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Inhalation of concentrated PM_{2.5} from Mexico City acts as an adjuvant in a guinea pig model of allergic asthma[☆]



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ABSTRACT

Exposure to Particulate Matter (PM) could function as an adjuvant depending on the city of origin in mice allergic asthma models. Therefore, our aim was to determine whether inhalation of fine particles (PM_{2.5}) from Mexico City could act as an adjuvant inducing allergic sensitization and/or worsening the asthmatic response in guinea pig, as a suitable model of human asthma. Experimental groups were Non-Sensitized (NS group), sensitized with Ovalbumin (OVA) plus Aluminum hydroxide (Al(OH)₃) as adjuvant (S + Adj group), and sensitized (OVA) without adjuvant (S group). All the animals were exposed to Filtered Air (FA) or concentrated PM_{2.5} (5 h/daily/3 days), employing an aerosol concentrator system, PM_{2.5} composition was characterized. Lung function was evaluated by barometric plethysmography (Penh index). Inflammatory cells present in bronchoalveolar lavage were counted as well as OVA-specific IgG1 and IgE were determined by ELISA assay. Our results showed in sensitized animals without Al(OH)₃, that the PM_{2.5} exposure (609 ± 12.73 µg/m³) acted as an adjuvant, triggering OVA-specific IgG1 and IgE concentration. Penh index increased ~9-fold after OVA challenge in adjuvant-sensitized animals as well as in S + PM_{2.5} group (~6-fold), meanwhile NS + FA and S + FA lacked response. S + Adj + PM_{2.5} group showed an increase significantly of eosinophils and neutrophils in bronchoalveolar lavage. PM_{2.5} composition was made up of inorganic elements and Polycyclic Aromatic Hydrocarbons, as well as endotoxins and β-glucan, all these components could act as adjuvant. Our study demonstrated that acute inhalation of PM_{2.5} acted as an adjuvant, similar to the aluminum hydroxide effect, triggering allergic asthma in a guinea pig model. Furthermore, in sensitized animals with aluminum hydroxide an enhancing influence of PM_{2.5} exposure was observed as specific-hyperresponsiveness to OVA challenge (quickly response) and eosinophilic and neutrophilic airway inflammation. Fine particles from Mexico City is a complex mix, which play a significant role as adjuvant in allergic asthma.

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1. Background

Asthma is a heterogeneous disorder characterized by airway inflammation (Flenley, 1986) and hyperresponsiveness, resulting in an exaggerated bronchoconstriction response (Postma and Kerstjens, 1998). This airway constriction is reversible, either spontaneously or in response to treatment (Flenley, 1986). The acute inflammatory response in allergic asthma involves IgE

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antibodies and Th2 cytokine production from activated lymphocytes (Lemanske and Busse, 2010). Some of the morphological changes that worsen asthma include airway remodeling, epithelium shedding, loss of ciliated cells, and goblet cell hyperplasia (Al-Muhsen et al., 2011). Epidemiological studies identify that >300 million persons suffer from this condition worldwide (Pawankar, 2014), with an estimated increase of 100 million more cases by 2025 (Bahadori et al., 2009). Human and animal studies suggest that air pollution could explain some of the increased asthma prevalence, but this process continues to remain unclear (Takizawa, 2011). In urban cities, we can find some gases (SO₂, NO₂, CO, CO₂, and Volatile Organic Compounds [VOC]) and a mixture of solids or liquid Particulate Matter (PM) (Brauer et al., 2002), which are ubiquitous pollutants suspended in the atmosphere (Curtius, 2006). PM originates from natural or anthropogenic sources. Natural sources include sea spray, volcanic eruptions, forest fires, geologic erosion, and aerobiological sources, such as animal dandruff, mites, pollen, bacteria, viruses, proteins, and carbohydrates (Curtius, 2006). Furthermore, urban anthropogenic sources emissions are mainly emissions from industrial activities and urban transportation (Calvo et al., 2013). In cities, the number of motor vehicles represents the main source of air pollution, including PM generated from the combustion of gasoline and diesel (Ulrich et al., 2012). PM can be inhaled, penetrating the respiratory tract, where they target different anatomical sites. Depending on its aerodynamic size, PM is categorized as PM₁₀ or coarse fraction, or as Fine Particles (FP) or PM_{2.5} (Nemmar et al., 2013). In the lung, particles are more easily deposited than gases (Shusterman, 2011) and can be adsorbed by the epithelium, inducing tissue damage (Zhang et al., 2001) by means of the production of Reactive Oxygen Species (ROS). Reported tissue damage includes lung endothelial-cell dysfunction, inflammation (Sancini et al., 2014), and lung cancer (Valavanidis et al., 2013), as well as decreased lung function (Möller et al., 2013). PM exposure also aggravates Chronic Obstruction Pulmonary Disease (COPD) (Valavanidis et al., 2013), pulmonary fibrosis, and allergic asthma (Jones and Richeldi, 2014). Some reports mention that Diesel Exhaust Particles (DEP) produce airway hyperresponsiveness in a dose-response manner (Li et al., 2007). Studies in mouse asthma models have supported the hypothesis that instilled PM_{2.5} fractions can induce or aggravate allergies, especially allergic asthma. Furthermore, Steerenberg et al. (2004) demonstrated that the instillation of PM collected in different European cities evoked adjuvant activity in a mouse model of allergic sensitization, which differed in severity according to city-of-origin (Steerenberg et al., 2004).

Thus, the aim of our study was to determine whether exposure to FP from Mexico City can induce an adjuvant effect on the airway hyperresponsiveness of allergic model in guinea pig, including changes such as bronchoconstriction, airway inflammation, and mucous metaplasia. The guinea pig model reproduces features of human asthma, such as airway hyperresponsiveness, inflammatory response, and bronchoconstriction better than any other rodent model.

2. Materials and methods

2.1. Locality

Mexico City is located in Valley of Mexico at 2240 m above sea level in a subtropical region (Velasco et al., 2005) surrounded by mountain ridges in its eastern, western, and southern sides with a broad opening to the north. As a result, mountain breezes occur and are frequently essential to local circulation. During daytime, the mountains heated up, producing up-slope winds from the city to the mountains. During nighttime, the mountains cool down,

resulting in strong down-slope winds from the mountains to the city (Tie et al., 2007). The city experiences mild weather, temperatures of >20 °C, and intense solar radiation all year (Velasco et al., 2005).

2.2. Animals

Thirty Hartley guinea pigs (weighing 450–500 g each) were maintained under conventional conditions [12 h/12 h light/dark cycles, filtered conditioned air (21 ± 1 °C; 50–70% humidity), and sterilized bedding] at our Institution's animal facilities. Animals were fed *ad libitum* with food pellets (Teklad Guinea Pig Diet 7006; Harlan Teklad, Madison, WI, USA) and drank sterilized water. The protocol was approved by the Scientific and Bioethics Committees of the Instituto Nacional de Enfermedades Respiratorias (INER) (approval #B17-12). Experiments were conducted according to the criteria of the Guiding Principles in the Care and Use of Animals published and approved by the American Physiological Society (APS). Guinea pigs were sensitized on day 1 by a single injection (1-mL) intraperitoneally (i.p.) (0.5-mL) and subcutaneously (s.c.) (0.5-mL) of 60 mg OVA₁ (OVA) (Sigma-Aldrich Co., St. Louis, MO, USA) as antigen and 1 mg of Aluminium hydroxide (Al(OH)₃) as adjuvant in 10 mL saline (0.9% NaCl). On days 8 and 15, the animals were nebulized with 3 mg/mL and 0.5 mg/mL OVA in saline during 5 min and 1 min, respectively. Aerosolized OVA was delivered utilizing an ultrasonic nebulizer (Model WH-802; Yuehua Medical Instrument Factory Co., Guangdong, China).

