

How far can we simplify *in vitro* diagnostics for Fagales tree pollen allergy? A study with three whole pollen extracts and purified natural and recombinant allergens

R. VAN REE, W. A. VAN LEEUWEN, J. H. AKKERDAAS and R. C. AALBERSE

C.L.B., Sanquin Blood Supply Foundation, and the Laboratory for Experimental and Clinical Immunology, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands

Summary

Background Current diagnostic tests for Fagales tree pollen allergy are often composed of mixtures of pollen of birch, alder and hazel. Their complex composition hampers accurate standardization.

Objective The aim of this study was to investigate whether mixtures of tree pollen extracts can be replaced by a single pollen species, and whether a single pollen species can be replaced by a limited number of purified natural or recombinant major allergens.

Methods Sera ($n=1725$) were selected on ground of a general suspicion for inhalant allergy, and tested in a RAST for birch, alder and hazel pollen. Sera with >0.5 RU/mL for any of the three species were tested in a RAST for natural Bet v 1 and Bet v 2 as well as for recombinant versions of both allergens.

Results Specific IgE antibodies (>0.3 RU/mL) against birch, alder and hazel were found in 242, 298 and 292 sera, respectively. All sera with a positive RAST for alder and/or hazel and a negative RAST for birch were low-responder sera on alder and hazel, only five sera having a RAST value >1.0 (all <2.0). For all sera with a RAST >0.5 RU/mL ($n=250$), the mean of individual ratio's alder/birch and hazel/birch was 1.02 and 0.54, respectively. Of 223 of these sera, 63.2% had specific IgE against natural Bet v 1 and 63.7% against natural Bet v 2. When responses to both allergens were combined 93.7% were positive. The mean ratios Bet v 1 + 2/extract were 1.00, 1.04 and 2.11 in case of birch, alder and hazel, respectively. For 211 sera the same analysis was performed with recombinant Bet v 1 and Bet v 2. Only six sera with Bet v 1-specific IgE (all <0.5 RU/mL) were negative (<0.3 RU/mL) on recombinant Bet v 1. For Bet v 2, 77/132 sera with specific IgE to the natural allergen did not react to the recombinant version. Twelve false-negatives had RAST values >1.0 RU/mL. The mean of the individual recombinant/natural ratios was 0.98 for Bet v 1 and 0.38 for Bet v 2 ($P<0.001$). The mean ratio rBet v 1 + 2/birch was 0.75 with 17.5% false-negatives on the combination of recombinant allergens.

Conclusion Reliable *in vitro* diagnosis is possible with a single tree pollen extract (birch or alder). The same is true for purified natural Bet v 1 and Bet v 2. A combination of recombinant molecules is slightly less efficient.

Keywords: tree pollen allergy, diagnostics, purified allergens, recombinant allergens, Bet v 1, Bet v 2

Clinical and Experimental Allergy, Vol. 29, pp. 848–855. Submitted 9 July 1998; revised 22 September 1998; accepted 26 October 1998.

Introduction

Both *in vivo* and *in vitro* diagnostic tests for Fagales tree pollen allergy are often performed with mixtures of tree pollen extracts from birch, alder and hazel. The same is true for application of tree pollen extracts in immunotherapy. The rationale behind this approach is to ensure that all species-specific IgE-binding epitopes are included. It is already well known that immune-reactivity with tree pollen of the order of the Fagales is of highly cross-reactive nature [1–6]. Patients with an IgE response, limited to pollen from a single species are extremely rare. By mixing different, largely cross-reactive species, any species-specific structure will be diluted in an excess of cross-reactive epitopes. It is therefore doubtful whether this approach results in sensitive detection of (or effective desensitization against) species-specific epitopes.

In recent years, regulatory authorities have been increasing their demands with respect to standardization and quality control of allergen products for *in vivo* use. Defining the composition of mixtures of pollen extracts, each consisting of many different (glyco)proteins and polysaccharides, is an almost impossible task. The trend in allergen standardization is slowly moving from biological standardization towards the measurement of major allergens [7,8]. To facilitate accurate measurement of major allergens, mixing of different species should be avoided. Is it possible to replace the present generation of allergen products, based on mixtures of tree pollen, by products based on pollen from a single tree species? The first part of this study addresses this question from the perspective of *in vitro* diagnostics.

Simplification could even go a step further. The two most important allergens of Fagales tree pollen are the group 1 (e.g. Bet v 1) and group 2 allergens (profilins). For group 1 allergens, a frequency of recognition by tree pollen-sensitive patients of 60% to >95% was reported [9–12]. For profilins, the reported figures range from 20% to 40% [10–12]. The second part of this study addresses the question whether *in vitro* diagnostics for tree pollen allergy can be performed with a combination of purified Bet v 1 and Bet v 2. This was investigated for both natural and recombinant molecules.

Materials and methods

Sera

From sera submitted for routine *in vitro* allergy diagnostics, a panel of 1725 sera was selected on the basis of the following criteria: they were submitted for measurement of total IgE, measurement of specific IgE against the most common inhalant allergens, or both. In this way sera originated from subjects with a general suspicion of inhalant allergy. For immunoblotting, four reference sera with

known IgE reactivities were used. One serum was reactive with Bet v 1, one with Bet v 2, and one with both allergens. The fourth serum was known to recognize carbohydrate epitopes.

