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Release of Bet v 1 from birch pollen from 5 European countries. Results from the HIALINE study

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A B S T R A C T

Exposure to allergens is pivotal in determining sensitization and allergic symptoms in individuals. Pollen grain counts in ambient air have traditionally been assessed to estimate airborne allergen exposure. However, the exact allergen content of ambient air is unknown. We therefore monitored atmospheric concentrations of birch pollen grains and the matched major birch pollen allergen Bet v 1 simultaneously across Europe within the EU-funded project HIALINE (Health Impacts of Airborne Allergen Information Network).

Pollen count was assessed with Hirst type pollen traps at 10 l min^{-1} at sites in France, United Kingdom, Germany, Italy and Finland. Allergen concentrations in ambient air were sampled at 800 l min^{-1} with a Chemvol® high-volume cascade impactor equipped with stages PM > 10 μm, 10 μm > PM > 2.5 μm, and in Germany also 2.5 μm > PM > 0.12 μm. The major birch pollen allergen Bet v 1 was determined with an allergen specific ELISA. Bet v 1 isoform patterns were analyzed by 2D-SDS-PAGE blots and mass spectrometric identification. Basophil activation was tested in an FcεR1-humanized rat basophil cell line passively sensitized with serum of a birch pollen symptomatic patient.

Compared to 10 previous years, 2009 was a representative birch pollen season for all stations. About 90% of the allergen was found in the PM > 10 μm fraction at all stations. Bet v 1 isoforms pattern did not vary substantially neither during ripening of pollen nor between different geographical locations. The average European allergen release from birch pollen was 3.2 pg Bet v 1/pollen and did not vary much between the European countries. However, in all countries a >10-fold difference in daily allergen release per pollen was measured which could be explained by long-range transport of pollen with a deviating

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allergen release. Basophil activation by ambient air extracts correlated better with airborne allergen than with pollen concentration.

Although Bet v 1 is a mixture of different isoforms, its fingerprint is constant across Europe. Bet v 1 was also exclusively linked to pollen. Pollen from different days varied >10-fold in allergen release. Thus exposure to allergen is inaccurately monitored by only monitoring birch pollen grains. Indeed, a humanized basophil activation test correlated much better with allergen concentrations in ambient air than with pollen count. Monitoring the allergens themselves together with pollen in ambient air might be an improvement in allergen exposure assessment.

1. Introduction

Allergies are the most prevalent chronic diseases in Europe with an >20% prevalence (Bauchau and Durham, 2004; Sunyer et al., 2004; Bousquet et al., 2007). Allergic diseases to airborne allergens have been steadily increasing over the past decades (Eder et al., 2006). This increase in prevalence is also due to replacement of older less sensitized individuals in the population by younger individuals with a higher degree of sensitization (Jarvis et al., 2005; Rönmark et al., 2009; Laatikainen et al., 2011). For developed countries a leveling-off of allergic disease prevalence was reported (Asher et al., 2006; Grize et al., 2006), but for some highly developed countries like Finland an end of this epidemic is not in sight (Laatikainen et al., 2011).

Allergen exposure determines sensitization (Olmedo et al., 2011) and allergy symptoms correlate with exposure (Corrigan et al., 2005; Brito et al., 2011). Exposure to outdoor airborne allergens is monitored by determining the concentration of pollen in ambient air with EAN (European Aeroallergen Network) database, a network of over 350 pollen traps spread over Europe (www.ean-net.org, accessed January 2012). However, humans react to the allergen and the concentration of airborne pollen (the pollen count) is a proxy of exposure. Indeed, several investigations imply that the pollen count might not be representative for allergen exposure, also because allergen was found in non-pollen bearing fractions of ambient air (Schäppi et al., 1997b; De Linares et al., 2010; Fernandez-Gonzalez et al., 2011).

Pollen grains release more immunologically active compounds than only allergen, like PALMS, adenosine and NADPH oxidase (Dharajiya et al., 2007; Gilles et al., 2009, 2011). These compounds can act as adjuvants, however the allergen from pollen is the dominant factor for evoking symptoms (Brito et al., 2011).

