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Seasonal and areal variability in $PM_{2.5}$ poses differential degranulation and pro-inflammatory effects on RBL-2H3 cells

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ABSTRACT

$PM_{2.5}$ pollution is a widespread environmental and health problem, particularly in China. Besides leading to well-known diseases in the respiratory system, $PM_{2.5}$ can also alter immune function to induce or aggravate allergic diseases. To determine whether there are temporal and spatial differences in the allergic responses to $PM_{2.5}$, monthly samples were collected from four regions (urban, industrial, suburban, and rural areas) through a whole year in Nanjing city, China. Inorganic chemical components (metals and water-soluble ions) of $PM_{2.5}$ were analyzed, and the rat basophil cells (RBL-2H3) exposed to $PM_{2.5}$ were assessed through quantitative measures of degranulation (β-hex and histamine) and pro-inflammation cytokine (IL-4 and TNF-α) expression. The highest levels of β-hex were measured in winter and spring $PM_{2.5}$ from urban and industrial areas, or autumn $PM_{2.5}$ from suburban and rural areas. With respect to histamine, autumn $PM_{2.5}$ samples were most potent irrespective of the location. Autumn and winter $PM_{2.5}$ induced higher levels of IL-4 than spring and summer samples. However, spring and autumn $PM_{2.5}$ caused higher levels of TNF-α. The concentrations of water-soluble ions (NH_4^+, K^+ and Cl^-), as well as heavy metals (Pb and Cr), were directly and statistically correlated to the inflammation observed *in vitro*. In general, the differences between regional and seasonal $PM_{2.5}$ in stimulating cell degranulation may depend on endotoxin and airborne allergen content of $PM_{2.5}$. The heavy metals and water-soluble ions in $PM_{2.5}$ were mostly anthropogenic, which increased the particles' mass-based cellular inflammatory potential, therefore, their health risks, e.g. from vehicular exhaust, coal, and biomass combustion, cannot be ignored.

1. Introduction

With the rapid industrialization and urbanization of the world, air pollution has become a global environmental problem and a serious public health crisis (Bourdrel et al., 2017; Landrigan, 2017; Song et al., 2017). Fine particulate matter with a diameter ≤2.5 μm ($PM_{2.5}$) is one of the most dangerous pollutants in the atmosphere. In general, ambient $PM_{2.5}$ originates from a wide range of sources and has a complex composition containing a variety of toxic and harmful components (Mukherjee and Agrawal, 2017). Due to its long transport distance and residence time in the atmosphere, $PM_{2.5}$ health effects are more prominent than coarse particles (Kroll et al., 2013). Undesirable cytotoxic, cell autophagy and apoptosis, oxidative stress, DNA damage, mutagenic, and pro-inflammatory cellular responses are some of the reported outcomes of $PM_{2.5}$ exposure (Bonetta et al., 2009; Chen et al., 2019; Huang et al., 2020; Wang and Tang, 2020). $PM_{2.5}$ can easily enter into alveoli and even the bloodstream through respiratory movements and deposit in large quantities to adversely affect the respiratory, cardiovascular, and immune systems (Cheng et al., 2021; Mei et al., 2018; Wang et al., 2017b; Xing et al., 2016). With regard to the circulatory system, $PM_{2.5}$ exposure has a serious impact on the body's blood-clotting abilities by increasing the concentrations of normal components (e.g. red blood cells) to cause thrombi (clots in blood vessels), and arterial accumulation of fat and calcium plaques that are prone to rupture and cause heart attacks (Kowalska and Kocot, 2016). With regard to the respiratory system, the damage of the airway epithelium caused by $PM_{2.5}$ can permit

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more invasion of inhaled xenobiotics, including allergens, that could lead to aggravation of respiratory diseases such as asthma (Chowdhury et al., 2018; Zhao et al., 2020). With regard to the immune system, He et al. (2016a) found that fine particulate matter harmed more than 500 million Chinese urban residents. Compared with adults, children are more vulnerable to air pollution hazards due to their immature respiratory structure and immune system, and relatively higher level of respiratory exposure per unit body weight (Bateson and Schwartz, 2008; Iskandar et al., 2012).

In order to further understand the related immune diseases caused by atmospheric $PM_{2.5}$ pollution and to achieve their timely prevention and treatment, it is necessary to discuss the role of $PM_{2.5}$ in the immune response in detail. Recently, the incidence of allergic diseases has increased rapidly, and this may be related to increased $PM_{2.5}$ exposure (Breton et al., 2012; Ding et al., 2017; Dunea et al., 2016). One study of 30,759 children in six cities of China has found that an increase of 10 mg/m^3 of the annual $PM_{2.5}$ was positively correlated with the prevalence of allergic rhinitis by an odds ratio (OR) of 1.20 and diagnosed asthma by an OR of 1.10. This study has also shown that long-term exposure to $PM_{2.5}$ may increase the risks of asthma and allergic diseases or symptoms in preschool children of China. Compared with children living in urban areas, children living in suburban or rural areas were at higher risk of $PM_{2.5}$ exposure (Chen et al., 2018a). Another study found that $PM_{2.5}$ related to heavy oil combustion, sea salt, and soil had a significant positive association with respiratory and allergic symptoms for the children in Fukuoka, Japan (Sugiyama et al., 2020). $PM_{2.5}$ can promote sensitization to common aeroallergens and promote the development or exacerbation of allergic diseases (Gavett et al., 2003). $PM_{2.5}$ exposure has been shown to alter the responses of basophils (Devouassoux et al., 2002), one of the primary effector cells in allergic inflammation (Marone, 1997; Obata-Ninomiya et al., 2007). Basophils circulate in blood vessels and travel to sites of tissue inflammation (e.g. skin or airways) where they bind to immune complexes. These immune complexes consist of an antigen (e.g. keyhole limpet hemocyanin) crosslinked to antibodies (e.g. IgE), and once bound to basophils, they cause the cells to secrete chemical mediators of allergic inflammation (e.g. histamine, catalytic enzymes, and cellular messenger molecules) (Diaz-Sanchez et al., 1999). Results from these studies indicated $PM_{2.5}$ plays an important role in IgE-mediated allergic reactions. Another study (Devouassoux et al., 2002) has found basophils induce IL-4 expression and histamine release in an IgE-allergen independent manner upon exposure to diesel exhaust particles (DEPs). DEPs are typical components of air pollutant and mainly composed of small particles less than 2.5 μm in diameter. Moreover, Yamada et al. (2012) found that, yellow sand aerosol extracts caused degranulation of RBL-2H3 cells. In view of these reported findings, the specific mechanism that drives $PM_{2.5}$-induced allergic reactions has not been clarified.

