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# Fine particulate matter ($PM_{2.5}$) enhances FcεRI-mediated signaling and mast cell function

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ABSTRACT

Persistent exposure to ambient fine particulate matter ($PM_{2.5}$) can exacerbate allergic diseases in humans. Mast cells play an important role in allergic inflammation in peripheral tissues, such as skin, mucosa, and lung. Engagement of the high-affinity Fc receptor leads to mast cell degranulation, releasing a variety of highly active mediators including histamine, leukotrienes, and inflammatory cytokines. How $PM_{2.5}$ exposure affects mast cell activation and function remains largely unknown. To characterize the effect of $PM_{2.5}$ on mast cells, we used bone marrow-derived mast cells (BMMCs) to examine whether $PM_{2.5}$ affected FcεRI-mediated signaling, cytokine production, and degranulation. Exposure to high doses of $PM_{2.5}$ caused pronounced apoptosis and death of BMMCs. In contrast, exposure to low doses of $PM_{2.5}$ enhanced mast cell degranulation and FcεRI-mediated cytokine production. Further analysis showed that $PM_{2.5}$ treatment increased Syk activation and subsequently phosphorylation of its substrates including LAT, PLC-γ1, and SLP-76. Moreover, $PM_{2.5}$ treatment led to activation of the PI3K and MAPK pathways. Intriguingly, water-soluble fraction of $PM_{2.5}$ were found responsible for the enhancement of FcεRI-mediated signaling, mast cell degranulation, and cytokine production. Our data suggest that $PM_{2.5}$, mainly water-soluble fraction of $PM_{2.5}$, could affect mast cell activation through enhancing FcεRI-mediated signaling.

## 1. Introduction

Air pollution in developing countries undergoing rapid urbanization has been recognized as a main cause of the onset and exacerbation of respiratory and allergic disorders [8,23]. High concentrations of air pollutants, such as ambient fine particulate matter with an aerodynamic diameter < 2.5 μm ($PM_{2.5}$), can deposit throughout the respiratory tract, particularly in small airways and alveoli, and have been demonstrated to have high toxicological effects on the respiratory system [8]. Short-term exposure to ambient $PM_{2.5}$ in prospective cohorts of asthmatic children and adults has been shown to associate with the asthma symptoms, especially in children with allergic sensitization [20,21]. Increased ambient concentration of $PM_{2.5}$ is considered as one of major causes for rising asthma hospitalization, especially in elderly population [14]. The animal studies also indicate that $PM_{2.5}$ inhalation renders individuals more susceptible to allergic asthma through initiating pulmonary allergic inflammatory responses [4,10]. The cellular mechanisms regulating hyper-allergic response caused by $PM_{2.5}$ have not been entirely elucidated.

Mast cells are derived from hematopoietic progenitors in the bone marrow and can be found in mucosal and epithelial tissues throughout the body. Mast cells play an important role in inflammatory and allergic responses by releasing histamine and other mediators after activation by IgE-allergen immune complexes. Anti-allergen IgE antibodies, which

*Abbreviations:* $PM_{2.5}$, fine particulate matter; BMMCs, bone marrow-derived mast cells; FBS, fetal bovine serum; WSF, water-soluble fraction; WIF, water-insoluble fraction; SEM, standard error of the mean; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; TNF-α, tumor necrosis factor-α; DEP, diesel exhaust particles; ROS, reactive oxygen species

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are produced in allergic individuals, bind to the high affinity Fc receptor, FcεRI, on mast cells. IgE-allergen immune complexes interact with the FcεRI and initiate activation of protein tyrosine kinases, such as Lyn, Fyn, and Syk, and subsequent phosphorylation of signaling proteins, such as LAT, SLP-76, and PLC-γ1, eventually leading to mast cell degranulation [15,17,27,28]. Through degranulation, mast cells release a variety of highly active mediators including histamine, serotonin, heparin, prostaglandins, leukotrienes, neutralproteases, and inflammatory cytokines [15]. Mast cells are well known to play an important role in allergic inflammation after engagement of the FcεRI receptor in peripheral tissues, such as skin, mucosa, and lung [16]. Published studies also indicate the involvement of mast cells in $PM_{2.5}$-induced lung allergic inflammation [3,18]. However, how $PM_{2.5}$ exposure affects mast cell activation remains unknown.

In this present study, we analyzed the effect of $PM_{2.5}$ on FcεRI-mediated signaling and mast cell function using bone marrow-derived mast cells (BMMCs) with a well-defined immune complexes, DNP (dinitrophenyl)-HSA (human serum albumin)/anti-DNP IgE. Our data indicated that $PM_{2.5}$ exposure could enhance FcεRI-mediated signaling, cytokine production, and degranulation.

## 2. Materials and methods

### 2.1. $PM_{2.5}$ collection and preparation

$PM_{2.5}$ was collected onto glass fiber filters in Xinxiang Medical University, Henan, China, as described previously [12,13]. The particles were recovered by sequential sonication of the filter (four cycles/20 min each) in sterile water; the particle suspension was lyophilized, resuspended in PBS in a concentration of 10 mg/ml, and stored at 4 °C. $PM_{2.5}$ suspension was sonicated again before being used to treat mast cells.

In regard to the preparation of water-soluble fraction (WSF) and water-insoluble fraction (WIF) of $PM_{2.5}$, 1 ml $PM_{2.5}$ stock suspension was sonicated for four cycles of 20 min each and then centrifuged at 8000 rpm for 5 min at 4 °C. The supernatant was collected as WSF, and the pellet was resuspended again in 1 ml PBS as WIF.

### 2.2. BMMC culture

Mast cells (BMMCs) used in this study were derived from the bone marrow cells from C57BL/6J mice in IL-3 medium (IMDM supplemented with 10% fetal bovine serum (FBS) and 5 ng/ml recombinant IL-3) as described previously [29]. After cultured in the IL-3 medium for 3–4 weeks, mast cells were stained with antibodies against FcεRIα and c-Kit and analyzed by flow cytometry for their purity.

### 2.3. Examination of cell morphology and apoptosis

BMMCs were seeded in a 96-well plate ($2 \times 10^4$/well) and treated with 0, 0.5, 1.25, 2.5, 5.0, 10.0, 25.0, 50.0, 100.0, or 200.0 μg/$cm^2$ $PM_{2.5}$ for 48 h. These cells were then analyzed by H&E staining. For apoptosis, BMMCs were stained with anti-c-Kit and Annexin V and analyzed by FACS.