Almost all patients allergic to birch pollen are allergic to Bet v 1, sometimes accompanied by a sensitization to Bet v 2 or Bet v 4 (Moverare et al., 2005). The Bet v 1 content of birch pollen is not constant (Buters et al., 2010), and geographical variation was described (Buters et al., 2008). Climate change with increasing concentrations of CO_2 results in higher pollen production as CO_2 is both an airborne fertilizer and a greenhouse gas. Changes in allergen release per pollen would be in addition to the changed load of airborne pollen (Estrella et al., 2006; Rogers et al., 2006; Shea et al., 2008; Ziska and Beggs, 2012). We therefore determined the variation in the release of the major birch pollen allergen Bet v 1 with an immunochemical ELISA method in the project HIALINE (Health Impacts of Airborne Allergen Information Network) and confirmed this independently in selected cases with a bio-assay using FcεR1-humanized rat basophils. We evaluated whether the used methods, Chemvol® and ELISA, were suited for an allergen-release measuring network. We also investigated whether meteorological factors could govern allergen release from pollen, in an effort to predict the effect of climate change on the allergenicity of pollen.

2. Materials and methods

2.1. Pollen count

Airborne concentrations of pollen were sampled with volumetric spore traps of the Hirst design (Hirst, 1952) and examined by light microscopy as described before (Smith et al., 2009). Pollen counting methods vary historically between countries and three different methods were included in this study. Three stations used a technique where slides were examined along three (France) or four (Germany and Italy) longitudinal transects (Sikoparija et al., 2011). In the UK pollen grains were counted along twelve latitudinal transects (Smith et al., 2009). The analysis method used in Finland is random sampling of microscopic fields which has been shown to give parallel results to the counts carried out with the two other methods (longitudinal and latitudinal transects) (Mäkinen, 1981). Difference between methods was eliminated by correction for surface counted (Comtois et al., 1999). Pollen counts for the 10-years average were obtained from the EAN (European Aeroallergen Network, https://ean.polleninfo.eu, accessed January 2012). The pollen season was calculated as described in the legend of Table 1. The pollen index, the total exposure to pollen from one season at

Table 1
Characterization of the different stations in 2009 (unless stated otherwise).

	UK	France	Germany	Finland	Italy
Pollen-flight					
Maximum birch pollen count (grains/m^{-3})	278	622	495	253/250	31
Day of highest peak	15.04	14.04	11.04	27.04/16.05	8.4
Cumulative annual sum (Σ grains m^{-3})	2586	1705	3144	2923	235
Average sum (1999-2009) (Σ grains m^{-3})	3103	1724	6848	10,160	773
Season length days[a]	24	28	19	30	48
Bet v 1					
Maximum Bet v 1 (pg Bet v 1 m^{-3})	755	986	2299	978	82
Cumulative Bet v 1 (Σ pg Bet v 1 m^{-3})	5969	7046	13,030	7634	681
Bet v 1 per pollen (pg Bet v 1/pollen)[b]	2.260	3.057	3.914	2.617	2.569
Temperature (°C)[c]					
Average	9.0	11.1	7.9	4.2	13.7
Average daily min	5.8	5.8	3.5	−0.03	8.9
Average daily max	13.9	16.6	12.8	8.9	18.6
Days above 2 °C[d]	28	28	27	24	28
Days below 0 °C	0	0	1	1	0
Humidity (%)[c]	71.2	68.2	68.4	73.5	60.8
Solar radiation at surface (MJ m^{-2})[c,e]	349.8	435.2	403.9	422.8/548.7	414.7
Precipitation (mm day^{-1})[c]	0.49	0.52	1.68	0.40	10.4

[a] Season was defined as 1% till 95% of all birch pollen for that season, according the recommendations of the European Aeroallergen Network (EAN.polleninfo.eu).
[b] From linear regression.
[c] During the 4 weeks before birch pollen peak.
[d] Birch flower growing days, after van Vliet, Int. J. Climatol. 22, 1757–67, 2002.
[e] Extracted from ECMWF archive of operational forecasts.