Effect of PM2.5 Exposure On Gestational Hypertension and Fetal Size in Preeclampsia-Like Rats

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Abstract

Studies have reported that gestational $PM_{2.5}$ exposure is associated with preeclampsia (PE) and fetal growth restriction (FGR). However, whether maternal exposure to $PM_{2.5}$ causes adverse pregnancy outcomes is still largely unknown. Pregnant Sprague-Dawley rats were exposed to either filtered (FA) or $PM_{2.5}$ air during the whole pregnant period. A PE-like rat model was established by intraperitoneal injection of L-NAME (300 mg/kg) from GD12 to until GD20. Systolic blood pressure (SBP), weight gain, pup weight and placental weight were measured. The percentages of rat Treg/Th17 cells and Th17-related cytokines were examined by flow cytometry. Gene expression profiles were analyzed by microarray, and the expression of differentially expressed genes were validated by qRT-PCR. The results showed that maternal $PM_{2.5}$ exposure had no effect on SBP but was associated with LBW and a higher labyrinth/basal zone ratio. The percentages of splenic Th17 cells from the $PM_{2.5}$ group in PE-like rats were higher than those from the FA or $PM_{2.5}$ groups in healthy controls. A significantly decreased Treg/Th17 cell ratio was found in the $PM_{2.5}$ group in PE-like rats. The mRNA expression of Foxp3 was downregulated, while the mRNA expression of ROR_{α} and $ROR_{\gamma\tau}$ was upregulated after $PM_{2.5}$ exposure. Furthermore, we observed that both the mRNA and protein expression of TNF-a, CCL2, CCL3 and CCR1 increased in the $PM_{2.5}$ groups. Our study suggested that systemic inflammation may contribute to the development of FGR associated with $PM_{2.5}$ exposure throughout pregnancy.

Introduction

Air pollution is a major global public health issue and is estimated to cause 4.2 million premature deaths globally (Cohen et al. 2017). The composition of air pollutants is complex and changeable, and particles with an aerodynamic diameter of 2.5 μm or less ($PM_{2.5}$) are the main pollutants affecting urban air quality worldwide. Because of its smaller size, larger surface area, easier enrichment of toxic chemicals and various microorganisms, the harm of $PM_{2.5}$ to public health has become a social hot point.

There is increasing epidemiological evidence that maternal exposure to $PM_{2.5}$ is associated with high risks of pregnancy complications and adverse pregnancy outcomes, including preeclampsia (PE) (M Sun et al. 2020; Zhang et al. 2021), low birth weight (LBW) (Yuan et al. 2020), less than gestational age and fetal growth restriction (FGR) (Dadvand et al. 2013; Fleischer et al. 2014; Lee et al. 2013; Li et al. 2017). Due to the differences in accuracy and method of exposure assessment, composition of exposure pollutant, study population and study design, studies have shown mixed results on the effects of $PM_{2.5}$ on PE. Animal studies suggest that gestational exposure to concentrated ambient $PM_{2.5}$ increases a risk for obstetric consequences(JL Blum et al. 2017; Dang et al. 2018). Most of animal studies use inhalation of concentrated $PM_{2.5}$ (de Melo et al. 2015) or intratracheal instillation (Liu et al. 2016), which does not represent real-world exposure and may overestimate the hazards of exposure. Hence, the relationship between maternal exposure to $PM_{2.5}$ and adverse pregnancy outcomes and its underlying mechanism need to be further explored.

Several studies have suggested that maternal $PM_{2.5}$ exposure may elicit systemic inflammation and promote the release of inflammatory mediators, such as proinflammatory cytokines and acute phase proteins, to the blood stream, which interfere with maternal and fetal growth (Wang et al. 2021; Xia et al. 2019). Previous studies reported that $PM_{2.5}$ exposure impair differentiation of Treg cells, promote differentiation of Th17 cells, and aggravate the inflammatory response (Cong et al. 2020; L Sun et al. 2020). Meanwhile, the balance between Treg and Th17 cell immunity is particularly important for normal placental development and pregnancy maintenance. It has been shown that Treg/Th17 imbalance is involved in PE and FGR pathogenesis. However, fewer studies have explored the role of $PM_{2.5}$ exposure and Treg/Th17 cells in PE and FGR.

In the present study, we evaluated whether maternal $PM_{2.5}$ exposure changed the proportion and differentiation of Treg/Th17 cells in preeclampsia-like rats. Therefore, pregnant rats were exposed to either airborne $PM_{2.5}$ or filtered air (FA) throughout pregnancy in a whole-body exposure chamber. The effects of $PM_{2.5}$ exposure on preeclampsia and fetal outcome were investigated. We evaluated systematic and local inflammation after $PM_{2.5}$ exposure. The analyses of global gene expression profiles in placentas of $PM_{2.5}$ or FA exposed rats were performed using microarray.

Materials And Methods

Animals

Animal care and use were carried out in accordance with guidelines approved by Capital Medical University, Beijing, China. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at Beijing Luhe Hospital, Capital Medical University. Sprague-Dawley rats purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) were used in this study.

Preparation of PE-like rat model

Female Sprague-Dawley (SD) rats weighing 200-250 g were raised in a light- and humidity-controlled room with free access to food and water. The day on which pregnancy was confirmed by the presence of vaginal spermatozoa was designated GD0. Pregnant rats were randomly divided into four groups as follows: filtered-air+NaCl (FN), $PM_{2.5}$-air+NaCl (PN), filtered-air+L-NAME (FL), and $PM_{2.5}$-air+L-NAME (PL) groups (n=5 each group). Rats in FL and PL group were injected with 300 mg/kg L-NAME from GD12 to GD20 to establish preeclamptic models.

$PM_{2.5}$ exposure

Pregnant rats were exposed to either ambient $PM_{2.5}$ or FA from GD0 to GD20 (September-October 2016) in a “real-world” airborne $PM_{2.5}$ exposure system for 24 h per day and 7 days per week as described in previous studies (Guan et al. 2019; Liu et al. 2020), which is located in the Institute of Neuroscience of Beijing Luhe Hospital. For $PM_{2.5}$, a cyclone was used in the $PM_{2.5}$-exposed group to remove particles

larger than 2.5 μm. For FA, a high-efficiency particulate air filter (HEPA) was positioned in the inlet valve to the exposure system to remove all of the particles from the air stream. Real-time $PM_{2.5}$ concentration were measured continuously using monitoring equipment (JK-PDR1500, Jinan Qianya Purification Equipment Co., Ltd.) installed inside and outside the exposure facility.

Blood pressure measurement

The blood pressure of rats was measured by the noninvasive blood pressure methodology (Zhongshi Scientific Technology Co., Ltd., Beijing, China), which consists of placing a cuff on the animal's tail to occlude blood flow. All rats were weighed and had their blood pressure measured every 3 days until delivery. Three blood pressure values were adopted within the variation <5 mmHg, and the average was taken for the blood pressure values.

