

Allergic Response to Birch and Alder Pollen Allergens Influenced by Geographical Location of Allergic Subjects

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Abstract. A detailed analysis was made of the reactivity patterns of birch pollen-allergic subjects from Norway and Australia to the various IgE-binding components of pollens from several different birch and alder species. Typically, with each of the pollens examined, the Norwegian subjects exhibited a specific limited response pattern in which only a few of the 18 possible allergenic bands were recognized. A single, major IgE-binding band of approximate molecular weight 16-17 kD was recognized in each pollen by all the Norwegian patients used in the study. In contrast to this situation, there was no 'typical' recognition pattern amongst the Australian patients. Although recognition of the 16- to 17-kD components occurred in some cases, it was not dramatically superior to any other single component. Recognition of higher molecular weight components occurred more frequently than with the Norwegian group. Reasons for the differences in the response patterns of the Australian and Norwegian groups are discussed with reference to the exposure of the populations to different allergenic sources and genetic variation.

Introduction

The antigenic and allergenic profiles of pollen from the birch tree, *Betula pendula* (syn. *B. verrucosa*) [12], was defined in detail recently by protein blotting [7]. A preliminary comparison of the antigenic and allergenic reactivities of three birch and one alder species was also made in an attempt to identify pollen components responsible for the allergic cross-reactivity existing among the members of the *Betulaceae* tree family. The pollens from birch and alder appear to be related antigenically and allergenically [3-5, 9, 10], and a number of protein bands with similar electrophoretic mobilities were detectable in pollen extracts from these species following sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). In *B. pendula*, the major IgE-binding reactivity was associated with the antigen(s) in the region 16-17 kD. The allergic response to birch and alder pollens exhibited extensive heterogeneity amongst individual subjects, although from the analysis carried out, the existence of different patterns of response that could be attributed to the geographical location of the allergic population could not be established conclusively [7].

This paper continues the assessment of the allergenicity of birch and alder pollens by analyzing patient response patterns in the light of the geographical location of both the subjects and the pollen samples. Our observations suggest that whilst the specific allergenic components from a given birch pollen source tend to remain constant regardless of geographical origin, the response of allergic subjects does not. Distinct patterns in the frequency of recognition of specific allergenic bands can be distinguished for different geographical populations, even though the same complement of allergenic proteins is recognized by both. The origin of these differences and the extent to which they reflect the allergenic exposure of the subjects are discussed.

Materials and Methods

Birch and Alder Pollen Extracts

Pollen was obtained from *B. pendula* supplied by Allergon A/S (Sweden) and grown in Scandinavia, and pollens from Australian *B. pendula*, *Betula davurica*, and *Alnus incana* were obtained from trees grown in Canberra (Australia). The antigenic components of the pollens were extracted with 0.001 M ammonium bicarbonate by rotating at 4°C for 24 h. Insoluble material was removed by centri-