### 2.4. Antibodies and flow cytometry analysis

The following antibodies were used for Western blotting: anti-pTyr (4G10, Millipore Biotechnology, Inc.), pPLC-γ1 (Tyr783), pSrc (Tyr416), pSyk(Tyr525/Tyr526), Syk, pAkt(Ser473), Akt, pERK1/2(Thr202/Tyr204), pp38, p38, pJNK (Cell Signaling Biotechnology, Inc.), PLC-γ1 (Upstate Biotechnology, Inc.), pSLP-76 (Tyr113), pSLP-76 (Tyr128), pSLP-76 (Tyr145) (BD Biosciences, Inc.), SLP-76 (Abcam Biotechnology, Inc.), LAT, ERK2, and JNK1 (Santa Cruz Biotechnology). The antibodies used in FACS analysis were the following: PE-conjugated-anti-c-Kit, PE-Cy7-anti-FcεRIα, APC-anti-CD107α, PE-Cy7-anti-TNF-α, APC-anti-IL-6, APC-Annexin V (Biolegend, Inc.), 7AAD (Life Technologies, Inc.), and Pacific Blue-live&dead (Invitrogen, Inc.). Flow cytometry measurement was performed using the Becton Dickinson FACS Canto and analyzed by the FlowJo software.

### 2.5. Examination of mast cell degranulation, activation and Western blotting

For short-term $PM_{2.5}$ exposure, BMMCs ($5 \times 10^6$/ml) were sensitized with anti-DNP IgE (1 μg/ml, SPE-7 mAb, Sigma) in IMDM medium without IL-3 for 2 h before addition of $PM_{2.5}$ at different doses(0, 12.5, 25.0, 50.0 μg/$cm^2$). After 4 h treatment, BMMCs were harvested and washed with IMDM, and then stimulated with different concentrations of DNP-HSA (1–1000 ng/ml) for 10 min in Tyrode's buffer.

For long-term $PM_{2.5}$ exposure, BMMCs ($2 \times 10^6$/ml) were treated with $PM_{2.5}$ at 0, 1.25, 2.5, 5.0, 10.0 μg/$cm^2$, WSF or WIF fractions for 24 h in IL-3 IMDM medium before sensitized with anti-DNP IgE. BMMCs were washed with IMDM and stimulated with 100 ng/ml DNP-HSA for 5 min.

To profile the time course of $PM_{2.5}$-induced cell deregulation, BMMCs after sensitization were exposed to 25.0 μg/$cm^2$ $PM_{2.5}$ for 60, 120, or 180 min, washed with IMDM, and then stimulated with different concentrations of DNP-HSA (1–1000 ng/ml). Degranulation of BMMCs was determined by measuring the release of β-hexosaminidase into the culture supernatants as described previously [27].

For Western blotting analysis, sensitized BMMCs were treated with different concentrations of $PM_{2.5}$ and followed by stimulation with 100 ng/ml DNP-HSA for 5 min before lysis. For phosphorylation kinetics, these BMMCs were stimulated with DNP-HSA for 0, 2, 5, 10, and 20 min before lysis. Protein samples were separated by SDS-PAGE and transferred to nitrocellulose membranes. After incubation with primary antibodies, nitrocellulose membranes were washed three times and probed with either anti-mouse or rabbit IgG conjugated to AlexaFluor 680 (Molecular Probes) or IRDye800 (Rockland). Membranes were then visualized with the LI-COR Bioscience Odyssey system (LI-COR).

### 2.6. Measurement of cytokine production

BMMCs were treated with $PM_{2.5}$ at 0, 1.5, 3.0, or 6.0 μg/$cm^2$ for 24 h in IMDM medium with IL-3 before being sensitized with anti-DNP IgE (1 μg/ml) for 4 h. These cells were then stimulated with 100 ng/ml of DNP-HSA overnight. Next day, the cell supernatants were harvested. The concentrations of IL-6 and MCP-1 in the supernatants were determined by ELISA. Expression of IL-6 and tumor necrosis factor-α (TNF-α) in these cells was analyzed by intracellular staining using antibodies against these cytokines (Biolegend, Inc.).

### 2.7. Statistical Analysis

All experiments were repeated at least three times. Data were presented as mean ± standard error of the mean (SEM). SPSS21.0 (IBM, Chicago, IL, USA) was used for statistical analysis. Data comparison was analyzed by one-way ANOVA followed by two-tailed Student's *t*-test. All graphs were prepared by using GraphPad Prism 6.0 (GraphPad software Inc., San Diego, CA, USA). A two-tailed $P$ value $< .05$ was considered statistically significant.

## 3. Results

### 3.1. Cytotoxicity of $PM_{2.5}$ exposure on mast cells

Mast cells derived from the bone marrow of C57BL/6J mice were grown in the presence of IL-3. First of all, we determined whether $PM_{2.5}$ posed toxic effect on mast cells. BMMCs were incubated with different doses of $PM_{2.5}$ (0 to 200 μg/$cm^2$) for 48 h before H&E staining for cell morphology and FACS analysis for cell apoptosis and death. As shown

