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Serum Neurofilament Light Polypeptide is a Biomarker for Inflammation in Cerebrospinal Fluid Caused by Fine Particulate Matter

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ABSTRACT

Epidemiological and toxicological evidence indicates that fine particulate matter ($PM_{2.5}$, particulate matter < 2.5 μm in aerodynamic diameter) causes cardiopulmonary toxicity; however, its neurotoxic effects remain unclear. The objective of this study was to investigate the role of the neurofilament light (NEFL) serum polypeptide in neurotoxicity. 6-month-old male Sprague Dawley (SD) rats were exposed to traffic-related PM_1 (< 1 μm in aerodynamic diameter; 16.3 μg m^{-3}) and gaseous pollutants (via high-efficiency particulate air, HEPA) for 3 and 6 months through a whole-body exposure system. According to our observations, the levels of interleukin (IL)-4, IL-10, and tumor necrosis factor (TNF)-α in the serum of the rats significantly increased ($p < 0.05$) after 3 months of exposure to PM_1, whereas that of NEFL polypeptide significantly increased ($p < 0.05$) after 3 and 6 months of exposure. Additionally, increases in the IL-2, IL-6, IL-10, IL-17a, TNF-α, and interferon (IFN)-γ levels after 3 and/or 6 months of exposure to this pollutant ($p < 0.05$) were observed in the cerebrospinal fluid (CSF). In terms of their respective levels, the IL-6 correlated well with the CSF IL-2 and IL-10; the TNF-α correlated well with the CSF IL-6, IL-17a, TNF-α, and IFN-γ; and the NEFL polypeptide correlated well with the CSF IL-2, IL-4, IL-6, IL-10, IL-17a, TNF-α, and IFN-γ. In summary, systemic neuroinflammatory and immune responses in rats occurred after chronic exposure to PM_1. Hence, NEFL polypeptide in serum may be a suitable biomarker for neurotoxicity caused by chronic exposure to this pollutant.

Keywords: Air pollution; Central nervous system; Neurotoxicity; Oxidative stress; Particulate matter.

INTRODUCTION

Neurodegenerative diseases are emergent chronic diseases in aging populations nowadays. According to the National Institute of Neurological Disorders and Stroke, there are more than 600 neurodegenerative disease, which affect approximately 50 million Americans each year (Brown *et al.*, 2005). In Taiwan, the average age-standardized prevalence of neurodegenerative diseases, such as Parkinson's disease (PD), per 100,000 population were 84.8 in 2004 and 147.7

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in 2011, representing a 7.9% annual increase (Liu *et al.*, 2016). Notably, accumulating epidemiological evidence indicates that pulmonary exposure to particulate air pollution is associated with increased risks of neurodegenerative diseases (Power *et al.*, 2011). For example, exposure to high levels of coarse particulate matter ($PM_{2.5-10}$, particulate matter with an aerodynamic diameter of 2.5–10 μm) and fine particulate matter ($PM_{2.5}$) is associated with increased risks of cognitive decline (Weuve *et al.*, 2012). Increased risks of neurodegenerative diseases such as PD were associated with chronic exposure to air pollution (Kioumourtzoglou *et al.*, 2016; Ritz *et al.*, 2016). It is worth noting that the effects of air pollution on the overall risk of neurodegenerative disease may be considerably higher than previously thought (The Lancet Neurology, 2018). Therefore, understanding the impacts of PM on the initiation of neurodegenerative disease is an urgent public health issue.

Our previous studies observed that chronic exposure to traffic-related air pollution caused cerebral edema and brain impairment in adult rats (Shih *et al.*, 2018). Oxidative stress and inflammation occurred in rat brains after PM exposure (Bai *et al.*, 2018; Li *et al.*, 2019), which led to central nervous system (CNS) impairment. The neuroinflammation could be due to microglia activation by ambient particulate matter (PM) exposure (Bai *et al.*, 2019a). Also, exposure of PM caused the formation of amyloid-β (Aβ) plaque (Cacciottolo *et al.*, 2017) as well as tau accumulation (Bai *et al.*, 2018; Calderon-Garciduenas *et al.*, 2018) in the brain. Exposure to traffic-related air pollution disrupted the brain's microvascular integrity in a high-fat diet animal model (Suwannasual *et al.*, 2018). Oxidative-inflammatory responses may cause the extracellular accumulation of fluid due to increased permeability of the blood-brain barrier (BBB). However, there are no biomarkers available to assess and evaluate conditions of the brain environment after PM exposure.