RBL-2H3 cells are a basophil subline isolated from a Wistar rat strain with basophilic leukemia. In addition, RBL-2H3 cells, a tumor analog of mast cells, display many of the characteristics and functions of mucosal-type mast cells and express hundreds of thousands of FceRI on the membrane surface (Yamada et al., 2012). FceRI is a multimeric cell-surface receptor that binds the Fc fragment of IgE with high affinity. Therefore, they are often used as an alternative model of mast cells for the research of allergic diseases (Passante et al., 2009). β-hexosaminidase (β-hex), which is stored in the secretory granules of mast cells is released simultaneously with histamine when mast cells are immunologically activated (Soto et al., 1988). Upon activation, the mast cells release several inflammatory mediators, including Tumor Necrosis Factor Alpha (TNF-α) and Interleukin-4 (IL-4) (Mastuda et al., 2002; Williams and Galli, 2000). Therefore, RBL-2H3 cells are considered as a good tool for studying the effects of environmental pollutants on the release activity of chemical media (Yamada et al., 2012).

Although there are reports that $PM_{2.5}$ can stimulate basophils in the mucosa of the human nasal cavity to produce IL-4, the effects of temporal and spatial $PM_{2.5}$ variability on basophils is still unknown. The aim of this study was to examine the effects of $PM_{2.5}$ in the ambient air from different seasons and regions on RBL-2H3 cells. Therefore, $PM_{2.5}$ samples in different functional areas (urban, industrial, suburban, and rural areas) of Nanjing were collected and analyzed. The effects of $PM_{2.5}$ on RBL-2H3 cell proliferation, activation of degranulation, and pro-inflammation responses were investigated by *in vitro* cell testing. We hypothesized that $PM_{2.5}$ collected in spring and autumn would enhance cell degranulation to a greater degree than $PM_{2.5}$ from summer and winter, while cold season (winter and spring) $PM_{2.5}$ will cause stronger cellular inflammation.

2. Materials and methods

2.1. Study areas and $PM_{2.5}$ sampling

Nanjing is a typical megacity in the Yangtze River Delta of eastern China. In the present study, four representative regions of Nanjing were selected as atmospheric $PM_{2.5}$ sampling locations (Fig. S1 with detailed site information in SI). These sampling areas included the Urban site (Nanjing Institute of Soil Science, Chinese Academy of Sciences, Xuanwu District); Industrial site (Nanjing University of Information Technology, Pukou District); Suburban site (Nanjing Medical University, Jiangning District), and Rural site (Fujiabian Agricultural Park, Lishui District). From March 2018 to February 2019, $PM_{2.5}$ samples (3 samples per season at each sampling site, 12 samples per sampling site, a total of 48 samples) were collected once a month for 23 h at each location by a high-volume (1000 L min^{-1}) air sampler (Wuhan Tianhong Environmental Protection Industry, CN). According to the local meteorological conditions, March to May was spring, June to August was summer, September to November was autumn, and December to February was winter. Before sampling, new quartz microfiber filters (QMA, 203 mm × 254 mm, Whatman, UK) were baked at 400 °C for 4 h to remove organic substances, and weighed after being equilibrated under constant temperature and humidity conditions for 24 h (Luo et al., 2017). After each collection event, the used (sample) filters were equilibrated and weighed, as mentioned above, to determine the collected $PM_{2.5}$ mass (post-sampling weight minus pre-sampling weight), and stored at −20 °C in the dark until further use. In total, 49 $PM_{2.5}$ filters (including a blank filter) were cut into subsamples by ceramic scissors for following cell testing and chemical analysis as described in Sections 2.2 and 2.6, respectively.

2.2. Preparing the $PM_{2.5}$ suspension for cell exposure

Each subsample filter loaded with $PM_{2.5}$ was cut into small pieces and placed in centrifuge tubes with 35 mL of ultra-pure water (UPW), and placed in an ultrasonic water bath and sonicated for 3 h (30 min × 6, cycle in turn). After sufficient ultrasonic elution, the $PM_{2.5}$ suspension was filtered through 2.6 μm mixed cellulose ester (MCE) microporous membrane to remove the pieces of QMA (high purity quartz microfiber) materials. The suspension was frozen and freeze-dried to obtain whole $PM_{2.5}$ particles, which were stored in a freezer at −20 °C for later use.

Using the freeze-dried $PM_{2.5}$ above, 800 mg L^{-1} $PM_{2.5}$ stock solution was prepared with sterile PBS. Then $PM_{2.5}$ stock solution was diluted with Dulbecco's Modified Eagle Medium (DMEM, Gibco, USA) without phenol red to 80 mg/L (according to preliminary experiments) for *in vitro* $PM_{2.5}$ assays.