2.3. Particle exposure

The concentrator system is localized in the North of Mexico City, at CINVESTAV-IPN Zacatenco. Experiments were conducted during the rainy season in October 2013 (from 8 a.m. to 1 p.m.) during 3 days (1–3 October). The aerosol concentrator system is allocated within an enclosed laboratory that has a secondary barrier enclosure (Bio-Bubble Systems, Inc., Fort Collins, CO, USA). The bio-bubble barrier is a double air-lock entry system that is operated under negative pressure to reduce airborne particulates to Class 100 clean-room conditions; therefore, all conditions within the laboratory were controlled. Particle exposures were performed in whole-body chambers associated with this particle concentrator, where temperature and humidity were constant in each exposure chamber. The particulate concentrator had a particle cut-off size of 2.5 µm. To estimate particulate chamber concentration, we performed PM_{2.5} ambient air monitoring using 37-mm Teflon filters that were weighed and equilibrated (PTFE 2-mm pore; Gelman Science, Ann Arbor, MI, USA) in a Mini-Vol sampler (Air Metrics, Eugene, OR, USA) with a 5.0 L/min air flow: the air ambient monitoring period followed the same schedule as that of the animal exposures. Particulate concentration in the chambers was estimated using a 37-mm teflon filter (PTFE 2-mm pore; Gelman Science) allocated in a holder inlet that received a 2.5-L/min constant airflow, the same air flow supplied in each chamber. Body mass was considered between control and the PM_{2.5} group as air volume displacement; it was adjusted to 1.1 ± 0.2 kg per chamber at the beginning of each weekly experiment. Each chamber had a volume of 18 L/min.

2.4. Mass concentrator and inorganic analysis

Teflon filters inside the aerosol concentrator system and the Mini-Vol were weighed and equilibrated. During 3 days, the filters were stored at 4 °C and, at the end of the exposure, the filters were removed from cassettes or Mini-Vol, then weighed again to determine mass. Both the aerosol concentration system and the Mini-Vol

filters were used to determine PM_{2.5} inorganic composition was done by means of x-ray fluorescence following the procedure previously reported by [Espinosa et al. \(2012\)](#).

2.5. Polycyclic aromatic hydrocarbon analysis

Polycyclic Aromatic Hydrocarbons (PAH) were analyzed by Gas Chromatography-quadrupole Mass Spectrometry (GC-qMS) (model 6890 plus/5973N; Agilent Technologies, CA, USA) following a procedure developed by [Amador-Muñoz et al. \(2014\)](#) ([Amador-Muñoz et al., 2014](#)) and [Villalobos-Pietrini et al. \(2006\)](#) ([Villalobos-Pietrini et al., 2006](#)).

2.6. Endotoxin and glucan analysis

Endotoxins and (1-3)- β -D-Glucan were evaluated by a Pyrochrome, Kinetic Chromogenic Method (Associates of Cape Cod, Inc., East Falmouth, MA, USA). All PM samples were resuspended in pyrogen-free 50 mM Tris buffer (Lonza, Walkersville, MD, USA),

1 mg/mL, employing an UltraSound (US) water bath for 1 h at 22 °C, with 1-min intervals of agitation in the vortex during 15 min. Two serial 1:10 dilutions from each suspension were prepared. Control Standard Endotoxin (CSE) derived from *Escherichia coli* 0113:H10 (Lot 139; Associates of Cape Cod, Inc.) with a potency of 18 EU/ng and (1-3)- β -D-Glucan standard 200 pg/mL (Lot 1189027; Associates of Cape Cod, Inc.) was used as reference. The samples were analyzed in duplicate utilizing a pyrogen-free tube reader and incubated at 37 °C in the Pyros Kinetix Flex Instrument (Associates of Cape Cod, Inc.). Optical Density (OD) was recorded at a wavelength of 405 nm. The digitized data were transmitted from the instrument to a computer and analyzed with Pyros EQS ver. 1.2 software.

2.7. Experimental design

To evaluate the adjuvant effect of PM_{2.5} in the guinea pig asthma model, we followed the next experimental design: All animals inhaled FA or PM_{2.5} for 5 h daily during 3 days. Non-Sensitized (NS)

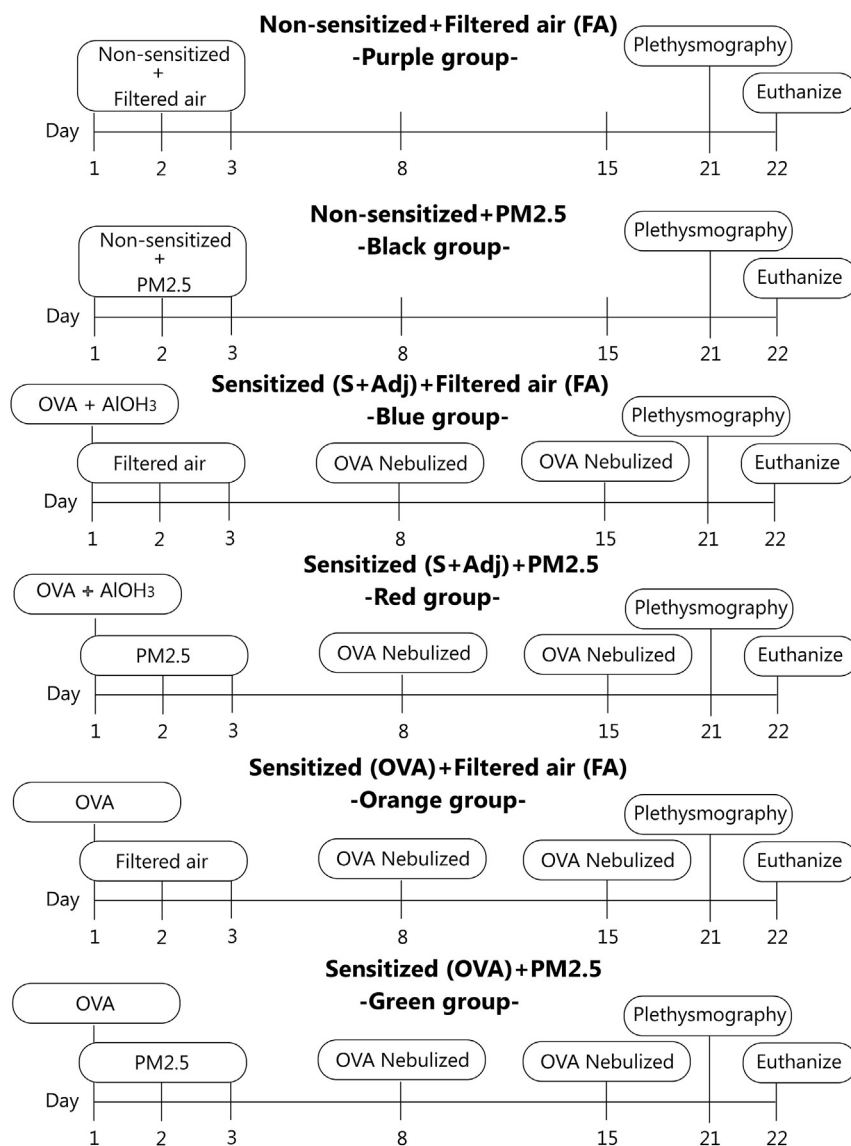


Fig. 1. Experimental design. Day 1, animals were sensitized with a mix of OVA + Al(OH)₃ or with Ovalbumin (OVA) alone. Also, they inhaled Filtered Air (FA) or Particulate Matter (PM)_{2.5} during 3 days: days 1, 2, and 3. On days 8 and 15, we applied OVA inhalation alone during 5 min and 1 min, respectively. On day 21, we performed the plethysmography test on all animals, and on day 22, we euthanized the animals. Two columns.

from the baseline value obtained prior to sensitization (100%). The result was reported as a percentage representing degree of hyperresponsiveness.