Sample collection and histological analysis

On GD21, the animals were euthanized, and the blood, spleen and placentas were rapidly removed for further analyses immediately after delivery. The following parameters of pregnancy outcomes were evaluated: fetal weight and placental weight. Sections of rat placentas at 5 μm thickness were stained with hematoxylin and eosin (H&E) according to the standard H&E protocol. All sections were from the middle of the respective placentas. The areas of the labyrinth and basal zone were determined using sections with the maximum parts for the layer of the whole placenta in ImageJ (NIH, USA). A single observer blinded to the experimental conditions at the time of the assessments performed all ImageJ assessments and quantification. The assessments were repeated a second time with a second blinded observer to confirm all results. The mean area per group was calculated from five individuals.

Flow cytometry

The rat spleen tissues were gently teased to release cells in PBS. The splenic mononuclear cells were isolated by density centrifugation. Isolated splenic mononuclear cells were stimulated and incubated with Cell Stimulation Cocktail (plus Protein Transport Inhibitor) (eBioscience, San Diego, CA, USA) for 6 h. Then, the cells were fixed and permeabilized for 30 min at room temperature in the dark with Fixation/Permeabilization Diluent (eBioscience, San Diego, CA, USA). The cells were stained with anti-mouse/rat Foxp3-PE (eBioscience, San Diego, CA, USA) or anti-mouse/rat IL-17A-APC (eBioscience, San Diego, CA, USA) antibodies. Finally, the cells were resuspended in 500 μl of PBS for subsequent flow cytometric analysis. All data were acquired on a FACScalibur (BD Biosciences, San Jose, CA) and processed using BD FACS Diva software (BD Biosciences, San Jose, CA). Serum levels of IL-17A, IL-6 and IL-22 were measured by flow cytometry using a multi-LegendPlex kit (Biolegend) according to the instructions of the manufacturer.

Microarray analysis and series test of cluster (STC) analysis

For microarray analysis, placental samples from the four groups were used to extract total RNA. Total RNA was quantified by a NanoDrop ND-2000 (Thermo Scientific), and RNA integrity was assessed using an Agilent Bioanalyzer 2100 (Agilent Technologies). The expression profiles of the placental mRNAs were detected using an Agilent SurePrint G3 Rat GE V2.0 Microarray (OE Biotech, Shanghai, China). The threshold for up- and downregulated genes was a fold-change (FC) value of 2.0 or greater. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were applied to determine the roles of these differentially expressed mRNAs that exhibited a significance value of $P<0.05$. The series test of cluster (STC) algorithm of gene expression was performed to enrich the expression tendency of the genes and narrow the target genes with greatly significant differential expression among the four groups.

RNA isolation and real-time RT-PCR

Total RNA was isolated from the placenta using RNeasy kits (Qiagen) according to the manufacturer's protocol, and 1 μg of total RNA was reverse transcribed with TransScript II All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (Transgene, Beijing, China). cDNA was quantified using SYBR Green PCR Master Mix (Applied Biosystems, USA). The sequences of the primers used for real-time PCR are shown in Table 1. The quantitative fold changes in mRNA expression were determined relative to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA levels in each corresponding group.

ELISA

Placental tissues were weighed, placed in lysis buffer, homogenized and centrifuged at 10 000 ×g for 5 min. The supernatants were collected, and the ELISA assays were conducted by quantitative sandwich enzyme immunoassays using commercial ELISA kits according to the manufacturer's protocol (Cloud-Clone Corp, Mbbiology, Wuhan, China). For each sample, the assay was conducted in triplicate, with duplicate standard curves.

Statistics

Data are presented as the mean ± standard error of the mean. Statistical analysis was performed using Statistical Package for Social Science (SPSS) for Windows (version 22.0 software, SPSS Inc., Chicago, IL, USA) and GraphPad Prism software (version 7.0, GraphPad). The differences were analyzed by ANOVA with Student-Newman. A p value less than 0.05 was considered significant.

Results

PM2.5 concentration in FA and PM2.5 chamber

The $PM_{2.5}$ exposure model and experimental protocol are outlined in Figure1A. The mean concentrations of $PM_{2.5}$ in the FA and $PM_{2.5}$ chamber were 7.29 ± 0.38 and 62.81 ± 8.65 μg/m^3, respectively, during the whole pregnancy period (Figure 1B).

$PM_{2.5}$ exposure during pregnancy has no effect on maternal SBP or gestational weight gain

As shown in Figure 2A, there was no difference in the baseline SBP among the four groups. The FN group had a relatively consistent SBP throughout the entire gestation period (SBP of 107.00±7.04 mmHg on GD8 and of 109.90±4.82 mmHg on GD17) (Table 2). Among the four groups, three of the groups (PN, FL, and PL) demonstrated similar maternal systolic blood pressure changes during pregnancy, with an increase in blood pressure from GD8 to GD20. The SBP of the PN group slightly increased from 111.20±3.96 mmHg on GD8 to 126.60±6.34 mmHg on GD17; however, there was no significant difference between the PN and FN groups. There was a significant increase in SBP in both the FL and PL groups (from 114.3±6.79 mmHg on GD8 to 132.10±3.15 mmHg on GD17 and from 104.2±2.83 mmHg on GD8 to 131.50±8.15 mmHg on GD17, respectively) compared with that in the FN group, which suggests that administration of L-NAME successfully induced a PE-like animal model (Figure 2). However, there was no significant difference between the PL and FL groups. These findings indicate that maternal $PM_{2.5}$ exposure during pregnancy did not increase the blood pressure of either pregnant or preeclamptic rats.

As shown in Figure 2B, we also observed less net weight gain in the other three groups than in the FN group, but the differences were not significant (Table 2). The results indicated that maternal $PM_{2.5}$ exposure has no effect on gestational weight gain.

Maternal $PM_{2.5}$ exposure results in decreased pup weight and an altered labyrinth-to-basal zone ratio

To evaluate the effect of $PM_{2.5}$ exposure on the fetus, we evaluated the development of the fetus and placenta on GD21. Pups delivered from the PL group were characterized by fetal growth restriction and malformation (Figure 3A). The PL group had a significantly lower pup weight than both the FN group ($p<0.01$) and PN group ($p<0.01$) (Figure 3B), whereas $PM_{2.5}$ exposure only throughout the gestational period did not significantly affect pup weight. Additionally, we did not observe any significant difference in placental weight. These data suggested that ambient $PM_{2.5}$ exposure during pregnancy might affect birth weight.