2.3. Cell culture for in vitro $PM_{2.5}$ exposure

RBL-2H3 cells (ATCC) used for *in vitro* $PM_{2.5}$ exposure assays were cultured in Minimum Essential Medium (MEM, Gibco, USA) supplemented with 15% fetal bovine serum (FBS, Hyclone, USA) and 1% antibiotic penicillin-streptomycin (100 U/mL penicillin, 100 U/mL streptomycin), in a humidified atmosphere of 5% CO_2, at 37 °C. The cells were cultured in cell culture flask (430639, Corning, USA), when the

density of cell reached 80–90%, then 1 mL 0.25% Trypsin-EDTA was added to remove cells from the plate. The cells used for $PM_{2.5}$ assays were collected during the exponential phase of growth. RBL-2H3 cells in their logarithmic growth phase were centrifuged at 1000 rpm for 8 min to enable the decantation and disposal of supernatant. The cells were then resuspended in complete medium and counted.

2.4. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay

The MTT assay is a sensitive and quantitative colorimetric assay that is used to determine cell viability (Yamada et al., 2007). The cell concentration was adjusted to 8×10^4 cells/mL, and the cells were seeded in 96-well plates (100 μL/well) in a humidified atmosphere with 5% CO_2 at 37 °C. The plates were incubated for 24 h, and the medium was discarded. Different $PM_{2.5}$ suspensions were added to the plates as the experimental groups (5 wells/group, n = 5), and the control group (no $PM_{2.5}$), and the blank group (no $PM_{2.5}$, no cells) were set up. After culturing for 8 h, the supernatant was discarded, and 90 μL of MEM and 10 μL of MTT solution (5 mg/mL) was added to each well. After culturing for 4 h, the supernatant was removed, and 100 μL of DMSO (dimethyl sulfoxide) solution was added to each well. The plates were shaken at low speed (5 Hz, amplitude 15 mm) at room temperature for 10 min to dissolve the formazan and develop color. The optical density (OD) of each well, which is used as an indirect measurement of cell viability, was determined at a wavelength setting of 490 nm using a microplate reader (Thermo Multiskan FC, USA). The microplate reader essentially quantifies how much a given substance reflects or absorbs light. In addition, the viability of cells exposed to $PM_{2.5}$ was calculated as a percentage relative to that of the control group, whose viability was deemed to be 100%. Cell viability = (OD experimental group - OD blank group) ÷ (OD control group - OD blank group) × 100%.

2.5. β-hex basophil degranulation assay

β-hex activity is a standard indicator of RBL-2H3 cell degranulation (Tang et al., 2012). The cell suspension (1×10^5 cells/mL) was seeded in 24-well plates with 500 μL/well. The plates were incubated for 24 h, after which the medium was discarded, and 400 μL of $PM_{2.5}$ suspension was added to each well. Control and blank groups were also added at the same time for incubation. After incubation for 8 h, the reaction was terminated on ice for 10 min. β-hex released into supernatants was quantified by hydrolysis of 4-nitrophenyl-N-acetyl-β-D-glucosamide (N9376, Sigma, USA) in 0.1 M sodium citrate buffer (pH 4.5) for 1 h at 37 °C (Wang et al., 2017a), including the following specific steps: the 50 μL supernatant of each well was transferred into a 96-well culture plate, and 50 μL substrate (4-nitrophenyl-N-acetyl-β-D-glucosaminide (1 mmol L^{-1})) was added. After incubation at 37 °C for 1 h, a 200 μL/well termination solution (0.1 mol L^{-1} Na_2CO_3/$NaHCO_3$ (pH 10.0)) was used to stop the reaction. The absorbance of each well reaction solution was measured using a microplate reader at 405 nm. The levels of β-hex in the supernatant was calculated for each experimental group according to the formula: levels of β-hex (%) = (OD experimental group-OD blank group)/(OD control group-OD blank group) × 100%.

2.6. Quantification of histamine and pro-inflammatory cytokines

The concentration of cell suspension was adjusted to 2×10^5 cells/mL and seeded in 96-well plates at 100 μL/well. After a 24-h cell incubation period, the medium was discarded, 100 μL of $PM_{2.5}$ suspension was added to each well, and the cells were incubated for an additional 8 h. After this 8-h exposure period, cell supernatant was collected for quantification of extracellular (released) histamine and pro-inflammatory cytokines (TNF-α and IL-4) by enzyme-linked immunosorbent assay (ELISA) kits (Jiangsu Enzymatic Biotechnology, CN). The OD values were measured at 450 nm by a microplate reader (Rönkkö et al., 2018).

2.7. $PM_{2.5}$ chemical composition analysis

Subsamples of air $PM_{2.5}$ filters were taken for the analysis of bulk heavy metals and water-soluble ions. For water-soluble components, membrane samples immersed in UPW were ultrasonically treated for 1.5 h in a pre-cooled ultrasonic cleaner and then filtered through 0.22-μm membrane to obtain the liquid fraction. The concentrations of main anions (Cl^-, NO_3^- and SO_4^{2-}) were analyzed by Dionex ICS-1100 (Thermo Fisher Scientific, USA), and the main cations (Mg^{2+}, Ca^{2+}, K^+, NH_4^+ and Na^+) were determined by Dionex DX-600 (Thermo Fisher Scientific, USA) (Huang et al., 2020). For the metal contents, $PM_{2.5}$ filter subsamples were digested by concentrated HNO_3–$HClO_4$–HF acids and dissolved in 5% high-purity HNO_3. The metal concentrations were measured by inductively coupled plasma-optical emission spectrometer (ICP-OES, Optima 8000, PerkinElmer). The recovery rates of each element were between 90% and 110%.