2.9. Bronchoalveolar lavage fluid

On day 22 after the OVA challenge, the animals were euthanized with a single injection (i.p.) of pentobarbital (35 mg/kg). The trachea was cannulated to administer 5 mL of saline solution (37 °C) by instillation twice. The recovered Bronchoalveolar Lavage Fluid (BALF) was centrifuged at 1500 rpm for 10 min at 4 °C. The supernatant was stored at −20 °C for the Enzyme-Linked Immunosorbent Assay (ELISA) assay. Cells were resuspended in 1 mL saline and stained with Trypan blue for total cell count using a Neubauer chamber (Hauser Scientific, Horsham, PA, USA). After, were added 1 mL of saline solution per each 1 000, 000 counted cells and we performed a bronchoalveolar lavage smear on a slide using a

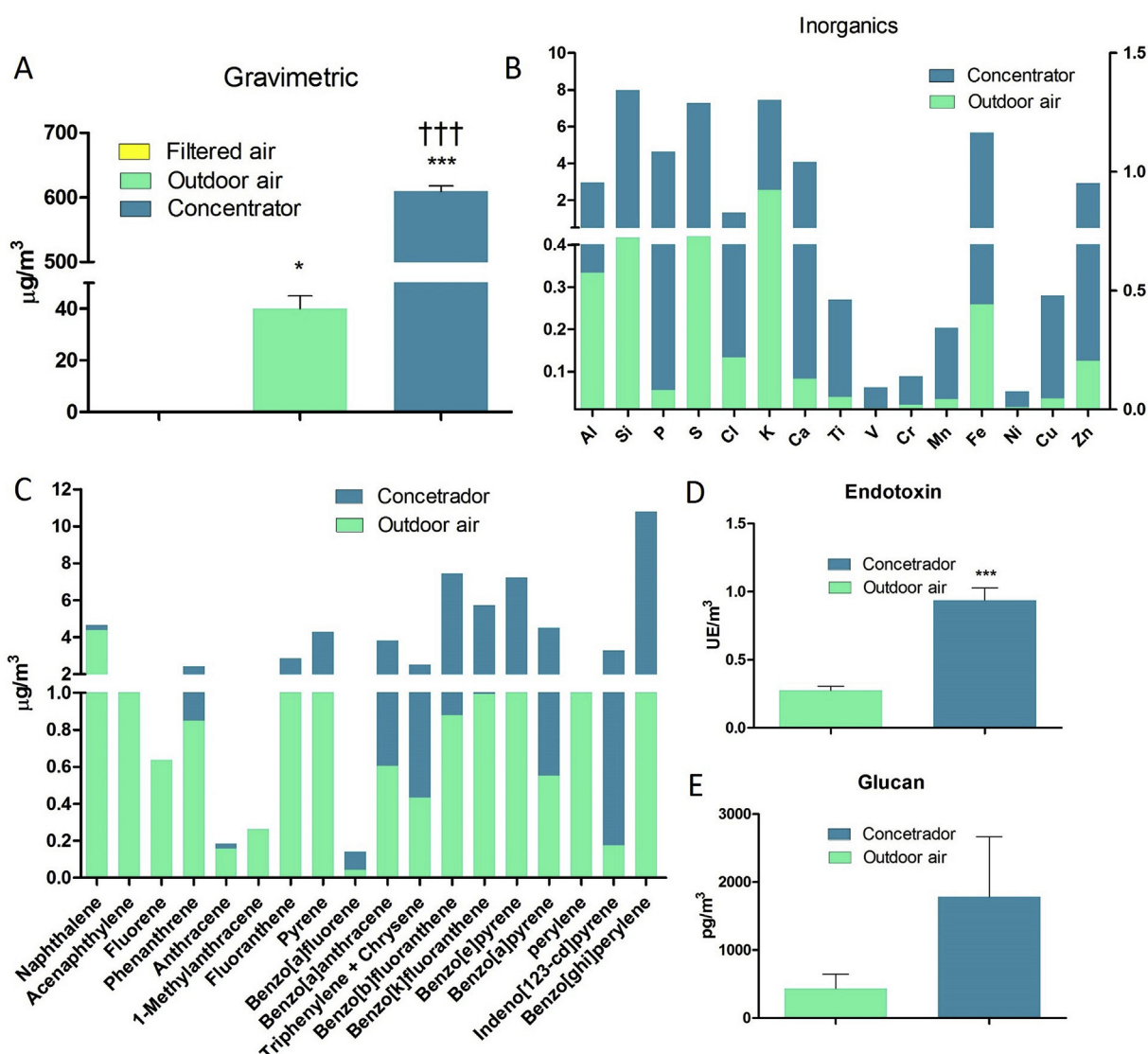


Fig. 2. PM_{2.5} mass, composition of outdoor air, and concentrated air samples during the study period. (A) During this time, the animals inhaled Filtered Air (FA), whereas animals exposed to fine Particulate Matter (PM) inhaled a total average 600 µg/m³ when outdoor PM_{2.5} was around 40 µg/m³. (B) Several elements present in the PM_{2.5} sample were concentrated at >1 µg/m³ (Al, Si, P, S, Cl, K, Ca, Fe, and Zn), whereas others were found at <0.5 µg/m³ (Ti, V, Cr, Mn, Ni, and Cu). (C) Only 14 Polycyclic Aromatic Hydrocarbons (PAH) of the 18 considered were identified as concentrated in the PM_{2.5} sample. Eleven PAH were concentrated at <2 µg/m³. Endotoxin (D) and glucan (E) were concentrated during this experiment. However, the only endotoxin exhibited differences. Values are reported as mean ± Standard Deviation (SD). Statistical analysis was performed using Student *t*-test (****p* < 0.001). Two columns.

cytochrome (Cyto-cent, Wescor, Inc., UT, USA). After this the slides were stained with Giemsa. Total cell count was expressed as cell/mL, and differential cell counts were expressed as a percentage of total cells.

2.10. Histological evaluation and morphometric analysis

After BALF, the lungs were fixed by instilling, intratracheally (i.t.) 10% buffered formalin and were immersed in the same fixative. The fixed tissue was paraffin-embedded and cut into 3.5- μ m sections. After this, the tissue was stained with Periodic Acid Schiff (PAS) for evaluation. We assessed goblet cell metaplasia by examining the bronchioles and counting the number of PAS-positive cells present in 20 bronchioles. Results are presented as percentage of PAS-positive cells of the total count of epithelial cells in each bronchiole.

2.11. ELISA assay

The stored supernatants were used to determine the presence of unspecific Immunoglobulin G (564155), specific-OVA IgG1 (746691), specific-OVA Immunoglobulin E (IgE) (701476), and CC16 protein (724602) (MyBioSource, San Diego, CA, USA) by ELISA utilizing duplicates and following the manufacturer's instructions.

2.12. Statistical analysis

Multiple group comparisons were analyzed using ANOVA (ANOVA) (Tukey) and pair of groups using the Student *t*-test from GraphPad Prism ver. 5 software (USA). We considered $p < 0.05$ as statistically significant.

3. Results

Animals inhaled concentrated PM_{2.5} or FA for 5 h during 3 days. The average concentrated air particle in the chambers was $609 \pm 12.73 \mu\text{g}/\text{m}^3$, and outdoor-air PM_{2.5} collected $40 \pm 7.07 \mu\text{g}/\text{m}^3$,

representing ~15-fold enrichments (Fig. 2A). Elemental and Polycyclic Aromatic Hydrocarbons (PAH) PM content also increased in the concentrated outdoor air (Fig. 2B and C), as well as endotoxins (Fig. 2D), and β -glucan (Fig. 2E). No PM_{2.5} was present in FA chambers.