Tree pollen extracts

Tree pollen from the following species were extracted as described before [13]: *Betula verrucosa* (birch), *Alnus glutinosa* (alder) and *Corylus avellana* (hazel). Of each dialysed and lyophilized extract, 25 mg (dry weight) was coupled to 1 g of CNBr-activated Sepharose 4B (Pharmacia & Upjohn, Uppsala, Sweden) for application in RAST (-inhibition) assays.

Purified natural and recombinant allergens

Natural Bet v 1 and Bet v 2 were purified from birch pollen extract by means of affinity chromatography as described before. In case of Bet v 1, a Sepharose-coupled Bet v 1-specific monoclonal antibody (5H8) was used [6]. For Bet v 2, the affinity of profilin for poly(L-proline) was exploited [14]. The resulting profilin preparation was checked for purity by SDS-PAGE in conjunction with silver-staining. No impurities were observed (not shown). Purified recombinant Bet v 1 (rBet v 1) and Bet v 2 (rBet v 2), produced in *Escherichia coli*, were obtained from Biomay (Linz, Austria) [15,16].

For application in RAST, Bet v 1, Bet v 2, rBet v 1, and rBet v 2 were coupled to Sepharose: 100 µg protein on 100 mg of Sepharose for Bet v 1, Bet v 2 and rBet v 1, and 50 µg for rBet v 2. Amounts were chosen in such way that saturating allergen concentrations were achieved in RAST (0.25–0.5 µg/test). Protein was determined with BCA Protein Reagent (Pierce, Rockford, IL, USA) and BSA as protein standard.

RAST/RAST-inhibition

RAST was performed as described previously [17]. Briefly, serum (50 µL) was incubated overnight with 0.5 mg of Sepharose-coupled allergen in a final volume of 300 µL PBS, 0.3% BSA, 0.1% Tween-20. After washing away unbound serum components, radiolabelled sheep antibodies, directed to human IgE, were added. After overnight incubation and washing, bound radioactivity was measured. Results were expressed as RAST units/mL (RU/mL), calculated by means of a total IgE standard curve. This curve was obtained by incubating serial dilutions of a serum with known IgE content with Sepharose-coupled sheep antibodies against human IgE instead of Sepharose-coupled allergen. A result of >0.3 RU/mL was regarded as positive.

For RAST inhibition, serum was preincubated for 1 h with serial dilutions of inhibitor (allergen extract: 80 µg/mL–10 mg/mL or purified natural or recombinant allergen: 80 ng/mL–120 µg/mL), prior to addition of Sepharose-coupled allergen. For the uninhibited value, serum was preincubated with PBS, 0.3% BSA, 0.1% Tween-20. Subsequent steps were identical to those described for the RAST. Results were expressed as percentage of the uninhibited value.

Proteinase K digestion

Proteinase K digestion of birch pollen extract was carried out as described elsewhere [18]. Briefly, birch pollen extract (25 mg/mL) was digested overnight at 50 °C with proteinase K (100 µg/mL). Digestion was stopped by heating the sample for 5 min at 100 °C. As a control, birch pollen extract was also subjected to the same protocol in the absence of proteinase K. Digestion was monitored by measuring residual Bet v 1 and profilin content with specific antibody reagents. Less than 1% of the control value was left. In addition, digest and control were analysed by SDS-PAGE/silver-staining. Digests did not show any protein bands. For application in RAST, digested extract was coupled to Sepharose at concentrations equivalent to undigested extract.

SDS-PAGE/immunoblotting

Pollen extracts were separated by SDS-PAGE using Excel-Gel 8–18% gradient gels (Pharmacia & Upjohn) according to the manufacturer's instructions, and transferred (semidry) to nitrocellulose on a NovablotTM electrophoretic transfer

apparatus (Pharmacia & Upjohn). After blocking with PBS, 1.8% (w/v) BSA, 0.1% (v/v) Tween 20, the blots were cut into strips prior to immunoprobings with 150 µL human serum. Radiolabelled sheep antibodies against human IgE were used for detection.

Statistical analysis

Statistical analysis was performed on log-transformed data by a student's *t*-test. Geometric means and SEs were calculated, using only sera with at least one positive test with any of the Sepharose-coupled allergens. On the basis of means of individual ratios and the 95% confidence intervals (CI) of these means, it was judged whether IgE responses found in various RASTs, were significantly different. A response is significantly different when the 95% CI does not include a ratio of 1.

Results

IgE responses to pollen of birch, alder and hazel

Of the 1725 sera tested for IgE reactivity with tree pollen from the order Fagales, 242 (14.0%), 298 (17.3%), and 292 (16.9%) were positive for birch, alder and hazel, respectively (Fig. 1). False-negatives on birch were low-responders on alder and hazel, with a mean ($\pm$ SE) RAST of 0.45 ± 0.02 and of 0.44 ± 0.02 , respectively. For alder only 4/56 sera had a RAST > 1.0 RU/mL (all < 1.5 RU/mL), for hazel 2/50 (both < 2.0 RU/mL). These sera were tested on immunoblot for birch, alder and hazel, but no IgE binding was detected. Two sera with a clearly lower (but positive) RAST to birch than to alder and hazel demonstrated IgE binding to a band between 55 and 60 kDa (Fig. 2). This band was recognized in all three tree pollen extracts, but reactivity to birch was much weaker than to alder and to hazel. RAST-inhibition analysis with the same sera confirmed that IgE was cross-reactive between the three pollen species, and that birch pollen was 10-fold weaker as inhibitor (not shown).