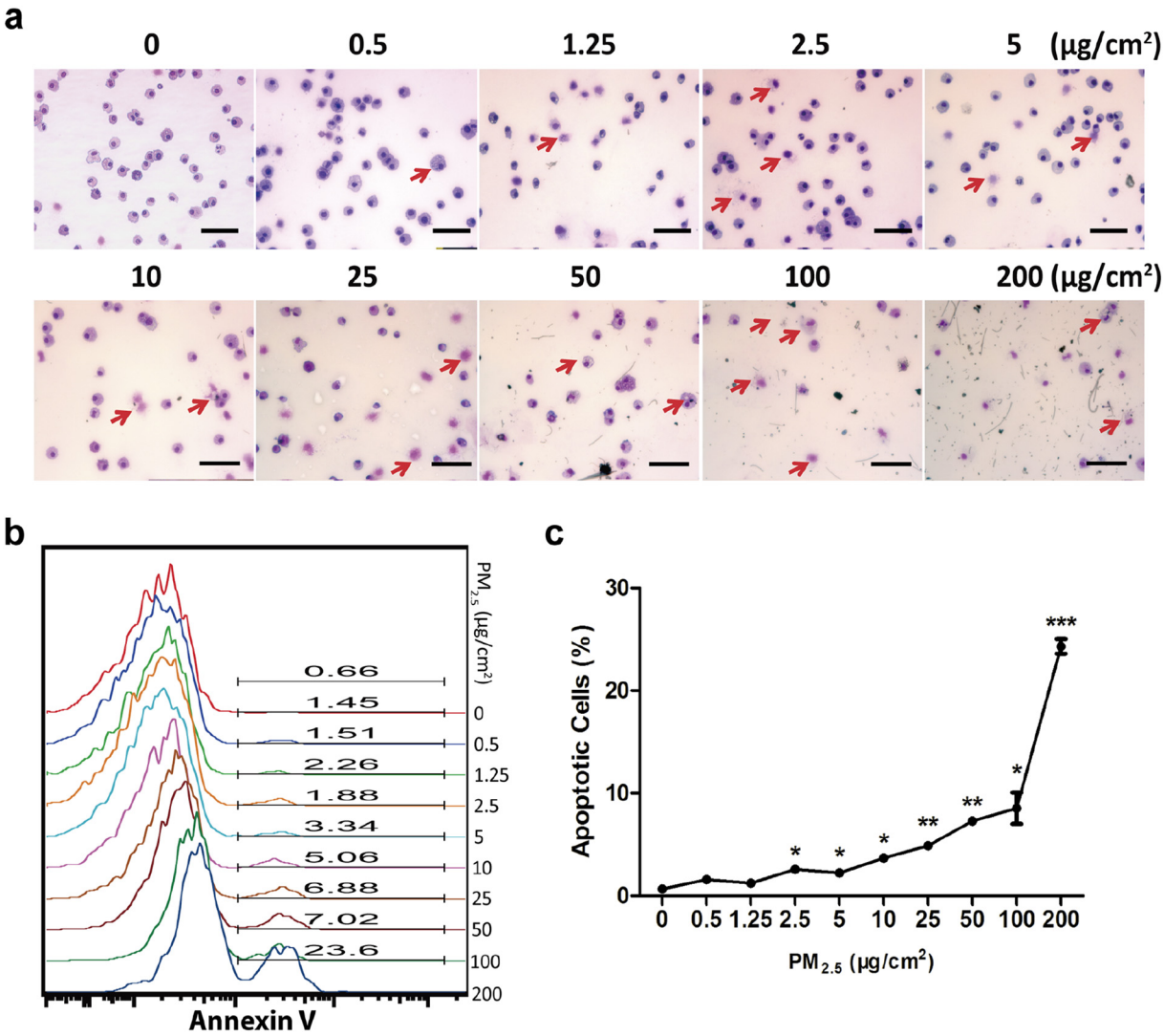


**Fig. 1.** $PM_{2.5}$ exposure induces morphological alterations and apoptosis of mast cells. BMMCs were seeded in 96-well plate ($2 \times 10^4$/well) and treated with different concentrations of $PM_{2.5}$ for 48 h before analysis. (a).H&E staining. (b). The percentage of apoptotic cells. (c). The effect of $PM_{2.5}$ exposure on apoptosis. Data shown are representative of three separate experiments. $^*P < 0.05$, vs. 0 μg/cm$^2$ ($n = 3$); $^{**}P < 0.01$, vs. 0 μg/cm$^2$ (n = 3); $^{***}P < 0.001$, vs. 0 μg/cm$^2$ (n = 3).

in Fig. 1a, upon exposure to high doses of $PM_{2.5}$, BMMCs exhibited cytoplasmic vacuolization and rupture of cell membranes (as indicated by red arrows). FACS analysis showed that the percentages of dead cells or apoptotic cells (Annexin V positive cells) were increased following $PM_{2.5}$ treatment (Fig. 1b and c). These results suggested that exposure to high doses of $PM_{2.5}$ could induce pronounced toxicity to mast cells and caused cell apoptosis. In contrast, at low doses (< 2.5 μg/cm$^2$), $PM_{2.5}$ treatment showed minimal toxic effects on BMMCs.

### 3.2. $PM_{2.5}$ exposure on FcεRI-mediated mast cell degranulation

To determine whether $PM_{2.5}$ exposure could affect FcεRI-mediated signaling and mast cell function, BMMCs were first sensitized with anti-DNP IgE for 2 h before addition of $PM_{2.5}$ at different doses (0, 12.5, 25.0, 50.0 μg/cm$^2$). After 4 h treatment, BMMCs were washed and then stimulated with different concentrations of DNP-HSA. Degranulation was assayed by measuring the release of β-hexosaminidase by mast cells into the culture supernatants. As shown in Fig. 2a, $PM_{2.5}$ exposure at 12.5, 25 or 50 μg/cm$^2$ enhanced FcεRI-mediated degranulation in BMMCs upon stimulation by DNP-HSA. In addition, $PM_{2.5}$ exposure at 2.5, 5, and 10 μg/cm$^2$ also enhanced FcεRI-mediated degranulation (Fig.1S). Sensitized BMMCs were also treated with 25 μg/cm$^2$ $PM_{2.5}$ for 0, 60, 120, and 180 min before stimulation with different concentrations of DNP-HSA to induce degranulation. As shown in Fig. 2b, 25 μg/cm$^2$ $PM_{2.5}$ exposure increased FcεRI-mediated degranulation in BMMCs at the time of 120 min.

To confirm above results, the expression of CD107α on the cell surface of BMMCs was examined after activation. CD107a is a marker for granule exocytosis [6] and upregulated on mast cells undergoing degranulation. As shown in Fig. 2c, $PM_{2.5}$ exposure up-regulated CD107α surface expression in stimulated BMMCs at the dose of 12.5 and 25 μg/cm$^2$. At 50 μg/cm$^2$ or higher, we did not observe significant differences in CD107α possibly due to toxicity of $PM_{2.5}$. Taken together, these results indicated that $PM_{2.5}$ exposure could render mast cell hypersensitive to stimulation via the FcεRI.

### 3.3. PM2.5 on FcεRI-mediated cytokine production

Mast cell activation leads to production of inflammatory cytokines, such as IL-6 and TNF-α. We next determined the effect of $PM_{2.5}$ on FcεRI-mediated cytokine production by intracellular staining an ELISA. As shown in Fig. 3a and b, after DNP-HSA stimulation, the percentage of cells expressing IL-6 and TNF-α were increased in the presence of $PM_{2.5}$. Further experiments showed that expression of IL-6 and TNF-α