Cerebrospinal fluid (CSF) is produced by specialized ependymal cells in the choroid plexuses of the ventricles of the brain, and it is present in the brain and spinal cord. Clinically, CSF is used to represent the condition of the brain for diagnoses (Molinuevo *et al.*, 2014; Vogelgsang *et al.*, 2018). Increasing numbers of studies have investigated the effects of air pollution on the brain based on CSF evidence. For example, a series of studies indicated that exposure to air pollution increased CSF inflammatory markers such as interleukin (IL)-6 and IL-2 (Calderon-Garciduenas *et al.*, 2013), which are associated with increased risks of Alzheimer's disease (AD) and PD (Calderon-Garciduenas *et al.*, 2016). The effects on the brain were more significant due to smaller PM such as ultrafine PM (Gonzalez-Maciel *et al.*, 2017).

Increasing evidence revealed that proteins from the CSF and serum are able to improve clinical diagnoses for neurodegenerative diseases (Pereira *et al.*, 2017). The neurofilament light (NEFL) polypeptide, for example, was observed to be present in both the CSF and the serum, and its levels between the CSF and serum were well correlated (Norgren *et al.*, 2003). Clinically, the serum NEFL polypeptide is considered a marker of brain damage and ongoing disease activity (Kuhle *et al.*, 2017). However, expression of the NEFL polypeptide in the serum after exposure to $PM_{2.5}$ remains unclear. The objective of this study was to investigate expression of the NEFL polypeptide in serum after chronic exposure to PM_1. CSF and serum inflammatory cytokines were examined for correlations with serum NEFL polypeptide levels to investigate the underlying mechanisms after chronic exposure to PM_1.

METHODS

Experimental Design

Male 6-month-old Sprague Dawley (SD) rats obtained from the National Laboratory Animal Center (Taipei, Taiwan) were used throughout the study. Rats were randomly selected for three groups: high-efficiency particulate air (HEPA; exposed to gaseous pollution only; n = 12; New Taipei City, Taiwan), and PM_1 exposure to traffic-dominated air pollution (exposed to particulate and gaseous pollution; n = 12; New Taipei City, Taiwan). The HEPA group (gaseous pollution only) served as control, whereas the PM_1 (particulate and gaseous pollution) served as exposure. The exposure site was a traffic-dominant area, which was near a highway (~710 m) and an expressway (~76 m). The distance to the closest major road was approximately 62 m. Rats were housed at a constant temperature of 22 ± 2℃ and a relative humidity (RH) of 55 ± 10% with a 12:12-h light:dark cycle throughout the study. After 3 and 6 months of exposure, rats were necropsied, and serum and CSF were collected (n = 6/group for 3 and 6 months; Li *et al.*, 2009; Nirogi *et al.*, 2009). Animal experiments were conducted in compliance with the animal and ethics review committee of the Laboratory Animal Center at Taipei Medical University (Taipei, Taiwan).

Whole-body Exposure to PM_1

Rats were whole-body exposed to PM_1 (particulate matter with an aerodynamic diameter of < 1 μm) using a whole-body exposure system for 3 and 6 months as previously reported (Shih *et al.*, 2018). Data of the gaseous pollution were collected from the nearby Taiwan Environmental Protection Administration Yonghe air quality monitoring station, and the concentrations and physical properties of PM were characterized by the collocated instruments. In the system, a tapered element oscillating microbalance (TEOM; Model 1400a; Thermo Scientific, USA) was used to determine PM_1 mass concentrations. A Scanning Mobility Particle Sizer (SMPS; Model 3936; TSI Inc., USA) and an Aerodynamic Particle Sizer (APS; Model 3321; TSI Inc., USA) were used to examine the submicron particle size distribution (PSD) and supermicron PSD, respectively. A Nanoparticle Surface Area Monitor (NSAM; Model 3550; TSI Inc., USA) was used to monitor lung deposition surface area (LDSA) concentrations. It was reported that measurements of black carbon (BC) by continuous monitors, such as an Aethalometer, showed good correlations with concentrations of traffic exhaust particles or with traffic counts (Wu *et al.*, 2007; deCastro *et al.*, 2008; Patel *et al.*, 2009). Therefore, an Aethalometer (AE33; Magee Scientific, Berkeley, CA, USA) was used to measure the BC mass concentration. Additionally, the numbers of cars were manually calculated to obtain passenger car units (PCU) during the study period.

