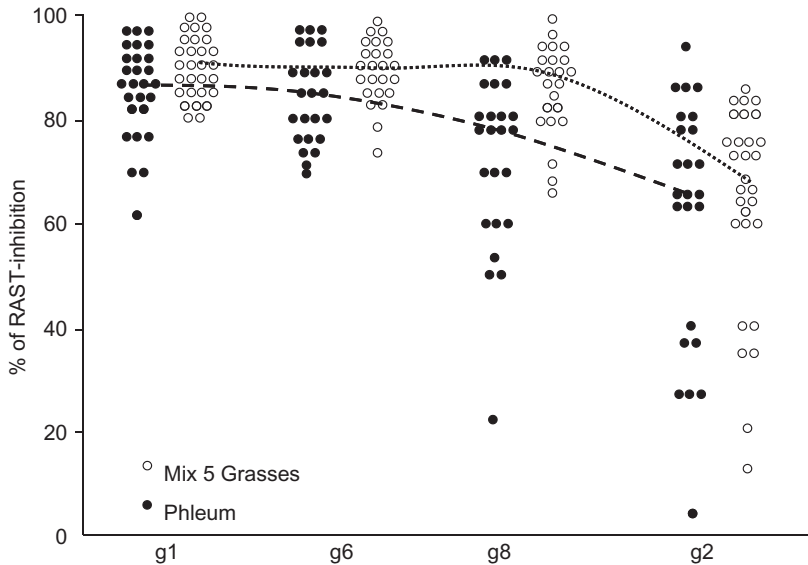


Figure 1: Results of RAST-inhibition of Mix 5 grasses vs. *Phleum pratense* in sera from grass-allergic patients.

(Andersson et al., 2003; Hrabina et al., 2008). Only four of these groups – 4, 7, 11 and 12 – are not grass-specific. Considering the classification of allergens as major or minor, depending on their recognition by IgE antibodies from approximately 50% of sensitised patients, Group 1 and Group 5 contain the major grass pollen allergens. Group 1 allergens are acidic glycoproteins, with an m.w. of 31-35 kDa, and have high homology (about 90% of the amino acid sequence) (Duffort et al., 2008). Group 5 allergens are proteins showing ribonuclease activity, occurring in two non-glycosylated isoforms, with an m.w. of 27-33 kDa, which have a lesser homology due to a 25-30% divergence in amino acid sequence (Hrabina et al., 2008). Due to the complex allergen repertoire of grass pollen, the pattern of IgE response of sensitised individuals is largely variable, especially concerning the recognition of the different epitopes expressed in the various allergens.

The most appropriate approach to investigate the matter is the RAST-inhibition technique, which evaluates *in vitro* the degree of cross-reactivity and affinity of specific IgE from allergic patients to different allergen extracts (Gleich et al., 1976). By using RAST-inhibition, van Ree and co-workers demonstrated significant quantitative and qualitative differences in the IgE from sera of grass-allergic patients to both Group 1 and Group 5 allergens in eight grass species (van Ree et al., 1992).

In the present study the comparison regarded a 5-grass mix extract versus a *Phleum pratense* extract, as this is the species believed to have the most representative allergen grass profile. A significantly higher ability to bind IgE

from grass-allergic patients was demonstrated by the 5-grass extract. Remarkably, when *Phleum pratense* alone was used in solid phase for RAST-inhibition, the 5-grass extract showed a similar binding affinity in liquid phase. The lack of differences in RAST-inhibition against *Cynodon dactylon* of both 5-grass and *Phleum* extracts is probably due to the fact that *Cynodon dactylon* belongs to the Panicoideae subfamily, while *Phleum pratense* and all grass extracts included in the 5-Mix belong to the Pooideae subfamily, thus accounting for a similar and low cross-reactivity *vs Cynodon*.