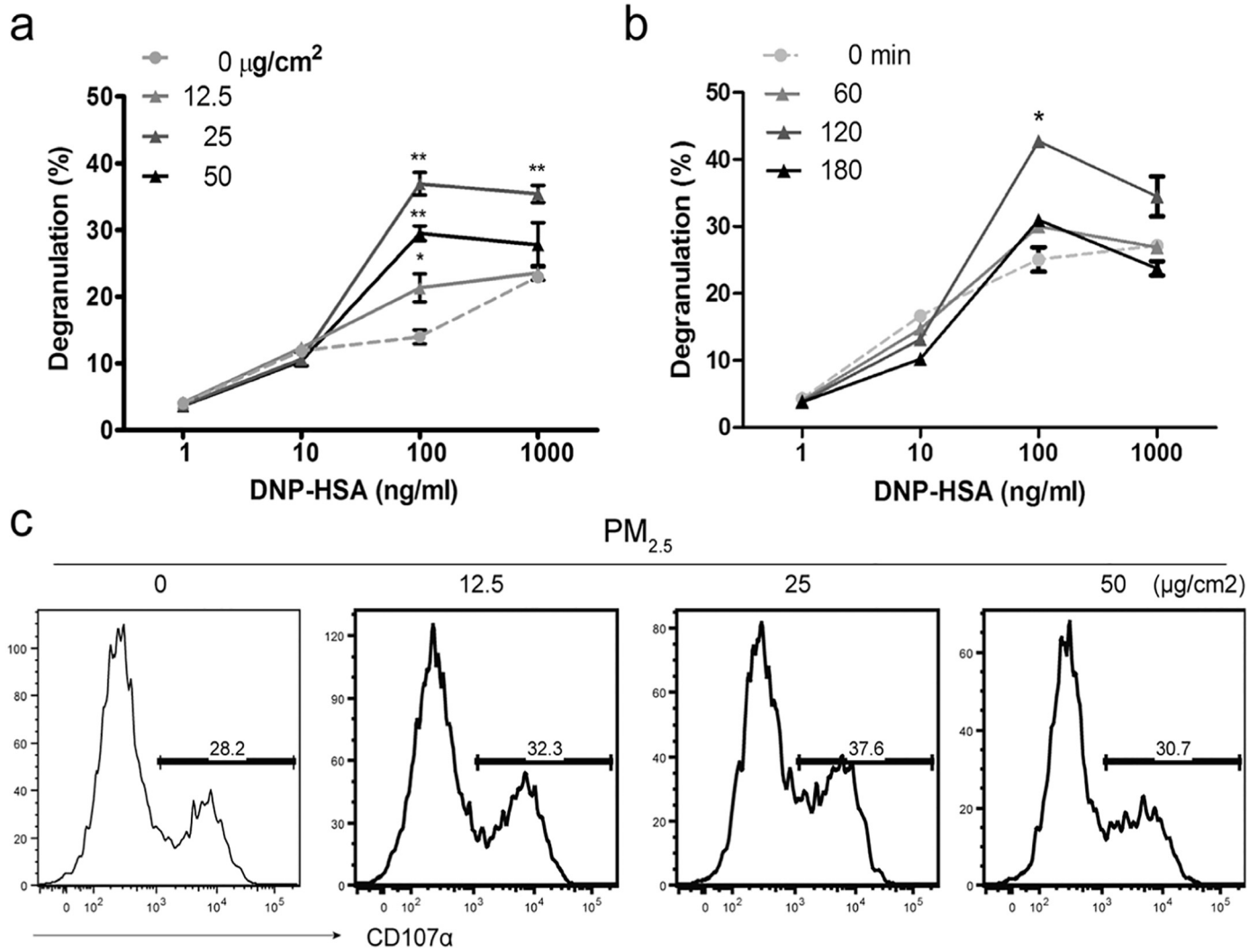


**Fig. 2.** Effect of $PM_{2.5}$ exposure on FcεRI-mediated mast cell degranulation. BMMCs were sensitized with anti-DNP IgE before treatment with $PM_{2.5}$ at indicated doses and then stimulated with various concentrations of DNP-HSA for 10 min. Degranulation of BMMCs was determined by measuring the release of β-hexosaminidase into the culture supernatants. (a). Different doses of $PM_{2.5}$ exposure on FcεRI-mediated mast cell degranulation. (b). Different exposure time of $PM_{2.5}$ on FcεRI-mediated mast cell degranulation. BMMCs were treated with 25 μg/cm$^2$ dose of $PM_{2.5}$ for 0, 60, 120, and 180 mins before stimulation. (c). Effect of $PM_{2.5}$exposure on CD107α surface expression. Sensitized BMMCs were treated with different concentrations of $PM_{2.5}$ before activation and FACS analysis. Data shown are representative of three separate experiments. *$P < 0.05$, vs. 0 μg/cm$^2$ or 0 min ($n = 2$); **$P < 0.01$, vs. 0 μg/cm$^2$ and 0 min (n = 2).

was significantly increased when these cells were treated with $PM_{2.5}$ at 25 or 50 μg/cm$^2$ (Fig. 3c and d). In addition, more IL-6 (Fig. 3e) and MCP-1 (Fig. 3f)were secreted by stimulated BMMCs in the presence of $PM_{2.5}$. Together, our data indicated that in addition to enhanced degranulation, $PM_{2.5}$ exposure also promoted FcεRI-mediated mast cell cytokine production.

### 3.4. $PM_{2.5}$ on FcεRI-mediated signaling

Next, we investigated whether $PM_{2.5}$ affected FcεRI-mediated signaling events, leading to increased expression of inflammatory cytokines. BMMCs were sensitized, treated with different concentrations of $PM_{2.5}$, and stimulated with DNP-HSA. Cell lysates were analyzed by Western blotting with different antibodies. For biochemical analysis, we chose to treat these BMMCs with low doses of $PM_{2.5}$ at 0, 1.25, 2.5, 5, and 10 μg/cm$^2$ for 24 h. At these concentrations of $PM_{2.5}$ treatment, mast cell survival was not significantly affected.

As shown in Fig. 4a, overall tyrosine phosphorylation of proteins after engagement of the FcεRI was similar before and after these cells were treated with $PM_{2.5}$. Phosphorylation of LAT, which was normalized to total LAT protein, in $PM_{2.5}$-treated BMMCs was increased when cells were treated with 2.5 and 5.0 μg/cm$^2$ $PM_{2.5}$. This result was reproducible in three independent experiments. To determine whether differences in protein phosphorylation is due to the difference of phosphorylation kinetics. In the case of particulate allergens, they could prolong FcεRI -mediated signaling and induce much stronger cytokine response compared to soluble allergens [11]. To this end, we stimulated sensitized mast cells in the presence or absence of $PM_{2.5}$ with DNP-HSA for 0, 2, 5, 10, and 20 mins before lysis. As shown in Fig.4c, the phosphorylation kinetics was similar in untreated and $PM_{2.5}$ -treated cells.

