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Semiquantitative and qualitative analysis of pre-seasonal airborne birch pollen allergens in different particle sizes

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Semiquantitative and qualitative analysis of pre-seasonal airborne birch pollen allergens in different particle sizes

Background information for allergen reports

EILA MATIKAINEN and AULI RANTIO-LEHTIMÄKI

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Bet v 1, the major allergen of birch pollen, has been reported to be synthesized in pollen grains shortly before anthesis. In order to obtain a better understanding of the causative agents of allergic symptoms which appear before the actual pollen season, the activity birch pollen antigen in outdoor air have been studied, using polyclonal antibodies against birch pollen, and monoclonal antibodies against Bet v 1. Using a size-selective bioaerosol sampler (SSBAS), birch pollen antigen concentrations were analysed separately in different particle size classes, down to the molecular size range.

About a week before the anthesis of alder, antigens were already detected in outdoor air samples. They were found mainly in the smallest particle size classes and might have had their origin in the developing leaves and flower structures of Betulaceae trees. At the beginning of birch anthesis Bet v 1 activity in the air increased markedly. The smallest particles may have been orbicules (2–4 µm) and starch granules (0.5–2 µm) from inside the pollen and present among the other particles. Birch pollen antigens were found throughout the whole study season. Peaks in activity preceding the start of alder pollination were most probably due to cross-reactivity between the main allergens Aln g 1 of alder and Bet v 1. The results support the idea that all airborne allergens of Betulaceae pollen could for practical purposes be monitored by using monoclonal antibodies against Bet v 1.

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Aerobiological studies have reported the presence of pollen antigenic particles in outdoor air both before and after the local pollen season (Spieksma et al. 1990, Takahashi et al. 1991, 1993, 1995; Rantio-Lehtimäki et al. 1994, Pehkonen & Rantio-Lehtimäki 1994, 1995; Ekeboom et al. 1996). Some non-local pollen is observed every year before the pollen season begins, and in some years strong air currents may carry considerable amounts of pollen over distances of several hundreds of kilometres (Hjelmroos 1991). This pollen is often called “long-distance transported pollen”. There is clinical confirmation that allergic symptoms may occur before the commencement of the local pollen season (Wallin et al. 1991).

In Finland, in early spring, the general pollen monitoring centres frequently report an absence of pollen from the air; at the same time, however, as allergic symptoms of highly sensitized individuals have clearly started. It has been shown that occasionally, although the pollen count is zero, there may be a high concentration of pollen antigen in the air (Rantio-Lehtimäki et al. 1994, Ekeboom et al. 1996). This often happens before the pollen season but may also occur during it when pollen has been washed down by heavy rain (Pehkonen & Rantio-Lehtimäki 1994, 1995). Sites of antigenic proteins inside the developing anthers and pollen grains vary during the particular stages of the maturation processes (El-Ghazaly & Grafström 1995). Paucimicronic and submic-

ronic (<1 µm) particles may be emitted from catkins and other parts of the plant (D’Amato et al. 1991, Fountain et al. 1992), or from dehiscing anthers (El-Ghazaly et al. 1995, Takahashi et al. 1995) and from within the pollen (Grote et al. 1993, Vrtala et al. 1993, Swoboda et al. 1995, Emilson et al. 1996, El-Ghazaly et al. 1996). Orbicules (Ubisch bodies) are detached from the inner anther wall, the tapetum (El-Ghazaly & Grafström 1995, El-Ghazaly et al. 1995). Some allergologically important species have starch granules in the cytoplasm of their pollen grains (Knox et al. 1993, Swoboda et al. 1995, Emilson et al. 1996, El-Ghazaly et al. 1996), which can be released when pollen grains burst, especially when they are exposed to water (Bellomo et al. 1992, Knox et al. 1993). Starch granules are 0.5–2 µm and orbicules are 2–4 µm in aerodynamic diameter (El-Ghazaly & Grafström 1995, El-Ghazaly et al. 1995, 1996) which is also the size of the most problematical allergen particles. The periods immediately before pollen release may be especially difficult for allergic persons, if allergen proteins are occurring in the small particle sizes which effectively penetrate deep into the respiratory organs, down to the alveoli.