3.1. Plethysmography measurements

The Penh index measured before and after the OVA challenge on day 21 showed that PM_{2.5} inhalations in sensitized animals (S + Adj) induced a significant ~9-fold increase above the Penh baseline. In the S + PM_{2.5} group, there was a nearly ~6-fold increase in the Penh index (Fig. 3A). Penh index response in time exhibited hyperresponsiveness in S + Adj + PM_{2.5} observed at 3 min after the OVA challenge. In contrast, the Penh index in animals from the S + PM_{2.5} group increased 9 min after the OVA challenge, demonstrating a slower response (Fig. 3B). In the S + Adj + PM_{2.5} group, the OVA challenge demonstrated a typical response, presenting higher Penh after 9 min in the OVA challenge. Non-Sensitized animals (NS + FA, NS + PM_{2.5}, or S + FA) lacked response.

3.2. Cellular count

Giemsa stain analysis revealed (Fig. 4A–F) that exposure to PM_{2.5} significantly elevated the percentage of macrophages in the NS + PM_{2.5} group. However, in the S + Adj + FA and S + PM_{2.5} groups, only eosinophils demonstrated an increase. In the case of animals from the S + Adj + PM_{2.5} group, both eosinophils and neutrophils showed an increase. Total cell count only exhibited statistically significant differences in the S + PM_{2.5} vs. the S + FA group (Fig. 4G).

3.3. ELISA assay

The non-specific IgG antibody levels lacked significant differences among groups (Fig. 5A). Sensitized animals receiving Al(OH)₃

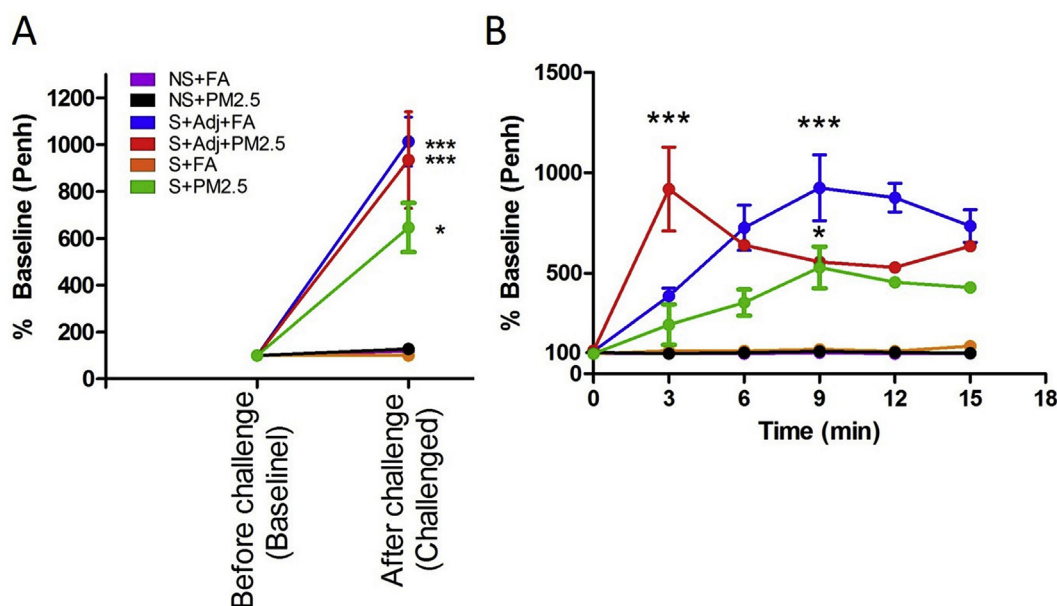


Fig. 3. Barometric plethysmography before and after the OVA challenge. (A) S + Adj + PM_{2.5} or S + Adj + FA raised the bronchoobstruction index by around 1000%, ANOVA (Tukey) test vs. NS + FA ($***p < 0.001$). Also, S + PM_{2.5} also increased the Penh index up to 600%, ANOVA (Tukey) test vs. NS + FA ($*p < 0.05$). (B) Over time, exposure to Fine Particles (FP) in S + Adj + PM_{2.5} produced great bronchoobstruction at 3 min after the OVA challenge, ANOVA (Tukey) test vs. NS + FA ($***p < 0.001$). The same occurred in S + Adj + PM_{2.5} but, at 9 min, a typical sensitization response was demonstrated. S + PM_{2.5} also responded at 9 min, similar to the previous group, ANOVA (Tukey) test vs. NS + FA ($*p < 0.05$). Each value is reported as mean $\pm$ Standard Deviation (SD) $n = 5$. Two columns.