Enzyme-linked Immunosorbent Assay (ELISA)

The NEFL polypeptide was determined in serum using an ELISA kit (Bioassay Technology Laboratory, Shanghai, China) in accordance with the manufacturer's instructions.

Multiplex Assay for Serum

IL-4, IL-6, IL-10, and tumor necrosis factor (TNF)-α in serum samples were determined by an AimPlex™ multiplex assay (St. Louis, MO, USA), according to the manufacturer's instructions. A BD LSRFortessa™ cell analyzer (NJ, USA) was used to determine complexes of beads and the studied proteins labeled with phycoerythrin antibodies.

Cytometric Bead Array (CBA) for CSF

Levels of IL-2, IL-4, IL-6, IL-10, IL-17A, interferon (IFN)-γ, and TNF-α in CSF were examined by a CBA (BD Bioscience, CA, USA), according to the manufacturer's instructions. Complexes of beads were determined with a BD LSRFortessa™ cell analyzer.

Statistical Analysis

A nonparametric Mann-Whitney *U*-test was used to examine the difference between groups. We defined as extreme outlier cytokine values ten times higher than the value of the 99th percentile. There were no significant outliers identified from the samples. Pearson's correlation coefficient was used to examine correlations of biomarkers between serum and CSF. Statistical analyses were performed using GraphPad vers. 5 for Windows. The level of significance was set to $p < 0.05$. Data are expressed as the mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Chronic Exposure to PM_1

Exposure to Air pollution has been linked to increased risks of neurodegenerative diseases (Malek *et al.*, 2015; Lee *et al.*, 2019). To study NEFL polypeptide expression in serum after chronic exposure to PM_1, an *in vivo* whole-body exposure system was established for chronic exposure to PM_1. Traffic is one of the most prominent sources contributing to airborne PM in urban environments, and a trailer-type monitoring site was established which was located in a neighborhood of the two major crossing/commuting avenues in the Shuang Ho area (New Taipei City, Taiwan), a typical urban environment in Taiwan. The average daily traffic flows in these major commuting routes are all over 50,000 PCU day^{-1}. The average daily traffic volume was 444 cars h^{-1} and 4731 motorcycles h^{-1} for evening rush hour (17:00–20:00) and 462 cars h^{-1} and 2183 motorcycles h^{-1} for morning rush hour (06:00–08:00) (Supplementary Material Fig. S1).

Sampling inlets were located at the top of the roof of the trailer station at about 6 m above the ground. The sampled traffic-related air pollution was delivered to two modified individual ventilated cage (IVC) systems. Rats were whole-body exposed to PM_1 using a whole-body exposure system for 6 months as reported previously (Shih *et al.*, 2018). One system with filters was considered exposure to gaseous pollutants only, and the other system without filters was the experimental group that was exposed to PM_1 as well as gaseous pollutants.

As shown in Fig. 1, the penetration of particles in the IVC without a filter was 75% over the entire particle size range, and the average particle penetration was 80% for particles of < 550 nm in size. In the particle size ranging 550–1000 nm, the average particle penetration was 34% and gradually decreased to about 20%. In other words, the major particles to which the mice were exposed were PM_1. For the IVC with filters, the average penetration of particles was 4% over the entire particle size range. Particularly for particles of > 600 nm, the overall removal efficiency of particles could be as high as 99%. The typical exposure PSDs measured in the exposure chamber/cabinet of the IVC are shown as a number and on a mass basis (Figs. 2(a) and 2(b)). The number PSD demonstrates the typical single Aitken-mode traffic characteristics with a major peak size of around 49 nm and a total number concentration of around 10^4–10^5 particles cm^{-3}. When an identical PSD was shown in the volume concentration, the typical tri-mode for PM_1 was observed, and the peak size of the volume PSD was located at 400 nm. Thus, the total mass concentration of PM_1 should largely be attributed to these accumulation modes, and the main chemical components could be sulfate, nitrate, ammonium, organic carbon, elemental carbon, and heavy metals (Tsai *et al.*, 2012; Liang *et al.*, 2013; Lin *et al.*, 2015).