2.8. Statistical analysis

Excel 2010 and SPSS Statistics 25 were used for statistical analysis and Origin (2018) was used for plotting. Pearson product-moment correlation coefficient was adopted for the relationships between pro-inflammatory responses and $PM_{2.5}$ components. The difference between the control and experimental group was statistically analyzed by T-test. Statistical significance was determined at p-values < 0.05. P-values<0.01 were considered extremely significant. Separate one-way analyses of variance (ANOVA) was used to determine the effect of season in each region. Post hoc Fisher's least significant difference (LSD) tests were used to determine significant differences between specific groups exposed to regional PM collected during different seasons.

3. Results

3.1. Cell viability of RBL-2H3 cells exposed to $PM_{2.5}$ from distinct regions in different seasons

The survival rates of RBL-2H3 cells after an 8-h $PM_{2.5}$ exposure are shown in Fig. 1. The overall cell viability after exposure to urban area

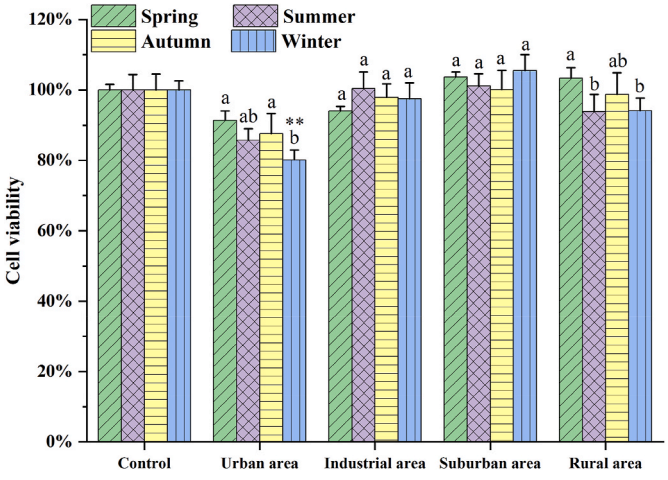

Fig. 1. The effects of seasonal $PM_{2.5}$ variability on the viability of RBL-2H3 cells. Cells were exposed to seasonal (spring, summer, autumn, or winter) $PM_{2.5}$ from one of four representative regions of Nanjing city, China. The letters, a and b, are classifications determined by Fisher's LSD tests. For each region, groups marked with different letters are statistically different (i.e., a = ab; b = ab; a ≠ b). $^{**}p < 0.01$ relative to the control group. N = 5 wells/group.

$PM_{2.5}$ was lower than that from other regions. Overall, the results suggested exposure to $PM_{2.5}$ at 80 mg/L for 8 h did not produce statistically significant decrements in cell viability except for cells exposed to urban winter PM.

3.2. Degranulation of RBL-2H3 cells induced by $PM_{2.5}$ samples from distinct functional areas of the city and different seasons

The level of extracellular β-hex measured in the supernatant of $PM_{2.5}$-exposed RBL-2H3 cells is shown in Fig. 2. Despite the minimal change in cell viability (Fig. 1), most PM-exposed groups had statistically significant levels of extracellular β-hex relative to controls, except for cells exposed to summer and autumn PM in industrial, winter PM in suburban, and spring, summer, winter PM in rural area (Fig. 2). Spring and winter samples appeared to produce the strongest degranulation effects of the industrial and urban PM. Though the effect of the urban winter PM was not statistically different from those of the summer and autumn samples, an upward trend was evident (Fig. 2). In the suburban and rural areas, $PM_{2.5}$ from autumn appeared to produce the strongest degranulation effects (statistically and non-statistically, by trend).

The level of extracellular histamine measured in the supernatant of $PM_{2.5}$-exposed RBL-2H3 cells is shown in Fig. 3. As with the β-hex findings, most PM-exposed groups had higher ($p < 0.05$) histamine levels than control cells. However, the patterns of histamine release were similar across regions, with PM from autumn ≥ spring ≥ winter > summer in terms of potency.

3.3. Inflammatory cytokines released by RBL-2H3 exposed to $PM_{2.5}$ from distinct functional areas of the city and different seasons

Supernatant levels of IL-4 and TNF-α are shown in Fig. 4a and b, respectively. IL-4 was significantly ($p < 0.05$) elevated relative to controls in all groups except those exposed to industrial or suburban, spring or summer PM (Fig. 4a). In general, for this endpoint, autumn and winter samples appeared most potent across regions (Fig. 4a).

With regard to extracellular TNF-α, significant differences relative to control were limited to cells exposed to urban (any season), suburban (autumn), or rural (spring or autumn) PM (Fig. 4b). The results suggested urban PM may have been the most potent stimulator of TNF-α

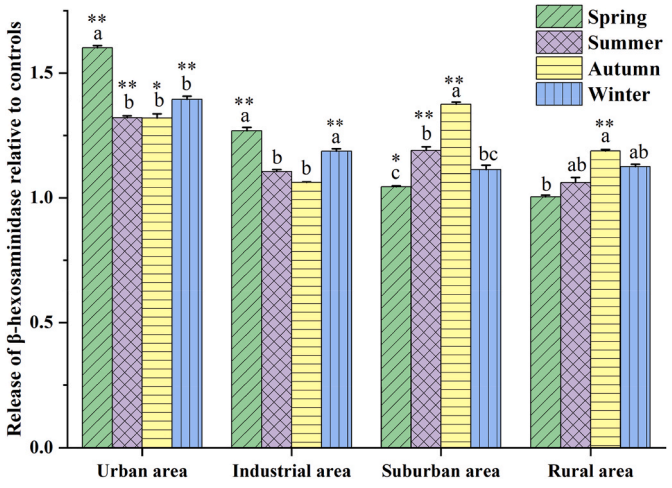

Fig. 2. The effects of seasonal $PM_{2.5}$ variability on the degranulation of RBL-2H3 cells as measured by extracellular β-hexosaminidase. Cells were exposed to seasonal (spring, summer, autumn, or winter) $PM_{2.5}$ from one of four representative regions of Nanjing city, China. The letters a, b, and c are classifications determined by Fisher's LSD tests. For each region, groups marked with different letters are statistically different (i.e., a = ab; a = ac; b = ab; b = bc; c = ac; c = bc; a ≠ b; a ≠ c; b ≠ c). *$p < 0.05$, **$p < 0.01$ relative to the control group. N = 5 wells/group.