Histology of the placenta was performed to compare the morphology of the labyrinth and basal zones in all groups. The relative area of the labyrinth zone showed no significant change in the other three groups compared to that in the FN group. The relative area of the basal zone decreased from the FN to the PL group (Figure 3C). The ratio between the labyrinth and the basal zone was increased in the FL and PL groups compared to that in the FN group (Figure 3D). Additionally, the labyrinth zones of the placentas from the $PM_{2.5}$ exposure groups had fewer red blood cells than those from the FN group, suggesting the potential for less efficient oxygen and nutrient exchange (Figure 3E). The basal zones of the placentas from the $PM_{2.5}$ exposure groups had larger and more dispersed trophoblast giant cells than those from the FN group, which also displayed more intense neutrophil infiltration (Figure 3E). Together, these results suggest that maternal $PM_{2.5}$ exposure could lead to changes in placental structure and subsequently decrease pup weight.

Maternal $PM_{2.5}$ exposure results in a reduced Treg/Th17 ratio in spleen tissue in PE-like rats

To assess the effect of $PM_{2.5}$ exposure on Th17 cell expansion, spleen samples were collected for flow cytometry analysis. Th17 cells were defined as $CD4^+IL\text{-}17A^+$. Gating strategies for Th17 cells are shown in Figure 4A. The prevalence of Th17 cells refers to the ratio of $CD4^+IL\text{-}17A^+$ cells to the total amount of $CD4^+$ T lymphocytes. As shown in Figure 4B, the prevalence of splenic Th17 cells showed an increase in the other three groups compared with that in the FN group, but the differences were not significant. We further determined the Treg/Th17 cell ratio and found a significant decrease in the Treg/Th17 cell ratio in the PL group compared with that in the FL group. These results revealed that maternal $PM_{2.5}$ exposure resulted in a reduced Treg/Th17 ratio in PE-like rats.

In the present study, the serum levels of IL-17A, IL-6 and IL-22 were measured in four groups using multifactor flow cytometry. The levels of IL-17A in the PL group (49.60±3.42 pg/ml) were significantly higher than those in the FN (34.25±3.19 pg/ml) and PN groups (35.07±2.47 pg/ml). The levels of IL-6 and IL-22 in the PL group (401.90±240 pg/ml, 42.33±4.51 pg/ml) were significantly higher than those in the FN group (46.99±7.99 pg/ml, 26.24±2.41 pg/ml). However, the levels of IL-6 and IL-22 in the PN and FL groups were not significantly different from those in the FN group.

Levels of systemic inflammatory reactions are increased in rats exposed to $PM_{2.5}$, especially after L-NAME treatment

Fine particulate matter exposure may promote systemic inflammation reactions. To explore changes in mRNA expression after $PM_{2.5}$ exposure, we conducted microarray analyses on the placentas from 4 groups and examined the most differentially expressed genes (DEGs) using bioinformatic analyses. Figure 5A shows the heatmap representation of the top 1000 DEGs among the 4 groups. Red represents upregulated genes, and blue represents downregulated genes. A total of 597 genes showed upregulation, and 473 genes showed downregulation between the PN and FN groups, whereas, as shown in Figure 5B, 1168 genes were upregulated and 1057 were downregulated between the PL and FL groups. The results of GO analysis showed that the upregulated DEGs were significantly enriched in the cellular response to interferon-gamma, neutrophil chemotaxis, monocyte chemotaxis and immune response (Figure 5C). STC analysis results of the screened DEGs are presented in Supplemental Figure 1. A total of 12 significant profiles were identified. Our STC analysis showed that DEGs in Profile 40 were persistently upregulated from the FN to PL group (Figure 6A), which included some classic inflammatory genes, such as TNFa, CCL2, CCL3 and CCR1. Figure 6B shows the heatmap representation of 13 DEGs from Profile 40.

To examine whether the expression of the characteristic transcription factors of Treg and Th17 cells underlies the reduced Treg/Th17 cell ratio in preeclamptic rats after $PM_{2.5}$ exposure, we further determined Foxp3, ROR_{α} and $ROR_{\gamma\tau}$mRNA expression using real-time qRT-PCR. As shown in Figure 6C, Foxp3 mRNA expression was the lowest in the placentas of the PL group, while ROR_{α} and $ROR_{\gamma\tau}$mRNA expression showed increased expression from the FN to the PL group. Furthermore, we observed that

both the mRNA and protein expression of TNF-a, CCL2, CCL3 and CCR1 increased from the FN to the PL group.

Discussion

In this study, we explored the effect of $PM_{2.5}$ on the blood pressure of PE-like rats and their perinatal outcomes. $PM_{2.5}$ is a heterogeneous mixture of fine particulate matter and is more harmful to health than other air pollutants. Recently, epidemiologic studies revealed positive associations between maternal exposure to $PM_{2.5}$ during pregnancy and adverse pregnancy outcomes, especially gestational hypertension (Xu et al. 2014; Zhu et al. 2017), gestational diabetes mellitus (Hu et al. 2015), low birth weight (Erickson et al. 2016) and preterm birth (CS Malley et al. 2017).

Two meta-analyses showed statistically significantly increased risk for hypertensive pregnancy disorders in association with exposure to $PM_{2.5}$ during pregnancy, and the odds ratios were 1.15 and 1.47, respectively, with a 5 μg/m^3 increase in $PM_{2.5}$ (Hu et al. 2014; Pedersen et al. 2014). However, Rudra et al reported nonsignificant associations between $PM_{2.5}$ and preeclampsia across all exposure windows in an analysis of 3,509 western Washington women who delivered infants between 1996 and 2006 (Rudra et al. 2011). Similarly, Savitz DA assessed the association between air pollution exposure across New York City and hypertensive disorders of pregnancy in 2008–2010 and found that there is lack of evidence of a positive association between $PM_{2.5}$ and gestational hypertension (Savitz et al. 2015). Prior animal studies have suggested that air pollution may increase the risk of gestational hypertension and preeclampsia (Weldy et al. 2014). In the present study, we observed that the BP of the PN group was slightly higher than that of the FN group in late pregnancy, while the difference was not significant. Furthermore, there was no difference between the PL and FL groups, which suggests that maternal exposure to $PM_{2.5}$ does not increase the risk of preeclampsia. Differences in exposure protocols may in part explain some of these differing findings. The major methods of $PM_{2.5}$ exposure in previous in vivo experiments are intratracheal inhalation and intratracheal instillation. Both methods have some common weaknesses, such as high dosage exposure, which cannot simulate real-world human exposure.