According to Swoboda et al. (1995), Bet v 1, the major allergen of birch, is synthesized in the pollen grains at a very late stage, immediately before anthesis. Particles released from birch trees weeks before flowering mainly contain antigens other than Bet v 1 (Swoboda et al. 1995). To gain

ALLERGENS 1996, polyclonal

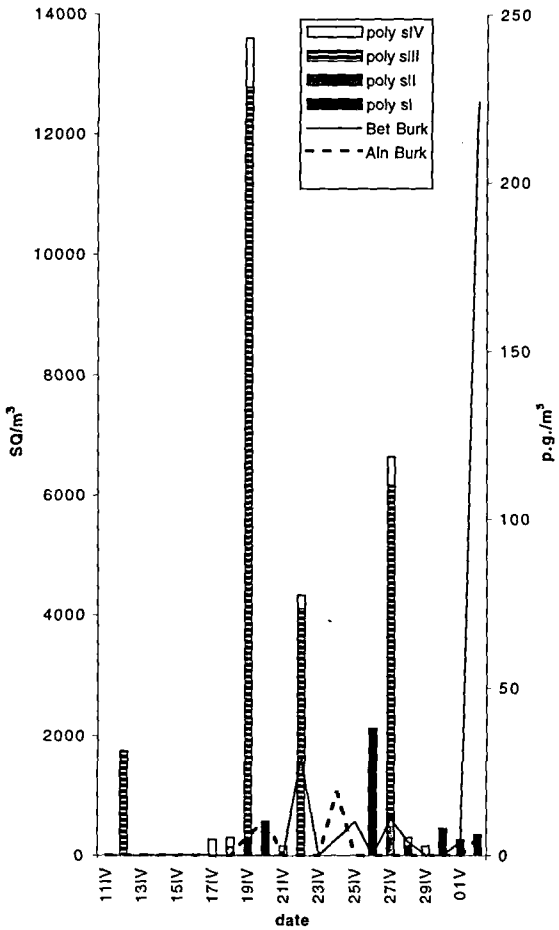


Fig. 1. Antigenic activity (SQ units m^{-3} , bars) in IgG-ELISA analyses with Pab against *Betula* pollen. Four particle sizes, see Table I. Airborne alder and birch pollen counts m^{-3} of air in Burkard samples (pollen grains, lines) in spring 1996.

a better understanding of the causes of early symptoms before the actual pollen season, airborne allergens were studied in pre-season periods using polyclonal antibodies (Pab) against birch pollen antigens and monoclonal antibodies (Mab) against Bet v 1. Using a particle size fractionating sampling method (Kauppinen et al. 1989), allergen concentrations were analysed separately in different particle sizes, including those where orbicules and starch granules might occur.

MATERIALS AND METHODS

Particle samples were collected in the outdoor air between April 11 and May 2 1996, before and during the flowering of alder (very low scores in 1996 compared to the average; see Finnish Pollen Bulletin 21, 1996) and thus also before and during the start of the birch pollen season. The size-selective bioaerosol sampler, SSBAS, was operating nearly continuously for 20–23 hours each day. The sampling height was 15 m above ground level and the air intake volume was 18.5 l/min. The sampler divides particles into four size classes by aerodynamic diameter: stage I $>7.2 \mu\text{m}$, stage II

BET V 1 1996

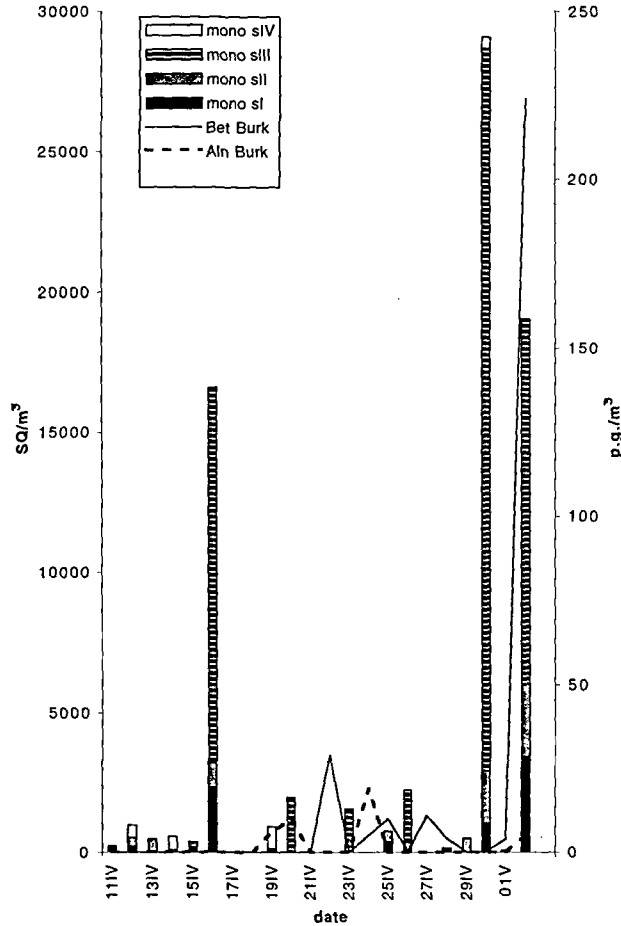


Fig. 2. Antigenic activity (SQ units m^{-3} , bars) in IgG-ELISA analyses with Mab against Bet v 1. Airborne alder and birch pollen counts m^{-3} of air in Burkard samples (pollen grains, lines) in spring 1996.