Table 1 shows the air pollution to which rats were exposed during the study period. The PM_1, PNC, BC, and LDSA were 16.3 ± 8.2 μg m^{-3}, 11,257 ± 4388 particles cm^{-3}, 1800 ± 784 ng m^{-3}, and 55.1 ± 21.7 μm^2 cm^{-3}, respectively, which were previously reported (Shih *et al.*, 2018). In the present study, the rats were exposed to relatively lower levels of PM_1 than the WHO $PM_{2.5}$ guideline (25 μg m^{-3} for a 24-h average) (WHO, 2006). The PNC and BC presented higher levels during the experiment period than previous measurements (Chuang *et al.*, 2017). This observation suggested that traffic-related particulate pollutants were the main source of exposure for rats. As to pollutants obtained from the air quality station, the PM_{10}, $PM_{2.5}$, NO_x, SO_2, and O_3 levels were all below WHO guidelines (WHO, 2006). Therefore, the exposure conditions provided a good environment to study the effects of long-term exposure to lower levels of air pollution on neurotoxicity *in vivo*.

Biomarkers Determined in Serum

Systemic inflammation occurring after chronic exposure to air pollution was previously reported (Neophytou *et al.*, 2013; Lee *et al.*, 2016). In the present study, we determined levels of IL-4, IL-6, IL-10, TNF-α, and the NEFL polypeptide in serum of rats after 3 and 6 months of exposure to PM_1 (Fig. 3). We observed that IL-4, IL-10, and TNF-α significantly ($p < 0.05$) increased with 3 months of exposure to PM_1. We observed that immune responses of IL-4 and TNF-α were activated after 3 months of PM_1 exposure. Consistently, a study by Shakya *et al.* (2019) showed that levels of IL-4, IL-10, and TNF-α in the blood of humans were elevated after roadside exposure. Our previous study observed that air pollution activated an immune response in pneumonia (Bai *et al.*, 2019b). Also, $PM_{2.5}$ exposure increased the risk of pneumonia in patients with chronic obstructive

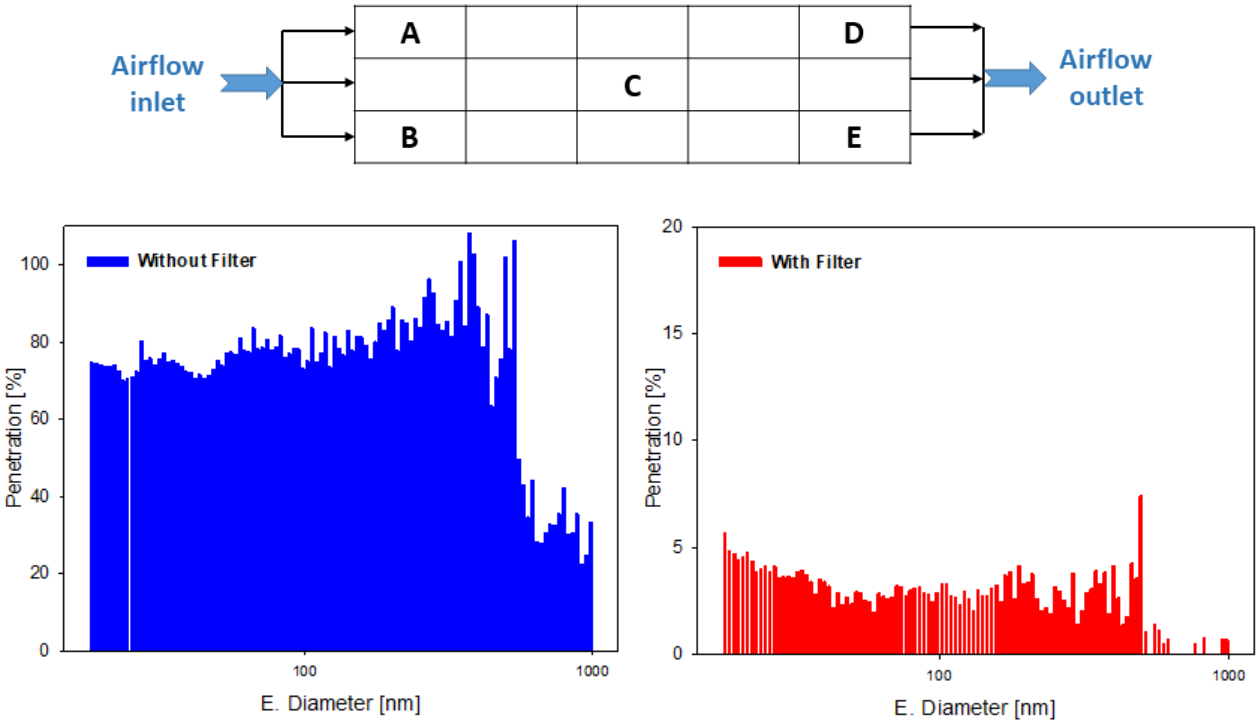


Fig. 1. Illustration of the airflow of the whole-body exposure system for the *in vivo* experiment. Average penetration rates of the filtered group and unfiltered group at different particle size ranges.

