

IgE Profiles of Bermuda Grass Pollen Sensitised Patients Evaluated by *Phleum Pratense* Allergens Phl P 1, 2, 4, 5, 6, 7, 11, 12

Renato E Rossi¹, Giorgio Monasterolo², Paolo Prina³, Giuseppe Coco², Daniela Operti² and Lucilla Rossi⁴

ABSTRACT

Background: Despite the difference in geographical dominance of certain grasses, a high degree of allergenic similarity or cross-reactivity between Bermuda grass pollen (BGP) and timothy grass pollen (TGP) has been previously demonstrated.

The aim of the present study was to ascertain the sensitisation to TGP in 411 patients known for their reactivity to BGP extracts by analysing their reactivity to crude timothy pollen extract and timothy pollen purified allergens, establishing their specific IgE-profiles.

Methods: Using the immunoenzymatic CAP method we evaluated IgE-specific antibodies for BGP- and TGP-extracts and the timothy recombinant (r) and natural (n) allergens rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5, rPhl p 6, rPhl p 7, rPhl p 11, and rPhl p 12.

Results: BGP-IgE positive patients (median = 8.0 kUA/l, 2.8–22.2 kUA/l 25th–75th percentile) simultaneously had IgE positive results for TGP (100% of subjects) (median = 48.9 kUA/l, 19.8– > 100 kUA/l 25th–75th percentile) and high prevalence of sensitization to 6/8 *Phleum pratense* allergens (Phl p 1, 2, 4, 5, 6, 11, markers of genuine sensitisation to TGP) other than profilin and calcium binding protein. More than 72% of BGP allergic patients were co-sensitised to rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5, rPhl p 6. A decrease of total and specific IgE with patients' age was observed.

Conclusions: Our data show that all BGP-allergic patients simultaneously exhibit higher IgE antibody levels to recombinant and natural *P. pratense* allergens as well as to crude TGP extract. This suggests that when choosing an immunotherapeutic regimen for BGP-sensitised patients (after establishing their IgE profile via purified TGP-allergens), subcutaneous or sublingual TGP-extract vaccines in appropriate doses, in order to influence T epitope specificity, might be beneficial. Though extremely uncommon, in cases where a patient is exclusively BGP allergen-sensitised, BGP-extract therapy is the appropriate therapeutic response.

KEY WORDS

Bermuda grass, recombinant allergens, timothy grass

INTRODUCTION

Grass pollens are a common cause of respiratory allergy in the industrialized world but a limited number of grass species are recognized as major causes of respiratory symptoms. Those affected by grass pollen sensitization are believed to account for as much as

20% of the general population and up to 40% of the atopic population.

Grasses are the group of plants studied in most detail, which are confined to a single taxonomic family, Poaceae. Up to now 71 allergens from 15 species distributed among 10 protein families have been isolated and the most relevant grass pollen allergens have al-

¹Allergy Unit, National Health Service, Rete di Allergologia, Regione Piemonte, ²Dipartimento di Analisi Chimico-Cliniche e Microbiologia, Ospedali di Fossano e Savigliano, ³Department of Otorhinolaryngology, Ospedale SS Annunziata, Savigliano and ⁴Department of General Pathology, University of Genoa, Italy.

Correspondence: Renato E Rossi, Rete di Allergologia Regione Piemonte, 14 Via Carlo Boggio, Cuneo 12100, Italy.

Email: immunoway@tin.it

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Table 1 Clinical picture of 411 patients with specific IgE to *Cynodon dactylon* and *Phleum pratense* extracts, arranged by age decade

Symptoms	<11 yrs (N = 87)	>10 <21 yrs (N = 123)	>20 <31 yrs (N = 93)	>30 <41 yrs (N = 63)	>40 <51 yrs (N = 22)	>50 <61 yrs (N = 14)	>60 <71 yrs (N = 9)
Rhinoconjunctivitis	65.5% N = 57	69.9% N = 86	74.1% N = 69	71.4% N = 45	72.7% N = 16	78.5% N = 11	77.7 % (N = 7)
Asthma	34.5% N = 30	29.1% N = 37	25.8% N = 24	28.6% N = 18	27.3% N = 6	21.5% N = 4	28.3% N = 2
Oral Allergy Syndrome	4.6% (N = 4)	8.1% (N = 10)	8.6% (N = 8)	9.5% (N = 6)	13.6% (N = 3)	7.1% (N = 1)	11.1% (N = 1)

