Introduction

In the last decades the number of people with allergies against at least one of the major allergens like birch, grass, ragweed pollen or domestic cat steady increases [1]. There are various investigation methods available with which new insights were gained regarding the allergy potential, the concentration or position of specific allergens at various sample types. A more sophisticated method is immunogold electron microscopy, which enables to locate specific proteins which are responsible for an allergic reaction. Because of their high spatial accuracy and the used small gold particle size (approximately 10 – 60 nm), this method is typically applied for transmission electron microscopy (TEM) investigation [2]. The investigations presented were performed to obtain information about the presence of specific allergens at particles deposited at fine dust filters. Because of the sample size they have to be examined in a scanning electron microscope (SEM). Unfortunately, several examinations have shown that typical TEM labelling protocols are not directly transferable for SEM investigations. Therefore the immunogold labelling protocol was adopted to enable labelling at the samples under investigation. The so developed strategy involves the extraction of the particles with subsequent labelling by the obtained protocol).

Methodology and results

A Particle extraction

Particles were collected on the bases of either a) hour or b) diurnal mean on fine dust filters. To enable the immunogold labelling investigation has been shown, that the particles have to be extracted from the filters. Therefore two different strategies were found: For the first particles were extracted by c) immersing the filter into liquid, ultrasonic it for about 5 min and subsequently evaporate the liquid on a carbon tape, see e). The second strategy d) uses compressed air to directly extract particles from the upside down clamped filter onto the tape, see f). Results show that the extraction efficiency is higher for the ultrasonic strategy, but a possible germination of possibly deposited pollen by contact with liquids can be neglected for the second strategy.

B Immunogold labelling protocol

By an iterative sample preparation process a labelling protocol was successfully achieved. At first the prepared sample underwent an fixation/immobilisation step by the usage of bovine serum albumin (BSA), to fix the proteins under investigation at its initial position. Subsequently, a blocking step with glutaraldehyde (GA) was performed to suppress a unwanted labelling of background. The labelling of the allergens of interest is performed by using appropriate monoclonal primary antibodies, which are already covalent bound to the secondary antibody and thus gold-nanoparticle. After final washing steps this samples then can be directly investigated in the environmental SEM (ESEM).

C Results, discussion and outlook

Initially the obtained labelling protocol was tested at cryo-milled birch and grass pollen for identifying the presence of the major grass pollen allergen Phl p5. As shown in Img. 1 a high signal (immunogold concentration) can be found at the cryo-milled grass pollen at a), while only a small signal was detected at the appropriate birch pollen at b), representing the background labelling and specificity of the used primary antibody.

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Contact

www: www.felmi-zfe.at
e-mail: manfred.nachtnebel@felmi-zfe.at