Upon engagement of TCR or FcεRI, LAT is phosphorylated and interacts with Grb2, PLC-γ1, SLP-76, and other signaling molecules [26]. Thus, we next investigated whether phosphorylation and activation of proteins upstream and downstream of LAT were also affected by $PM_{2.5}$ using Western blotting with antibodies against phosphorylated Src, Syk, PLC-γ1, and SLP-76. As shown in Fig. 4b, phosphorylation (activation) of PLC-γ1, Syk, and SLP-76 in stimulated BMMCs was enhanced after treatment with 1.25, 2.5, 5.0 μg/cm$^2$ $PM_{2.5}$ for 24 h. On the other hand, phosphorylation of Src kinase was not increased by $PM_{2.5}$ treatment.

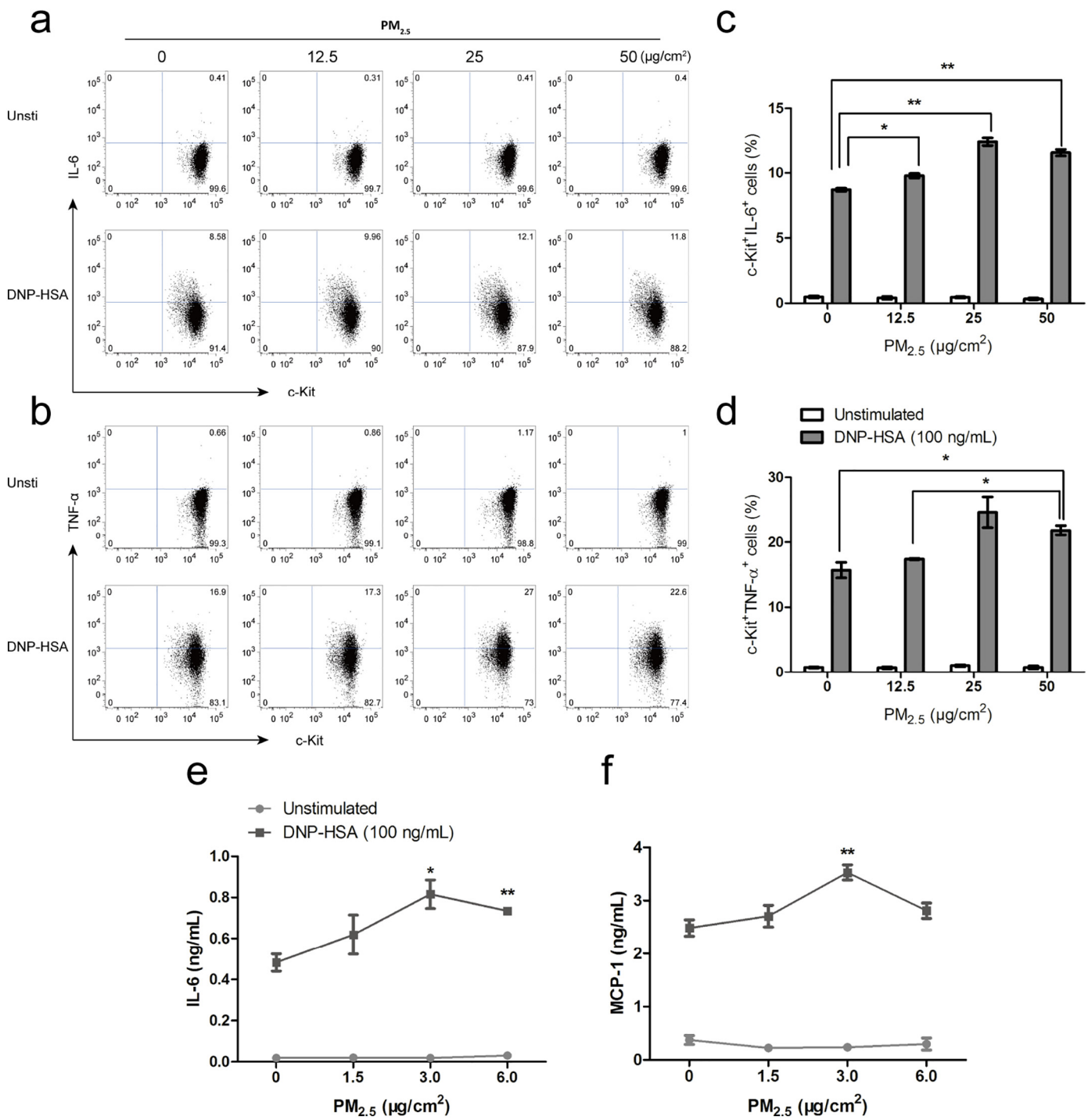


**Fig. 3.** $PM_{2.5}$ exposure enhances FcεRI-mediated cytokines production. (a-b). Intracellular staining of IL-6 and TNF-α. (c-d). Statistical analysis of IL-6 and TNF-α production. Sensitized BMMCs treated with $PM_{2.5}$ were stimulated with 100 ng/ml DNP-HSA for 4 h in the presence of monensin before permeabilization and intracellular staining for IL-6 and TNF-α. Cells were gated on live cells. The percentages of c-Kit$^+$IL-6$^+$ or c-Kit$^+$ TNF-α$^+$ cells were used in statistical analysis to compare cells treated with $PM_{2.5}$. to those untreated cells. (e-f). Secretion of IL-6 and MCP-1 by mast cells. Sensitized BMMCs with $PM_{2.5}$ treatment were stimulated with 100 ng/ml DNP-HSA for overnight, and then the cell supernatants were harvested for determination of IL-6 (e) and MCP-1 (f) concentration by ELISA. Data shown are representative of three separate experiments. $^*P < 0.05$, vs. 0 μg/cm$^2$ ($n = 3$); $^{**}P < 0.01$, vs. 0 μg/cm$^2$ (n = 3).

The same effect on FcεRI-mediated proximal signaling was also seen when higher doses of $PM_{2.5}$ were used (data not shown). Taken together, our results suggested that $PM_{2.5}$ exposure could augment FcεRI-mediated proximal signaling. $PM_{2.5}$ exposure may enhance Syk activation and subsequently phosphorylation of its substrates, including LAT, PLC-γ1, and SLP-76.