ready been identified.¹

In allergy diagnosis and allergen extract-based immunotherapy, pollen extracts from about 20 grasses are currently employed. Andersson and Lidholm reported that, *Phleum pratense* and twelve other grasses (*Anthoxatum*, *Dactylis*, *Festuca*, *Lolium*, *Poa*, *Agrostis*, *Bromus*, *Secale*, *Holcus*, *Avena*, *Triticum*, *Alopecurus*) contain homologous allergen components.² Their study also showed that four species (*Cynodon dactylon*, *Phragmites communis*, *Sorghum halepense* and *Paspalum notatum*), stood out as different from the corresponding data for *P. pratense*, but when the two groups were then plotted against each other, two species (*S. halepense* and *P. notatum*) were found to be similar.

Pure natural or recombinant allergens are currently used in diagnostic tests.^{3,4}

Bermuda grass (*Cynodon dactylon*) pollen (BGP) is one of the most common causes of airway allergic disease, and has been shown to contain over 12 allergenic proteins (i.e. Cyn d 1, 2, 4, 5, 6, 7, 10, 11, 12, 13, 15, 22, 23) on 1-D immunoglobulin E (IgE) immunoblots.^{5,6} However, of these allergens only a few have been identified and characterized. Recently, a pathogenesis-related protein 1, Cyn d 24, was identified from BGP.⁷ Despite the difference in geographical dominance of certain grasses, there are extensive studies^{2,5,8} highlighting a high degree of allergenic similarity or cross-reactivity between BGP and timothy grass pollen (TGP). In the present study, we selected a large number of patients already known to be sensitized to BGP and their IgE profiles were evaluated with a panel of TGP allergen molecules.

METHODS

STUDY POPULATION

This retrospective study used the sera of 411 consecutive patients, mean age 22.7 years, range 2–67 years, with specific IgE towards BGP extract >0.7 kUA/l. All subjects lived in or near the city of Cuneo in north-western Italy, and therefore presumably were exposed to the same allergen sources. The patients had a history of seasonal (April–July) pollen allergy with symptoms of rhinitis ($n = 273$) and/or asthma ($n = 138$). A fraction of them ($n = 33$) suffered

from oral allergy syndrome after eating fruit (OAS). None of the patients had received any form of specific immunotherapy to pollen extracts, although some received topical steroids, antihistamines and antileukotrienes. The patients' clinical picture, arranged by age decade is shown in Table 1.

DETERMINATION OF ALLERGEN-SPECIFIC IGE ANTIBODIES

IgE-specific antibodies for BGP-, TGP-extracts and the timothy recombinant (r) and natural (n) allergens rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5, rPhl p 6, rPhl p 7, rPhl p 11, and rPhl p 12 were evaluated using the immunoenzymatic CAP method (Phadia, Uppsala, Sweden) according to the manufacturer's instructions. The results were expressed in classes of positivity from 0 to 6, where class 0 corresponds to <0.35 kUA/l; class 1 0.35–0.7 kUA/l; class 2 0.7–3.5 kUA/l; class 3 3.5–17.5 kUA/l; class 4 17.5–50 kUA/l; class 5 50–100 kUA/l; and class 6 >100 kUA/l.

STATISTICAL ANALYSIS

Nonparametric statistical methods were used. The Mann-Whitney *U* test was used for comparison of the two unpaired groups. The correlation index (*r*) was determined with the Spearman rank correlation coefficient matrix. Patients were also evaluated with a mixed design analysis of variance (ANOVA). $P < 0.05$ was considered to indicate significant differences.

RESULTS

Patients in our study were selected for their known reactivity to BGP extracts and were further characterized by their reactivity to crude timothy pollen extract; in fact 100% of our subjects tested positive to TGB. Moreover, those patients who had detectable serum IgE levels towards BGP (median = 8.0 kUA/l, 2.8–22.2 kUA/l 25th–75th percentile) simultaneously had higher TGP IgE levels (median = 48.9 kUA/l, 19.8– > 100 kUA/l 25th–75th percentile). In addition, BGP positive patients had a high prevalence of sensitization to 6/8 *P. pratense* allergens other than profilin and calcium binding protein (more than 68%) (Fig. 1). Furthermore, in BGP/TGP-double positive patients the values obtained through the sum of the individual