To investigate whether mixtures of tree pollen extracts facilitate detection of species-specific IgE responses, seven sera with a negative RAST for birch, and a positive for alder and/or hazel, were tested in a RAST with a mixture of the three pollen species. With this mixture, six out of seven sera were negative (Fig. 3).

All sera with a RAST > 0.5 RU/mL, with one or more of the Sepharose-coupled tree pollen extracts ($n = 250$), were further analysed. The geometric mean ($\pm$ SE) of all RAST values for birch, alder and hazel were 2.1 ± 0.3 , 2.2 ± 0.2 and 1.2 ± 0.1 RU/mL. Responses to birch and alder were not significantly different, the mean ratio (95% CI) of all individual RAST values (alder/birch) being 1.02 (0.93–1.12).

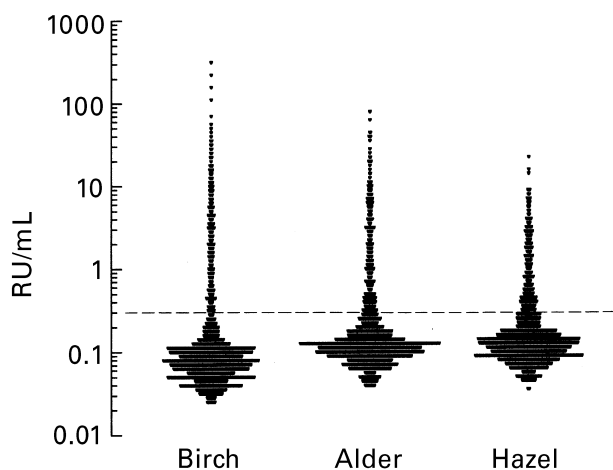


Fig. 1. RAST for birch, alder and hazel pollen. Sera of 1725 subjects were tested for IgE antibodies against extracts of birch, alder and hazel pollen. Results are expressed in RU/mL. Results > 0.3 RU/mL were regarded as positive.