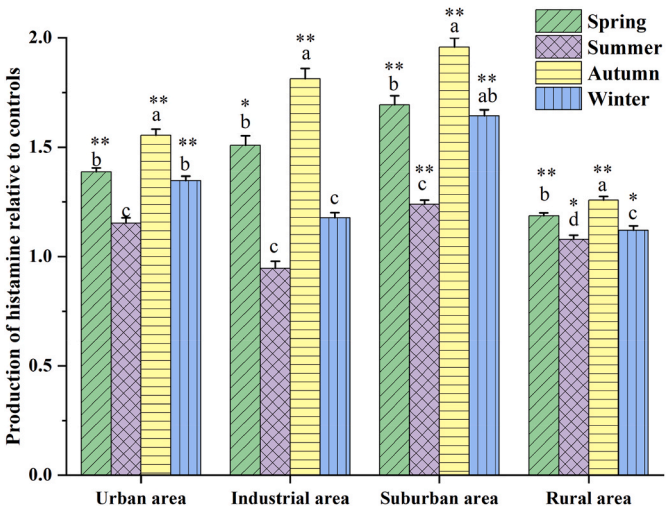

Fig. 3. The effects of seasonal $PM_{2.5}$ variability on the degranulation of RBL-2H3 cells as measured by extracellular histamine. Cells were exposed to seasonal (spring, summer, autumn, or winter) $PM_{2.5}$ from one of four representative regions of Nanjing city, China. The letters a, b, and c are classifications determined by Fisher's LSD tests. For each region, groups marked with different letters are statistically different (i.e., a = ab; a = ac; b = ab; b = bc; c = ac; c = bc; a ≠ b; a ≠ c; b ≠ c). *$p < 0.05$, **$p < 0.01$ relative to the control group. N = 5 wells/group.

irrespective of season. In general, the TNF-α trends across regions were similar to those for histamine, with PM from autumn ≥ spring ≥ summer > winter in terms of potency.

3.4. Chemical compositions of $PM_{2.5}$ from different seasons and functional regions in Nanjing city, China

As shown in Fig. 5, there were seasonal and spatial differences in the metal element content of the collected $PM_{2.5}$ samples. Differences in iron (Fe), the most abundant measured metal overall, were most obvious. Except for the rural region, the mass concentration of Fe in $PM_{2.5}$ samples appeared higher in summer and autumn than in spring and winter (Fig. 5). The high Fe concentration measured from the industrial region was consistent with smelting operations common to the area (Wen et al., 2011). Arsenic (As) mainly comes from coal combustion (Tian et al., 2010). Its concentration in industrial and urban PM was higher than that in suburban and rural PM. The concentration of As was generally higher in summer than in other seasons. The reason may be that the power generation mode of Nanjing was mainly thermal power, and the hot weather in summer and the increase of residential electricity consumption led to the increase of industrial coal consumption. Atmospheric lead (Pb), copper (Cu), and zinc (Zn) are mainly from vehicle exhaust, non-tailpipe emissions (e.g. tire and brake wear) and coal combustion (Duan and Tan, 2013; Huang et al., 1994). The concentrations of these elements were higher in urban and industrial PM than in suburban and rural PM. Apart from the industrial area, metal element content was highest in summer $PM_{2.5}$ samples.

Temporal and spatial differences were also evident for the water-soluble anions and cations in the various $PM_{2.5}$ samples (Fig. 6). In general, the cumulative water-soluble ion content measured in urban and industrial $PM_{2.5}$ samples was higher in spring and winter than in summer and autumn. In the suburban and rural samples, the water-soluble ion content was highest in spring and autumn. Among them, NH_4^+, NO_3^- and SO_4^{2-} are mainly converted into aerosol components by secondary reactions. The concentrations of measured SO_4^{2-} and NO_3^- were the highest. NH_4^+ and NO_3^- appeared higher in spring and winter, especially in urban and industrial PM samples, while those in suburban and rural areas were higher in autumn. The contents of measured Ca^{2+}