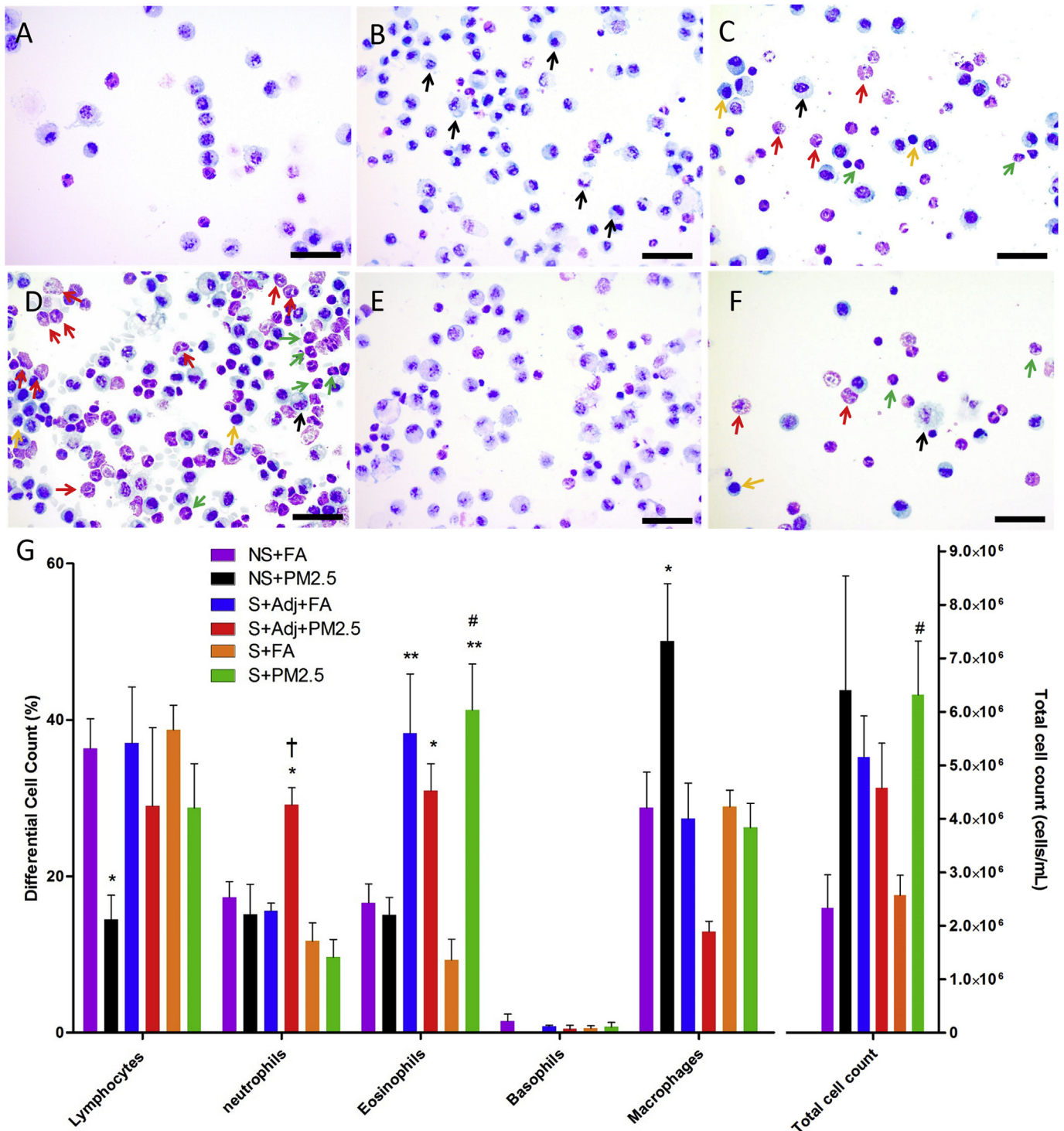


Fig. 4. Cell count in bronchoalveolar lavage fluid. (A) NS + FA; (B) NS + PM_{2.5}; (C) S + Adj + FA; (D) S + Adj + PM_{2.5}; (E) S + FA, and (F) S + PM_{2.5}. Arrows indicate inflammatory cells; Macrophages (black); Lymphocytes (yellow); Eosinophils (red); Neutrophils (green). (G) Only PM_{2.5} exposure raised macrophages, Student *t*-test (***p* < 0.01 vs. NS + FA). Percentage of cell count. Neutrophils, Student *t*-test (**p* < 0.05 vs. NS + FA), and eosinophils, Student *t*-test (***p* < 0.01 vs. NS + FA) increased in S + Adj + PM_{2.5}. S + PM_{2.5} increased eosinophils, Student *t*-test (***p* < 0.01 vs. NS + FA). However, S + Adj + PM_{2.5} raised neutrophils more than its counterpart, Student *t*-test (†*p* < 0.05 vs. S + Adj + FA). Total cell count only increased in S + PM_{2.5}, Student *t*-test (#*p* < 0.05 vs. S + FA). Each value is reported as mean ± Standard Deviation (SD), *n* = 5. Two columns.

adjuvant (S + Adj + FA and S + Adj + PM_{2.5} groups) exhibited significantly raised OVA-specific IgG1 levels. In sensitized animals with no additional adjuvant (S + PM_{2.5}), these antibodies were also increased, indicating an adjuvant role of PM_{2.5} (Fig. 5B).

Furthermore, OVA-specific IgE levels were positive in the same three groups (Fig. 5C). CC16 protein had constitutive concentrations in the NS + FA group, while in the NS + PM_{2.5} group, animals showed a non-statistically significant increase in CC16 protein. In

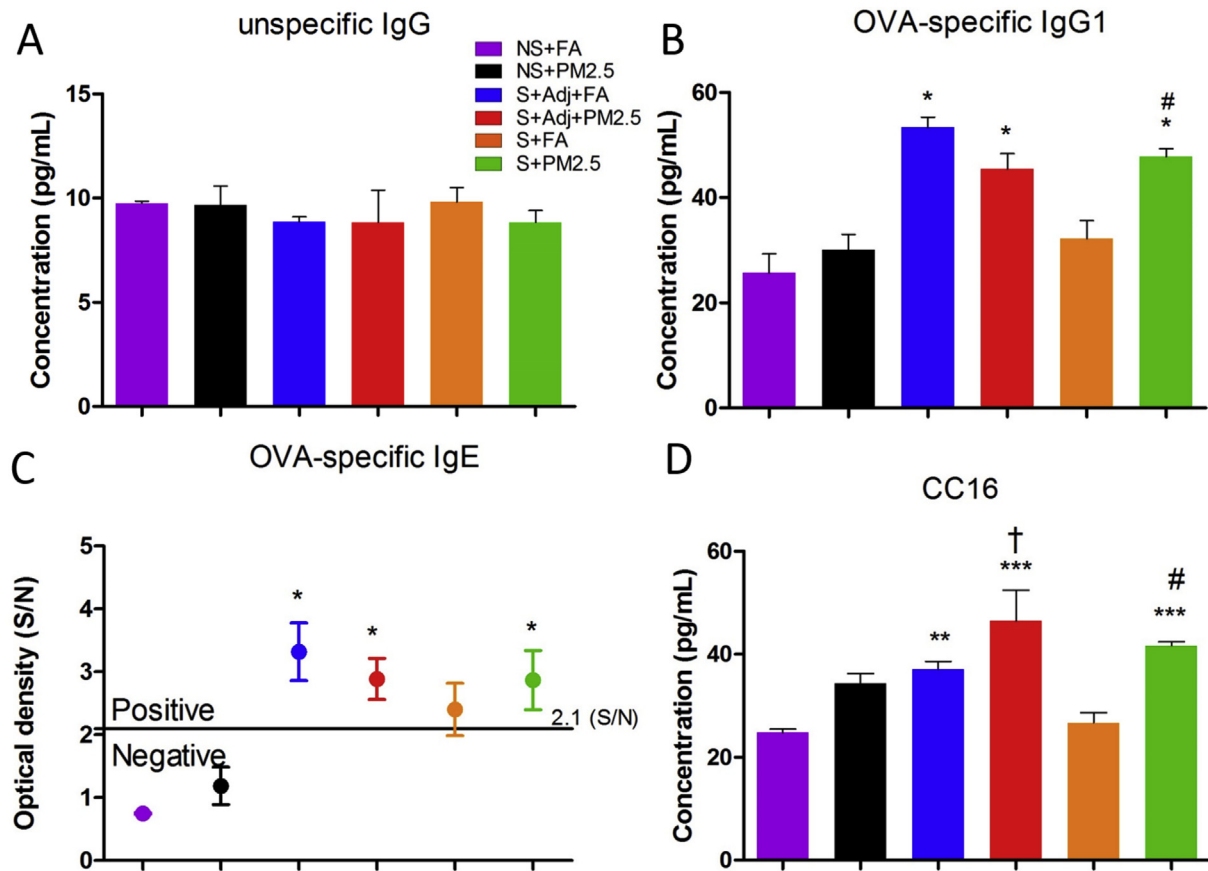


Fig. 5. Damage and sensitization biomarkers. (A) IgG exhibited null differences among groups. (B) OVA-specific IgG1 levels rose in S + Adj + FA or S + Adj + PM_{2.5}, and in S + PM_{2.5}, Student *t*-test (**p* < 0.05 vs. NS + FA). This latter group was also different from their counterparts, Student *t*-test (#*p* < 0.05 vs. S + FA). (C) OVA-specific IgE levels also increased in the same groups, Student *t*-test (**p* < 0.05). (D) CC16 protein as damage biomarker demonstrated significant differences in S + Adj + FA, S + Adj + PM_{2.5}, and S + PM_{2.5}, Student *t*-test (***p* < 0.01, ****p* < 0.001 vs. NS + FA). However, PM_{2.5} inhalations increased damage in S + Adj group animals (†*p* < 0.05 vs. S + Adj + FA). S + PM_{2.5} also rose significantly, Student *t*-test (#*p* < 0.05 vs. NS + FA). Each value is reported as mean ± Standard Deviation (SD), *n* = 5. Two columns.

the S + Adj + FA group, CC16 protein levels increased and were even higher in animals that inhaled PM_{2.5} (Fig. 5D).

3.4. Histology analysis

The S + Adj + FA group had an increment in the number of bronchiolar mucous cells (40% of cells in the epithelium) vs. the 3–5% observed in the NS + FA group (Fig. 6A–F). Exposure to PM_{2.5} raised the number of mucous cells to >70% and 60% in the S + Adj and S groups, respectively (Fig. 6G).