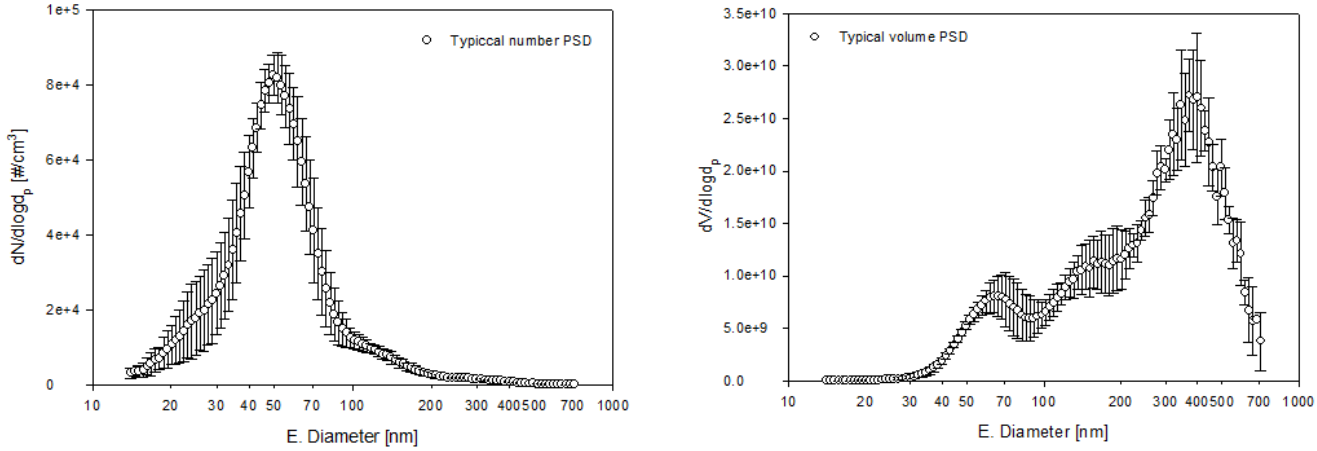


Fig. 2. (a) Typical exposure number particle size distribution (PSD); (b) typical exposure volume PSD.

pulmonary disease (COPD) (Ho *et al.*, 2019). Therefore, PM_1 exposure may alter systemic immune responses after subchronic exposure in rats.

We noted that serum IL-10 had increased after 3 months of PM_1 exposure compared to the controls ($p < 0.05$), whereas there was no significant difference in IL-6 between the controls and the PM_1-exposed group (Fig. 3). The results indicated that an anti-inflammatory reaction was activated to mitigate the PM_1-induced systemic inflammation in rats. Inflammation was resolved by the self-repair/defense response; thus, persistent low-grade inflammation in serum after chronic exposure to PM_1 was observed in our study. Notably, we investigated levels of the NEFL polypeptide in serum after PM_1 exposure (Fig. 3). The NEFL polypeptide is unique to neuronal cells, and it is shed to the CSF and is detectable at low concentrations in peripheral blood (Disanto *et al.*, 2017). The presence of increasing levels of the NEFL polypeptide in the CSF is considered to be an indicator of axonal damage in the CNS (Norgren *et al.*, 2003). Recently, the NEFL polypeptide was detected in serum in the presence of a neurodegenerative disease (Norgren *et al.*, 2003). Levels of the NEFL polypeptide showed good correlations between CSF and serum samples, both of which are able to represent the disease progress of neurodegenerative diseases (Disanto *et al.*, 2017). In the present study, the NEFL polypeptide had significantly increased ($p < 0.05$) after 3 and 6 months of exposure to PM_1 compared to the controls. Increased levels of the NEFL polypeptide suggested that chronic exposure to

Table 1. Characterization of air pollution for whole-body exposure and ambient levels during the study period.