### 3.5. The effect of $PM_{2.5}$ on the PI3K and MAPK pathways

FcεRI-mediated signaling leads to activation of the PI3K and MAPK pathways [7]. Therefore, we further investigated whether these pathways were also affected in $PM_{2.5}$-treated BMMCs. BMMCs were treated and activated as depicted above and protein lysates were subjected to Western blotting using antibodies against phospho-Akt, ERK1/2, JNK,

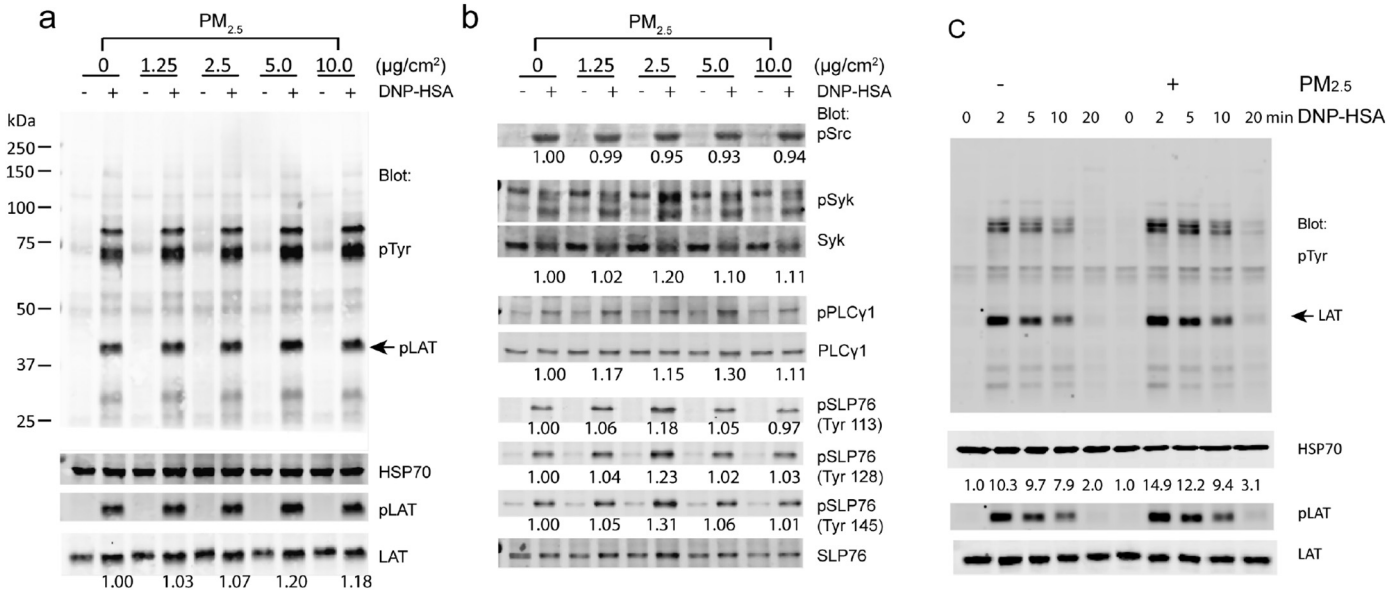


**Fig. 4.** Effect of $PM_{2.5}$ on FcεRI-mediated proximal signaling. (a).Overall tyrosine phosphorylation of proteins. Sensitized BMMCs treated with $PM_{2.5}$ were stimulated with 100 ng/ml DNP-HSA for 5 min before lysis. Tyrosine phosphorylation of proteins in whole cell lysates were analyzed by Western blotting with anti-pTyr. (b). Phosphorylation of different signaling proteins. The numbers shown in Fig.4a-b were relative intensities for the phosphorylated forms of proteins in $PM_{2.5}$-treated cells to those in untreated cells after normalization by non-phosphorylated forms. pSrc was normalized by HSP-70. Data shown are representative of three separate experiments. (c). Kinetics of tyrosine phosphorylation. Sensitized BMMCs with or without $PM_{2.5}$ (5 μg/cm$^2$) treatment were stimulated with DNP-HSA for 0, 2, 5, 10, and 20 mins before lysis. The numbers shown were the relative intensity for the phosphorylated form of LAT in DNP-HSA stimulated cells to that in unstimulated cells after normalization by non-phosphorylated LAT.

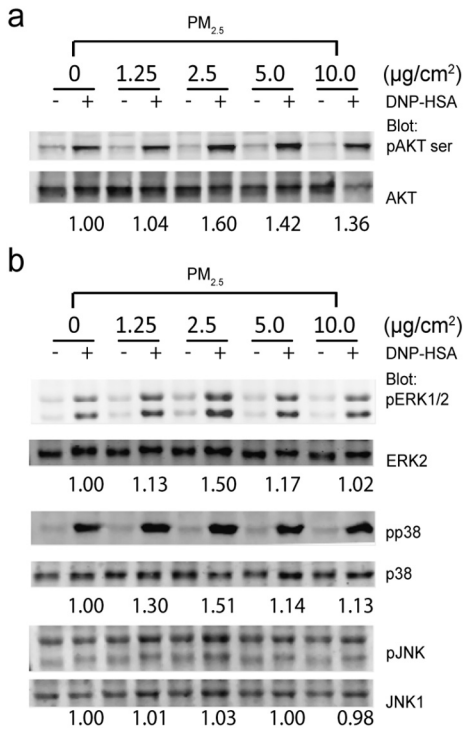


**Fig. 5.** Effect of $PM_{2.5}$ exposure on PI3K and MAPK pathways. Sensitized BMMCs were treated with different concentrations of $PM_{2.5}$ and followed by stimulation with 100 ng/ml DNP-HSA for 5 min before lysis. The whole cell lysates were blotted with antibodies against the phosphorylated and non-phosphorylated forms of Akt, ERK1/2, p38 and JNK proteins. (a). Akt activation. (b). MAPK activation. The numbers shown were relative intensities for the phosphorylated form of proteins normalized by non-phosphorylated form. Data shown are representative of three separate experiments.

orp38, respectively. The corresponding antibodies against non-phosphorylated forms of these proteins were determined in parallel for normalization. As shown in Fig. 5a, Akt phosphorylation in stimulated BMMCs was enhanced following exposure to $PM_{2.5}$ at 2.5, 5, and 10 μg/cm$^2$ $PM_{2.5}$ for 24 h. In addition, ERK and p38 phosphorylation (Fig. 5b) were also increased. On the other hand, JNK activation was not changed. Higher doses of $PM_{2.5}$ also had similar effects on the PI3K and MAPK pathways (data not shown). Taken together, these results indicated that $PM_{2.5}$ treatment could enhance activation of the PI3K and MAPK pathways.