One of the most important findings is that we observed that PE-like rats exposed to $PM_{2.5}$ have a significantly reduced fetal weight and alterations in placental histology compared to those of pregnant rats exposed to $PM_{2.5}$. An increasing number of studies have demonstrated that maternal exposure to $PM_{2.5}$ has an adverse effect on fetal development and is closely related to adverse birth outcomes, such as preterm birth, still birth, term low birth weight and small for gestational age (Basu et al. 2017; Li et al. 2017; C Malley et al. 2017; Stieb et al. 2016). A meta-analysis by Li et al. showed that maternal exposure to $PM_{2.5}$ increases the risk of term low birth weight. The polled OR for the association between $PM_{2.5}$ exposure per interquartile range increment and term low birth weight was 1.03 (95% CI: 1.02–1.03) (Li et al. 2017). Blum JL et al. reported that exposure of pregnant mice to 160 μg of concentrated ambient $PM_{2.5}$/m^3 throughout pregnancy decreased birth weight by 11.4% compared with that with filtered air, and particle concentration and relative compositions are important for the magnitude of decrease in birth

weight (J Blum et al. 2017). Altered placental structure and function after $PM_{2.5}$ exposure may account for the low birth weight. In this study, an inverse relationship between $PM_{2.5}$ exposure and the labyrinth/basal zone ratio was found. A study of $PM_{2.5}$ from traffic exposure of pregnant mice in Brazil showed that fetal weight was remarkably decreased after exposure during pregnancy due to reduced vasculature volumes, luminal diameters and surface area of blood spaces on the maternal face of the placenta (Veras et al. 2008). Wylie BJ et al reported that an increase in the adjusted odds of fetal thrombotic vasculopathy (FTV) with increasing $PM_{2.5}$ (adjusted odds ratio = 5.5, 95% CI: 1.1–26.8) and fetal thrombosis may account for the adverse outcomes associated with $PM_{2.5}$, including stillbirth and low birth weight (Wylie et al. 2017).

Previous studies demonstrated that the balance between Treg and Th17 cells plays a critical role in the pathogenesis of PE and FGR (Cotechini et al. 2014). Prins JR reported that the expression of IL-6, which stimulates the differentiation of Th17 cells and inhibits the generation of FOXP3 + Treg cells, increased in chorionic villous tissues of women who develop preeclampsia associated with FGR. Another study using the reduced uterine perfusion pressure (RUPP) rat model of preeclampsia found that adoptive transfer of RUPP Th17 cells induced intrauterine growth restriction and increased proinflammatory cytokines in normal pregnant rats. The systemic inflammatory is also considered to be the most important mechanism underlying $PM_{2.5}$-induced adverse health problems. In this study, we investigated the distribution of T cell subtypes in a preeclamptic rat model after $PM_{2.5}$ exposure and found that Th17 cells were increased in both the PN and PL groups. The Treg/Th17 ratio was significantly decreased in the PL group compared with that in the FL group. Furthermore, our study demonstrated a significant increase in the levels of proinflammatory cytokines, including IL-17A, IL-6 and IL-22, after $PM_{2.5}$ exposure. As important proinflammatory cytokines, IL-17A and IL-22 are secreted by Th17 cells, and IL-6 is involved in inducing the development of Th17 cells and inhibiting Treg differentiation. The collective pattern of changes in serum cytokines may indicate that systemic inflammation increased in PE-like rats exposed to $PM_{2.5}$ due to exacerbating the imbalance of Treg and Th17 cells. Therefore, $PM_{2.5}$ exposure exacerbated Treg and Th17-mediated immunological dysfunction.

Our placental microarray data revealed that the differentially regulated genes were involved in more than 20 biological processes that can be clustered into the inflammatory response and neutrophil chemotaxis. The transcription factors Foxp3 and $ROR_{\gamma\tau}$ are the lineage-specific markers for the differentiation of Treg and Th17 cells, respectively. Our data showed that Foxp3 mRNA decreased, while ROR_{α} and $ROR_{\gamma\tau}$ mRNA significantly increased in placental tissues from the PL group. Furthermore, we observed that exposure to $PM_{2.5}$ increased the mRNA and protein expression of TNF-α, CCL2/MIP-1α and CCL3/MCP-1 in placental samples in PE-like rats, which could induce the accumulation of neutrophils and monocytes, consistent with the results observed by H&E staining. Additionally, MIP-1a and MCP-1 also play an essential role in regulating Th cell differentiation in vivo. Alahakoon reported that the number of inflammatory monocytes for both PE groups (with or without IUGR) was significantly increased, which correlated with the increased level of proinflammatory factors (MIP-1α and MCP-1) (Alahakoon et al. 2018; Ma et al. 2019). These data

indicate that maternal $PM_{2.5}$ exposure aggravates systemic and local inflammatory reactions and leads to placental inflammation, which impairs placental function.

Conclusion

In summary, this study demonstrates that maternal exposure to $PM_{2.5}$ may decrease pup weight in PE-like rats. The characteristic patterns of changes in cytokines in the PE-like rats exposed to $PM_{2.5}$ reported in our study suggested that gestational $PM_{2.5}$ exposure may promote systemic and local inflammation in PE-like rats and result in placental insufficiency by exacerbating the imbalance of Treg and Th17 cells. These findings shed light on the potential mechanisms by which $PM_{2.5}$ may cause or exacerbate FGR.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Medical Faculty, Beijing Luhe Hospital, Capital Medical University, and all experiments were performed in accordance with approved guidelines (The Guide for Care and Use of Laboratory Animals; National Institute of Health publication 85-23).

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interest.

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Author's contributions

JG designed and executed the study, analyzed the data and prepared the manuscript. ML, SZ, HW, PX and XL collected the relevant data and performed the analysis. WM conceived the study and CL edited the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1 Primer sequences for real-time RT-PCR

Gene	Sequence	Product size (bp)
FOXP3	F: GCTTGTTTGCTGTGCGGAGAC	218
	R: GTTTCTGAAGTAGGCGAACAT	
RORa	F: CCCGATGTCTTCAAATCCTTAGG	89
	R: TCAGTCAGATGCATAGAACACAAACTC	
RORc	F: TCATCAGCTCCATATTCGACTTTTC	92
	R: GATGAGAACCAAGGCCGTGTAG	
TNF-α	F: ACCACGCTCTTCTGTCTACTG	169
	R: CTTGGTGGTTTGCTACGAC	
IL-6	F: CTGATGTTGTTGACAGCCAC	175
	R: CAGAATTGCCATTGCACAAC	
CCL2(MCP-1)	F: CAGAAACCAGCCAACTCTCA	144
	R: GTGGGGCATTAACTGCATCT	
CCL3(MIP-1α)	F: GACTGCCTGCTGCTTCTC	77
	R: AAAGGCTGCTGGTCTCAAA	
CCR1	F: AAGTACCTTCGGCAGCTGTTTC	71
	R: ACAGAGAAGAAGGGCAGCCAT	
CXCL14	F: TGTTCCCGGAAGGGGCCCAA	127
	R: GGCCGCGGTACCTGGACATG	
CXCL16	F: TCTGCCAGGCGATGGCAACC	198
	R: ACCCACTGGTCTTGGCTTCCTCC	
GAPDH	F: CAAAGTTGTCATGGATGACC	195
	R: CCATGGAGAAGGCTGGGG	