Meteorological and pollutants (unit)	Mean ± SD
Particulate matter[1]	
PM_1 (μg m^{-3})	16.3 ± 8.2
PNC (particles cm^{-3})	11,257 ± 4388
BC (ng m^{-3})	1800 ± 784
LDSA (μm^2 cm^{-3})	55.1 ± 21.7
Ambient pollutants[2]	
PM_{10} (μg m^{-3})	36.6 ± 15.2
$PM_{2.5}$ (μg m^{-3})	19.7 ± 9.8
NO_x (ppb)	32.9 ± 16.4
SO_2 (ppb)	2.5 ± 1.0
O_3 (ppb)	29.7 ± 11.0

[1] PM data referenced from Shih *et al.* (2018).
[2] Gaseous pollution was measured by the Environmental Protection Administration Yonghe air quality monitoring stations. PM_1: particulate matter of < 1 μm in aerodynamic diameter; PNC: particle number concentration; BC: black carbon; LDSA: lung deposition surface area; PM_{10}: particulate matter of < 10 μm in aerodynamic diameter; $PM_{2.5}$: particulate matter of < 2.5 μm in aerodynamic diameter; NO_2: nitrogen dioxide; SO_2: sulfur dioxide; O_3: ozone.

PM_1 led to an increase in its levels in the serum and CSF, which could be associated with the development of neurodegenerative diseases by PM_1. However, more studies are required to investigate the role of the NEFL polypeptide in response to air pollution exposure.

Biomarkers Determined in CSF

CSF is commonly used to determine levels of neuroinflammation and oxidative stress and to represent the healthy or diseased statuses of the brain (Guest *et al.*, 2014). In the present study, we observed that IL-17a and TNF-α had increased with 3 months of PM_1 exposure, and IL-6 had increased with 6 months of PM_1 exposure ($p < 0.05$; Fig. 4). Both 3 and 6 months of exposure to PM_1 caused increases in IL-2, IL-10, and IFN-γ levels in the CSF compared to the controls ($p < 0.05$; Fig. 4). The results indicated that neuroinflammation occurred due to chronic exposure to PM_1 as observed in the CSF. Similarly, alterations in CSF inflammatory markers due to air pollution were investigated in children and adults in Mexico City (Calderon-Garciduenas *et al.*, 2016). They observed that $PM_{2.5}$ exposure increased the risk of AD and PD. A previous study further observed that neuroinflammation occurred in children of Mexico City after exposure to severe air pollution, leading to IL-6 and IL-2 increases in CSF (Calderon-Garciduenas *et al.*, 2013). Smaller combustion-derived particles, especially ultrafine PM, induced more-significant impacts in the brains of children (Gonzalez-Maciel *et al.*, 2017). Therefore, alterations in inflammatory responses of CSF may be able to represent impairment of the brain caused by PM_1. However, the contributions of physicochemistry of PM to CSF inflammation require further investigation.

Correlations of Biomarkers between Serum and CSF

CSF biomarkers were correlated with levels of inflammation in serum. In the present study, associations of biomarkers between serum and CSF were examined as shown in Table 2. We observed that serum TNF-α was correlated with serum NEFL polypeptide ($r = 0.477$, $p < 0.05$). Serum IL-6 was correlated with CSF IL-2 ($r = 0.520$, $p < 0.01$) and IL-10 ($r = 0.478$, $p < 0.05$). Serum TNF-α was correlated with CSF IL-6 ($r = 0.522$, $p < 0.01$), IL-17a ($r = 0.519$, $p < 0.01$), TNF-α ($r = 0.519$, $p < 0.01$), and IFN-γ ($r = 0.407$, $p < 0.05$), whereas serum NEFL polypeptide was correlated with IL-2 ($r = 0.529$, $p < 0.01$), IL-4 ($r = 0.571$, $p < 0.01$), IL-6 ($r = 0.486$, $p < 0.05$), IL-10 ($r = 0.653$, $p < 0.01$), IL-17a ($r = 0.683$, $p < 0.01$), TNF-α ($r = 0.683$, $p < 0.01$), and IFN-γ ($r = 0.596$, $p < 0.01$). The results indicated that immune responses and inflammatory markers were correlated between serum and CSF. Previous reports showed that CSF NEFL polypeptide was well correlated with serum levels (Norgren *et al.*, 2003), which also increased after exposure to air pollution in the serum and CSF of children (Calderon-Garciduenas *et al.*, 2016). Notably, we observed that serum NEFL polypeptide had good correlations with all markers determined in CSF (IL-2, IL-4, IL-6, IL-10, IL-17a, TNF-α, and IFN-γ). Our observations suggest that serum NEFL polypeptide levels are a good biomarker to represent immune-inflammatory responses in CSF that occur due to PM_1 exposure.