### 3.6. Different $PM_{2.5}$ fractions on mast cells

Ambient $PM_{2.5}$ is a complex mixture of different chemical and microbial components, originating from different sources [9]. Next, we investigated whether $PM_{2.5}$ components differentially affected FcεRI-mediated mast cell signaling and subsequent activation. $PM_{2.5}$ suspension was separated into two fractions after sonication in PBS, WSF and WIF as referred to previously [22]. BMMCs were treated with 2.5 μg/cm$^2$ of $PM_{2.5}$, or WSF and WIF of $PM_{2.5}$ for 2 h or 24 h., respectively. Cell lysates were subjected to Western blotting using different antibodies.

As shown in Fig. 6a, overall tyrosine phosphorylation of proteins in BMMCs treated with 2.5 μg/cm$^2$ $PM_{2.5,}$ WSF or WIF of $PM_{2.5}$, respectively, showed similar patterns. Again, LAT as well as PLC-γ1, Syk, or SLP-76 phosphorylation was detected to increase when cells were treated with $PM_{2.5}$. Interestingly, this effect was also detected in the cells treated with WSF (Fig.6b). In addition, WSF treatment also enhanced ERK and p38 activation (Fig. 6c). These results demonstrated that WSF, not WIF of $PM_{2.5}$ could augment FcεRI-mediated mast cell signal pathways.

We next investigated whether WSF of $PM_{2.5}$ could affect FcεRI-mediated mast cell degranulation and cytokines production. As shown in Fig. 6d, CD107α surface expression was elevated in BMMCs treated with WSF corresponding to 12.5 or 25 μg/cm$^2$ of $PM_{2.5}$. Moreover, WSF also increased production of IL-6, and MCP-1(Fig. 6e). Together, these

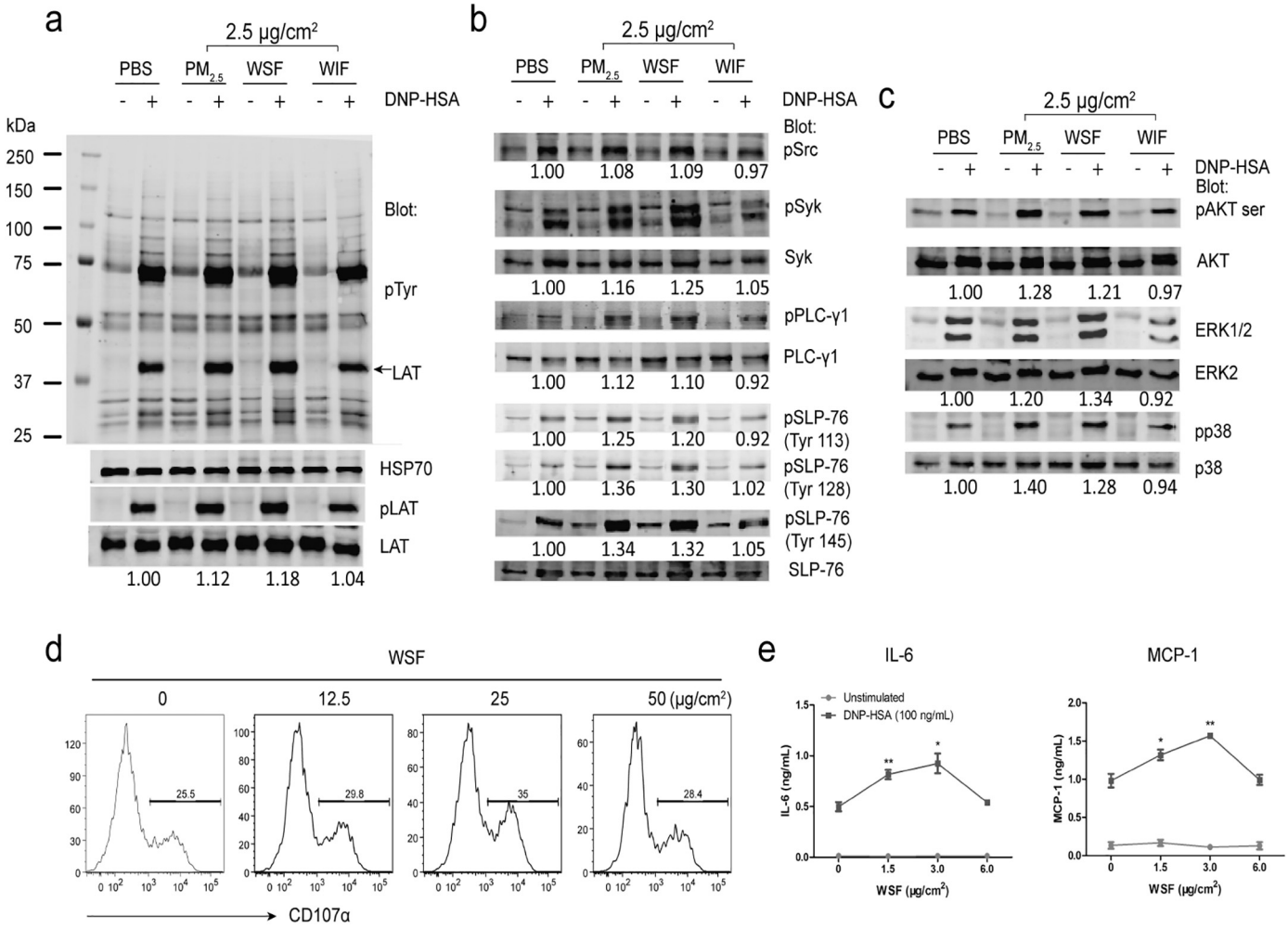


**Fig. 6.** Effect of WSF of $PM_{2.5}$ on FcεRI-mediated mast cell signaling. Sensitized BMMCs with $PM_{2.5}$ and its extracts treatment were stimulated with 100 ng/ml DNP-HSA for 5 min before lysis. Whole cell lysates were blotted with different antibodies. (a). Overall tyrosine phosphorylation of proteins. (b). Phosphorylation of Src, Syk, PLC-γ1 and SLP-76. (c). Akt and MAPK activation. The numbers shown were relative intensities for the phosphorylated form of proteins normalized by non-phosphorylated form. (d). The WSF of $PM_{2.5}$ on FcεRI-induced up-regulation of CD107α surface expression. Sensitized BMMCs with $PM_{2.5}$ extracts treatment were stimulated with 100 ng/ml DNP-HSA for 5–10 min before FACS analysis. (e). Cytokines secretion. Sensitized BMMCs with WSF or WIF treatment were stimulated with 100 ng/ml DNP-HSA for overnight, and then the cell supernatants were harvested for measurement of IL-6 and MCP-1 concentrations by using ELISA. Data shown are representative of three separate experiments. $^{*}P < 0.05$, vs 0 μg/cm$^2$ ($n = 3$); $^{**}P < 0.01$, vs 0 μg/cm$^2$ (n = 3).$^{***}P < 0.001$, vs 0 μg/cm$^2$ (n = 3).

results indicated that the WSF of $PM_{2.5}$ played an important role in enhanced FcεRI-mediated signaling, mast cell degranulation, and cytokines production.