4. Discussion

In the present study, we evaluated the adjuvant potential of Mexico City PM_{2.5} and its pulmonary toxicological manifestations in a guinea-pig allergic asthma model. Our results revealed that a 3-day exposure to PM_{2.5} alone was not capable of producing changes in allergic asthma biomarkers. Instead, both sensitized groups receiving adjuvant and PM_{2.5} (S + Adj + PM_{2.5}) or without adjuvant (S + PM_{2.5}), responded with severe airway hyperresponsiveness, showing a faster response. PM_{2.5} exposure induced severe airway hyperresponsiveness in animals sensitized with adjuvant (S + Adj + PM_{2.5}), and the sensitized group (S + PM_{2.5}) evidenced a slow hyperresponsiveness response. In both groups, OVA-specific IgG1 and IgE levels were increased. Previous studies have suggested that in animals sensitized with antigen alone, the IgG1 antibody can be detected in the serum, but when it is administered

with an adjuvant, both IgG1 and IgE are present in the serum (Andersson, 1980), as occurred in S + PM_{2.5} groups. Similar findings were observed in an allergic-asthma mouse model with exposure to DEP, where the latter acted as an adjuvant, elevating OVA-specific IgG and IgE (Hao et al., 2003). Also, in a mouse model, intranasal (i.n.) instillation of fine particles gave rise to a stronger effect on OVA-specific IgE, IgG1, or IgG2a antibodies than coarse particles (Steerenberg et al., 2006). Additionally, Li et al. (2009) reported a greater increase in both antibodies when animals were OVA-sensitized and exposed to UltraFine Particles (UFP) in comparison to those that only received OVA sensitization (Li et al., 2009). However, this difference was not found in the present study, suggesting a differential effect related with the particle size tested.

Furthermore, it is known that exposure to endotoxins increased this antibody, although neither glucan nor the endotoxin + glucan mixture changed these antibody levels (Thorn and Rylander, 1998), as occurred in our analysis, which presented high levels of endotoxin and glucan, the result of which showed the same levels of non-specific IgG in our groups. It is clear that our results were obtained in animals exposed to very high PM_{2.5} concentrations (609 ± 12.73 µg/m³ for 3 days). Nevertheless, similar levels exist in some cities of Asia and the Middle East (Zhang and Cao, 2015; UNEP, 2016).

A characteristic of asthma or the sensitization process comprises the presence of pulmonary eosinophilia (Foster et al., 1996). However, in our study, S + Adj + PM_{2.5}-group animals increased not

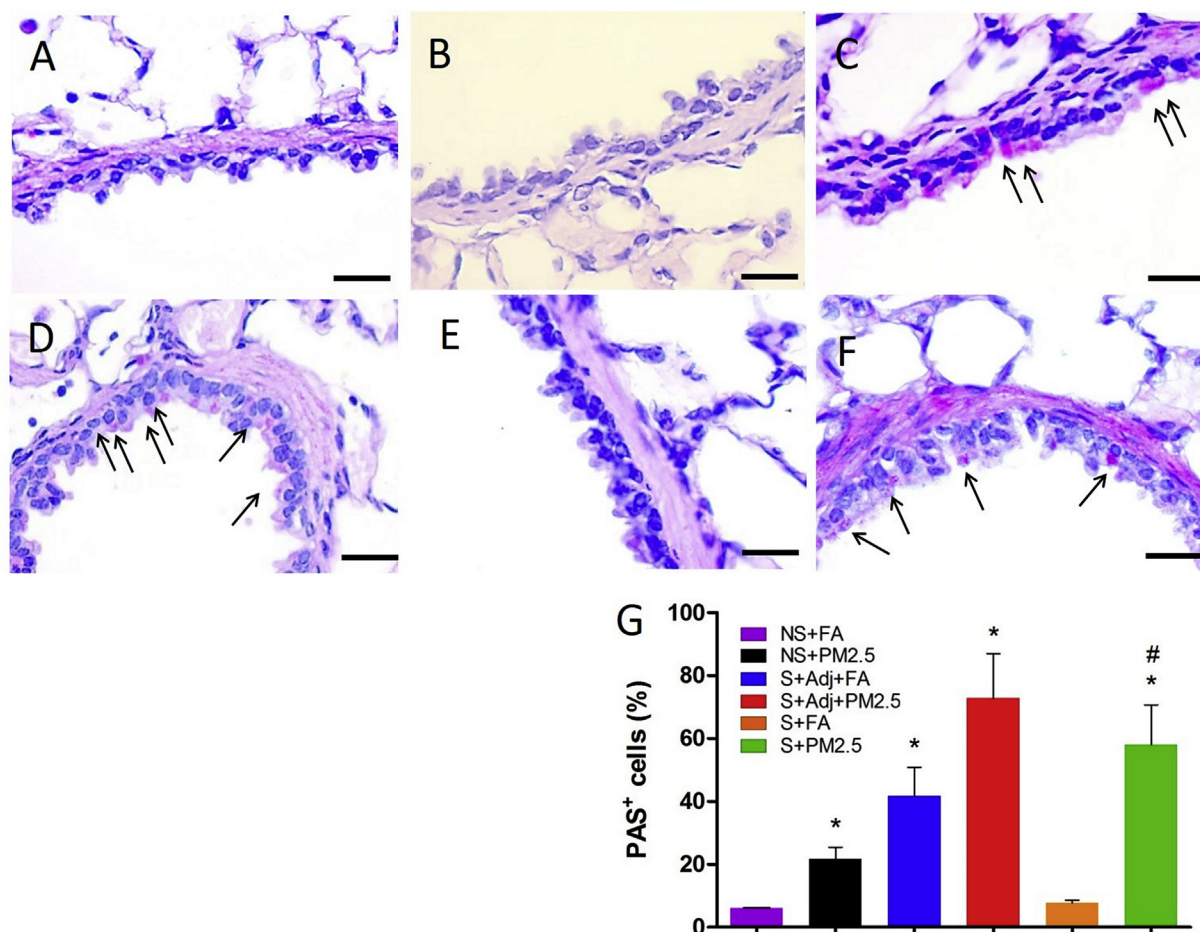


Fig. 6. Mucous metaplasia. (A) NS + FA; (B) NS + PM_{2.5}; (C) S + Adj + FA; (D) S + Adj + PM_{2.5}; (E) S + FA, and (F) S + PM_{2.5}. Arrows indicate mucous cells in terminal bronchioles of the epithelium. Differential cell count (G). S + Adj + FA increased mucous cells, Student *t*-test (**p* < 0.05 vs. NS + FA). In addition, exposure to PM_{2.5} in S + Adj or S animals raised mucous metaplasia, Student *t*-test compared vs. NS + FA (**p* < 0.05) or vs. S + FA (#*p* < 0.05), respectively. Each value is reported as mean ± Standard Deviation (SD), *n* = 5. Two columns.

only in eosinophils but also in neutrophils. Similar results were found by Steerenberg et al. (2005) when comparing PM from five European cities (Rome, Oslo, Amsterdam, Zilk, and Lodz) in the mouse model. Only particles from Lodz increased eosinophils, neutrophils, and monocytes in BALF (Steerenberg et al., 2005). Another study, UFP + DEP + OVA in a mouse model, raised mononuclear cells and eosinophils (Farraj et al., 2006; Li et al., 2009). It has been observed that eosinophilic inflammation is related with frequent asthma exacerbations, while a neutrophilic increase is responsible for severe airflow limitation (Lee et al., 2014). This effect was observed in our physiologic analysis by plethysmography in S + Adj + PM_{2.5} animals, in which a high percentage of eosinophils and neutrophils was reported. Furthermore, in these animals, inhalation of PM_{2.5} altered the bronchiolar epithelium, as demonstrated in a higher concentration of CC16 in the BALF than that observed in the S + Adj + FA group. Some chemicals, such as smoke, ozone, and PM, induced lung damage represented by an increase in CC16 protein due to disturbance of the integrity or permeability of the bronchiolar epithelium (Broeckaert, 2000). This protein appears to protect the respiratory tract against oxidative stress and inflammation, as demonstrated in experiments using CC16-deficient mice (Broeckaert, 2000). Studies in human revealed a positive correlation between PM₁₀ exposure and CC16 protein increments: for each 5-μg/m³ PM₁₀ increase, a 0.52-μg/L rise in CC16 protein was observed in serum (Provost et al.,

2014). Also, in rats and in a mouse model, acute exposure to ozone produces a transient dose-dependent elevation of CC16 in serum (Broeckaert, 2000).