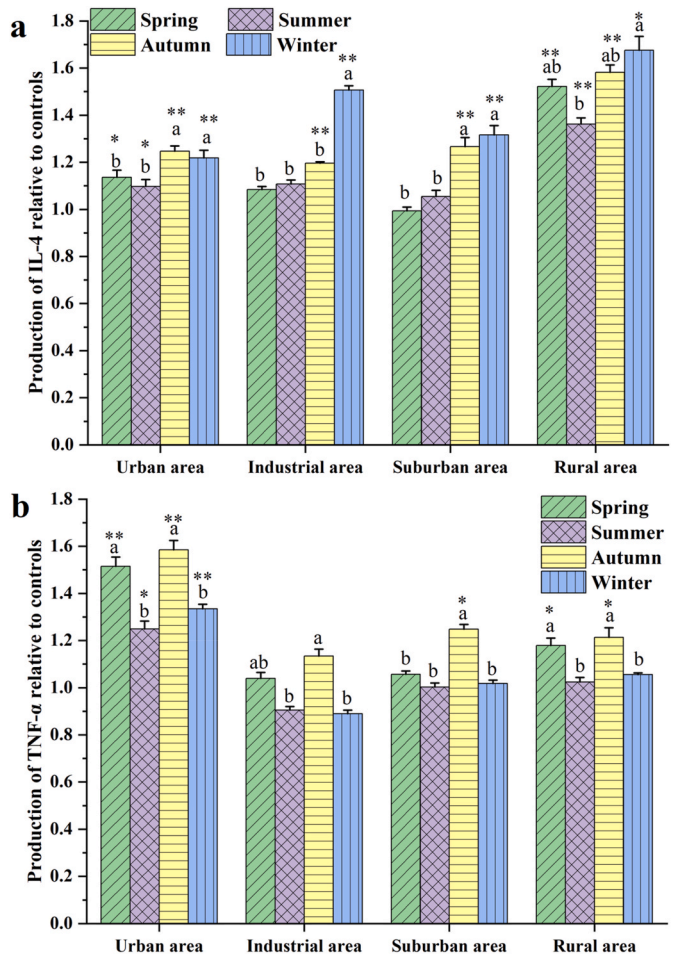

Fig. 4. The effects of seasonal $PM_{2.5}$ variability on the release of inflammatory cytokines by RBL-2H3 cells. Cells were exposed to seasonal (spring, summer, autumn, or winter) $PM_{2.5}$ from one of four representative regions of Nanjing city, China. The letters, a and b, are classifications determined by Fisher's LSD tests. For each region, groups marked with different letters are statistically different (i.e., a = ab; b = ab; a ≠ b). *$p < 0.05$, **$p < 0.01$ relative to the control group. N = 5 wells/group.

and Mg^{2+} in suburban and rural $PM_{2.5}$ samples were higher in spring and summer. In the urban samples, Ca^{2+} and Mg^{2+} appeared highest in summer; in industrial samples, the content of Ca^{2+} was highest in winter, and the content of Mg^{2+} was highest in autumn. Ca and Mg are markers of crustal elements, mainly from soil and construction dust. Their presence in the atmosphere is most likely due to large-scale and road-construction projects associated with the recent urbanization and expansion of Nanjing in suburban and rural areas. The measured concentrations of K^+ and Cl^- were highest in urban winter and industrial summer $PM_{2.5}$. In suburban and rural samples, the contents of K^+ were highest in autumn, and the contents of Cl^- were the highest in winter. K^+ mainly comes from human sources and is related to human activities such as biomass burning and waste incineration (Jacob and Wofsy, 1988; Weitkamp et al., 2005). The measured concentrations of K^+ in suburban and rural PM samples were higher in autumn and winter than in spring and summer.

3.5. *Correlations between cellular inflammatory responses and inorganic chemical components of $PM_{2.5}$*

The correlations between different chemical components (metals, water-soluble ions) and cellular responses (production of IL-4 and TNF-α) of $PM_{2.5}$ were analyzed (Table S1). Among the water-soluble ions, K^+ and Cl^- were positively correlated ($p < 0.05$) with IL-4. The NH_4^+ in the industrial area was also significantly correlated with TNF-α. Among the metal elements, Pb in the industrial area and Cr in the suburban area were positively correlated ($p < 0.05$) with inflammatory mediators (Table S1).

4. Discussion

MTT assay results suggested the exposure paradigm (concentration and duration) was insufficient to produce cytotoxic responses in the tested RBL-2H3 cells (Fig. 1). Relative to control cells, these cells were 80% viable on average, a level that increased degranulation (Figs. 2 and 3) and inflammatory response (Fig. 4) of RBL-2H3 cells without loss of cell viability (D'Evelyn et al., 2021; Manzano-León et al., 2013; Yamada et al., 2012).

Histamine, released from mast cells stimulated by antigens or other degranulation inducers, is usually identified as a degranulation marker in allergic reaction experiments performed *in vitro*. β-hex is stored in secretory granules of mast cells and released with histamine when mast cells are immune activated. The activity of this enzyme in culture medium is used as a marker of mast cell degranulation (Mastuda et al.,

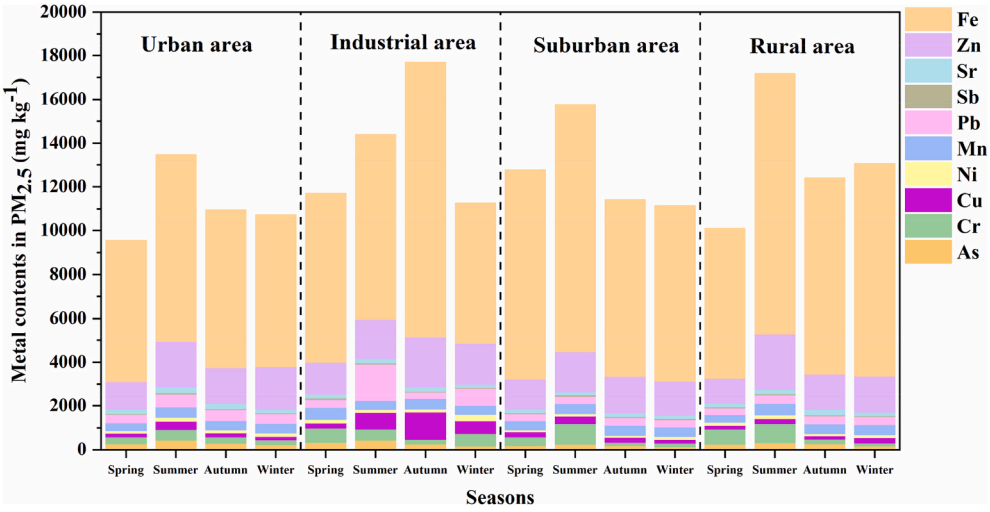

Fig. 5. Metal contents (mg kg^{-1}) of $PM_{2.5}$ samples from different functional areas of Nanjing city during four seasons of a year. Iron (Fe), Zinc (Zn), Strontium (Sr), Antimony (Sb), Lead (Pb), Manganese (Mn), Nickel (Ni), Copper (Cu), Chromium (Cr), Arsenic (As).