These observations could have important consequences when specific immunotherapy is considered to treat grass-pollen allergy. This treatment is aimed at modifying the immunologic response to the administered allergen(s) by eliciting changes in the Th lymphocyte subpopulations, in their cytokine pattern, and in the synthesis of allergen-specific IgG instead of IgE antibodies (Akdis et al., 2007). Until now, controlled trials on SCIT or SLIT used mix-grass extracts as well as single extracts of grasses such as *Dactylis glomerata*, *Phleum pratense*, or *Lolium perenne*, with no apparent differences in clinical efficacy.

However, one must consider that the clinical results of such trials are reported as mean scores of symptoms and use of rescue medications, and data from single patients are not considered. It therefore remains unexplored whether a lack of efficacy in a given patient is caused by incomplete interaction between the administered allergen extract and the patient's IgE profile, with unchanged reactivity to allergens or epitopes that are not present in the administered extract. This is the clinical equivalent of a RAST-inhibition performed with pooled sera instead of sera from single patients, which could mask the individual variability (van Ree et al., 1992). Also concerning strictly related allergen sources, a difference in allergen profile could result in inefficacy of immunotherapy. This is true for giant and short ragweed as it has been demonstrated that ragweed pollen-allergic patients, not responding to SCIT by a giant ragweed extract, had an excellent outcome when they were shifted to short ragweed immunotherapy. The immunologic background was the inability to inhibit *in vitro* the IgE reactivity to some short ragweed allergens by pre-absorption of sera with giant ragweed extract (Asero et al., 2005).

A further factor to consider is the different pattern of sensitisation in populations from different geographical areas, which is influenced by genetic background as well as by dissimilar allergen exposure. A recent study on grass allergic patients mostly from northern Europe found, with different *in vitro* techniques, that immunotherapy with *Phleum pratense* extract resulted in a high degree of correlation of IgE and IgG4 recognition to extracts of other grass species (Hejl et al., 2009).

In recent years, the model of patient-tailored immunotherapy has been advocated, based on the increasing availability of diagnostic techniques able to

detect all epitope specificities for a given allergen in single patients, thus indicating the optimal content of the allergen preparation for immunotherapy (Cramer et al., 2006). However, it is hard to foresee when such an ideal approach will be really applicable. Currently, using a 5-grass mix pollen extract, as has already been suggested on a theoretical basis as preferable to single grass extracts (Moingeon et al., 2008), seems to be the optimal choice when applying specific immunotherapy in grass pollen-allergic subjects from the Mediterranean area.

Declaration of interest: F. Marcucci and C. Incorvaia are scientific consultants for Stallergenes Italia, the other authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

- Abramson, M.J., Puy, R.M., Weiner, J.M. (2003). Allergen immunotherapy for asthma. *Cochrane Database Syst Rev.* (4):CD001186.
- Akdis, M., Akdis, C.A. (2007). Mechanisms of allergen-specific immunotherapy. *J. Allergy Clin. Immunol.* 119:78–7–91.
- Andersson, K., Lidholm, J. (2003). Characteristics and immunobiology of grass pollen allergens. *Int. Arch. Allergy Immunol.* 130:87–107.
- Asero, R., Weber, B., Mistrello, G., Amato, S., Madonini, E., Cromwell, O. (2005). Giant ragweed specific immunotherapy is not effective in a proportion of patients sensitized to short ragweed: analysis of the allergenic differences between short and giant ragweed. *J. Allergy Clin. Immunol.* 116:1036–1041.
- Bousquet, J., Clark, T.J., Hurd, S., Khaltaev, N., Lenfant, C., O'Byrne, P., Sheffer, A. (2007). GINA guidelines on asthma and beyond. *Allergy*. 62:102–112.
- Bousquet, J., Lockey, R., Malling, H.J. (1998). Allergen immunotherapy: therapeutic vaccines for allergic diseases. *Allergy* 53(suppl. 54):4–30.
- Bousquet, J., Lockey, R., Malling, H.J. (1998). World Health Organization Position Paper. Allergen immunotherapy: therapeutic vaccines for allergic diseases. *Allergy* 53 (suppl. 52):3–15.
- Bousquet, J., Van Cauwenberge, P., Khaltaev, N.; Aria Workshop Group; World Health Organization. (2001). Allergic rhinitis and its impact on asthma. *J. Allergy Clin. Immunol.* 108(suppl. 5):147–334.
- Calderon, M., Alves, B., Jacobson, M., Hurvitz, B., Sheikh, A., Durham, S. (2007). Allergen injection immunotherapy for seasonal allergic rhinitis. *Cochrane Database Syst Rev.* (1):CD001936.
- Cramer, R., Rhyner, C. (2006). Novel vaccines and adjuvants for allergen-specific immunotherapy. *Curr. Opin. Immunol.* 18:761–768.
- D'Amato, G., Cecchi, L., Bovini, S., Nunes, C., Annesi-Maesano, I., Behrendt, H., Liccardi, G., Popov, T., van Cauwenberge, P. (2007). Allergenic pollen and pollen allergy in Europe. *Allergy* 62:976–990.
- Dreborg, S. (ed). (1989). Skin tests used in type I allergy testing. Position paper of the European Academy of Allergology and Clinical Immunology. *Allergy* 44 (suppl 10):1–59.

