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Maternal exposure to airborne particulate matter causes postnatal immunological dysfunction in mice offspring

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ABSTRACT

Evidence suggests that prenatal exposure to air pollution affects the ontogeny and development of the fetal immune system. The aim of this study was to investigate the effect of maternal exposure to airborne particulate matter (PM) on immune function in postnatal offspring.

Pregnant female ICR mice were intralaryngopharyngeally administered with 30 μl of phosphate buffered solution (the control group) or resuspended PM of Standard Reference Material 1649a at 0.09 (low), 0.28 (medium), 1.85 (high) or 6.92 (overdose) μg/μl once every three days from day 0 to 18 of pregnancy (n = 8–10). Offspring were sacrificed on postnatal day 30. Interleukin-4 and interferon-γ levels in plasma and splenocytes, splenic lymphocyte proliferation, and expressions of GATA-3 and T-bet mRNA in the spleen were tested. The spleen and thymus were histopathologically examined.

The offspring of the medium, high and overdose PM-exposed dams showed significantly suppressed splenocyte proliferation. Decreased interferon-γ and increased interleukin-4 levels in the blood and splenocytes, and lowered T-bet and elevated GATA-3 mRNA expressions were found in the spleen in the medium, high and overdose groups when compared with the control or low dose group (P < 0.05). Histopathology revealed prominent tissue damage in the spleen and thymus in the overdose group.

These results suggest that exposure of pregnant mice to PM modulates the fetal immune system, resulting in postnatal immune dysfunction by exacerbation of Thl/Th2 deviation. This deviation is associated with altered T-bet and GATA-3 gene expressions.

1. Introduction

As a result of extensive urbanization and an increasing number of vehicles, air pollution has become a severe problem in many cities within economically emerging countries (Zhang, 2011). Epidemiologic evidence has shown a significant increase in immunologic diseases in severely polluted areas (Nastos et al., 2010; Brauner et al., 2010). Additionally, the prevalence of immunologic diseases in early life has been on the rise over the last few years (Price et al., 2012). The exposure to air pollution in utero has also been shown to be potentially devastating, as the first signs of some immune disorders, such as the infantile anaphylactic diseases, neonatal lupus erythematosus and some cancers, can develop in infants shortly after birth (Yu et al., 2006; Izmirly et al., 2010; Kozyrskyj et al., 2011). Thus, the high prevalence of immunologic diseases in children may be putatively associated, at least in part, with the consequent maldevelopment of the prenatally impaired immune system (Dietert, 2011). If this is true, it will suggest that exposure to air pollution in utero may well affect the development of the fetal immune system.

Airborne particulate matter (PM) is one of the main contributors to ambient air pollution throughout the world, especially in developing countries (Kan et al., 2009). PM is a common air pollutant that could have a significant impact on the immune system during both pre- and postnatal periods of life, potentially resulting in system

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malfunction in early life (Svendsen et al., 2007). In particular, particulate matter that is <2.5 μm in diameter ($PM_{2.5}$) has been shown to influence fetal immune system development and appears to result in altered immunoglobulin E (IgE) (Herr et al., 2011) and lymphocyte distributions (Herr et al., 2010) in cord blood among neonates. Maternal exposure to air pollution before and during pregnancy can also alter immunity in offspring, thus increasing a child's risk to develop health conditions like asthma and allergies later in life (Baïz et al., 2011). In summary, data from epidemiologic surveys and clinical observation suggests that the fetal immune system is highly vulnerable to environmental pollutants – much more so than the more developed systems of infants and older children.

Exposure to ambient levels of PM generally causes no appreciable effects, but impalpable alteration that results from prenatal PM exposure may come to exert a more evident impact on later development throughout childhood or even adulthood (Corson et al., 2010). Nonetheless, the results are not always consistent regarding the immune system, in spite of the fact that infants who later develop immune disorders show some altered neonatal immune responses (Prescott, 2006). We hypothesized that in utero exposure to PM may affect the development of the fetal immune system, resulting in its abnormal phenotype in early life following birth. In the present work, we investigated in utero PM exposure on childhood immune function in a subacute murine model with administration of resuspended standard PM. We tested T-bet and GATA-3, both of which are the transcription factors that induce cell differentiation of T helper cell subgroup 1 (Th1) and subgroup 2 (Th2), in addition to systemic and local biochemical measurements and morphological changes in the immune organs.