7.2–2.4 μm , stage III $<2.4 \mu\text{m}$ and stage IV submicronic antigen-bearing particles in a condenser unit (Pehkonen & Rantio-Lehtimäki 1994). The first three samples are on filters, the fourth is in condensation liquid.

Filter discs (0.5 cm in diameter, three duplicates) were immersed in 125 μl sodium carbonate buffer (pH 9.5) in microwells and incubated overnight at 37°C. The plates were washed with phosphate-buffered saline, PBS (3 times, pH 7.4, 200 μl /well) and incubated on a shaker in PBS-BSA (0.5% BSA, bovine serum albumine) for one hour at RT (room temperature, ca 20°C). The condensed samples from stage IV were lyophilized, dissolved in approximately 5 ml sodium carbonate buffer and lyophilised again in a tube. Before analyse the samples were dissolved in 3 ml sodium carbonate buffer; 125 μl aliquots were treated in the same way as the elutions of the filter discs.

The bound birch antigen was detected with Pab antibodies against *Betula* pollen produced in rabbit (ALK A/S, Copenhagen, Denmark, dilution of 1:1 000, 125 μl /well) and Mab against Bet v 1, likewise produced in rabbit (ALK A/S). The incubation time was 2 h at RT. HRP (horseradish peroxidase)-conjugated swine antibodies against rabbit IgG (DAKO A/S, Copenhagen) (1:1 000 dilution in PBS-BSA) were added and incubated 1 h at RT. The bound HRP-conjugate was determined using o-phenyldiamine substrate diluted

Table I. Allergenic activity expressed as percentages of the total activity, maximum values and standard deviations in SQ units in IgG-ELISA analyses in different particle size classes.

Stages I-IV (stage I >7.2 µm, II 2.4–7.2 µm, III <2.4 µm) in SSBAS with polyclonal antibody, Pab and monoclonal antibody, Mab against Bet v I. Total activity is in SQ units m⁻³ of air; n=number of samples.

	sI, <7.2 µm	sII, 2.4–7.2 µm	sIII, <2.4 µm	sIV, molecular	tot SQ unit
Pab	14%	3%	76%	7%	
max SQ unit	2121	633	12,493	814	31,256
stand dev	464	140	2996	218	
Mab Bet v I	11%	10%	76%	3%	
max SQ unit	3447	2562	25,744	788	75,447
stand dev	865	659	6439	238	
n Pab/Mab	22/22	22/22	22/22	18/17	84/83

Table II. Spearman correlation coefficients for each variable: Stages (sI–sIV): stage I >7.2 µm, II 2.4–7.2 µm, III <2.4 µm, IV molecular size.

Air samples analysed with Pab and Mab against Bet v I, pollen counts of alder (=Aln Burk) and birch (=Bet Burk). Burk = Burkard trap; precip = precipitation.

	n	sIPab	sIIPab	sIIIPab	sIVPab	Aln Burk	sIMab	sIIMab	sIIIMab	sIVMab	BetBurk
sIPab	22	1.000									
sIIPab	22	-0.211	1.000								
sIIIPab	22	-0.059	0.297	1.000							
sIVPab	18	-0.018	0.375	*0.553	1.000						
Aln Burk	22	0.416	-0.148	0.108	0.133	1.000					
sIMab	22	0.222	-0.272	-0.049	-0.415	0.011	1.000				
sIIMab	22	0.138	-0.272	-0.255	*-0.496	-0.133	*0.503	1.000			
sIIIMab	22	*0.569	-0.191	-0.282	-0.485	0.210	0.333	*0.476	1.000		
sIVMab	17	0.278	-0.187	0.397	-0.052	0.131	0.376	0.205	-0.027	1.000	
Bet Burk	22	0.048	0.167	0.245	0.203	0.211	0.145	-0.078	-0.122	-0.236	1.000
precip	22	0.017	0.158	-0.024	0.065	0.246	0.209	0.016	-0.078	-0.236	***0.721

in citrate buffer (pH 5.5), 75 µl/well, incubated for 30–45 min. The colour development was stopped by the addition of 125 µl 1M HCl. Optical absorbance was measured at 492 nm with a Multiscan[®] microplate reader (Flow Laboratories Inc.). Birch pollen extract of 100,000 SQ (standard quality) units/ml (ALK A/S, Copenhagen, Denmark (Arntzen et al. 1987)), in dilutions of 1:5000, 1:10,000 and 1:100,000, was used as the standard. The antigenic activities of each stage were converted to correspond to SQ units m⁻³ of air.