Table 2 Effect of PM2.5 exposure on L-NAME-induced PE-like symptoms in rat models. Data are analyzed by one-way ANOVA, and presented as mean±SEM. *p<0.05, **p<0.01 vs. SBP on

	FN group (n=5)	PN group (n=5)	FL group (n=5)	PL group (n=5)
SBP on D2 (mmHg)	96.73 ± 5.51	95.47 ± 5.18	94.63 ± 3.76	95.37 ± 3.02
SBP on D5 (mmHg)	102.90 ± 5.47	98.11 ± 7.52	104.00 ± 3.53	101.90 ± 3.39
SBP on D8 (mmHg)	107.00 ± 7.04	111.20 ± 3.96	114.30 ± 6.79	104.2 ± 2.83
SBP on D14 (mmHg)	106.70 ± 6.45^{ab}	121.90 ± 5.95	130.60 ± 4.59^{a}	135.00 ± 4.92^{b}
SBP on D17 (mmHg)	109.90 ± 4.82^{cd}	126.60 ± 6.34	132.10 ± 3.15^{c}	131.50 ± 8.12^{d}
SBP on D20 (mmHg)	105.20 ± 5.61^{ef}	117.30 ± 3.57	130.50 ± 10.92^{e}	130.00 ± 4.25^{f}
Weight gain throughout pregnancy (g)	125.60 ± 19.55	92.80 ± 14.63	89.40 ± 13.26	84.80 ± 18.79
Weight gain after D12 (g)	66.40 ± 16.31	52.20 ± 11.89	29.40 ± 13.04	33.40 ± 14.13

Figures

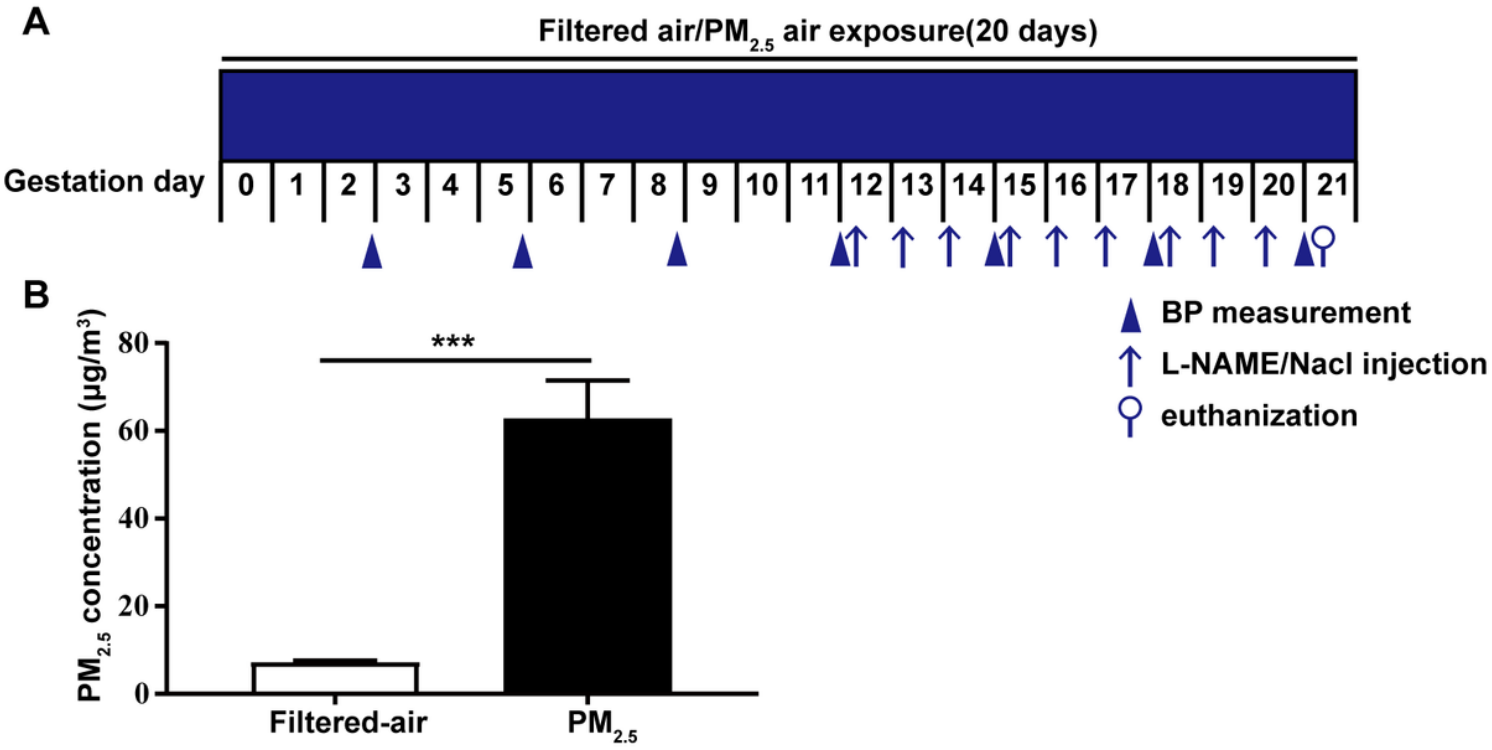

Figure 1

Daily concentrations of PM2.5 measured in polluted chambers during the experimental period. (A) Experimental design: rats were exposed to filtered-air or PM2.5 air during the whole pregnancy. Blood pressure was measured every three days. From GD12 to GD20, 300 mg/kg body weight L-NAME was subcutaneously injected every day. At GD21, rats were euthanized for spleen harvesting. (B) The mean concentrations of PM2.5 chambers were significantly higher than those of filtered-air chambers. ***p<0.001.