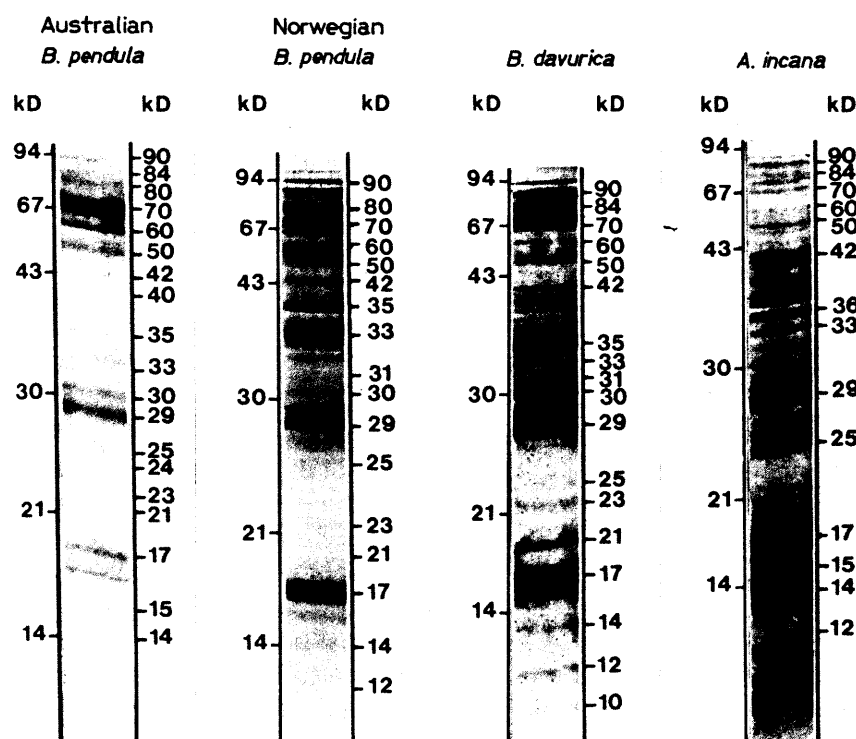


Fig. 1. Protein stains for pollen components separated by SDS-PAGE. The standard molecular weight markers are given on the left of each stain and the IgE-binding components on the right.

fugation at 6,000 rpm at 4°C for 50 min, and the clear supernatant was dialyzed against water and then lyophilized.

Subjects

Sera from Norwegian birch pollen allergic subjects were from patients who gave positive skin prick tests to a partially purified birch pollen extract. The sera were radioallergosorbent test (RAST) positive (class 2–4) using Pharmacia birch (*B. verrucosa*) pollen RAST discs and collected at the University Hospital of Bergen (Norway). Sera from Australian patients presenting with allergic symptoms and having positive skin test and RAST to birch pollen were collected in Canberra. Sera were also obtained at the Allergy Clinic, Royal North Shore Hospital, from other allergic patients not allergic to birch pollen.

Gel Electrophoresis and Protein Blotting

The pollen components were separated by SDS-PAGE after denaturation of the pollen extracts with 2-mercaptoethanol (Sigma) at 100°C for 5 min. The conditions and materials for electrophoresis have been described previously [11]. Eighteen percent SDS-PAGs (8.0 × 6.0 × 0.15 cm) were used in conjunction with a Tris-glycine-SDS buffer (pH 8.4). Molecular weight markers (Pharmacia or Bio-Rad) were included for the estimation of the molecular weights of pollen components. Approximately 100 µg protein was loaded per gel, and electrophoresis was carried out at 150 V for 2 h. Proteins were detected in the gels by Coomassie brilliant blue (Sigma). Following SDS-PAGE, the proteins were transferred to 0.1 µm nitrocellulose (Schleicher and Schuell) at 100 V for 2.5 h using 20% methanol in a Tris-glycine buffer (pH 8.4). After transfer, the unbound membrane sites were blocked by immersion with rocking in 0.1% Tween 20 (Sigma) in phosphate-buffered saline for 1 h at room

temperature. Proteins transferred to nitrocellulose were detected with amido black (Sigma).

Detection of IgE-Binding Components

Nitrocellulose strips (3 mm in width) were incubated overnight with atopic sera (1:10). After washing with Tween 20 (0.05%), the strips were incubated for 6 h with ¹²⁵I-anti-IgE (50,000 cpm; Kallestad). After washing again, the strips were air-dried and loaded into a Kodak X-Omatic cassette (KP68375) with a photographic film (Fuji Rx-safety) and stored at -70°C for 3–7 days prior to development.

Radioallergosorbent Test

RAST was performed using birch pollen allergen (*B. verrucosa*) paper discs (Pharmacia). Fifty microlitres of undiluted atopic serum was incubated with each disc for 6 h, followed by washing and overnight incubation with ¹²⁵I-antihuman IgE (50,000 cpm; Kallestad). The discs were washed again before being counted in a gamma counter.

Results

Electrophoresis of Pollen Extracts

The antigenic profiles of the pollens from *B. pendula*, *B. davurica*, and *A. incana* following separation by SDS-PAGE are shown in figure 1. The bands which bound IgE in the immunoblots from these gels are given on the right-hand side of the protein stains.