- Duffort, O., Quintana, J., Ipsen, H., Barber, D., Polo, F. (2008). Antigenic similarity among group 1 allergens from grasses and quantitation ELISA using monoclonal antibodies to Phl p 1. *Int. Arch. Allergy Immunol.* 145:283–290.
- Erbas, B., Chang, J.H., Dharmage, S., Ong, E.K., Hyndman, R., Newbiggin, E., Abramson, M. (2007). Do levels of airborne grass pollen influence asthma hospital admissions? *Clin. Exp. Allergy* 37:1641–1647.
- Frankland, A.W., Augustin, R. (1954). Prophylaxis of summer hay-fever and asthma: a controlled trial comparing crude grass-pollen extracts with the isolated main protein component. *Lancet* 266:1055–1057.
- Freye, H.B. (2008) The role of pollens in allergy. *Med. Health R. I* 91:174–175.
- Gleich, G.J., Leiferman, K.M., Jones, R.T., Hooton, M.L., Baer, H. (1976). Analysis of the potency of extracts of June grass pollen by their inhibitory capacities in the radioallergosorbent test. *J. Allergy Clin. Immunol.* 58:31–38.
- Hejl, C., Wurtzen, P.A., Kleine-Tebbe, J., Johansen, N., Broge, L., Ipsen, H. (2009). Phleum pratense alone is sufficient for allergen-specific immunotherapy against allergy to Pooideae grass pollens. *Clin. Exp. Allergy* 39:752–759.
- Hrabina, M., Peltre, G., van Ree, R., Moingeon, P. (2008). Grass pollen allergens. *Clin. Exp. Allergy Rev.* 8:7–11.
- Moingeon, P., Hrabina, M., Bergmann, K.C., Jaeger, S., Frati, F., Bordas, V., Peltre, G. (2008). Specific immunotherapy for common grass pollen allergies: Pertinence of a five grass pollen vaccine. *Int. Arch. Allergy Immunol.* 146:338–342.
- Noon, L. (1991). Prophylactic inoculation against hay fever. *Lancet* 1:1572–1573.
- Penagos, M., Passalacqua, G., Compalati, E., et al. (2008) Meta-analysis of the efficacy of sublingual immunotherapy in the treatment of allergic asthma in pediatric patients, 3 to 18 years of age. *Chest* 133:599–609.
- van Ree, R., Driessen, M.N., van Leeuwen, W.A., Stapel, S.O., Aalberse, R.C. (1992). Variability of cross-reactivity of IgE antibodies to group I and V allergens in eight grass pollen species. *Clin. Exp. Allergy* 22:611–617.
- Wilson, D.R., Torres-Lima, M., Durham, S. (2005) Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. *Allergy* 60:4–12.

Copyright of Immunological Investigations is the property of Taylor & Francis Ltd and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.