Specific IgE to Grass Pollen Allergens

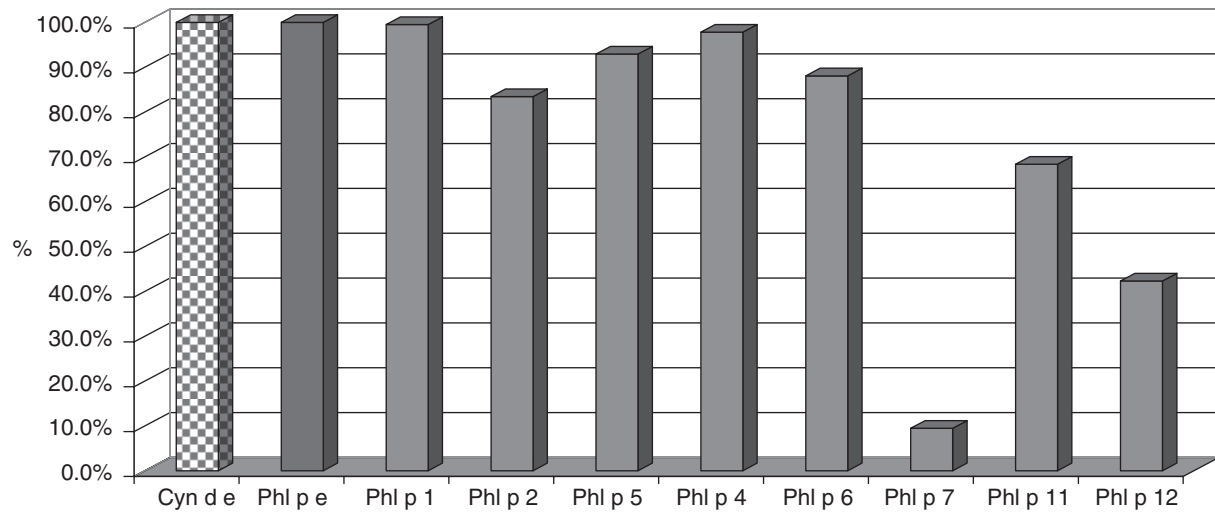


Fig. 1 Frequency of sensitisation to timothy allergens in 411 patients sensitised to Bermuda grass pollen (*Cynodon dactylon*).

Cyn d e, *Cynodon dactylon* extract; Phl p e, *Phleum pratense* extract.

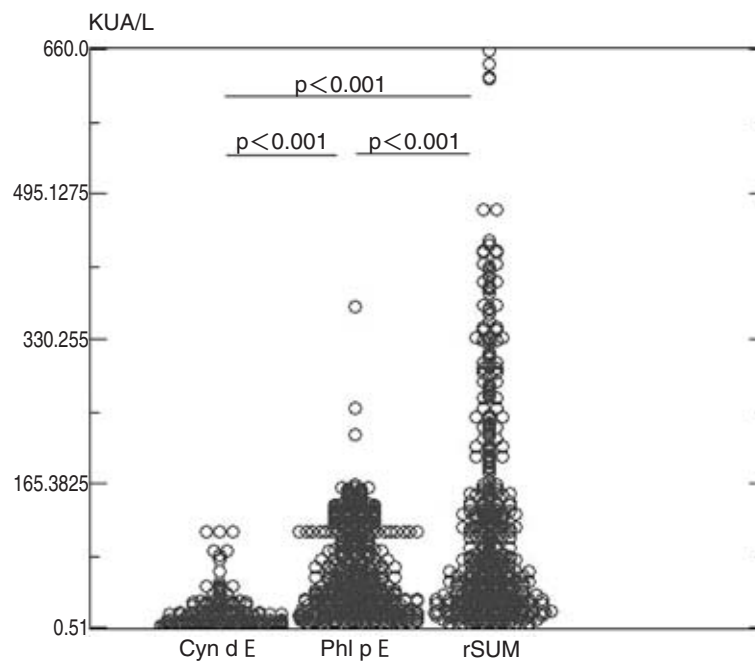


Fig. 2 Median value of specific IgE to *Cynodon dactylon* extract (Cyn d E)(median = 6.68 kUA/l, 2.8–18.2, 25th–75th percentile), *Phleum pratense* extract (Phl p E)(median = 42.4 kUA/l, 19–84.8, 25th–75th percentile), and the sum of single values of specific IgE towards rPhl p 1, rPhl p 2, nPhl 4, rPhl p 5, rPhl p 6, rPhl p 7, rPhl p 11, rPhl p 12 (rSUM)(median = 63.9 kUA/l, 27.5 – 129.6, 25th – 75th percentile), in 411 Bermuda grass allergic patients.