2. Materials and methods

2.1. Animals

Sixty females and 30 males specific pathogen free (SPF) ICR nonparous mice of 6-week-old were purchased from Fujian Center for Disease Control and Prevention (Certificate number: SCXK2011-0001, Fuzhou, China), of which the female mice weighed 29 ± 2 g and the male mice 32 ± 2 g for copulation. The mice were housed in a climate controlled room at 23 ± 1 °C with 55 ± 7% humidity, in addition to a 12/12-h light/dark cycle and standard rodent chow with water ad libitum. The experiments were performed in accordance with the guidelines of the "Principles of Laboratory Animal Care" by the National Society for Medical Research in China. The experimental protocol was approved by the Scientific Research Committee of Fuzhou General Hospital.

2.2. PM exposure

Airborne PM of Standard Reference Material (SRM) 1649a was purchased from the National Institute of Standards and Technology (Gaithersburg, MD, USA). This particulate matter is an atmospheric material collected in an urban area with particle diameters ranging 6.7–100 μm (averaged 12.9 μm). Substances that deposit on the surface of these particle cores include polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl (PCB) congeners and chlorinated pesticides, inorganic salts, heavy metals, bioactive components such as lipopolysaccharides, and other trace elements, many of which, especially the organic components that dominate the deposits, are noxious to human health (Benner, 1998; Chiu et al., 2001; Albinet et al., 2006). Prior to exposure, SRM 1649a was resuspended in sterile phosphate buffered saline (PBS) to obtain a final concentration of 0.09, 0.28, 1.85 or 6.92 μg/μl and was stored at 4 °C. The suspension, which contains the indissoluble (e.g., PAHs), poorly dissoluble (e.g., PCB and chlorinated pesticides) and dissoluble (e.g., inorganic constituents) components, was thoroughly mixed and sonicated for at least half an hour in an ultrasonic vibration instrument prior to administration.

Mice were allowed one week adjustment prior to the experiments. The female mice were placed with the male mice (2:1) overnight and pregnancy was confirmed by the presence of vaginal plug valve (referred to as gestation day 0). The pregnant mice were weighed, numbered, and randomly divided into one of the five groups (12 mice per group): experimental groups, which were administered with SRM 1649a suspension at concentration of 0.09, 0.52, 1.85 or 6.92 μg/μl (low, medium, high, or overdose group), and the control group, which received the same volume of PBS without particles.

The instillation procedure to deliver resuspended particles to the mice was performed as described previously, by which 77.5–88.2% particles would enter the lung (Rao et al., 2003). The female mouse was briefly anesthetized in a glass container filled with isoflurane. The mouse's mouth was opened using rubber bands with the tongue pulled gently aside by a pair of forceps. A 30-μl SRM 1649a suspension or the same volume of PBS alone was pipetted to the base of the tongue, in which the mouse was held steady for at least two deep breaths (less than 15 s). The particle suspension was administered beginning from day 0 and repeated at day 3, 6, 9, 12, 15 and 18 with a total of 18.9 (low), 109.2 (medium), 388.5 (high) or 1453.2 μg (overdose) of SRM 1649a per mouse. After the last SRM 1649a administration at day 18, the mice were housed individually and were allowed to deliver. Shortly after parturition, the pups, together with their dam in a cage, were transferred to an airborne particle-eliminated chamber for 30 days before the experiment was performed. Mice with the onset of delivery within day 20–22 of pregnancy and with number of pups within 8–12 per litter were allowed to enter the experiment.