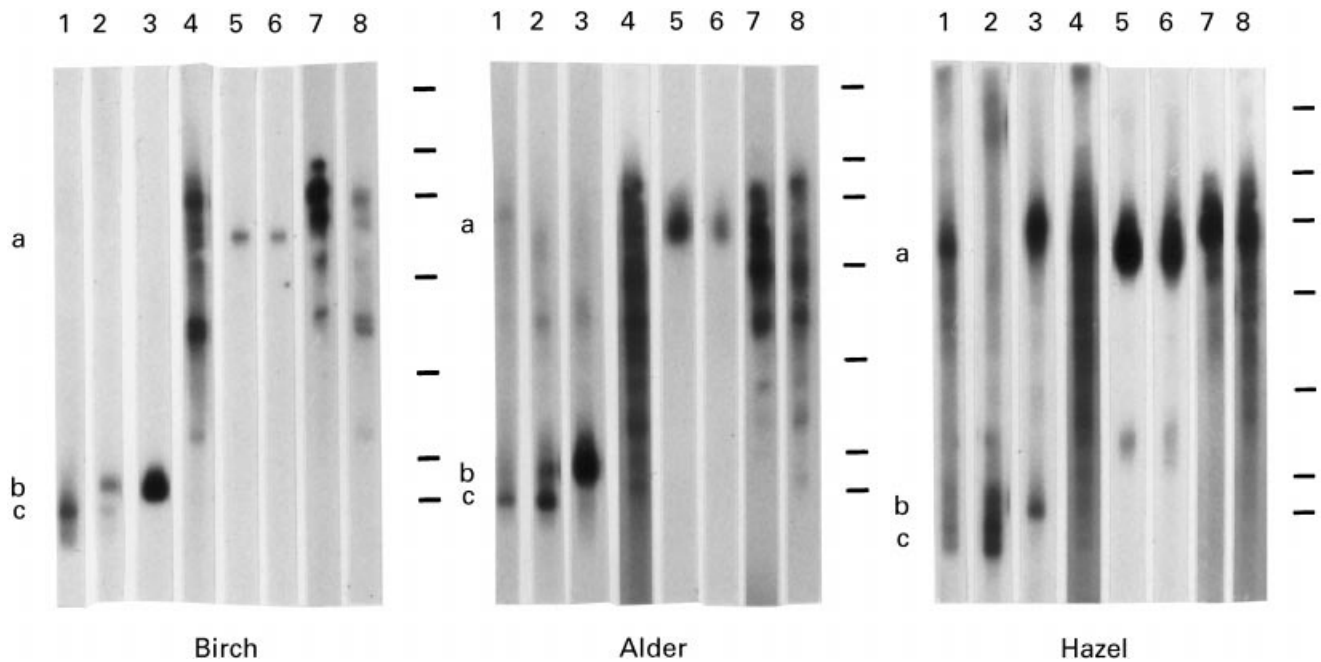


Fig. 2. Birch, alder and hazel immunoblots. Lanes 1–4 contain control sera with known IgE specificities; lane 1: anti-Bet v 2, lane 2: anti-Bet v 1 + 2, lane 3: anti-Bet v 1, lane 4: antihydroxy. In lanes 5 and 6 two sera are shown that had a higher response to alder/hazel than to birch; lane 5: 0.4 RU/mL for birch, 2.0 RU/mL for alder, and 1.7 RU/mL for hazel; lane 6: 0.9 RU/mL, 2.9 RU/mL, and 1.2 RU/mL, respectively. These sera recognize a 55–60-kDa band. In lanes 7 and 8 two sera are shown with a negative RAST to Bet v 1 and Bet v 2; lane 7: 2.6 RU/mL for birch, 5.4 RU/mL for alder, and 7.5 RU/mL for hazel; lane 8: 2.1 RU/mL, 3.6 RU/mL, and 3.8 RU/mL, respectively. These sera recognize a broad spectrum of high-molecular weight bands, typical for antihydroxy IgE. Bands corresponding to the 55–60-kDa allergen, Bet v 1 (or homologues) and profilin are indicated with the codes a, b and c, respectively. M_w markers are indicated by stripes, from top to bottom: 200, 97, 68, 43, 29, 19, and 15 kDa.

The responses to hazel were significantly lower ($P < 0.001$) than to birch and alder with mean ratios of 0.54 (0.47–0.63) and 0.53 (0.49–0.59), respectively. In a Spearman rank correlation the closest correlation was found for birch–alder: $R_s = 0.90$ (95%CI: 0.87–0.92), followed by alder–hazel with $R_s = 0.79$ (0.70–0.81), and birch–hazel with $R_s = 0.63$ (0.55–0.70).

IgE reactivity to purified natural Bet v 1 and Bet v 2

A RAST for natural Bet v 1 and Bet v 2 was performed for the 223 sera from which a sufficient volume was available and that had RAST values > 0.5 RU/mL for whole extracts. For Bet v 1, 141/223 (63.2%) were positive. For Bet v 2 this figure was 142/223 (63.7%). The geometric mean (SE) of all positive RAST values was 4.20 RU/mL (3.69–4.77) for Bet v 1 and 0.90 (0.83–0.98) for Bet v 2. The mean of individual ratios (95% CI) Bet v 1/birch and Bet v 2/birch were 0.44 (0.37–0.51) and 0.19 (0.14–0.22), respectively.

The combination of both purified allergens resulted in 209/223 (93.7%) positive diagnoses. Of the 14 false-negatives nine, seven and eight sera had a RAST result < 1.0 RU/mL for birch, alder and hazel, respectively. The

remaining sera were all < 5.0 RU/mL. The mean RAST results for the three pollen and for the combination of Bet v 1 and Bet v 2 ($n = 223$) are listed in Table 1. The response to the combination of both purified allergens was not significantly different from that to birch or alder. The mean (95% CI) of individual ratios (Bet v 1 + 2/extract) was

Table 1. RAST was performed for birch, alder and hazel and for purified Bet v 1 and Bet v 2 ($n = 223$). Results for Bet v 1 and Bet v 2 were added for each serum. Geometric means were calculated. Responses to birch, alder and the combination of purified allergens did not differ significantly. The response to hazel was twofold lower than the response to the other pollen and to the purified allergens ($P < 0.001$)

	Mean RAST	(SE)
Birch pollen	2.64	(2.38–2.92)
Alder pollen	2.53	(2.33–2.75)
Hazel pollen	1.26	(1.17–1.34)
Bet v 1 + Bet v 2	2.64	(2.38–2.93)

1.00 (0.91–1.11) for birch and 1.04 (0.93–1.17) for alder. A significant difference ($P < 0.001$) was observed in favour of the purified allergens in case of hazel, the ratio being 2.11 (1.78–2.50).

IgE reactivity to purified recombinant Bet v 1 and Bet v 2

RAST analysis with rBet v 1 and rBet v 2 was performed on 211 sera that were already tested on the natural allergens and of which still a sufficient volume was available. Only 6/136 sera (4%) with a positive RAST for natural Bet v 1 were negative on rBet v 1. RAST values of these six sera for the natural allergen were < 0.5 RU/mL. For Bet v 2 the number of false-negatives was 77/132 (58.3%). The geometric mean ($\pm$ SE) RAST of these false-negative sera for natural Bet v 1 was 0.8 ± 0.1 . The geometric mean ($\pm$ SE) of all RAST values for rBet v 1 was $4.1 (\pm 0.5)$, and $0.38 (\pm 0.03)$ for rBet v 2. The mean (95% CI) of all individual ratio's recombinant/natural was 0.98 (0.94–1.03) for Bet v 1. For Bet v 2 the response to recombinant was significantly ($P < 0.001$) lower: 0.38 (0.35–0.42). In a Spearman rank correlation responses to natural and recombinant Bet v 1 indicated a strong correlation: $R_s = 0.99$ (95% CI: 0.98–1.00). For Bet v 2 the correlation was weaker: $R_s = 0.68$ (0.58–0.76).