Damage to the integrity or permeability of the bronchiolar epithelium produces an increase in the sensitization process (Broeckaert, 2000) and in hyperresponsiveness. Moreover, during this exposure, the bronchiolar epithelium underwent mucous cell metaplasia, similar to our finding in the present study. Although mucous cells typically develop in the bronchiolar epithelium as part of the sensitization process (Reader et al., 2003), this effect increases when sensitized animals are exposed to particles. In bronchioles, the high levels of CC16 protein levels within some Club cells are transformed into a mucoid phenotype (Evans et al., 2004), whose degranulation is regulated by neutrophil elastase (Agusti et al., 1998). In this investigation, both mucous metaplasia and its neutrophils worsened bronchial obstruction, as was reported in our plethysmography studies.

Aluminum hydroxide worked as an excellent adjuvant in our experimental model, in that, if not administered, the animals would never have become “asthmatics” (results of our S + FA group). Aluminum hydroxide in allergen immunotherapy is associated with the induction of the Th2 response and the production of InterLeukin (IL)-4 and IL-5, and the specific IgG1 antibody (Brewer et al., 1999). Furthermore, exposure to Aluminum (Al) causes a pro-inflammatory effect mediated by the formation of ROS, which is

potentiated when Al is combined with another metal such as iron, producing the reduction of Fe III into Fe II and auto-oxidation of the latter (Exley et al., 2010). PM_{2.5} is a big mixture of chemicals that recruits inflammatory and ROS formation in the airways (Dworski, 2000). Also, it is known that some PM_{2.5} constituents affect the initial priming process and the subsequent allergen challenge, which enhances the production of specific IgE (Mahony et al., 2013) by the switching of B cells (Parnia et al., 2002), but only in co-stimulated B cells (Pandya et al., 2002). Some studies have recognized elements or compounds that play a major role as adjuvant, such as nickel (Gilmour et al., 2000), Silicon as SiO₂ (mesoporous silica nanoparticles) (Mahony et al., 2013), anthracene, fluoranthene, benzo(a)pyrene (Pandya et al., 2002), and phenanthrene (Tsien et al., 1997). In our study, S + Adj + PM_{2.5} and S + PM_{2.5} groups inhaled high levels of a mix: Al; Si; Fe; Zn; benzo(a)anthracene; benzo(b,k)fluoranthene; benzo(a,e)pyrene; benzo(ghi)perylene; fluoranthene, and phenanthrene, which functioned as adjuvants, associating the activation of the type-2 inflammatory response and IgE and IgG production within the lungs in acute inhalation in the allergic guinea pig.

5. Conclusions

Our study demonstrated that acute inhalation during 3 days of fine particles from Mexico City acts as an adjuvant, similar to aluminum hydroxide, producing allergic asthma in a guinea pig model in animals sensitized with ovalbumin in the absence of an adjuvant. Furthermore, in sensitized animals that received aluminum hydroxide as an adjuvant, exposure to fine particles worsens bronchoconstriction, airway inflammation, and pulmonary damage. Fine particulate matter from Mexico City comprises a compound mixture. Knowing more about its composition is crucial to determine which of the chemical components play a significant role as adjuvants in allergic asthma.

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References

- Agusti, C., Takeyama, K., Cardell, L.O., Ueki, I., Lausier, J., Lou, Y.-P., Nadel, J.A., 1998. Goblet cell degranulation after antigen challenge in sensitized Guinea pigs: role of neutrophils. *Am. J. Respir. Crit. Care Med.* 158, 1253–1258.
- Al-Muhsen, S., Johnson, J.R., Hamid, Q., 2011. Remodeling in asthma. *J. Allergy Clin. Immunol.* 128, 451–462.
- Amador-Muñoz, O., Vázquez Santos-Medina, G., Jazcilevich-Diamant, A., Valle-Hernández, B., 2014. Dispositivo integrado por múltiples deflexiones para la recuperación de compuestos orgánicos debido a la evaporación de disolventes orgánicos por arrastre de vapor. Mexican patent 32 5625 2014.
- Andersson, P., 1980. Antigen-induced bronchial anaphylaxis in actively sensitized Guinea-pigs: anti anaphylactic effects of sodium cromoglycate and aminophylline. *Br. J. Pharmacol.* 69, 467–472.
- Bahadori, K., Doyle-Waters, M.M., Marr, a, C., Lynd, L., Alasaly, K., Swiston, J., FitzGerald, J.M., 2009. Economic burden of asthma: a systematic review. *BMC Pulmonary Med.* 9, 24.
- Brauer, M., Hoek, G., Van Vliet, P., Meliefste, K., Fischer, P.H., Wijga, A., Koopman, L.P., Neijens, H.J., Gerritsen, J., Kerkhof, M., Heinrich, J., Bellander, T., Brunekreef, B., 2002. Air pollution from traffic and the development of respiratory infections and asthmatic and allergic symptoms in children. *Am. J. Respir. Crit. Care Med.* 166, 1092–1098.
- Brewer, J.M., Conacher, M., Hunter, C.A., Mohrs, M., Brombacher, F., Alexander, J., 1999. Aluminium hydroxide adjuvant initiates strong antigen-specific Th2 responses in the absence of IL-4-or IL-13-mediated signaling. *J. Immunol.* 163, 6448–6454.
- Broekaert, B., 2000. Clara cell secretory protein (CC16): characteristics and perspectives as lung peripheral biomarker. *Clin. Exp. Allergy* 30, 469–475.
- Calvo, A., Alves, C., Castro, A., Pont, V., Vicente, A., Fraile, R., 2013. Research on aerosol sources and chemical composition: past, current and emerging issues. *Atmos. Res.* 120, 1–28.
- Curtius, J., 2006. Nucleation of atmospheric aerosol particles. *Comptes Rendus Phys.* 7, 1027–1045.
- Dworski, R., 2000. Oxidant stress in asthma. *Thorax* 55, 51–53.
- Espinosa, A., Reyes-Herrera, J., Miranda, J., Mercado, F., Veytia, M., Cuautle, M., Cruz, J., 2012. Development of an x-ray fluorescence spectrometer for environmental science applications. *Instrum. Sci. Technol.* 40, 603–617.
- Evans, C.M., Williams, O.W., Tuvim, M.J., Nigam, R., Mixides, G.P., Blackburn, M.R., DeMayo, F.J., Burns, A.R., Smith, C., Reynolds, S.D., 2004. Mucin is produced by Clara cells in the proximal airways of antigen-challenged mice. *Am. J. Respir. Cell Mol. Biol.* 31, 382–394.
- Exley, C., Siesjö, P., Eriksson, H., 2010. The immunobiology of aluminium adjuvants: how do they really work? *Trends Immunol.* 31, 103–109.
- Farraj, A.K., Haykal-Coates, N., Ledbetter, A.D., Evansky, P.A., Gavett, S.H., 2006. Neurotrophin mediation of allergic airways responses to inhaled diesel particles in mice. *Toxicol. Sci.* 94 (1), 183–192.
- Flenley, D., 1986. Reversible airway obstruction: neurohumoral mechanisms and treatment. *Respiration* 50, 57–64.
- Foster, P.S., Hogan, S.P., Ramsay, A.J., Matthaei, K.I., Young, I.C., 1996. Interleukin 5 deficiency abolishes eosinophilia, airways hyperactivity, and lung damage in a mouse asthma model. *J. Exp. Med.* 183, 195–201.
- Gilmour, M., Selgrade, M., Lambert, A., 2000. Enhanced allergic sensitization in animals exposed to particulate air pollution. *Inhal. Toxicol.* 12, 373–380.
- Hao, M., Comier, S., Wang, M., Lee, J.J., Nel, A., 2003. Diesel exhaust particles exert acute effects on airway inflammation and function in murine allergen provocation models. *J. Allergy Clin. Immunol.* 112, 905–914.
- Jones, M.G., Richeldi, L., 2014. Air pollution and acute exacerbations of idiopathic pulmonary fibrosis: back to miasma? *Eur. Respir. J.* 43, 956–959.
- Lee, T.-H., Song, H.J., Park, C.-S., 2014. Role of inflammasome activation in development and exacerbation of asthma. *Asia Pac. Allergy* 4, 187–196.
- Lemanske Jr., R.F., Busse, W.W., 2010. Asthma: clinical expression and molecular mechanisms. *J. Allergy Clin. Immunol.* 125, 95–102.
- Li, N., Wang, M., Bramble, L.A., Schmitz, D.A., Schauer, J.J., Sioutas, C., Harkema, J.R., Nel, A.E., 2009. The adjuvant effect of ambient particulate matter is closely reflected by the particulate oxidant potential. *Environ. Health Perspect.* 117, 1116–1123.
- Li, Y.-J., Kawada, T., Matsumoto, A., Azuma, A., Kudoh, S., Takizawa, H., Sugawara, I., 2007. Airway inflammatory responses to oxidative stress induced by low-dose diesel exhaust particle exposure differ between mouse strains. *Exp. Lung Res.* 33, 227–244.
- Mahony, D., Cavallaro, A.S., Stahr, F., Mahony, T.J., Qiao, S.Z., Mitter, N., 2013. Mesoporous silica nanoparticles act as a self-adjuvant for ovalbumin model antigen in mice. *Small* 9, 3138–3146.
- Möller, A., Agius, R.M., Vocht, F., Lindley, A., Gerrard, W., Lowe, L., Belgrave, D., Custovic, A., Simpson, A., 2013. Long-term exposure to PM10 and NO2 in association with lung volume and airway resistance in the MAAS birth cohort. *Environ. Health Perspect.* 121, 1232–1238.
- Nemmar, A., Holme, J.A., Rosas, I., Schwarze, P.E., Alfaro-Moreno, E., 2013. Recent advances in particulate matter and nanoparticle toxicology: a review of the *in vivo* and *in vitro* studies. *BioMed Res. Int.* 2013, 1–22.
- Pandya, R.J., Solomon, G., Kinner, A., Balmes, J.R., 2002. Diesel exhaust and asthma: hypotheses and molecular mechanisms of action. *Environ. Health Perspect.* 110, 103–112.
- Parnia, S., Brown, J., Frew, A., 2002. The role of pollutants in allergic sensitization and the development of asthma. *Allergy* 57, 1111–1117.
- Pawankar, R., 2014. Allergic diseases and asthma: a global public health concern and a call to action. *World Allergy Organ. J.* 7, 1–12.
- Postma, D.S., Kerstjens, H.A.M., 1998. Characteristics of airway hyperresponsiveness in asthma and chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 158, 187–192.
- Provost, E.B., Chaumont, A., Kicinski, M., Cox, B., Fierens, F., Bernard, A., Nawrot, T.S., 2014. Serum levels of club cell secretory protein (Clara) and short- and long-term exposure to particulate air pollution in adolescents. *Environ. Int.* 68, 66–70.
- Reader, J.R., Tepper, J.S., Schelegle, E.S., Aldrich, M.C., Putney, L.F., Pfeiffer, J.W., Hyde, D.M., 2003. Pathogenesis of mucous cell metaplasia in a murine asthma model. *Am. J. Pathology* 162, 2069–2078.
- Sancini, G., Farina, F., Battaglia, C., Cifola, I., Mangano, E., Mantecchia, P., Camatini, M., Palestini, P., 2014. Health risk assessment for air pollutants: alterations in lung and cardiac gene expression in mice exposed to milano winter fine particulate