Selected samples of the immunoblots demonstrating the range of IgE-binding components detected are presented in figure 2. It is clear, as reported previously [7], that there is extensive similarity in the protein band patterns across birch species and between birch and alder. In each case, this pattern is dominated by three strong bands in the vicinity of 17, 30, and 35 kD and a fourth region around 70–80 kD. However, the 'fingerprint' of each species appears to be unique, a characteristic which may be useful as a means of species identification. Small differences observed between the protein stains of the Scandinavian and Australian *B. pendula* pollens, particularly in the region above 50 kD, suggest that the exact pollen composition may be influenced by environmental factors, resulting in variation amongst different strains of the same species.

Identification of IgE-Binding Bands

Allergenic bands/regions of similar electrophoretic mobilities tended to be recognized regardless of the birch pollen species or strain, and with the exception of the alder pollen, approximately the same numbers of components were recognized in each sample. In the allergenic profiles, 16 bands of the same or very close electrophoretic mobilities were common to the three birch samples, and 12 of these also appeared in the alder pollen. *B. davurica* pollen was the only sample in which a 10-kD IgE-binding band could be unequivocally identified. In both strains of *B. pendula* pollen, 17 of the 19 allergenic bands recognized in the Australian sample were common to the Scandinavian sample in which a total of 18 allergenic bands were detected. Two bands at 15 and 84 kD in the Australian strain and one at 12 kD in the Scandinavian strain were not recognized in both. These bands, however, were recognized by only a few sera.

Twenty-nine patients who showed IgE binding to at least 1 band in immunoblots of at least 1 of the 3 birch pollen extracts were used in this study. These patients also had a positive RAST with birch pollen discs and comprised 13 Norwegian and 16 Australian subjects. The individual allergic profiles for these subjects against each of the four pollens, with the exception of the 4 Australian patients 26–29, who were tested against the two *B. pendula* pollen strains only, are presented in tables I and II. These tables clearly show that the allergic spectrum of the Norwegian population with all four pollens is dominated by a single band at 16–17 kD, in contrast to the profile of

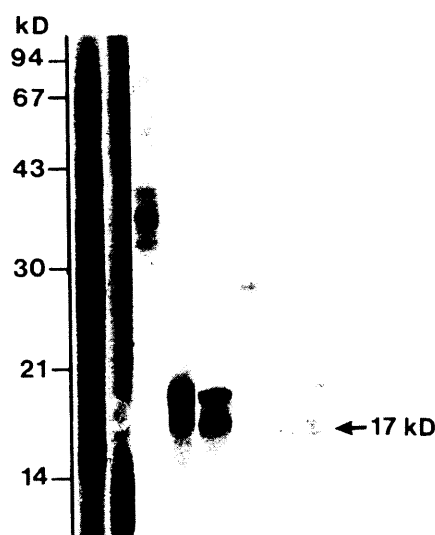


Fig. 2. Specific IgE binding by eight individual birch pollen allergic sera to *B. pendula* pollen extract separated by SDS-PAGE and transferred to nitrocellulose.

the Australian population in which there is extensive heterogeneity in the recognition pattern.

Characterization of Response Patterns of Norwegian and Australian Birch Pollen Allergic Subjects

The apparent differences between the responses of the Norwegian and Australian groups suggested in tables I and II prompted a more detailed analysis of the data. These results are summarized in figure 3, which presents the responses of the Norwegian and Australian subjects to each of the four different pollens, and in figure 4, in which the responses to the four pollens can be seen side-by-side for both geographical populations. These representations demonstrate that the Australian and Norwegian responses to the same allergen sources and allergenic bands are dramatically different, although they can be obscured if the response is analyzed without considering the population origin, as shown in figure 5. In the Norwegian group, 100% of the birch pollen allergic sera react with the 16- to 17-kD component in all the pollens. In addition, the allergic profile for each pollen was very similar. Neither of these characteristics was observed with the Australian group. Although the same IgE-binding bands were recognized, there was no single dominant band, and the response of the Australian group as a whole was distinct for each

Table 1. Reactions of sera from individual birch pollen allergic Norwegian patients with identified allergenic pollen components electroblotted onto nitrocellulose following SDS-PAGE

