Environmental Technology & Innovation 5 (2016) 60–66

Contents lists available at ScienceDirect

Environmental Technology & Innovation

journal homepage: www.elsevier.com/locate/eti

PM10 contains particle-bound allergens: Dust analysis by Flow Cytometry

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HIGHLIGHTS

- Flow Cytometry can be used to quantify antigen-loaded particles.
- The major birch pollen antigen Bet v 1 is bound to PM10 particles.
- PM10 samples from high pollen season contain significantly higher proportions of Bet v 1 positive particles than PM10 samples from low pollen season.
- PM10 from high pollen season but not from low pollen season induces basophil activation in a pollen allergic proband.

ARTICLE INFO

Article history:
Received 8 December 2014
Received in revised form 6 October 2015
Accepted 3 January 2016
Available online 6 January 2016

Keywords:
Basophil activation
Bet v 1
Birch pollen antigen
Flow Cytometry
Particulate matter
PM10

ABSTRACT

Exposure to inhalable particulate matter (PM10, particle size $\leq$10 μm in diameter) can be associated with a number of adverse health effects such as airway inflammation and aggravation of asthma. Asthma can be triggered amongst others by birch pollen allergens like Bet v 1, but due to their size whole birch pollen cannot enter the lower respiratory tract. However, PM10 may act as carrier of adsorbed allergens and we speculated that Flow Cytometry, a method widely used to characterize suspended solids >0.5 μm in diameter, can be used to quantify these allergen-loaded particles. We show here, that the major birch pollen antigen Bet v 1 is bound to PM10 particles and that PM10 samples from high pollen season contain significantly higher proportions of Bet v 1 positive PM10 > 0.5 μm particles than PM10 samples from low pollen season. This difference may be of biological relevance as PM10 from high pollen season but not from low pollen season induced basophil activation in a pollen allergic proband. These findings indicate that ambient particles can transport adsorbed allergens into the lower respiratory airways where they could cause allergic sensitizations or trigger allergic reactions. Furthermore, Flow Cytometry adds to the list of tools for PM10 characterization.

1. Introduction

Air pollution is being discussed as an environmental cause of the increased incidence and severity of respiratory allergies observed in recent decades (Cakmak et al., 2012; Barraza-Villarreal et al., 2008; Chen et al., 2012). In this regard, the distribution of common allergens in dust came into scientific focus (Cyprowski et al., 2013; Brough et al., 2013a). The exact impact of airborne pollen in the triggering of asthma still needs to be elucidated. Patients with nasobronchial allergy showed

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http://dx.doi.org/10.1016/j.eti.2016.01.004
2352-1864/

sensitizations towards animal, mite, grass and tree pollens in varying degrees as demonstrated in several studies (Warm et al., 2015; Toppila-Salmi et al., 2015; Balaban et al., 2014) and in a Swedish cohort study exposure to grass pollen was associated with a reduced forced expiratory volume in children, although no associations were found for birch or other pollen exposures (Gruzieva et al., 2015). Furthermore, thunderstorm-associated asthma underlines the allergenic potential of pollen allergens (D'Amato et al., 2015). In Europe, Birch pollen is one of the main triggers of hay fever and can also be an important cause of allergic asthma (Galli et al., 2008; World Health Organisation, 2003; Wuthrich et al., 1995).

Due to their size (22 μm in diameter) birch pollen are unlikely to be found in the PM10 fraction of particulate matter or in the lower respiratory airways. However, it has been demonstrated that birch pollen rupture can release an aerosol of allergen-containing particles (Grote et al., 2003) that range in size from 30 nm to 4 μm (Taylor et al., 2004). Furthermore, combustion particles in ambient air can act as carriers of adsorbed allergens (Namork et al., 2006).

So far, Bet v 1 allergen content in ambient air samples has been studied by extracting the respective allergen and subsequent quantification with ELISA (Schappi et al., 1997; Buters et al., 2010, 2012): Bet v 1 could be detected in the PM $<$ 10 μm fraction, but the vast majority of the measured allergen was found in the PM $>$ 10 μm fraction (Buters et al., 2012). In these studies the dissolved allergen was quantified after extraction from the sampling device and thus no information about the quantity of particle-bound allergens was available. Using scanning electron microscopy, particle-bound antigens could be visualized (Namork et al., 2006; Ormstad et al., 1998), but according to our knowledge there is no commercially available or scientifically reported method to quantify the proportion of allergen-loaded particles.

Flow Cytometry is a powerful method to investigate suspended solids larger than 0.5 μm. Therefore we hypothesized that this method can be used to quantify the proportion of antigen-positive, namely Bet v 1- positive, particles in the $>$ 0.5 μm fraction of PM10, further referred to as $PM10_{>0.5}$.

2. Material and methods

2.1. PM10 sampling

Between May 2012 and December 2013, nineteen PM10 samples were collected in- and outside of the Federal Environment Agency building, situated in a suburb of Berlin. Six PM10 specimens were sampled within low pollen season in autumn/winter and thirteen within high pollen season in May. For the *in vitro* spiking experiments, other six PM10 samples were collected within low pollen season in autumn/winter 2013/2014. Additionally, in 2015, 12 PM10 samples were collected with running times of 24 h, 48 h and 72 h respectively.

PM10 particles were collected on Teflon filters (Pall Life Sciences, USA, R2PL047, 47 mm, 1.0 μm) using a low volume sampler (Sven Leckel Ingenieur Büro GmbH, Germany, LVS3) with an air flow of 2.3 m^3/h. Running time was between 4 and 9 days if not stated otherwise. The filters were frozen at −20 °C until used.

2.2. PM10 removal and particle count

The filters were overlaid with 4 ml PBS (Phosphate-buffered saline, Biochrom, Germany, without Ca/Mg, low endotoxin) and brushed with an electrical toothbrush (Braun, Germany, Oral-B Vitality Sensitive) for 1 min. The concentration of PM10 particles greater than 0.7 μm was measured using a Casy cell counter (Schärfe System GmbH, Germany, Model TTC; range of detectable particle size: 0.7–45 μm).

2.3. Flow cytometric analysis

Flow cytometric analysis was done on a FACSCanto II (Becton Dickinson, USA) and the data was evaluated with FACS Diva Software v6.1.3 (Becton Dickinson). In all PM10 measurements, the forward scatter (FSC) threshold was set at the lowest value (200) and at least 10.000 particles were examined.

2.3.1. Scatter analysis

50 μl of PM10 suspension in PBS (8 × 10e6 particles $>$0.7 μm per ml) were coanalyzed together with: (i) BD Cytometer Setup and Tracking beads (Becton Dickinson, Lot: 22680), (ii) blank calibration particles (BD Biosciences, Lot: 63100) and (iii) peripheral blood mononuclear cells (PBMC) isolated from heparinized whole blood from a healthy donor by use of Ficoll (Ficoll Paque Plus, GE Healthcare Bio-Sciences AB, Sweden) density gradient centrifugation.

2.3.2. Bet v 1 staining

50 μl of PM10 suspension (8 × 10e6 particles $>$0.7 μm per ml, suspended in PBS plus 0.02% bovine serum albumin (BSA) (Sigma–Aldrich, USA)) were incubated with a monoclonal mouse IgG1 antibody against Bet v 1 (Indoor Biotechnologies, UK, clone MA-3B4) at a final concentration of 0.02 μg/μl for 60 min. After two washing steps the APC (Allophycocyanin)-labeled secondary anti-Mouse IgG1 antibody (Becton Dickinson, Lot: 2223986) was applied as described by the manufacturer. After another two washing steps the samples were analyzed for APC staining.