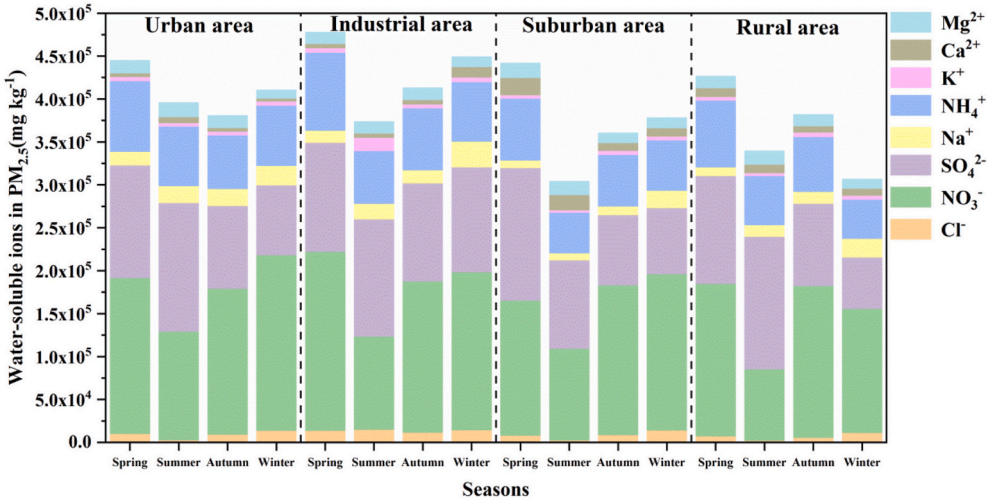

Fig. 6. Water-soluble cation and anion contents (mg kg^{-1}) of $PM_{2.5}$ samples from different areas of Nanjing city during four seasons of a year. Magnesium (Mg^{2+}), Calcium (Ca^{2+}), Potassium (K^+), Ammonium (NH_4^+), Sodium (Na^+), Sulphate (SO_4^{2-}), Nitrate (NO_3^-), Chloride (Cl^-).

2002). At least one study reported that when healthy volunteers were exposed to diesel exhaust, an increase of inflammatory cells and histamine was observed in their airway lavage specimens (Salvi et al., 1999). In another study, DEPs were shown to induce histamine release directly (Devouassoux et al., 2002). In the present study, the activated degranulation of RBL-2H3 cells was directly induced by $PM_{2.5}$ from multiple different regions. In general, the levels of β-hex released from RBL-2H3 cells exposed to urban or industrial $PM_{2.5}$ from cold seasons (winter and spring) were higher than those exposed to warm-season (summer and autumn) $PM_{2.5}$ from the same region (Fig. 2). For suburban and rural regions, autumn $PM_{2.5}$ samples were the most potent inducers of β-hex release (Fig. 2). With respect to histamine, autumn samples were most potent irrespective of the PM collection location (Fig. 3). Theoretically, histamine and β-hex can be released parallelly by RBL-2H3 *in vitro*, but there were differences between them in this study. Schwartz et al. (1987) reported that the half-life of histamine under physiological conditions was very short, and the time was only a few minutes. The reason for the instability of detection results in the present study was probably related to the short half-life of histamine itself and the instability of *in vitro* detection. Therefore, this study showed that $PM_{2.5}$ could directly induce a certain degree of RBL-2H3 cells activation and degranulation, and promote the release of β-hex and histamine, consistent with previous studies (Devouassoux et al., 2002). In the present study, the ability of $PM_{2.5}$ to induce cell degranulation was greatly affected by regional and seasonal composition differences. This process may depend on the contents of endotoxin and airborne allergen in $PM_{2.5}$ (Yamada et al., 2012).

Atmospheric pollution can also stimulate the expression of inflammatory cytokines and induce inflammatory reactions (Li et al., 2020). As the primary mediator of systemic and local inflammation, TNF-α is considered to be a marker of the inflammatory response (Yang et al., 2016). IL-4 is the main inflammatory mediator of RBL-2H3 cell degranulation (Mastuda et al., 2002). In the present study, the levels of IL-4 released by cells exposed to $PM_{2.5}$ from autumn and winter were higher than those exposed to spring or summer PM, and the $PM_{2.5}$ samples from spring and autumn led to higher levels of TNF-α production. Studies have shown that water-soluble components (including water-soluble organic carbon and secondary ions) in $PM_{2.5}$ are associated with pro-inflammatory cytokines (Pang et al., 2020; Wang et al., 2013). In the present study, NH_4^+, K^+ and Cl^- had significant positive correlations with inflammatory mediators (Table S1). K^+, in suburban and rural $PM_{2.5}$, and Cl^-, in urban and suburban $PM_{2.5}$ were positively correlated ($p < 0.05$) with IL-4. NH_4^+ in the industrial $PM_{2.5}$ was also significantly correlated with TNF-α. NH_4^+ mostly comes from fossil-fuel combustion and vehicle exhaust. K^+ is mainly produced by human sources, related to a series of human activities such as waste incineration and biomass combustion. Cl^- is associated with industrial activities (such as salt electrolysis) and coal combustion, and is a typical marker element for coal combustion (Tan et al., 2009). Metal elements account for a small proportion of $PM_{2.5}$, but exhibited serious threats to human health (Fan et al., 2021). Moreover, one study showed that the heavy metals (Cr, Ni, Cu, Cd, Pb, Zn, Mn, and Co)-containing $PM_{2.5}$ induced season-dependent apoptosis of cells through an inflammatory response mediated by reactive oxygen species (ROS) (Zhang et al., 2016). Some metals, such as Cd, can activate inflammation through tissue damage induction mediated by free radicals (Milnerowicz et al., 2015). Compared with summer, the inflammatory response caused by $PM_{2.5}$ in winter were more severe, which may be related to the high levels of some transition metals (Cu, Mn, Co) in winter $PM_{2.5}$ samples (Chen et al., 2018b). In the present study, Pb and Cr had significant positive correlations to inflammatory mediators (Table S1). Cr in the suburban $PM_{2.5}$ was positively correlated ($p < 0.05$) to IL-4, and Pb in the industrial $PM_{2.5}$ also significantly correlated to TNF-α. Airborne Pb and Cr are mainly emitted from coal combustion and vehicle emissions (Johansson et al., 2009; Wang et al., 2013). Therefore, $PM_{2.5}$ related to industrial coal combustion and vehicle exhaust on suburban main roads likely drove the significant positive association with the inflammatory reaction. Combined with the correlations between cellular inflammatory responses and inorganic chemical components of $PM_{2.5}$, it is speculated that vehicle exhaust, industrial production, and fossil fuel and biomass combustion have great impacts on inflammatory responses.