RAST %	Serum No.	Molecular weight of IgE-binding bands, kD																					
		10	12	14	15	17	21	23	24	25	29	30	31	33	35	40	42	50	60	70	80	84	90
3	1	□	● □ ■	● □ ■	■	○ ● □ ■	○ □	□	○	○ ● □ ■	○ ● □ ■	○ □	□	○ ● □ ■	□		○ ● □ ■	○ □ ■	○ ● □ ■	○ ● □ ■	○ □ ■		● □ ■
21	2					○ ● □ ■	□				■				○		●		○ ● ■	○ □			
18	3					○ ● □ ■																	
36	4					○ ● □ ■																	
15	5					○ ● □ ■																	
20	6				■	○ ● □ ■	□	○ ●		○ ● ■	□ ■	○ □	□	○ ● □ ■	■	□	○ ● □ ■	■	○ ● □ ■	○ ● □ ■	○ ● □ ■		○ ● □ ■
31	7					○ ● □ ■	●																
21	8					○ ● □ ■																	
39	9					○ ● □ ■																	
37	10					○ ● □ ■						●											
19	11				○	○ ● □ ■																	
32	12					○ ● □ ■									●								
21	13					○ ● □ ■																	

○ = Australian *B. pendula* pollen; ● = Norwegian *B. pendula* pollen; □ = *B. davurica* pollen; ■ = *A. incana* pollen.

Table II. Reactions of sera from individual birch pollen allergic Australian patients with identified allergenic pollen components electroblotted onto nitrocellulose following SDS-PAGE

RAST %	Serum No.	Molecular weight of IgE-binding bands, kD																					
		10	12	14	15	17	21	23	24	25	29	30	31	33	35	40	42	50	60	70	80	84	90
30	14									■	□			□	□	○		○	○	■	□	○	○
20	15			□		□	□	□			○			○		○		○	○	○			○
8	16			○		○	○	○	○	○	○			○	○	○	○	○	○	○	○	○	○
10	17			□		□	□	□		■	□	□	■	□	□	□	■	□	□	□	□	□	□
7	18		□	□		●	●	●		□		○	●	□	●			○	●	●	○	○	○
43	19					○			●														
9	20	□	□	□		●	●	●															
23	21		□	□			●	●				○							○	●			○
7	22			■		■		●				○			●		●		○	○			
4	23											○					○	○	○	○		■	
13	24			□			●	●		■				○	●		●	○	○	○	●		●
7	25					●													○				
37	26					○						○				○		○	○		○		
4	27								○	○	○	○		○		○		○	○	●	●	○	○
5	28											○				○		○	○	○			○
35	29																○		○	○		○	○

For explanation of symbols see footnote of table I.

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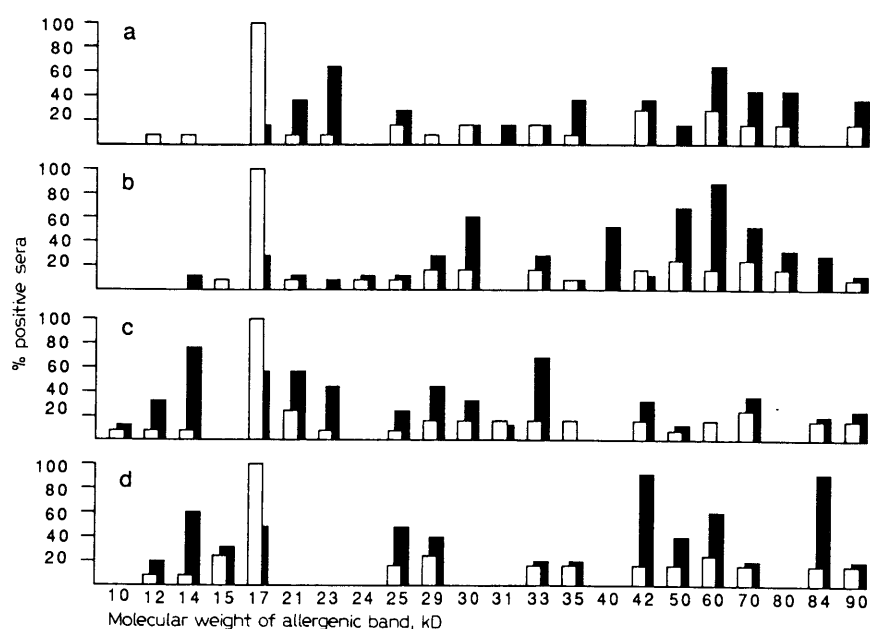