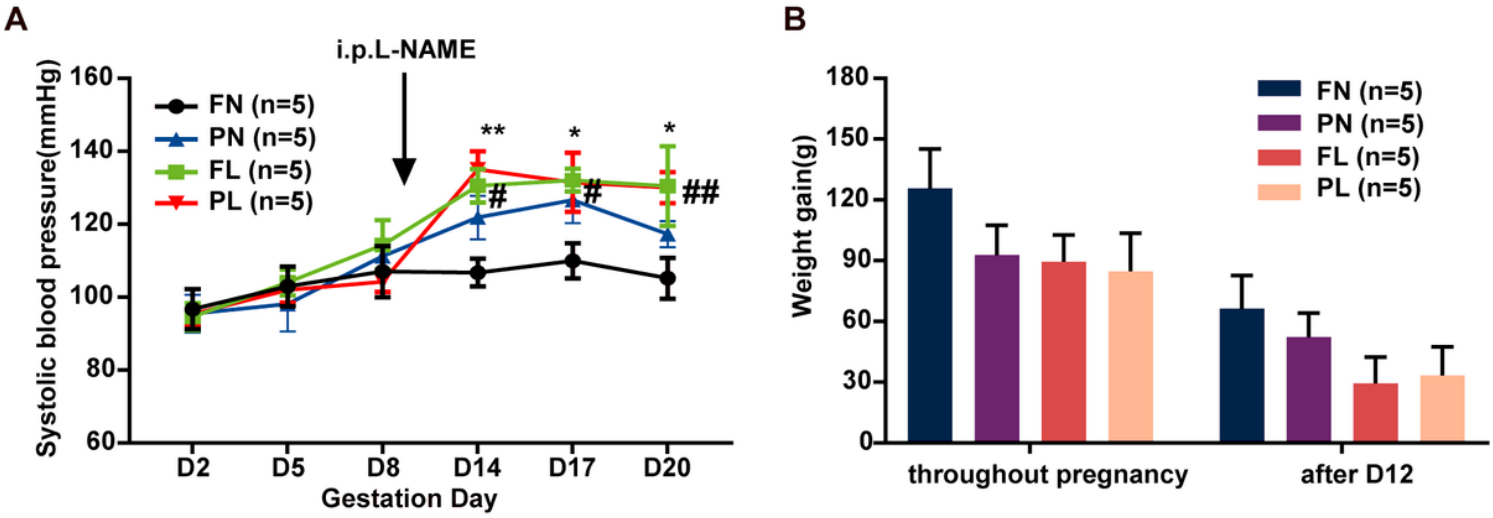

Figure 2

The variation tendency of SBP (A) and net weight gain (B) during pregnancy in the four groups. * p<0.05 # p<0.05 vs the FN group, ** p<0.01 ##p<0.01 vs the FN group. Values are expressed as the mean ±SEM. Groups: FN, rats treated with filtered air throughout pregnancy; PN, rats treated with PM2.5air throughout pregnancy; FL, rats treated with L-NAME (300 mg/kg body weight/day) starting from GD10 in a filtered-air chamber throughout pregnancy; PL, rats treated with L-NAME (300 mg/kg body weight/day) starting from GD10 in a PM2.5-air chamber throughout pregnancy. GD, gestational day; L-NAME, N-nitro-L-arginine methyl ester; SBP, systolic blood pressure. (B). The net weight gain of each rat was observed throughout pregnancy (the difference between GD20 body weight and GD0body weight) and after GD12 (the difference between GD20 body weight and GD12 body weight).

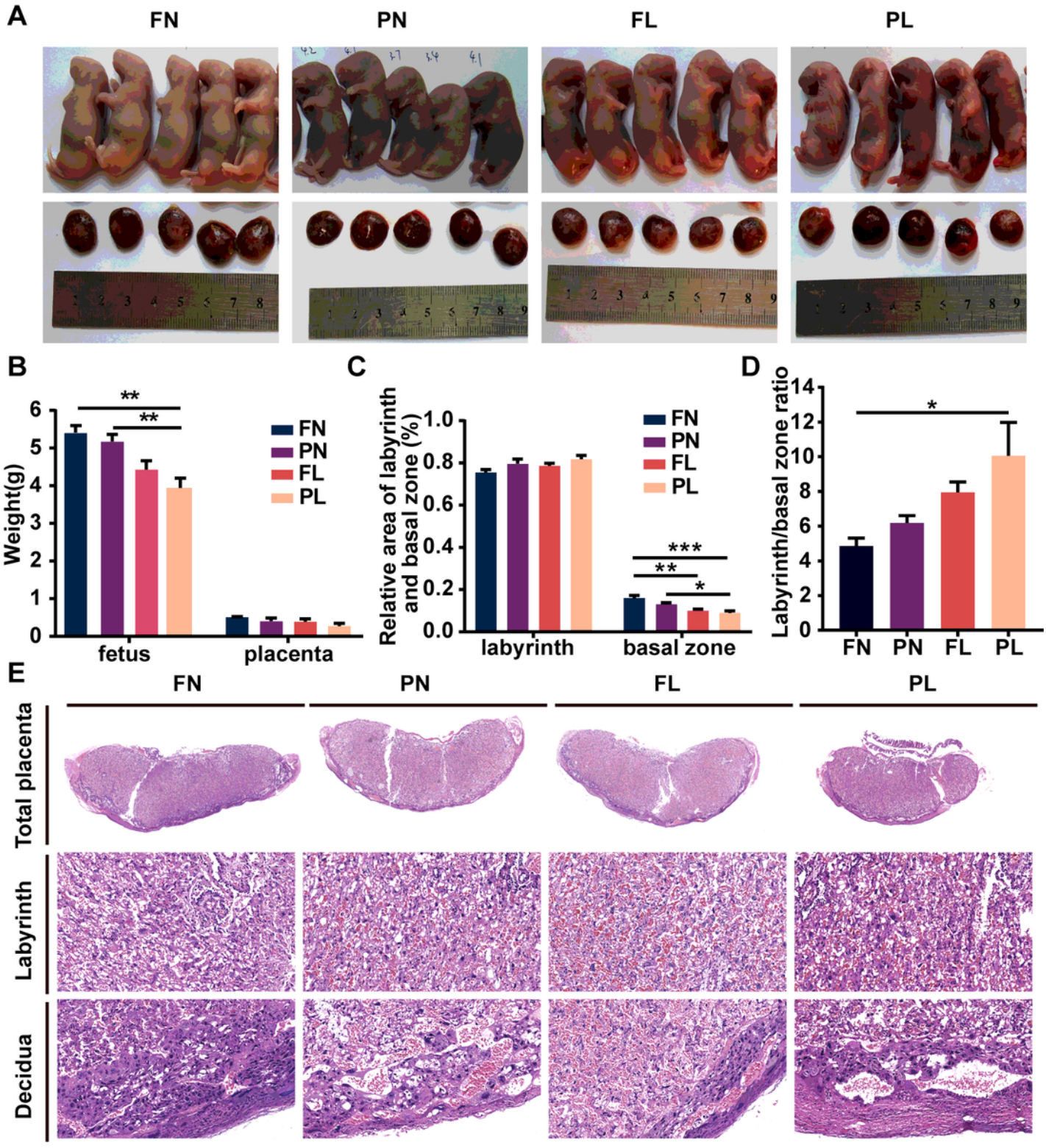

Figure 3

Effects of PM2.5 exposure on rat fetal outcomes. (A) Representative pictures of fetal congenital malformation and small placentas that were observed in preeclamptic rats. (B) Fetus weight and placenta weight in each group were measured. (C) The relative area of the labyrinth and basal zones. (D) The ratio of labyrinth to basal zone areas. (E) Hematoxylin and eosin (H&E) staining of placentas was subsequently performed in the FN, PN, FL and PL groups. Top row, lower magnification views. Bottom

two rows, higher magnification views of the labyrinth (middle row) and decidua (bottom row). Data are presented as the mean ± SEM. * p<0.05, **p<0.01, ***p<0.001.