To exclude that the observed difference between Bet v 2

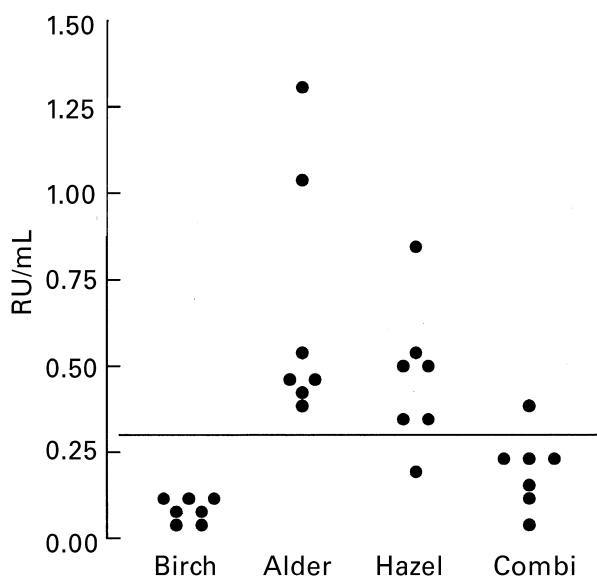


Fig. 3. RAST with pollen mixtures. Seven sera with a negative RAST for birch, but a positive RAST for alder and/or hazel were tested with a mixture of birch, alder and hazel (combi). For this purpose, equal amounts of Sepharose-coupled extracts were mixed and used in a RAST. In all cases, the same Sepharose quantity was used (0.5 mg/test). Six out of seven sera turn negative when a mixture is used.

and rBet v 2 was simply caused by non-saturating allergen concentrations in case of rBet v 2, 21 sera were re-tested with double and triple rBet v 2 amounts per test (500 ng and 750 ng, respectively). Only in three cases was there a moderate increase in RAST values at higher rBet v 2 concentrations. For the majority of the false-negatives no improvement was observed. This is illustrated for four sera in Table 2. False-negative sera were also analysed by RAST-inhibition (Fig. 4). The RAST for natural Bet v 2 was inhibited with natural Bet v 2 and with recombinant Bet v 2. For three sera no significant inhibition was observed with rBet v 2. In one case, 50% inhibition was reached at 50 μ g/mL of rBet v 2 (Fig. 4c). For a similar degree of inhibition only 1 μ g/mL of nBet v 2 was needed.

The combination of both recombinant allergens resulted in 37/211 (17.5%) false-negatives, an increase of 24 compared to the combination of natural Bet v 1 and Bet v 2. The mean of individual ratio's rBet v 1 + 2/birch was 0.75 (0.66–0.84).

IgE reactivity to carbohydrate epitopes

Both Bet v 1 and Bet v 2 lack N-linked glycosylation. Sera with a positive RAST to whole pollen extract but negative (or > 1.5 times lower) RAST to the combination of Bet v 1 and Bet v 2 ($n = 29$) were tested in a RAST with proteinase K-digested birch pollen extract as a measure for IgE reactivity to glycans. Almost all sera (28/29) had carbohydrate-specific IgE antibodies. When the carbohydrate-specific IgE responses were added to the RAST values for Bet v 1 and Bet v 2 (Fig. 5), the ratio (95% CI) of IgE responses to the combination of purified allergens and to birch pollen extract was 0.82 (0.65–1.04). Two sera were also analysed on immunoblot (Fig. 2, lanes 7 and 8). Multiple high-molecular weight bands were detected, confirming recognition of a broad range of glycoproteins with related IgE-binding glycans. Proteinase K-digested pollen extract was also separated by SDS-PAGE and blotted. Sera ($n = 5$)

Table 2. Sera with a negative RAST for rBet v 2 and a positive RAST for natural Bet v 2 were tested with two- and threefold higher concentrations of rBet v 2 test. No increase in RAST values was observed; results are expressed in RU/mL

n Bet v 2	r Bet v 2			
	250 ng/test	500 ng/test	750 ng/test	
500 ng/test				
3.2	0.7	0.7	0.7	
3.3	0.2	0.2	0.2	
5.2	0.3	0.2	0.2	
6.2	0.2	0.1	0.2	

with a high RAST value for digested birch pollen did not detect any bands on immunoblot, indicating that the reactivity in RAST is not caused by the presence of protease-resistant allergens (not shown).

Discussion

Intentionally, serum selection in this study was very broad: a general suspicion of inhalant allergy. This was done to avoid a bias towards any of the three pollen species at the start of the study. The aim of this investigation was to compare different *in vitro* approaches, not to determine the clinical relevance of a method. The clinical relevance of *in vitro* diagnostics based on whole pollen extracts is widely accepted. To compare established and new *in vitro* approaches in a reliable way, large panels of sera need to be screened. Of course, selection of several hundreds of sera from patients with clinical allergy in early spring would have been ideal, but was not feasible.