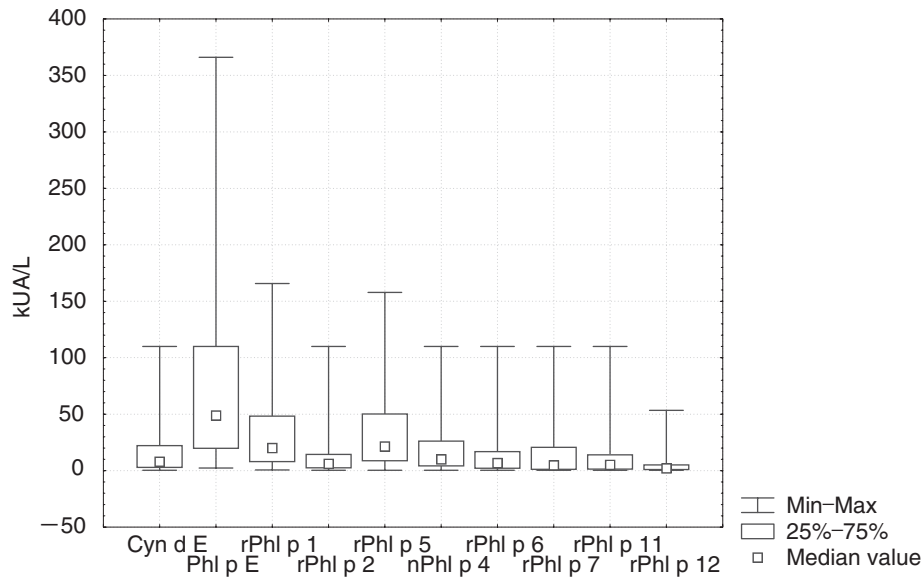


Fig. 3 Median value of specific IgE to *Cynodon dactylon* extract (Cyn d E), *Phleum pratense* extract Phl p E) and single values of specific IgE towards rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5, rPhl p 6, rPhl p 7, rPhl p 11, rPhl p 12 in 411 Bermuda grass allergic patients.

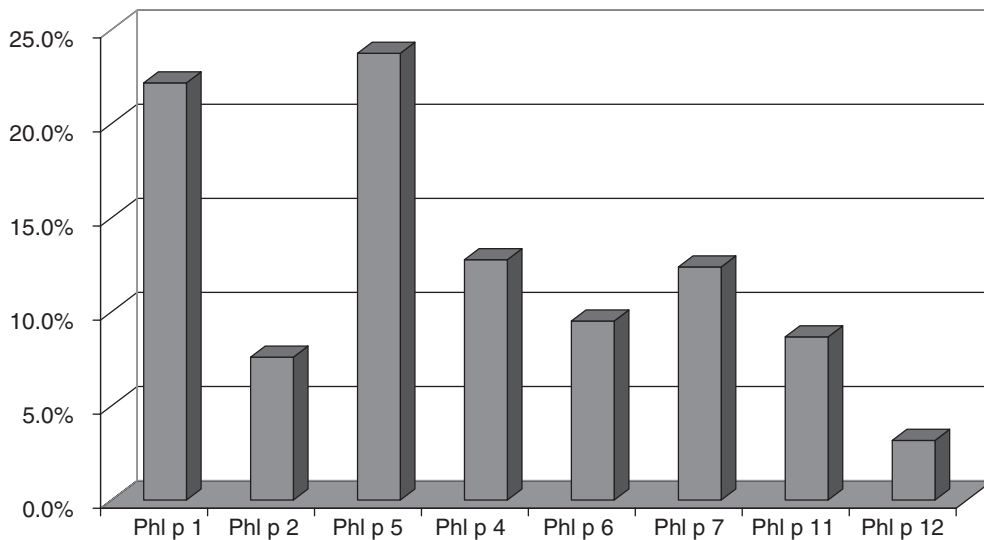


Fig. 4 Eight *Phleum pratense* allergens which contribute to the total sum of its IgE value, shown here in their percentage representations.

TGP allergen IgE levels (Phl p 1, 2, 4, 5, 6, 7, 11, 12) were almost twice higher than TGP-IgE levels and approximately 8 times higher than BGP IgE levels (Fig. 2). Serum specific IgE levels of the 8 TGP allergens are shown in Figure 3. Variations of total and specific serum IgE concentrations were observed depending on patients' ages (Figs. 4–7) with the exception of Phl p 7. Comparisons of different variables as viewed across 10-year age increments are shown in Table 2

and Figures 4–6.