We next found that most of the markers were correlated with each other in the CSF in the present study, except for IL-2 with IL-17a, IL-2 with TNF-α, and IL-5 with IL-10 (Table 2). These results suggest that a series of immune-inflammatory reactions were activated in the CSF after PM_1 exposure in rats. Neuroinflammatory responses play a role in regulation of defense or damage reactions. Inhaled pollutants can damage epithelial and endothelial barriers in the lungs, allowing their movement to the brain, which would be a robust trigger of tight junction and neural antibodies. After exposure, the produced molecules may be able to activate autoimmune responses, which potentially would contribute to neuroinflammation and the pathological hallmarks of neurodegenerative diseases (Calderon-Garciduenas *et al.*, 2015). $PM_{2.5}$ is able to directly and/or indirectly induce brain inflammation. More evidence is required to understand the underlying mechanisms of neuroinflammation and brain injury caused by PM.

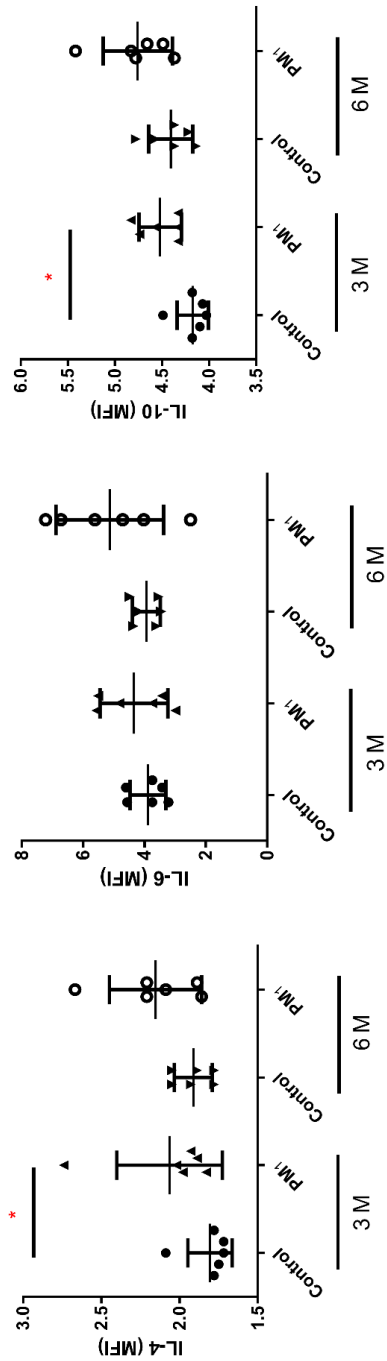
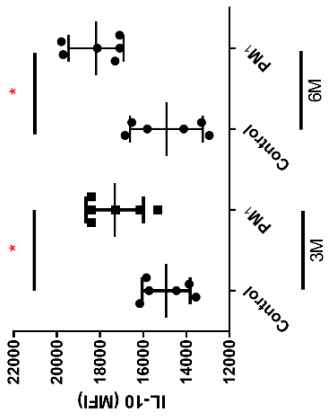
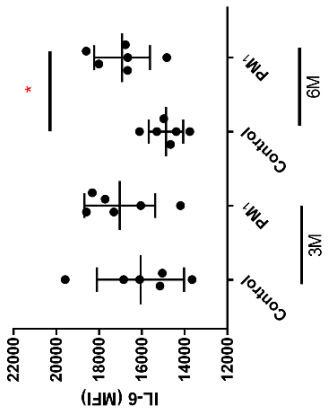
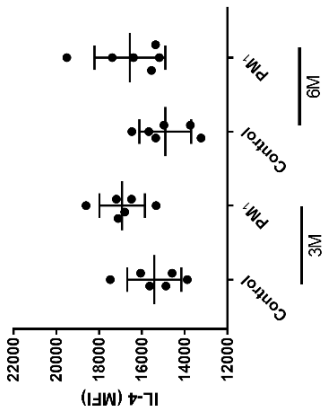
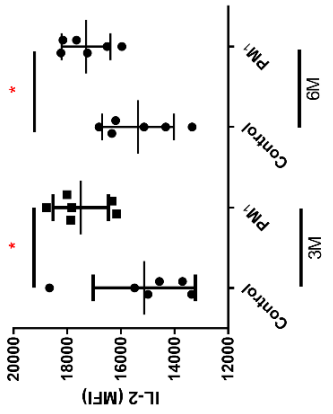