Fig. 3. Reactivity of birch pollen allergic patients from Norway (open columns) and Australia (dark columns) to IgE-binding components in pollens from *B. pendula* grown in Scandinavia (a), *B. pendula* grown in Australia (b), *B. davurica* (c), and *A. incana* (d).

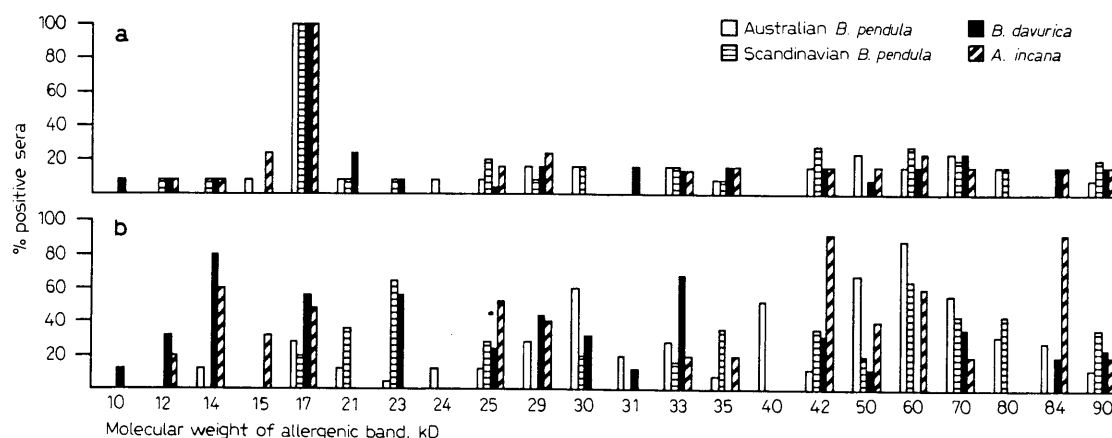


Fig. 4. Response of Norwegian birch pollen allergic patients (a) and Australian birch pollen allergic patients (b) to identified IgE-binding components present in pollen from *B. pendula* grown in Australia and Scandinavia, *B. davurica*, and *A. incana*.

pollen species. The overall response was extensively heterogeneous, as was the response of the same individual to each of the pollens. The Australian group also tended to recognize allergens in the higher molecular weight region more frequently than the Norwegian group.

Discussion

Birch and alder pollens demonstrate extensive similarity in their protein profiles (fig. 1) and cross-reactivity

in their antigenic composition [3, 7, 10]. This is reflected in their allergenic profiles in terms of a common complement of IgE-binding bands of similar electrophoretic mobilities. Such a phenomenon is not unique to birch pollens. For example, cross-reactivity amongst different species of related grass pollens is well known [1]. What distinguishes this investigation is the fact that the apparent cross-reactivity has been assessed on the basis of the reactivity of two geographically distinct birch pollen allergic populations who were exposed to geographically different strains of the same pollen species.

It is apparent from tables I and II and from figures 3 and 4 that differences exist in the response patterns of Australian and Norwegian birch pollen allergic patients to the allergens present in several birch and alder pollens, even though both groups recognize essentially the same complement of allergens in each source. The most dramatic differences are the variations in the frequency of recognition of the 16- to 17-kD allergenic region and the heterogeneity of the response to the entire complement of allergens. To a large extent, these differences are obscured if the two populations are analyzed together (fig. 5).