Of 411 BGP/TGP-positive subjects 298 (72.50%) were co-sensitised to rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5, rPhl p 6. The two main patient IgE profiles with relative prevalence in different age decades are depicted in Table 3. The sum of the specific IgE levels to *P. pratense* allergens within the study population is to a certain extent determined by Phl p 1 and Phl p 5 IgE values as is shown in Figure 4.

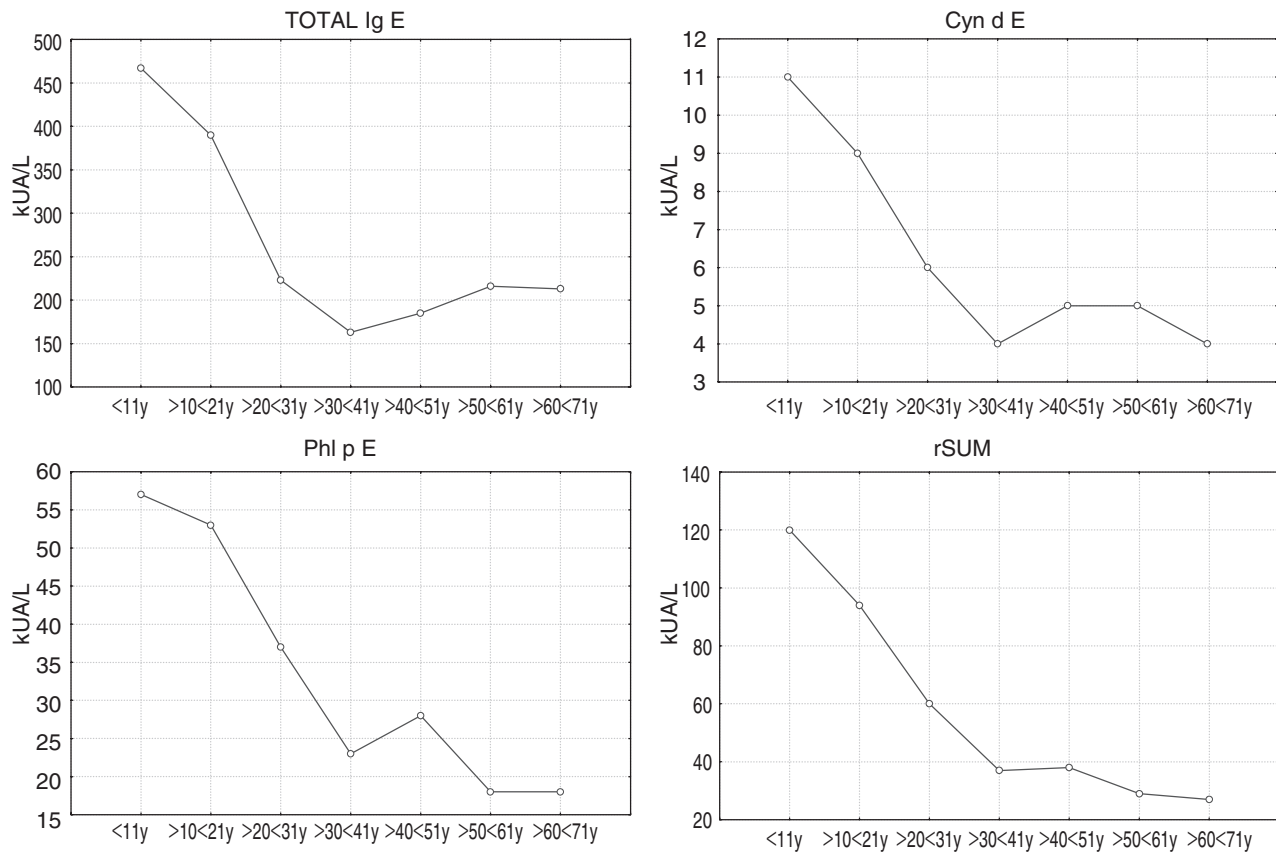


Fig. 5 Total IgE; *Cynodon dactylon*-extract specific (Cyn d E) IgE; *Phleum pratense*-extract specific (Phl p E) IgE; sum of Phl p (1, 2, 4, 5, 6, 7, 11, 12)-IgE value variation, in 411 Bermuda grass pollen allergic patients, shown by age (y = years).