This study has demonstrated that both birch and alder pollen extracts are suitable candidates for reliable *in vitro* diagnosis of Fagales pollen allergy. Hazel pollen extract clearly binds less IgE (by a factor of ≈ 2), although there is a small number of sera ($<1\%$) with a moderate response (between 1.0 and 2.0 RU/mL) against hazel and a low (<1.0 RU/mL) or negative response against birch. RAST-inhibition and immunoblotting demonstrated that this rare discrepancy is most likely caused by a cross-reactive allergen of ≈ 55 – 60 kDa that is under-represented in birch pollen extract. In general, sera with a negative RAST for birch pollen and a positive RAST for alder/and or hazel were low-responder sera with a mean RAST <0.5 RU/mL. Mixing of three whole pollen extracts did not result in a positive RAST for such sera, indicating that sensitivity for specific structures is decreased. Other members of the order of Fagales, like oak (*Quercus*), beech (*Fagus*) and hornbeam (*Carpinus*) were not included because they are known to be far less allergenic and largely, if not completely, cross-

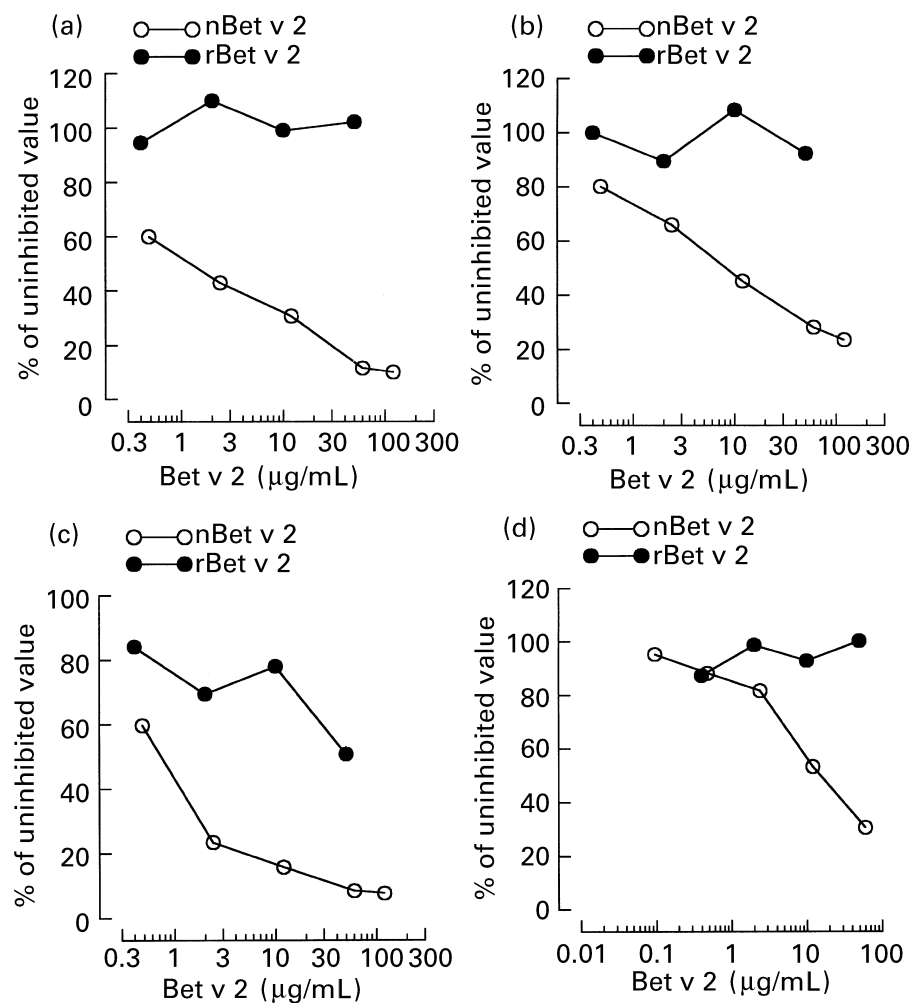


Fig. 4. Bet v 2 RAST-inhibition. Bet v 2 RAST inhibition was performed with natural Bet v 2 (homologous inhibition) and rBet v 2. With natural Bet v 2 almost complete inhibition was reached at ≈ 50 $\mu\text{g/mL}$. At similar concentrations, rBet v 2 did not result in significant inhibition for three sera. For one serum $\approx 50\%$ inhibition was observed with rBet v 2 (c).

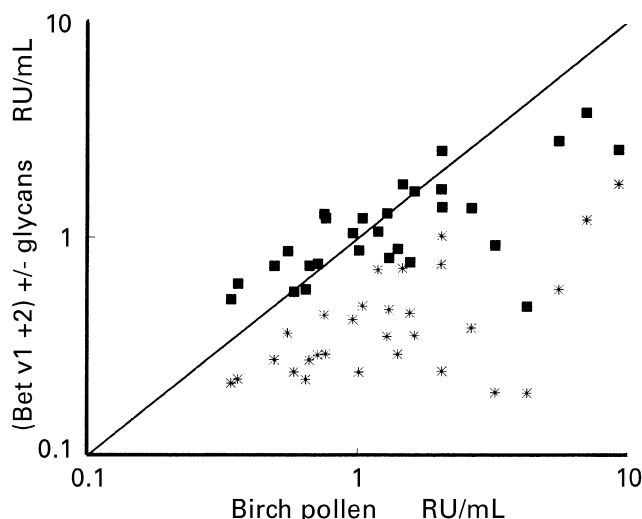


Fig. 5. Anti-carbohydrate IgE. Sera with a false-negative RAST for, or relatively low response to the combination of purified Bet v 1 and Bet v 2 were tested in a RAST for protease-digested birch pollen extract. The majority of the sera (28/29) demonstrated glycan-specific IgE antibodies. RAST values for whole birch pollen extract are compared with RAST values for the combination of Bet v 1 and Bet v 2 without (*) or with (■) glycans. Inclusion of glycan-specific IgE results in closer resemblance with IgE reactivity to whole birch pollen.