At present, the pollution levels and sensitization potential of atmospheric particulates, especially $PM_{2.5}$, are notable public health and research concerns. In one study (Zhao et al., 2012), acute exposure to $PM_{2.5}$ caused acute local and systemic inflammatory responses in mice. Dong et al. (2005a) also found that DEPs could enhance ovalbumin (OVA)-induced allergic reactions, and the interaction between DEPs and OVA increased the levels of ROS, nitric oxide (NO), and intracellular glutathione in macrophages and bronchoalveolar lavage fluid. Dong et al. (2005b) also suggested DEPs that elicit an adjuvant effect on the production of antigen-specific IgE and IgG. However, in the present study, $PM_{2.5}$ induced allergen-independent degranulation of basophils, and further studies are needed to prove whether particulate matter in different functional areas and seasons can act as an allergen adjuvant to promote IgE-mediated allergic reactions. Due to the differences in particle composition from different sources (Jaafari et al., 2021), subsequent studies should consider the synergistic effects of $PM_{2.5}$ from different sources (i.e. haze and sandstorm weather) and antigens on this

basis, and clarify the effects of $PM_{2.5}$ on cell sensitization and pro-inflammatory responses. Studies have shown that in macrophages, the resident immune and primary responder cells in the lungs, the biological responses to ultrafine PM, $PM_{2.5}$, and PM_{10} fractions were significantly different depending upon PM size, the specific gene measured, and exposure time (D'Evelyn et al., 2021). Since PM_{10} also includes biological aerosols, such as endotoxin, mold, and fungal spores (Kelly and Fussell, 2012; Robertson et al., 2019), the cellular immune response of particles to different particle sizes should be considered in future studies. One study reported that Zn and Pb in $PM_{2.5}$ are mainly derived from anthropogenic coal combustion (Zhang et al., 2016); these metals were the main elements in spring and winter $PM_{2.5}$ samples from the present study. Pb has also been shown to be responsible for greater potential health risks to children (Zhang et al., 2016). Another study suggested that exposure to organic-chemical-rich urban $PM_{2.5}$ and microbial-element-rich desert $PM_{2.5}$ is a significant risk factor for inflammatory and allergic lung diseases, and the desert-$PM_{2.5}$ may cause greater effects upon human respiratory health than organic-chemical-rich urban $PM_{2.5}$ (He et al., 2016b). The above studies support our finding that the immune and pro-inflammatory responses to $PM_{2.5}$ in different regions and seasons may be affected by the chemical composition of the $PM_{2.5}$, a finding that needs further study.

Nevertheless, there were still several limitations in our study. First, we did not measure the endotoxin and allergen content of the collected $PM_{2.5}$; these substances may influence the ability of $PM_{2.5}$ to induce degranulation of basophil cells. The detailed mechanisms behind the enhancement of allergic effect of $PM_{2.5}$ will be the subject of future studies (Dong et al., 2005a). Second, we did not determine the source apportionment of aerosol pollution from an atmospheric chemistry perspective. However, we classified the sources of these components based on reliable literature to explain results.

5. Conclusion

The present study was designed to deepen scientific understanding of the impacts of atmospheric particulates on human health by examining the mechanisms driving basophil degranulation, and pro-inflammatory responses upon exposure to temporally and spatially heterogeneous $PM_{2.5}$ samples. Given that variation in regional and seasonal atmospheric conditions may give rise to unique combinations of sensitizing aerosol components and different dispersal patterns, studies that include multiple PM variables may provide additional insight into potential synergistic effects of $PM_{2.5}$ components in the air. $PM_{2.5}$ from urban and industrial areas in winter and spring, and suburban and rural areas in autumn, were the most potent stimulators of the degranulation ability of basophilic cells under cell culture conditions. The $PM_{2.5}$ from cold seasons (winter and spring) were largely due to anthropogenic sources (e.g. vehicle exhaust, industrial production, and fossil fuel and biomass combustion) with the induction of strong pro-inflammatory effects. Therefore, while assessing $PM_{2.5}$ pollution on human health, besides the damage to the respiratory and cardiovascular systems, it is important to also consider that $PM_{2.5}$ in different regions and seasons may cause changes in inflammation and immune functions.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.130919.

Credit author statement

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