Science of the Total Environment 541 (2016) 365–371

Contents lists available at ScienceDirect

Science of the Total Environment

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

Impact of urban air pollution on the allergenicity of *Aspergillus fumigatus* conidia: Outdoor exposure study supported by laboratory experiments

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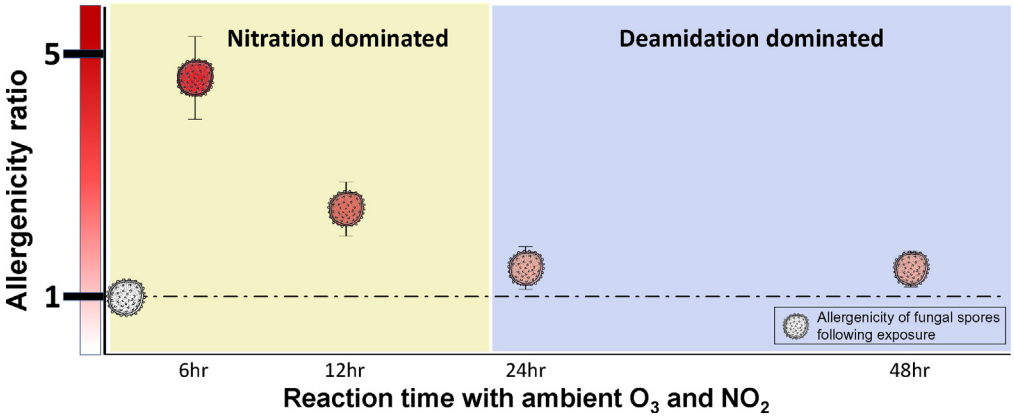
HIGHLIGHTS

- Short-term exposure to urban air pollution increases *A. fumigatus* allergenicity.
- The effect is most significant during the first hours due to nitration of proteins.
- At longer exposure time allergenicity reduces, possibly due to protein deamidation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 July 2015
Received in revised form 10 September 2015
Accepted 13 September 2015
Available online xxxx

Editor: D. Barcelo

Keywords:
Allergenicity
Urban air pollution
Fungal spore

ABSTRACT

Understanding the chemical interactions of common allergens in urban environments may help to decipher the general increase in susceptibility to allergies observed in recent decades. In this study, asexual conidia of the allergenic mold *Aspergillus fumigatus* were exposed to air pollution under natural (ambient) and controlled (laboratory) conditions. The allergenic activity was measured using two immunoassays and supported by a protein mass spectrometry analysis. The allergenicity of the conidia was found to increase by 2–5 fold compared to the control for short exposure times of up to 12 h (accumulated exposure of about 50 ppb NO_2 and 750 ppb O_3), possibly due to nitration. At higher exposure times, the allergenicity increase lessened due to protein deamidation. These results indicate that during the first 12 h of exposure, the allergenic potency of the fungal allergen *A. fumigatus* in polluted urban environments is expected to increase. Additional work is needed in order to determine if this behavior occurs for other allergens.

Abbreviation: PTM, post-translational modifications; HLA-DR, human leukocyte antigen class II; PDA, potato-dextrose agar; PBS-T, phosphate buffered saline with 0.05% tween 20; MCE, mixed cellulose esters; HEPA, high efficiency particulate air; BCA, bicinchoninic acid assay; SAFS, severe asthma with fungal sensitizations; ABPA, allergic bronchopulmonary aspergillosis; RH, relative humidity; DA, deamidation.

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http://dx.doi.org/10.1016/j.scitotenv.2015.09.058
0048-9697/

Aspergillus fumigatus
Allergenic protein
Protein modification

1. Introduction

Prevalence of allergies in children and adults is increasing worldwide (Asher et al., 2006; Nicolaou et al., 2005; Sicherer and Sampson, 2014; Wong et al., 2013; Zhang and Zhang, 2014). Several recent studies have linked the increased occurrence of allergies and asthma cases to higher susceptibility due to increased exposure to urban pollutants (Gruzieva et al., 2012; Kim et al., 2011; Kim et al., 2013; Ko et al., 2007). The globally mounting urbanization is accompanied by increased traffic-related air pollution that is characterized by high concentration of pollutants such as nitrogen oxides, ozone, sulfate, and particulate matter. Some of these pollutants can adversely affect the immune system, and modulate airway diseases such as asthma by increasing the release of inflammatory mediators from bronchial epithelial cells (Bayram et al., 2001; Chuang et al., 2007).

An additional hypothesized pollution effect is the induction of increased prevalence of allergies and asthma, through chemical alteration of the airborne allergens during their transport in the lower troposphere (Franze et al., 2005; Gruijthuijsen et al., 2006; Karle et al., 2012; Untersmayr et al., 2010), possibly by post-translational modifications (PTM). Previous studies have shown that Bet v 1.0101, an allergenic protein originated from birch pollen, undergoes nitration by mixtures of NO_2 and O_3 (Franze et al., 2003; Gruijthuijsen et al., 2006; Walcher et al., 2003). This was further supported by kinetic studies (Shiraiwa et al., 2011) and by a report on the effect of surface-to-volume ratio on this process (Kampf et al., 2015). These allergenic proteins were then shown to increase allergen-derived human leukocyte antigen class II (HLA-DR)-associated peptides in human dendritic cells (Karle et al., 2012). In a later work, it was shown that an increase in the level of nitration (produced by tetranitromethane) leads to changes in the molecular structure of the protein (Ackaert et al., 2014).