The sum value of specific IgE directed to TPB allergens is correlated as follows: IgE, $r = 0.649781$ ($P < 0.0001$); BGP, $r = 0.844994$ ($P < 0.0001$); TGP, $r = 0.962687$ ($P < 0.0001$); patient age (inversely correlated), $r = -0.38641$ ($P < 0.0001$). Out of 33 patients suffering from OAS after eating fruits and vegetables, 28 were sensitised to profilin (the remaining 5 patients with OAS were co-sensitised to the birch pollen major allergen Bet v 1).

DISCUSSION

In this study we documented that 411 consecutive patients with BGP-extract IgE positive results simultaneously had TGP-extract IgE positive results. Despite not having performed an IgE inhibition analysis of these patients' sera (this was a retrospective study) the selection of patients on the basis of their IgE positivity to BGP revealed that these same patients had simultaneous and higher specific IgE antibodies towards TGP allergens with respect to BGP specific IgE levels. This would seem to reinforce our premise that the presence of cross-reactive allergens is commonly represented in grass species, including BGP.

Moreover, we did not find patients with IgE positive results to BGP yet TGP negative results, nor patients with higher BGP IgE than TGP IgE. Therefore, in the present observation BGP positive patients were in reality sensitised to TGP. BGP-IgE levels were comparable with TGP-IgE levels in those subjects co-sensitised to calcium binding protein (Phl p 7) and/or profilin (Phl p 12). A previous study showed that BGP may contain up to 230 proteins, about 65 of which are IgE binding proteins and some of these are either isoforms or degraded allergen products.⁵ Currently extract-based diagnosis includes both BGP- and TGP-extract (or a mixture of grasses) in standard panels of allergens employed for allergy diagnosis. This is due to the assumption that BGP allergens differ from those contained in other grasses as previously indicated.^{2,5} The consequence of co-sensitisation to further allergens, other than allergen markers of genuine sensitisation to grass species, may contribute to an increase in the sum of single allergen-IgE values. We can assume that the sum of single allergen-IgE values from a given allergen source better reflects the overall IgE binding to allergen epitopes than IgE