For comparison, airborne pollen counts of birch and alder (daily averages of pollen grains per cubic metre of air) were obtained from a continuously operating Burkard trap (Burkard, Rickmansworth, UK) situated at 5 meters distance from the SSBAS.

RESULTS

Mab against Bet v I showed clearly higher activities in SQ units than Pab (Table I, Figs. 1. and 2.). The difference was, in general, about two to four-fold. Allergen activity measured with Mab was already relatively high four days before the onset of the local alder pollen season. As no pollen had been trapped at the beginning of study period before the local alder pollen season, long-distance transported pollen could not be the cause of this rise in allergenic activity.

Bet v I activities were very high in stage III for particles below 2.4 µm in diameter (76% of total Bet v I activity, Table I). This stage also had the highest maxima of Bet v I concentrations (Table I).

Total antigen activity including that of other antigens

besides Bet v I was particularly high in stage III representing exactly the same percentage, 76% of total activity (Table I).

Bet v I activity in stage II was detected throughout the whole study period, being dominant at the beginning of birch flowering. The orbicule size class (2–4 µm) for example is caught primarily in this fraction.

Large particles with Bet v I activity (>7.2 µm) were trapped throughout the sampling period (11% of total). With Pab the percentage was 14% of total antigenic activity. This size fraction covers intact pollen grains. Pollen grains are effectively washed down in the rain, as is also indicated by the positive correlation between the pollen counts in Burkard samples and the amount of precipitation (Table II).

Antigenic activity detected using Pab varied through the study season, and daily concentrations did not always inter-correlate with airborne pollen counts. Spearman correlation coefficients are shown in Table II. Correlations between allergenic activities in different size fractions analysed by Pab and Mab did not show any obvious logic (Table I).

DISCUSSION

The findings of 76% of total activity in the small particles <2.4 µm may indicate that these small particles are from dehiscing anthers and other parts of Betulaceae trees. The highest peaks of small particle sizes were obtained just before

(Pab) and at the beginning (Mab) of the season when birch pollen grains were recorded in the air samples. The polyclonal marker used in this study does not differentiate between Aln g 1 and Bet v 1. The peaks shortly preceding the start of alder pollen release and during it are most probably due mainly to Aln g 1 and cross-reactivity between main allergens, which has been shown to be high (Ipsen et al. 1991, Ipsen et al. 1992, Ebner et al. 1993, Ramirez et al. 1997).

Due to the high identity (60–70%) and higher degree of sequence homology between the major pollen allergens in the order Fagales (Ipsen et al. 1992), not only Aln g 1 but also Cor a 1 has been detected rather well by the analysis. Mab against Bet v 1 (1 µg/ml) in ELISA can pick up the other main pollen antigens of trees in the order Fagales as follows: *Betula verrucosa* 9.5%/total pollen proteins, *Alnus glutinosa* 6%, *Corylus avellana* 4%, *Carpinus betulus* 0.7%. For any other allergen tested the specificity was less than <0.0001% (Ramirez et al. 1997).

The release of antigenic and allergenic particles other than pollen grains, into the air seems to be a very common phenomenon; and so the pollen information service really needs the equipment and methods to detect these particles as well as intact pollen. It is important to inform allergy sufferers about increasing concentrations. A particle size selective sampler is not obligatory, but a quick immunochemical method should be available, using an inexpensive, commercial, possibly recombinant monoclonal antibody for each important major plant allergen. People who are allergic have much more use for information regarding the level of allergen concentrations in the air than they do for precise pollen counts, but first they need to learn to interpret the new information which is available. It is not yet possible to give threshold values for low, medium and high allergen concentrations in the same way as is commonly done for pollen counts. It is possible to provide information of when allergens are first observed and whether their concentration is increasing or decreasing. It is important to produce commercial immunochemicals, which are satisfactorily standardized for quantitative measurement of airborne allergens as soon as possible.

In the experience of the present authors monitoring of airborne allergens in Scandinavia has to be carried out from February onwards, because in very warm winters alder and hazel may start flowering in February or at least start releasing allergens in the small particle sizes. Both events require a temperature above zero on a number of consecutive days. Both Pab against *Betula* pollen and Mab against Bet v 1 are useful tools for monitoring the outdoor allergen concentration for information purposes.

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