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Basophil mediated pro-allergic inflammation in vehicle-emitted particles exposure

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ABSTRACT

Despite of the fact that engine manufacturers develop a new technology to reduce exhaust emissions, insufficient attention given to particulate emissions. However, diesel exhaust particles are a major source of air-borne pollution, contain vast amount of polycyclic aromatic hydrocarbons (PAHs) and may have deleterious effects on the immune system, resulting in the induction and enhancement of pro-allergic processes. In the current study, vehicle emitted particles (VEP) from 2 different types of cars (diesel - D and gasoline - G) and locomotive (L) were collected. Overall, 129 four-week-old, male SPF-class Kunming mice were subcutaneously instilled with either low dose 100, 250 or high dose, 500 mg/kg VEP and 15 mice were assigned as control group. The systemic toxicity was evaluated and alterations in the percentages of the CD3, CD4, CD8, CD16, CD25 expressing cells, basophils, eosinophils and neutrophils were determined. Basophil percentages were inversely associated with the PAH content of the VEPs, however basophil sensitization was more important than cell count in VEP exposure. Thus, the effects of VEP-PAHs emerge with the activation of basophils in an allergen independent fashion. Despite the increased percentage of CD4+ T cells, a sharp decrease in basophil counts at 500 mg/kg of VEP indicates a decreased inhibitory effect of CD16+ monocytes on the proliferation of CD4+ T cell and suppressed polarization into a Th2 phenotype. Therefore, although the restrictions for vehicles emissions differ between countries, follow up studies and strict regulations are needed.

1. Introduction

Vehicle emitted particulate (VEP) matter can have direct consequences for human and environmental health (Bonazza et al., 2007; Pope and Dockery, 2006). Although the vehicle gas emission and water soluble counterparts have been widely investigated, the effects of VEPs are ignored (Canagaratna et al., 2010; Cheung et al., 2009; Karjalainen et al., 2014). Exhaust gas is emitted as a result of the combustion of various kinds of fuels (Omidvarborna et al., 2014) and according to the type of the engine, it is discharged into the atmosphere and contributes to the air pollution (Golokhvast et al., 2015a, 2015b). Thus, the characteristics of the particulate matter that is emitted by each car differs. Caiazzo et al. indicated that 53,000 early deaths occur per year in the United States alone because of vehicle emissions (Caiazzo et al., 2013). Although the pollutants emitted by vehicles are typically regulated by governmental agencies, due to their undesirable effects, the interest in particulate matter (PM) emissions has grown substantially and subsequent issues arise regarding the necessity of regulations, in the last few years (Mazzoleni et al., 2010). In 2012, based on sufficient evidence, the International Agency for Research on Cancer

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(IARC) concluded that diesel engine exhaust exposure is associated with an increased risk for lung cancer. It is classified as Group 1, carcinogenic to humans (Attfield et al., 2012; Benbrahim-Tallaa et al., 2012; Silverman et al., 2012). Globally, the particle emission limitations for vehicles are under strong control. VEPs are restricted by standards which have quite wide variations between different countries. Thus, European Union restricted the VEPs total mass in 2009, and the number in 2014 (EC Treaty/Euratom Treaty, 2008; Martini et al., 2009). Although the bulk amount of investigations today is concentrated on the size of VEP and their chemical composition (Gillies, 2014; Hochmuth et al., 2013; Kalberer et al., 2014; Wichmann and Shapiro, 2006), it has been shown that the main particulate fraction of diesel exhaust can easily penetrate into the lung when inhaled, because of their small size as they mostly consist of fine particles. Also, their rough surfaces make it easy to bind to other toxins in the environment, thus increase their hazardous effects (Fenga et al., 2016; Omidvarborna et al., 2015; Piperigkou et al., 2016). On the one hand, it has been shown that diesel exhaust particles (DEP) are associated with the induction of free radical production and initiation of pro-inflammatory responses (Bai et al., 2001; Becker et al., 2005; Bostan et al., 2016; Salvi et al., 1999; Vitkina et al., 2016). On the other hand, DEP have been implicated in the increased incidence and morbidity of asthma and allergic rhinitis (Diaz-Sanchez et al., 1994; Lubitz et al., 2010). However, data regarding the gasoline and locomotive emitted particles are insufficient. Previously, our group determined the morphometric parameters and chemical composition of the VEPs from different car brands. This investigation revealed that the various size fractions and aerodynamic diameters may have differential spreading to the environment resulting in health consequences on exposed individuals (Golokhvast et al., 2015b; Sayapina et al., 2016). However, the exposure amounts differ between the countries or even in rural or urban areas (Gramsch et al., 2014; Nikolova et al., 2011). Indeed, it was found that the fetus of the mice who were orally exposed to various doses of DEP ranging from 31.25 mg/kg/day to as high as 500 mg/kg/day, had a significant increase in the frequency of DNA deletions (Reliene et al., 2005). Furthermore, the interactions of the low doses of particulate matter with the immune system and their toxicity status have been discussed by different research groups (Ernst et al., 2002; Miyata and van Eeden, 2011). In accordance with these evidences, the main goal of this work was to evaluate the immunological consequences of high dose exposure to VEPs that are collected from the locomotive, diesel, and gasoline powered cars in Russia.