- matter (pm_{2.5}). PLoS One 9, 10.
- Shusterman, D., 2011. The effects of air pollutants and irritants on the upper airway. *Proc. Am. Thorac. Soc.* 8, 101–105.
- Steerenberg, P.A., van Amelsvoort, L., Lovik, M., Hetland, R.B., Alberg, T., Halatek, T., Bloemen, H.J., Rydzynski, K., Swaen, G., Schwarze, P., 2006. Relation between sources of particulate air pollution and biological effect parameters in samples from four European cities: an exploratory study. *Inhal. Toxicol.* 18, 333–346.
- Steerenberg, P.A., Withagen, C.E.T., van Dalen, W.J., Dormans, J.A.M.A., Cassee, F.R., Heisterkamp, S.H., van Loveren, H., 2004. Adjuvant activity of ambient particulate matter of different sites, sizes, and seasons in a respiratory allergy mouse model. *Toxicol. Appl. Pharmacol.* 200, 186–200.
- Steerenberg, P.A., Withagen, E.T., van Dalen, W.J., Dormans, J.A.M.A., Heisterkamp, S.H., van Loveren, H., Cassee, F.R., 2005. Dose dependency of adjuvant activity of particulate matter from five European sites in three seasons in an Ovalbumin mouse model. *Inhal. Toxicol.* 17, 133–145.
- Takizawa, H., 2011. Impact of air pollution on allergic diseases. *Korean J. Intern. Med.* 26, 262–273.
- Thorn, J., Rylander, R., 1998. Inflammatory response after inhalation of bacterial endotoxin assessed by the induced sputum technique. *Thorax* 53, 1047–1052.
- Tie, X., Madronich, S., Li, G., Ying, Z., Zhang, R., García, A.R., Lee-Taylor, J., Liu, Y., 2007. Characterizations of chemical oxidants in Mexico City: a regional chemical dynamical model (WRF-Chem) study. *Atmos. Environ.* 41.9, 1989–2008.
- Tsien, A., Díaz-Sánchez, D., Ma, J., Saxon, A., 1997. The organic component of diesel exhaust particles and phenanthrene, a major polycyclic aromatic hydrocarbon constituent, enhances IgE production by IgE-secreting EBV-transformed human B cells *in vitro*. *Toxicol. Appl. Pharmacol.* 142, 256–263.
- United Nations Environment Programme (UNEP), 2016. A Review of Air Pollution Control in Beijing: 1998–2013. <http://www.bjepb.gov.cn/bjepb/323265/340674/4389403/2016052511264687868.pdf> (Accessed on 27 January 2017).
- Ulrich, A., Wichser, A., Hess, A., Heeb, N., Emmenegger, L., Czerwinski, J., Kasper, M., Mooney, J., Mayer, A., 2012. Particles and Metal Emissions of Diesel and Gasoline Engines - Are Particle Filters Appropriate Measures? 16th Conference on Combustion Generated Nanoparticles, Zürich, Switzerland.
- Valavanidis, A., Vlachogianni, T., Fiotakis, K., Loidas, S., 2013. Pulmonary oxidative stress, inflammation and cancer: respirable particulate matter, fibrous dusts and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms. *Int. J. Environ. Res. Public Health* 10, 3886–3907.
- Vargas, M.H., Sommer, B., Bazán-Perkins, B., Montaña, L.M., 2010. Airway responsiveness measured by barometric plethysmography in Guinea pigs. *Veterinary Res. Commun.* 34, 589–596.
- Velasco, E., Pressley, S., Allwine, E., Westberg, H., Lamb, B., 2005. Measurements of CO₂ fluxes from the Mexico City urban landscape. *Atmos. Environ.* 39 (38), 7433–7446.
- Villalobos-Pietrini, R., Amador-Muñoz, O., Waliszewski, S., Hernández-Mena, L., Munive-Colín, Z., Gómez-Arroyo, S., Bravo-Cabrera, J.L., Frías-Villegas, A., 2006. Mutagenicity and polycyclic aromatic hydrocarbons associated with extractable organic matter from airborne particles $\leq 10 \mu\text{m}$ in southwest Mexico City. *Atmos. Environ.* 40, 5845–5857.
- Zhang, Y.L., Cao, F., 2015. Fine particulate matter (PM_{2.5}) in China at a city level. *Sci. Rep.* 5, 14884.
- Zhang, Z., Kleinstreuer, C., Kim, C.S., 2001. Effects of curved inlet tubes on air flow and particle deposition in bifurcating lung models. *J. Biomechanics* 34, 659–669.