In the case of the Norwegian group, the single dominant allergenic band is the 16- to 17-kD component, regardless of the pollen species or origin. The frequency of recognition of this band by the Norwegian group is so high that it could be considered as a marker of birch pollen allergy in Norwegian patients. The importance of the 16- to 17-kD allergenic band in birch and alder pollens is supported by the results of earlier investigations, which have frequently described low molecular weight allergenic components in the region 10–20 kD [2, 6, 7, 9, 14] and by two independent studies which assigned the major allergenic activity of this fraction, and indeed of birch pollen generally, to the presence of heat-stable components in the vicinity of 17 kD [8, 13].

The Norwegian profile was also distinguished by a striking homogeneity in the response of individuals to different birch pollen species and strains of the same species. The simplicity of the allergic profile of the Norwegian group, as seen in figure 4a, is a direct consequence of this. Essentially the same allergic profiles were obtained for the same patients with all the pollen samples, suggesting clinical and immunological cross-reactivity uncomplicated by the presence of cross-reactions arising from exposure to allergens from non-birch pollen sources. In other words, we observe with most of the Norwegian group a single, primary allergy to pollen from the *Betulaceae* family.

Despite the similarity in protein stains and allergic profiles among the birch and alder pollens, it is apparent from the slight differences in the reactivities of the same patients for the different pollens that components of corresponding mobilities are chemically and immunologically distinct. Though it is highly probable that identical or closely related determinants responsible for the interspecies cross-reactivity exist on different components from the same species and on corresponding components from dif-

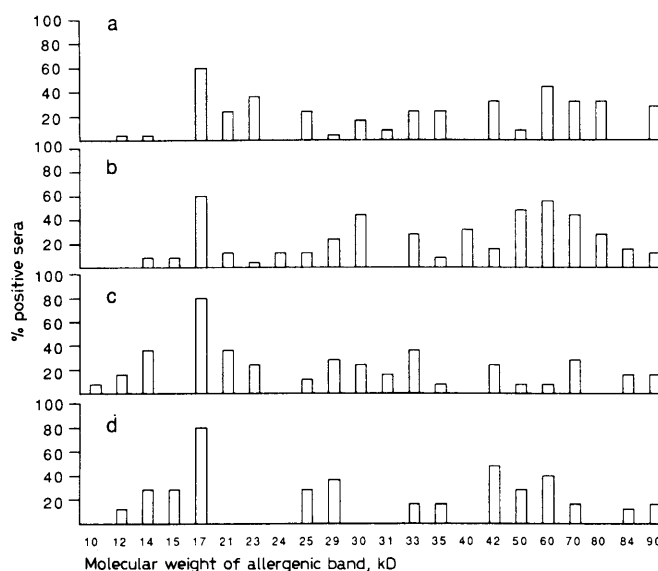


Fig. 5. Reactivity of birch pollen allergic sera with pollen from *B. pendula* grown in Scandinavia (a) and Australia (b), *B. davurica* (c), and *A. incana* (d).

ferent species, it is also clear that at least some IgE-binding paratopes or sites are unique and form the source of the variation in patient response.

In contrast to the selectivity of the allergic profile of the Norwegian group, the Australian group exhibited extensive diversity both in terms of the number of subjects who recognized multiple bands and the variation observed in the allergic profiles of the same individual for the different pollens. Birch pollen allergic Australian patients responded less frequently to the 16- to 17-kD band and also recognized many of the higher molecular weight allergens more frequently than their Norwegian counterparts. Only 1 Australian patient, subject No. 19, demonstrated a simple allergic profile analogous to that of the Norwegians. This patient was distinguished from the other Australian patients by the absence of reactivity with other allergens from different sources. In particular, subject No. 19 did not react with grass pollens. Most of the other Australian patients used in this study presented with multiple allergies which included a common reactivity to grass pollens.

It is possible that the apparent difference between the Australian and Norwegian groups may reflect genetically different immunological responses to the same allergens or differences in the original allergen exposure of the two populations. All 16 Australian sera showed clear positive reactions to birch pollen al-

lergens. However, the fact that more than 60% of the Australian patients who reacted with birch or alder pollens failed to recognize the major 17-kD band suggests that these people could be exhibiting clinical cross-sensitivity to birch pollen arising from an IgE antibody response to an allergenic source distinct from birch pollen but which shares common, cross-reacting determinant(s) with some of the minor allergens of birch pollen.

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