2.3. Collection and storage of organ and blood samples

After preclusion of the dams that did not match the condition, there were 8–10 dams (litters) enrolled in each group. Total of 40 fetal mice from the 8 to10 litters with each litter 4–5 fetuses in a group were randomized, weighed, and decapitated after eutherization. Blood samples from the randomly selected fetal mice in each litter, with each litter as one sample, were pooled into a heparin-containing tube (n = 8–10) and blood plasma was isolated and stored in −70 °C. The thoracic gland and spleen were identified, isolated and weighed. Ratios of organ weights summed from the selected 4 to 5 fetal mice in each litter over their summed body weights were calculated and compared among the groups (n = 8–10). The randomly chosen 20 spleen organs and 20 thoracic gland organs from the total 40 organs with every litter 2–3 spleen or thoracic gland organs in a group were processed for histopathological examination. Half of the remaining 20 spleen organs, with every litter 2–3 organs pooled to form one sample (n = 8–10), were cut into small pieces about 2–3 mm^3 in ice-cold saline and stored in RNA storage liquid in −70 °C, and the other half in 37 °C saline for preparation of spleen lymphocyte suspension.

2.4. Measurement of total immunoglobulin G1, G2a (IgG1, IgG2a) and IgE antibodies in plasma

Plasma samples were thermometrically balanced to room temperature. Total IgE antibodies in the plasma were measured by a sandwich technique using the ELISA kit according to the manufacturer's protocols (Yamasa Co., Chiba, Japan), in which two monoclonal rat anti-mouse IgE antibodies recognizing different epitopes on the FcεR fragment were used. Optical density at 450 and 550 nm was determined using a microplate reader. Total IgG1 and IgG2a antibodies in the plasma were also measured by ELISA. Anti-mouse Ig (BioLead Biology Sci & Tech Co. Ltd., Beijing, China) of 0.1 ml containing 10 μg/ml protein diluted by 0.05 mol/L coating buffer (pH = 9.6) was added to each reacting well in the microtiter plate and was incubated at 4 °C overnight. The plate was washed four times in PBS containing 0.1% Tween-20, added by diluted plasma at 37 °C for 2 h, and washed sequentially by PBS and PBS containing 0.1% Tween-20 (PBST) each for two times. One hundred microliters diluted horseradish peroxidase-labeled anti-mouse IgG1 or IgG2a (1:1000, BioLead Biology Sci & Tech Co. Ltd.) were added into each well, incubated at 37 °C for 1 h, washed for 5 times, added by 100 μl substrate buffer, and then incubated away from light at 37 °C for 30 min. The reaction was terminated by addition of 0.05 ml sulphuric acid (2 mol/L) and optical density was read at 450 nm with the microplate reader.

2.5. Spleen lymphocyte proliferation test

Spleen homogenates were filtered through a 200-screen mesh grit made of stainless steel to prepare a single-cell suspension. The erythrocytes in the suspension were lyzed by addition of ammonium chloride. The lymphocytes were harvested by centrifugation and re-suspended in RPMI 1640 (Sigma–Aldrich Co., St. Louis, MO, USA) containing 10% calf serum. The vial lymphocytes, confirmed by trypan blue staining, were adjusted to a density of 1×10^6/ml, and inoculated in 96-well culture plates with 200 μl in each well. Each sample was inoculated in 6 wells, 3 of which were added by 10 μl phytohemagglutinin and the other 3 were not to serve as the control. The mixture was cultured at 37 °C for 72 h under 5% carbon dioxide and saturated humidity, and was added by 10 μl tetramethylthiazole blue (Sigma, St. Louis, MO, USA) prior to the end of the culture. Supernatant of 100 μl was gently removed from each well and replaced by the same amount of DMSO. The mixture was then vibrated for 30 min and tested at 570 nm for optical density.

2.6. Assay for interferon-γ (IFN-γ) and interleukin (IL)-4 contents

The above spleen lymphocyte suspension preparation was inoculated in a 96-well culture plate, followed by 10 μl phytohemagglutinin per well, and cultured at 37 °C for 48 h under 5% carbon dioxide and saturated humidity. The suspension was then centrifuged at approximately 3000 × g at 4 °C for 10 min and the supernatant was used for the assay. Contents of IFN-γ and IL-4 in the supernatant and plasma were assayed by using a solid phase sandwich technique using a commercially available ELISA kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and were performed in duplicate. Results are expressed as pg/ml. Ratios of IL-4/IFN-γ were calculated.