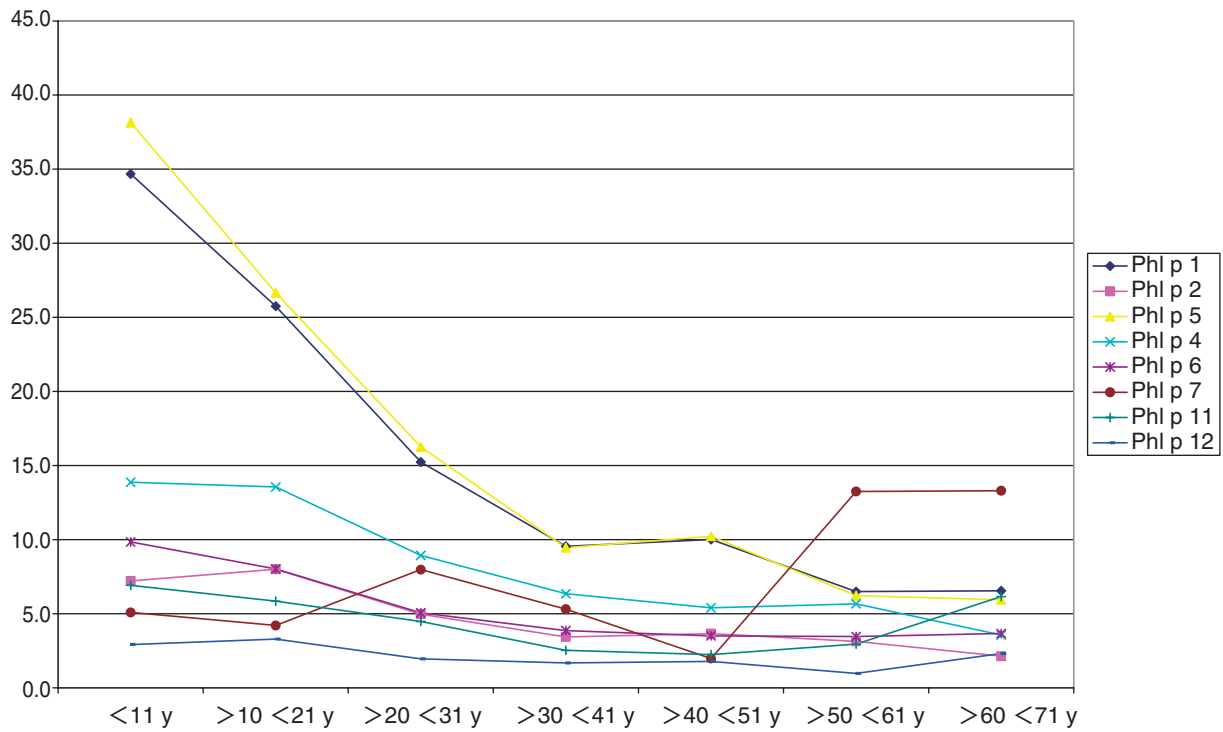


Fig. 6 Single Phl p 1, 2, 4, 5, 6, 7, 11, 12 IgE variation by age in 411 Bermuda grass pollen allergic patients.

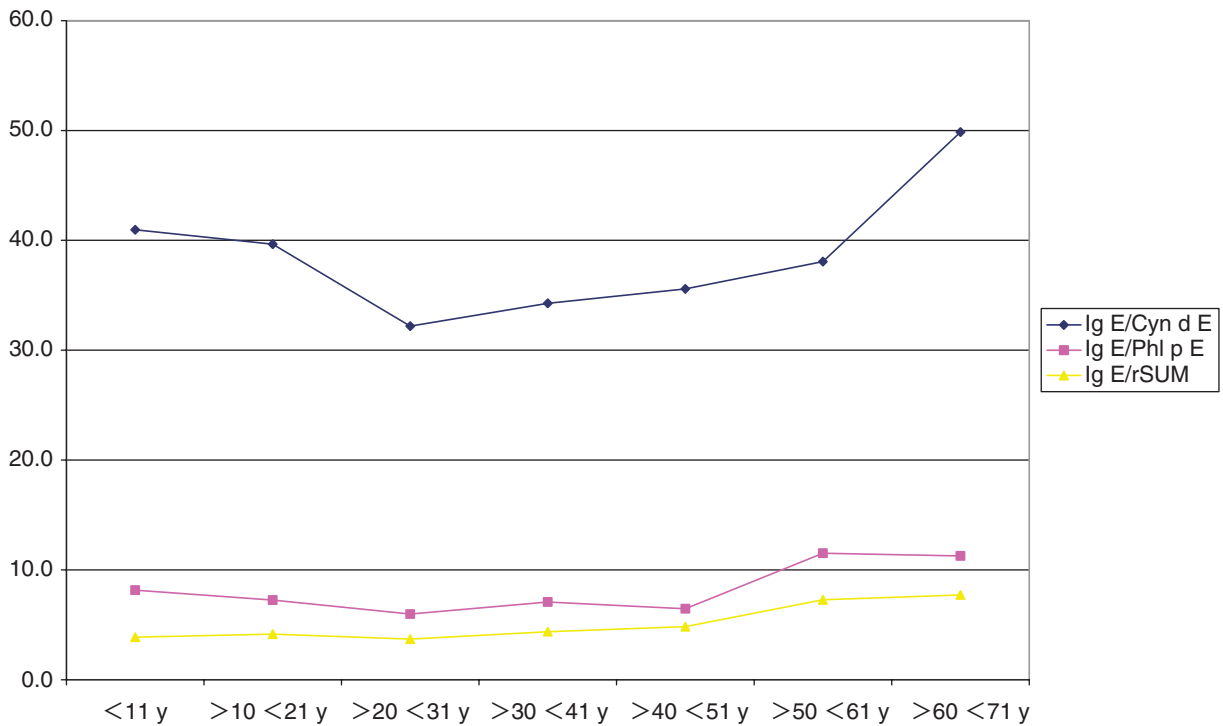


Fig. 7 Ratios for total IgE value and *Cynodon dactylon* extract (Cyn d E)-; *Phleum pratense* extract (Phl p E)-; and sum of rPhl p 1, rPhl p 2, nPhl 4, rPhl p 5, rPhl p 6, rPhl p 7, rPhl p 11, rPhl p 12 specific IgE-value in 411 Bermuda grass pollen allergic patients, ages shown by decades (y = years).