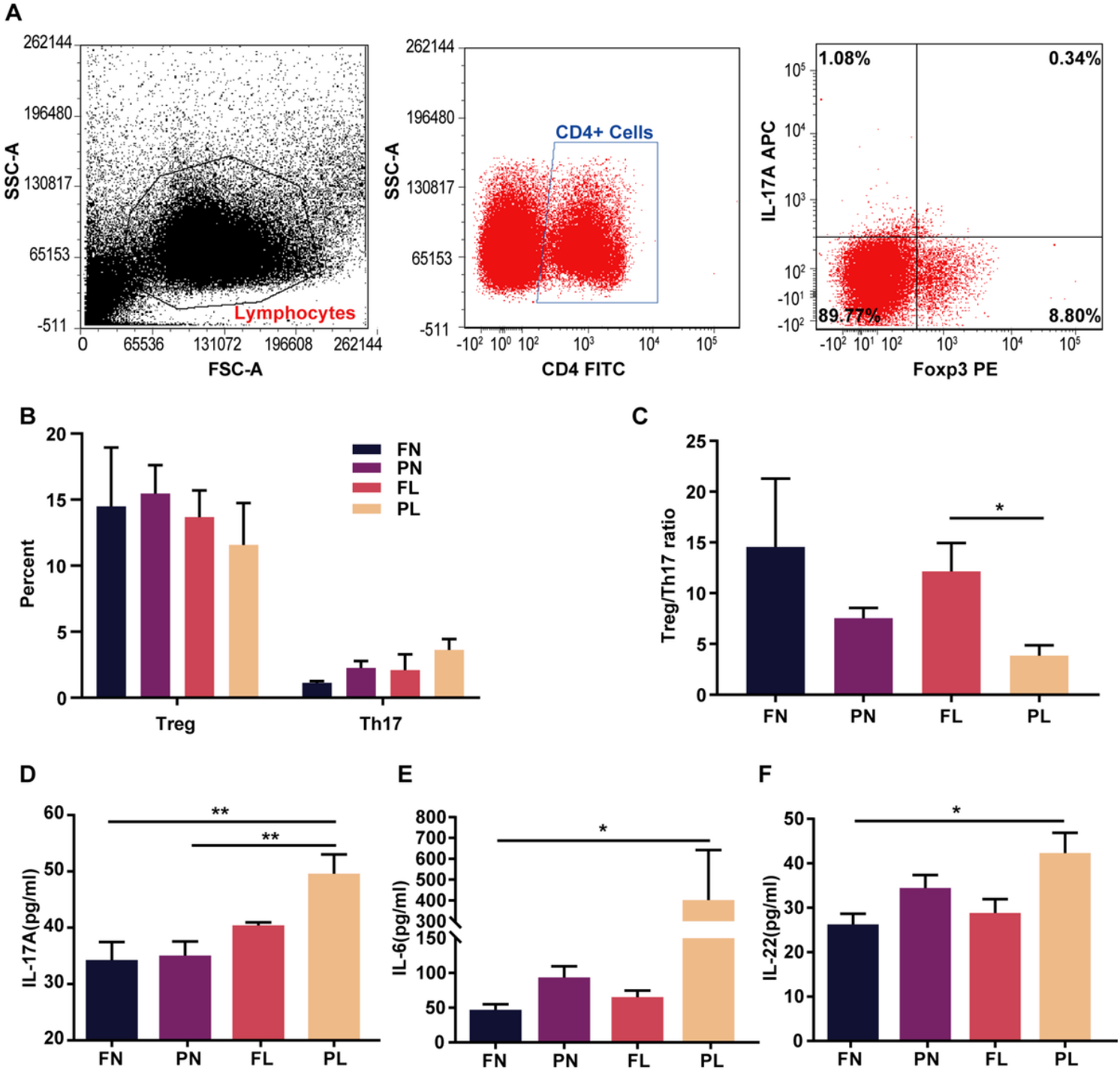

Figure 4

Determination of rat splenic Th17 cells in different groups by flow cytometry. (A) Gating strategies for Th17 cells used in our study. (B) The percentages of splenic Treg and Th17 cells were detected by flow cytometry in the four groups at the end of pregnancy (GD21). (C) The splenic Treg/Th17 ratio in the four groups. Circulating IL-17A (D), IL-6 (E), and IL-22 (F) levels were measured using a BioLegend multifactor flow assay. TNF, tumor necrosis factor. Data are presented as the mean ± SEM. * p<0.05, **p<0.01.