reactive with the pollen included in this study [19]. Of course, it can never be excluded completely that unique reactivities against such tree pollen exist.

A further simplification would be to replace birch pollen extract by a combination of Bet v 1 and birch profilin (Bet v 2). Products based on purified allergens would facilitate simpler standardization and increase batch-to-batch consistency. Stability could benefit as well, because crude allergen extracts often contain protease activity. Several studies have reported on the use of Bet v 1 and Bet v 2 for diagnosis of tree pollen allergy [9–12,20]. In all these reports recombinant molecules were applied. In this study, natural allergens as well as recombinant versions were tested for their applicability. With a combination of natural Bet v 1 and Bet v 2, 93.7% of all tree pollen-positive sera were detected. Most false-negative sera were low-responders on whole pollen extracts. Measurement of IgE antibodies against birch pollen-derived glycans demonstrated that an important factor in the observed discrepancies between RASTs with whole-pollen and with (non-glycosylated) purified allergens, is IgE reactivity to carbohydrate epitopes. Recent studies have shown that IgE antibodies against carbohydrates are clinically irrelevant [21]. A combination of purified Bet v 1 and Bet v 2 therefore is a good alternative for whole-pollen extracts.

Several reports focusing on the use of recombinant birch

pollen allergens have claimed that these molecules are suitable to replace pollen extracts for diagnostic purposes [9,10,20,22–27]. In some cases, this judgement was primarily based on immunoblotting data. At its best, this approach results in semiquantitative data. In our study, natural and recombinant allergens were compared by means of RAST and RAST-inhibition. Recombinant Bet v 1 demonstrated an almost perfect match with its natural counterpart. The mean response to the recombinant allergen was 98% (95% CI: 94–103) of that to natural Bet v 1. In a Spearman rank correlation both responses turned out to be almost identical: $R_s = 0.99$ (95% CI: 0.98–1.00). This analysis has not produced any evidence that a single isoform of Bet v 1 is insufficient for measurement of Bet v 1-specific IgE.

For Bet v 2, the recombinant allergen demonstrated a significantly lower (> twofold) IgE binding capacity than its natural counterpart. Several sera had a strong IgE response (> 5 RU/mL) against natural profilin but were not reactive with the recombinant allergen. It is not clear why the performance of recombinant Bet v 2 is poorer than that of its natural counterpart. Possibly, *E. coli*-derived recombinant Bet v 2 does not have the right conformation. Such structural differences between natural and recombinant allergens might have been overlooked in comparative studies based on immunoblotting under denaturing conditions. Perhaps, expression of profilin in eukaryotic expression systems is necessary to obtain recombinant molecules equivalent to the natural allergen. Another factor might be the existence of different isoforms of profilin. Both acidic and basic isoforms of some profilins have been reported [28]. Perhaps IgE antibodies can distinguish between different isoforms.

In summary, this study has demonstrated that diagnostics for Fagales tree pollen allergy can be simplified by replacing mixtures of birch, alder and hazel pollen by a single species. Both birch pollen and alder pollen are eligible candidates. A combination of purified natural Bet v 1 and Bet v 2 also covered the vast majority (93.7%) of all tree pollen-positive sera. The combination of recombinant Bet v 1 and Bet v 2 was slightly less efficient (82.5%).

Acknowledgements

This study was financially supported by the Dutch Ministry of Economic Affairs (grant nr. BIO 94058) and by HAL Allergen Laboratories (Haarlem, The Netherlands).

References

- 1 Rohac M, Birkner T, Reimitzer I et al. The immunological relationship of epitopes on major tree pollen allergens. *Mol Immunol* 1991; 28:897–906.