## 4. Discussion

High levels of $PM_{2.5}$ pollution are a serious public health problem in most developing countries [24]. Persistent or repetitive exposure to ambient $PM_{2.5}$ is known to result in exacerbation of allergic diseases [14,20]. However, how $PM_{2.5}$ affects allergy response in humans is not clear. Previous studies found that diesel exhaust particles (DEP) exposure could trigger mast cell degranulation [3]. DEP are one of the main contributors to atmospheric $PM_{2.5}$. In this study, we analyzed the effect of $PM_{2.5}$ on FcεRI-mediated signaling, mast cell activation, and effect or function. It is well acknowledged that mast cells are responsible for many of the symptoms of allergic disorders through rapid release of preformed inflammatory mediators, such as histamine, heparin, tryptase, and acid hydrolases [5]. Mast cells are mostly activated through the engagement of the FcεRI [7]. The results from this study showed that $PM_{2.5}$ exposure enhanced FcεRI-mediated signaling since FcεRI-mediated phosphorylation of Syk, LAT, SLP-76, PLC-γ1, Akt, ERK1/2 or p38 was increased. $PM_{2.5}$ exposure also led to increased mast cell degranulation and production of cytokines, such as IL-6, TNF-α and MCP-1. Therefore, we assume that $PM_{2.5}$-induced allergic effects was through enhancing mast cell function, leading to increased release of histamines, cytokines, and other important mediators. Histamine released from mast cells plays a central role in airway inflammation by promoting vasodilation, bronchoconstriction and airway mucus secretion [5,19] while cytokines produced by mast cells have the potential to recruit immune cells either directly or indirectly to the affected sites and further cause local inflammation [1,5]. While we observed enhanced FcεRI-mediated signaling in the presence of the low concentrations of $PM_{2.5}$ (1.25 and 2.5 μg/cm$^2$), phosphorylation of those signaling proteins in the presence of high concentrations of $PM_{2.5}$ (5 and 10 μg/cm$^2$) was actually reduced. While these results seem to contradict to each other, this is likely to due to increased cell death at higher concentrations of $PM_{2.5}$ as shown in Fig. 1. Dying cells do not respond well to stimulation from the FcεRI. On the other hand, it is also possible that $PM_{2.5}$ may contain substances that inhibit activation of tyrosine kinases, such as Lyn or Syk. At low concentrations of $PM_{2.5}$, the effect of these inhibitory substances might be minimal; however, at high concentrations of $PM_{2.5}$, they may inhibit kinases and reduce tyrosine phosphorylation of proteins.

To investigate how $PM_{2.5}$ enhanced mast cell effect or function, we

analyzed FcεRI-mediated signaling in BMMCs. Following FcεRI aggregation, Src family kinases, such as Lyn and Fyn, and Syk are activated, which subsequently phosphorylated downstream signaling molecules, such as LAT and SLP-76 [17,27,28]. Phosphorylated LAT binds Grb2, Gads, PLC-γ1, as well as the guanine exchange factors VAV and SOS, eventually leading to activation of the PI3K and MAPK-dependent pathways and production of cytokines [7,17,27,28]. This present study showed that $PM_{2.5}$ exposure enhanced FcεRI-mediated Syk activation, and LAT, SLP-76, and PLC-γ1 phosphorylation, but not Src implying that $PM_{2.5}$ might affect the activation of these pathways through enhancing Syk activity. The responsible component for $PM_{2.5}$-increased Syk activity was also investigated in this study, which indicated that water-soluble fraction of $PM_{2.5}$ could enhance FcεRI-mediated signaling and mast cell activation. Water-soluble fraction of $PM_{2.5}$ has been shown to exhibit higher toxicological effects on lung cells than insoluble fraction [22,30]. For example, it has been demonstrated that WSF of $PM_{2.5}$ could induce early response of reactive oxygen species (ROS) generation in A549 cells, and inhibition of ROS could abrogate IL-4 expression in DEP-exposed human basophiles after specific antigen activation [2]. The water-soluble fraction of $PM_{2.5}$ contains high concentrations of metal ions [22,30], which could potentially affect mast cell activation [25].

## 5. Conclusions

Our study suggests that $PM_{2.5}$, mainly water-soluble fraction of $PM_{2.5}$, could affect mast cell activation through enhancing FcεRI-mediated signaling. In the future, we will identify which element in water-soluble fraction of $PM_{2.5}$ is responsible for sensitization of mast cells. The resultant information will help to design strategies to alleviate $PM_{2.5}$-associated allergic disease.

## Authors' contributions

WZ, WW, YJ and MZ designed the experiments. FF and WW prepared $PM_{2.5}$. YJ, MZ, YG, DF, FF, WZ and WW performed the experiments. YJ, MZ, DF, WZ, WW and FF interpreted the data, prepared the figures, and wrote the manuscript. All authors read, commented on, and approved the final manuscript.

## Conflict of interest

The authors declare that they have no competing interests.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cellsig.2019.01.010.

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**Fine Particulate Matter ($PM_{2.5}$) Enhances FcεRI-mediated Signaling and Mast Cell Function**

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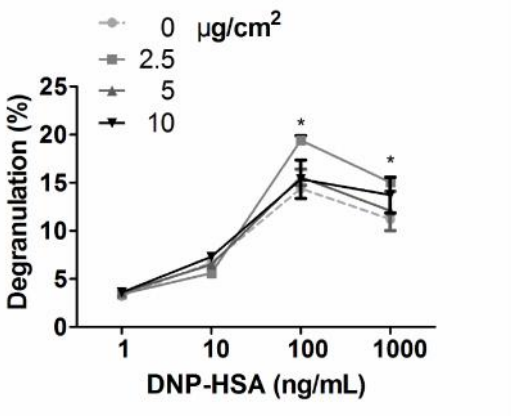


**Fig. S1. Effect of $PM_{2.5}$ on FcεRI-mediated mast cell degranulation after 24 hours exposure.** BMMCs were sensitized with anti-DNP IgE before treatment with $PM_{2.5}$ at indicated doses for 24 hours and then stimulated with various concentrations of DNP-HSA for 10 min. Degranulation of BMMCs was determined by measuring the release of β-hexosaminidase into the culture supernatants.