binding to crude allergen extract (with variable allergen content), as we have observed through our evaluation of the ratio between total IgE value and *P. pratense* extract IgE, and between total IgE value and the sum of *P. pratense* recombinant allergen IgE, respectively. These data seem to suggest that the patient sensitised to BGP who simultaneously has positive CAP-results towards TGP-recombinant allergens is suited to treatment with an allergen vaccine composed of a comprehensive extract of *P. pratense* containing all the relevant cross-reactive allergens to which a patient is sensitised. In our study, it is noteworthy that more than 72% of patients were co-sensitised to Phl p 1, 2, 4, 5, 6 which are contained in grass-pollen allergen-extract vaccines, as we have previously shown.^{9,10} As established in a previous report of total and specific IgE variation with age¹¹ our findings confirm the decrease of total and allergen specific IgE with age. A curious exception is the persistence of IgE antibodies toward Phl p 7 where sensitisation seems to be unaffected by age. Previously we demonstrated the lack of IgG antibodies specific for Phl p 7 in Phl p 7-IgE negative patients treated with grass pollen vaccine (only Phl p 7-IgE positive patients produced significant titres of specific Phl p 7-IgG4 antibodies after grass pollen vaccine).⁹ These findings together with the present observations sug-

gest a possible association of Phl p 7 reactivity with HLA class II phenotypes. As expected, the great majority of patients suffering from OAS were sensitised to profilin contained in many vegetables and fruits. A minority of patients were also co-sensitised to Bet v 1, cross reacting with homologous allergens contained in *Rosaceae* and *Umbrelliferae* plant foods.

It is important to remember that some T cell epitopes of BGP and TGP could be useful for immunotherapeutic strategies. It is well known that allergen specific T cell response in one individual, which is determined by T cell epitopes, depends on the intrinsic characteristics of their sequence which allow their binding to the MHC class II groove on antigen presenting cells. Homologous allergens from different allergen sources tend to display peptides which are immunologically dominant in relatively homogeneous sites of the protein, usually corresponding to the most conserved regions of the allergen. The mechanism of cross-recognition by T-cell of determinants belonging to different allergens has been described.^{12,13} Kolbe *et al.* indicated previously that immunization with minute doses of antigen leads to the production of antibodies with class-associated epitope specificities (designated epitopes G for IgG and E for IgE antibodies), and their study showed no class antibody competition. For IgG antibody production, G epitopes were immunodominant and IgG antibodies directed to E epitopes were produced only after immunization with an increased immunogen dose.¹⁴ This concept might be applied in the case of T epitopes as well, although IgG4 antibodies are shown to be dynamic molecules, undergoing Fab arm exchange *in vitro* and *in vivo*.¹⁵

In conclusion our data show that all BGP-allergic patients simultaneously exhibit higher IgE antibody levels to recombinant and natural *P. pratense* allergens as well as to crude TGP extract. This suggests that when choosing an immunotherapeutic regimen for BGP-sensitised patients (after establishing their IgE profile via purified TGP-allergens), subcutaneous or sublingual TGP-extract vaccines in appropriate doses, in order to influence T epitope specificity, might be beneficial. Though extremely uncommon, in cases where a patient is exclusively BGP allergen-sensitised, BGP-extract therapy is the appropriate therapeutic response.

Table 2 Differences among variables with age as evaluated by analysis of variance (ANOVA)

Variable	p-value
Total IgE	.007762
Specific IgE to <i>Cynodon dactylon</i> extract	.000348
Specific IgE to <i>Phleum pratense</i> extract	.000000
Sum of <i>P. pratense</i> allergen IgE values	.000000
Specific IgE to Phl p 1	.000000
Specific IgE to Phl p 2	.000378
Specific IgE to Phl p 4	.000001
Specific IgE to Phl p 5	.000000
Specific IgE to Phl p 6	.000107
Specific IgE to Phl p 7	.65625
Specific IgE to Phl p 11	.000048
Specific IgE to Phl p 12	.0321597

Table 3 Prevalence of the two most common specific IgE profiles shown by age in two sub-groups of patients sensitised to Bermuda grass living in the province of Cuneo, Italy.

Main IgE sensitisation profiles	<11 yrs	>10 <21 yrs	>20 <31 yrs	>30 <41 yrs	>40 <51 yrs	>50 <61 yrs
Phl p 1 + Phl p 2 + Phl p 4 + Phl p 5 + Phl p 6 + Phl p 7 – Phl p 11 + Phl p 12 –	31.0% N = 27	30.1% N = 37	16.1% N = 15	15.9% N = 10	13.6% N = 3	23.1% N = 3
Phl p 1 + Phl p 2 + Phl p 4 + Phl p 5 + Phl p 6 + Phl p 7 – Phl p 11 + Phl p 12 +	12.6% N = 11	28.5% N = 35	24.7% N = 23	20.6% N = 13	22.7% N = 5	7.7% N = 1

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