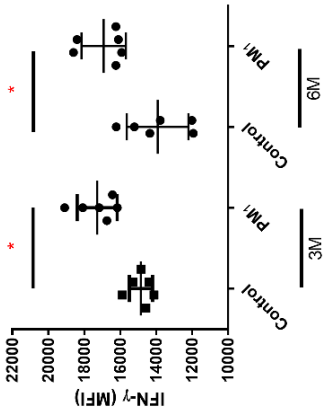
Fig. 3. Interleukin (IL)-4, IL-6, IL-10, tumor necrosis factor (TNF)-α, and the neurofilament light (NEFL) polypeptide were determined in the serum of rats in the control, HEPA (high-efficiency particulate air), and PM_1 (particulate matter with an aerodynamic diameter of < 1 μm) groups ($n = 6$). * $p < 0.05$.

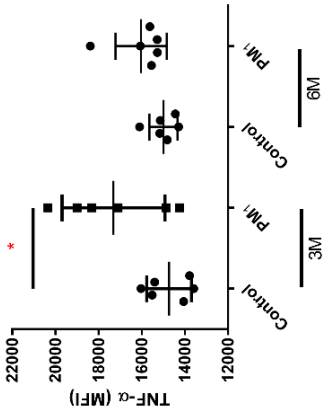












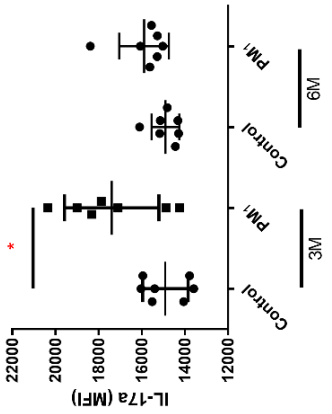


Fig. 4. Interleukin (IL)-2, IL-4, IL-6, IL-10, IL-17a, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ were determined in the cerebral spinal fluid (CSF) of rats in the control, HEPA (high-efficiency particulate air), and PM_1 (particulate matter with an aerodynamic diameter of <1 μm) groups ($n = 6$). * $p < 0.05$.

Table 2. Correlations of biomarkers between the serum and cerebral spinal fluid (CSF).

		Serum					CSF						
		IL-4	IL-6	IL-10	TNF-α	NEFL	IL-2	IL-4	IL-6	IL-10	IL-17a	TNF-α	IFN-γ
Serum	IL-4	1.000	0.103	0.222	0.093	0.260	0.337	0.378	0.292	0.284	0.033	0.033	0.355
	IL-6		1.000	0.160	0.231	0.381	**0.520****	0.337	0.310	**0.478***	0.263	0.263	0.170
	IL-10			1.000	0.292	0.210	0.179	0.253	0.290	0.371	0.313	0.313	0.356
	TNF-α				1.000	**0.477***	0.357	0.268	**0.522****	0.337	**0.519****	**0.519****	**0.407***
	NEFL					1.000	**0.529****	**0.571****	**0.486***	**0.653****	**0.683****	**0.683****	**0.596****
CSF	IL-2						1.000	**0.514***	**0.590****	**0.635****	0.354	0.354	**0.644****
	IL-4							1.000	**0.428***	0.389	**0.569****	**0.569****	**0.658****
	IL-6								1.000	**0.491***	**0.518****	**0.518****	**0.610****
	IL-10									1.000	**0.491***	**0.491***	**0.666****
	IL-17a										1.000	**0.878****	**0.635****
	TNF-α											1.000	**0.635****
	IFN-γ												1.000

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).
IL: interleukin; TNF: tumor necrosis factor; NEFL: neurofilament light polypeptide; IFN: interferon.

CONCLUSIONS

We successfully established a whole-body exposure system of natural exposure to respirable PM (i.e., PM_1) to investigate chronic *in vivo* impacts. To assess the exposure conditions, the levels of PM_1 were measured on-line. Our previous findings showed that chronic exposure to this pollutant caused short-term memory loss, brain edema, and neuroinflammation in rats. Microglia activation was also observed following exposure. The present study further demonstrates that systemic neuroinflammatory and immune responses occur in rats after chronic exposure to PM_1. Our results suggest that chronic exposure to PM_1 may play a larger role than gaseous pollutants in triggering neurotoxicity. Critically, NEFL polypeptide in serum may be a biomarker for this damage.

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DISCLAIMER

The authors declare that they have no conflicts of interest.

SUPPLEMENTARY MATERIAL

Supplementary data associated with this article can be found in the online version at http://www.aaqr.org.

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