- 2 Ipsen H, Hansen OC. The NH₂-terminal amino acid sequence of the immunochemically partial identical major allergens of alder (*Alnus glutinosa*) Aln g I, birch (*Betula verrucosa*) Bet v I, hornbeam (*Carpinus betulus*) Car b I and oak (*Quercus alba*) Que a I pollens. *Mol Immunol* 1991; 28:1279–88.
- 3 Valenta R, Breiteneder H, Pettenburger K et al. Homology of the major birch-pollen allergen Bet v I, with the major pollen allergens of alder, hazel and hornbeam at the nucleic acid level as determined by cross-hybridization. *J Allergy Clin Immunol* 1991; 87:677–82.
- 4 Ipsen H, Wihl JA, Nüchel Petersen B, Løwenstein H. Specificity mapping of patients IgE response towards the tree pollen major allergens Aln g I, Bet v I and Cor a I. *Clin Exp Allergy* 1992; 22:391–9.
- 5 Ebner C, Ferreira F, Hoffmann K et al. T cell clones specific for Bet v I, the major birch pollen allergen, crossreact with the major allergens of hazel, Cor a I, and alder, Aln g I. *Mol Immunol* 1993; 30:1323–9.
- 6 Akkerdaas JH, van Ree R, Aalbers M, Stapel SO, Aalberse RC. Multiplicity of crossreactive epitopes on Bet v I as detected with monoclonal antibodies and human IgE. *Allergy* 1995; 50:215–20.
- 7 American Academy of Allergy, Asthma and Immunology. Position statement: The use of standardized allergen extracts. *J Allergy Clin Immunol* 1997; 99:583–6.
- 8 Van Ree R. Analytic aspects of the standardization of allergenic extracts. *Allergy* 1997; 52:795–805.
- 9 Menz G, Dolocek C, Schönheit-Kenn U et al. Serological and skin-test diagnosis of birch pollen allergy with recombinant Bet v I, the major birch pollen allergen. *Clin Exp Allergy* 1996; 26:50–60.
- 10 Pauli G, Oster JP, Deviller P et al. Skin testing with recombinant allergens rBet v 1 and birch profilin, rBet v 2: diagnostic value for birch pollen and associated allergies. *J Allergy Clin Immunol* 1996; 97:1100–9.
- 11 Elfman L, Svensson M, Lidholm J, Pauli G, Valenta R. Different profiles in specific IgE to rBet v 1 and rBet v 2 in patients allergic to birch pollen from six countries. *Int Arch Allergy Immunol* 1997; 113:249–51.
- 12 Liappis N, Starke A. Qualitative and quantitative profile of specific IgE, antibodies against the recombinant allergens Bet v 1 and Bet v 2 (profilin) and Phl p 1, Phl p 2 and Phl p 5 in the sera of children. *Allergo J* 1997; 6:305–7.
- 13 Van Ree R, Clemens JGJ, Aalbers M, Stapel SO, Aalberse RC. Characterization with monoclonal and polyclonal antibodies of a new major allergen from grass pollen in the group I molecular weight range. *J Allergy Clin Immunol* 1989; 83:144–51.
- 14 Lindberg U, Schutt CE, Hellsten E, Tjäder A-C, Hult T. The use of poly (L-proline)-Sepharose in the isolation of profilin and profilactin complexes. *Biochim Biophys Acta* 1988; 967:391–400.
- 15 Breiteneder H, Pettenburger K, Bito A et al. The gene coding for the major birch pollen allergen, Bet v I, is highly homologous to a pea disease resistance response gene. *EMBO J* 1989; 8:1935–8.
- 16 Valenta R, Duchêne M, Pettenburger K et al. Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals. *Science* 1991; 253:557–60.
- 17 Aalberse RC, Koshte V, Clemens JGJ. Immunoglobulin E antibodies that crossreact with vegetable foods, pollen, and Hymenoptera venom. *J Allergy Clin Immunol* 1981; 68:356–64.
- 18 Van Ree R, Hoffman D, van Dijk W et al. Lol p 11, a new major grass pollen allergen, is a member of a family of soybean trypsin inhibitor-related proteins. *J Allergy Clin Immunol* 1995; 95:970–8.
- 19 Weber RW, Nelson HS. Pollen allergens and their interrelationships. *Clin Rev Allergy* 1985; 3:291–318.
- 20 Valenta R, Duchêne M, Vrtala S et al. Recombinant allergens for immunoblot diagnosis of tree-pollen allergy. *J Allergy Clin Immunol* 1991; 88:889–94.
- 21 Van der Veen MJ, van Ree R, Aalberse RC et al. Poor biological activity of cross-reactive IgE directed to carbohydrate determinants of glycoproteins. *J Allergy Clin Immunol* 1997; 100:327–34.
- 22 Scheiner O. Recombinant allergens: biological, immunological and practical aspects. *Int Arch Allergy Immunol* 1992; 98:93–6.
- 23 Mohapatra SS, Sehon AH. Therapeutic potential of recombinant allergens. *Int Arch Allergy Immunol* 1992; 99:265–70.
- 24 Valenta R, Dolocek C, Vrtala S et al. Recombinant tree and grass pollen allergens for diagnosis and therapy of type I allergy. *Allergo J* 1994; 3:90–5.
- 25 Valenta R, Kraft D. Recombinant allergens for diagnosis and therapy of allergic diseases. *Curr Opin Immunol* 1995; 7:751–6.
- 26 Scheiner O, Kraft D. Basic and practical aspects of recombinant allergens. *Allergy* 1995; 50:384–91.
- 27 Rossi RE, Monasterolo G, Operti D, Corsi M. Evaluation of recombinant allergens Bet v 1 and Bet v 2 (profilin) by Pharmacia CAP system in patients with pollen-related allergy to birch and apple. *Allergy* 1996; 51:940–5.
- 28 Binder M, Ortner S, Erben H et al. The basic isoform of profilin in pathogenic *Entamoeba histolytica*. cDNA cloning, heterologous expression, and actin-binding properties. *Eur J Biochem* 1995; 233:976–81.