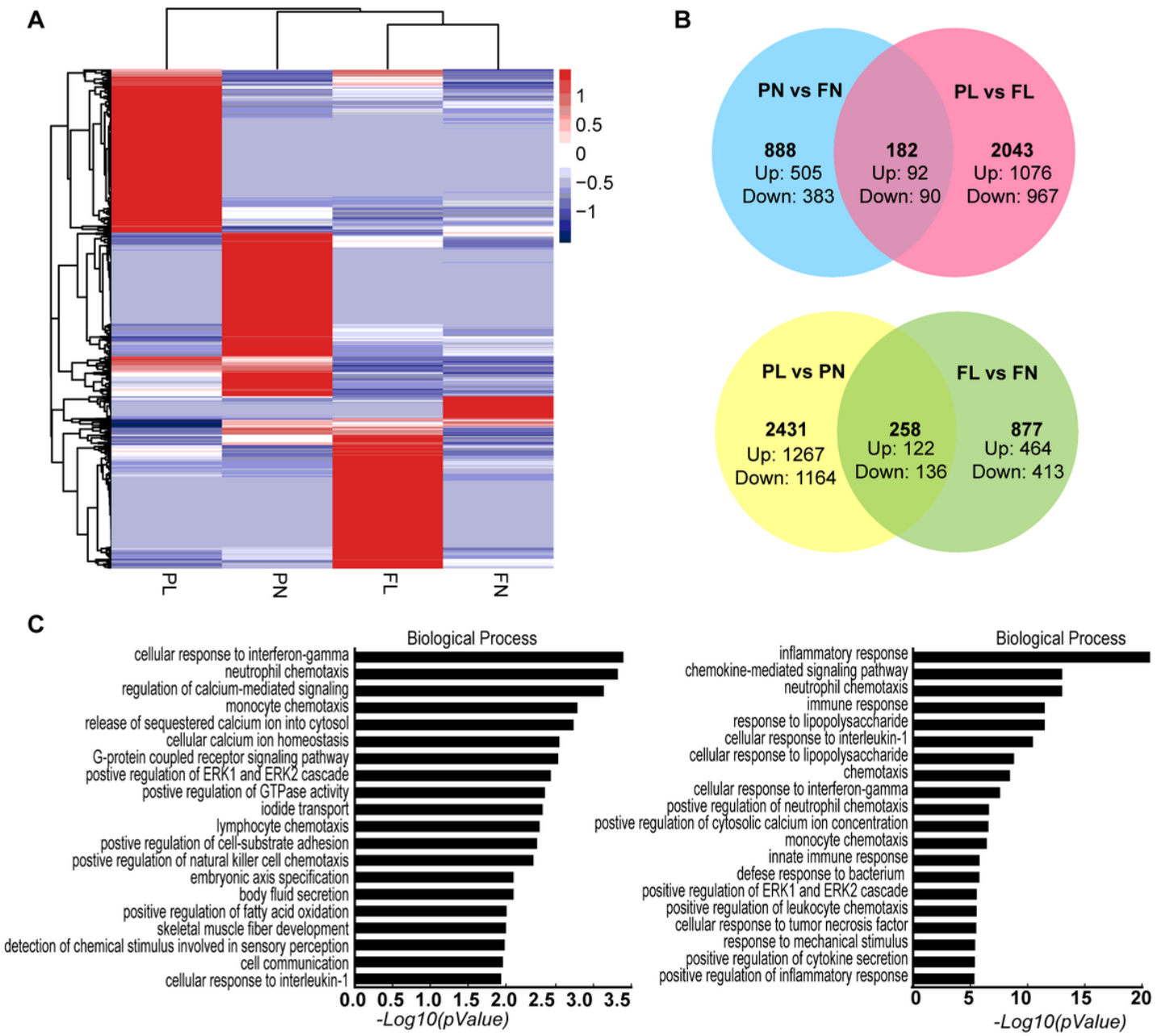

Figure 5

(A) Heatmap representation of the top 1000 DEGs among the 4 groups. (B) A total of 597 genes were upregulated, and 473 genes were downregulated between the PN and FN groups, whereas 1168 genes were upregulated, and 1057 were downregulated between the PL and FL groups. (C) The results of GO analysis between the PN and FN groups (left) and between the PL and FL groups (right).

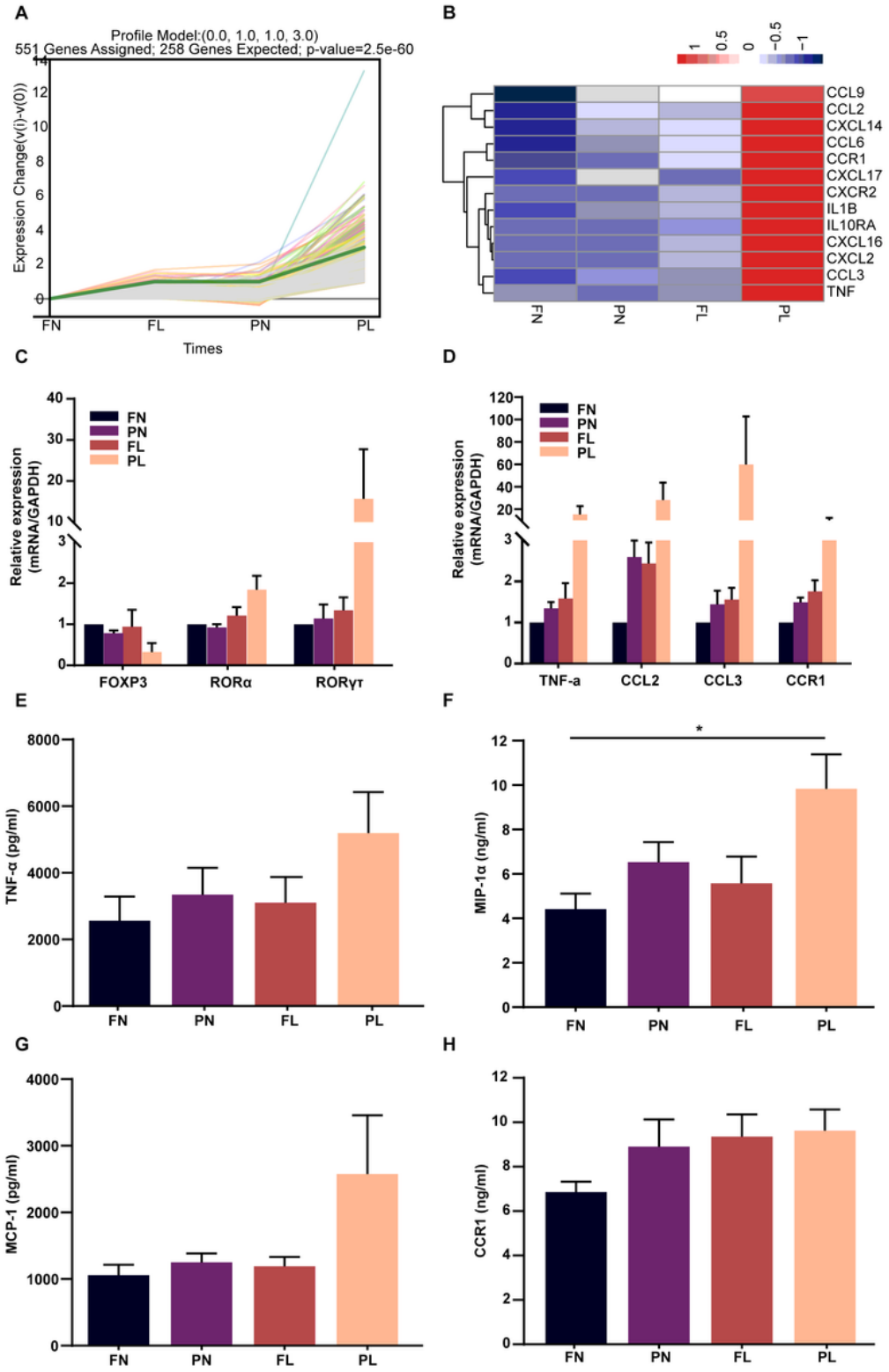

Figure 6

(A) STC analysis showing that DEGs in Profile 40 were persistently upregulated from the FN to PL groups. (B) Heatmap representation of the top 10 DEGs of Profile 40. (C) The relative mRNA expression of FOXP3, RORα and RORγτ was determined by real-time RT-PCR. (D-H) The relative mRNA and protein expression of TNF-a, CCL2, CCL3 and CCR1was determined by real-time RT-PCR and ELISA.

Supplementary Files

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- Supplementalfigure1.tiff