Fungal molds are known to be one of the asthma inducing factors (Denning et al., 2013; Karjalainen et al., 2001). According to global models, over 6.5 million people have severe asthma with fungal sensitizations (SAFS) and about 4.8 million adults have allergic bronchopulmonary aspergillosis (ABPA), both caused frequently by *Aspergillus fumigatus* (Chaudhary and Marr, 2011; Denning et al., 2014; Denning et al., 2013).

In this study we investigated whether exposure to urban air pollution affects the common fungal allergen, *A. fumigatus* (Chaudhary and Marr, 2011; Simon-Nobbe et al., 2008), and studied the changes in the allergenicity of its asexual conidial (spores), which contain at least 23 potential allergenic proteins (IUIS, 2014). We hypothesize that urban air pollution can induce changes in the allergenic potency of common fungal allergens, on the basis of PTM. Using a unique ELISA bioassay that evaluates the efficiency of the IgE-antigen interaction (Low et al., 2011), we show that exposure of *A. fumigatus* spores to ambient air pollution significantly increases their allergenicity during the first hours of exposure to ambient urban air pollution. The effect weakens after longer exposure periods, and higher cumulative gas exposure. We support this finding with laboratory experiments and protein mass spectrometry analysis and discuss possible mechanisms to explain these results.

2. Materials and methods

2.1. Fungal growth and sample preparation

A. fumigatus (ATCC, #1022) was cultivated on potato-dextrose agar (PDA, Difco) amended with 100 mg/l chloramphenicol (Sigma-Aldrich), and incubated at 25 °C in the dark. After 10 days of growth, spores were harvested using flocked, nylon fiber tipped swabs (Copan Diagnostics, Brescia, Italy), and re-suspended in 50 ml phosphate buffered saline with 0.05% tween 20, pH = 7.4 (PBS-T, Sigma-Aldrich). The conidial suspension concentration was determined using a hemocytometer. Conidial samples were prepared by pipetting 2 ml of the conidial suspension (2×10^8 conidia/ml) on Mixed Cellulose Esters (MCE) sterile filters (Millipore, Billerica, MA, USA), using a vacuum system, and stored at 4 °C under low pressure conditions until required.

2.2. Exposure to air pollution

Outdoor exposure was conducted in central Israel, in the city of Modi'in (35.0092N, 31.9082E) at a certified and calibrated governmental air quality monitoring station. This area is downwind from the metropolitan area of Tel Aviv and experiences relatively high levels of secondary pollutants such as O_3 and NO_2 (Boersma et al., 2009). Permission to sample in this location was provided from the Israel Ministry of Environmental Protection http://www.sviva.gov.il.

The experimental setup, detailed in Fig. 1A, is based on pumping ambient air onto the samples using an environmental pump (AirCon-2, Sensidyne Gilian, FL, USA) located at the end of the flow line, with a controlled flow rate of 300 $cm^3\ min^{-1}$. After removal of particles using high efficiency particulate air (HEPA) cap filters (Whatman, WA, USA), the air was directed into a chamber in which the spore-containing filters were placed, allowing reactive gases to interact with the spores. Air was then directed through a carbon-cap filter (Whatman, WA, USA), adsorbing the reactive gases, and then into a control chamber, in which duplicate spore-containing filters were placed. Experimental chambers were placed in a dark box, preventing light from entering. To reduce pressure fluctuations, a bulb was placed between the controlled chamber and the pump. It was verified that the pollutants were absorbed and that the control sample was not exposed to the ambient pollution.

The outdoor concentrations of ambient pollutants for the sampling period were obtained from the Israel Ministry of Environmental Protection (http://www.svivaaqm.net/Default.rtl.aspx) and are shown in Fig. 2 and Table S1.

Three complementary experiments were conducted in the laboratory to expose the spores in four exposure treatments: 1) exposure to synthetic air composed of pure N_2 and O_2 (98% purity); 2) exposure to O_3; 3) exposure to NO_2; 4) exposure to synthetic air containing both O_3 and NO_2, as described in Fig. 1B. Ozone was produced from O_2 using a UV-light source (Ozone Generator, 500 Jelight Company Inc., Ervine, CA, USA), and diluted with N_2 to 50 ± 8 ppb (see Table 1). The concentration was measured using a commercial O_3 monitor (Model 1180, Dasibi Environmental Corp., Glendale, CA, USA). Nitrogen dioxide (86 ppm, Linde) was diluted to 47 ± 3 ppb with N_2. To simulate tropospheric conditions, additional O_2 was added to the flow, maintaining an O_2:N_2 ratio of 21:79%. Each experiment (both outdoor and laboratory) was conducted at 4 time-intervals of exposure: 6, 12, 24 and 48 h with triplicates. In control laboratory experiments, exposure only to the synthetic air did not lead to any significant difference between the samples in the exposure chamber compared to the control chamber.

2.3. Protein extraction procedure

After exposure to air pollution or controlled gas flow, proteins were extracted from the spores as described by Low et al. (2011). Briefly, glass beads were added to 2 ml PBS-T (Sigma Aldrich) suspensions and spores were lysed by bead-beating (Mini-Beadbeater-16, BioSpec)