2. Materials and methods

2.1. Samples

The samples were collected from a diesel car (engine volume 2.5 lt) (D), a gasoline car (engine volume 4.3 lt) (G) and a diesel locomotive (2×3060 horsepowers) (L) (engine volume not available). The cars were fueled in the same gas station located in Vladivostok, Russia. The fuels of cars and locomotive were from the same manufacturer. All preparations were done by our methods, as described and patented by Golokhvast et al., previously (Golokhvast et al., 2015a, 2015b). Briefly, to collect the samples, the exhaust gas suspension (EGS) method was used. Exhaust gases were collected via a PVC hose and cooled by passing through water, so that up to 80% particulates retained in deionized water (Golokhvast et al., 2015a, 2015b). The particles sizes were ranging from 1 to 10 μm by volume count, and from 0.1 to 1 μm by number count.

2.2. Experimental animals

SPF-class Kunming mice (male; age, 4 weeks; weight, 15–20 g) were accommodated one week before experiments. They kept in a room, in plastic cages at room temperature 22–27 °C, relative humidity 55 ± 15% and 12 h dark/light cycle. Mice received a balanced diet and water unlimited. Male mice aged 5 weeks and weighing 20–25 g were used for all further experiments. The animals were randomly assigned to the groups. The study protocol was reviewed and approved by the Animal Care Committee of Far Eastern Federal University. The study followed guiding principles for experimental procedures of Declaration of Helsinki for animal experimentation.

2.3.1. VEP sample analysis

The methanol and water soluble fractions of VEP were analyzed. To extract the methanol soluble fraction, 10 mg of each sample was diluted in 1 ml of methanol and placed in an ultrasonic water bath for 10 min. After vortexing for 20 s, the supernatant was centrifuged at 14,000 rpm for 5 min and analyzed by Gas chromatography-Mass spectrometry (GC-MS).

In order to determine the water soluble contents, moreover, 4–20 mg of each sample was placed in Solid Phase Microextraction vials containing 1 ml of ultrapure water, 200 mg of NaCl and sealed with Polytetrafluoroethylene/silicon septum caps. Online extraction was performed with a 65 μm Polydimethylsiloxane/Divinylbenzene Metal Alloy type fiber, at 90 °C for 20 min with an agitation speed at 250 rpm. After the absorption of the analytes was complete, the fiber tip was inserted in the injection port of the GC-MS for 3 min

2.3.2. Instrumentation

Analysis was carried out by a GC-MS instrument (Shimadzu QP-2010) equipped with a split/splitless injection inlet and an AOC-5000 auto-sampler. Pure helium (99.999%) was used as flow gas (1 ml/min). The separation of the analytes was achieved by a Supelco Analytical SLBtm-5 ms capillary column of 30 m length, 0.25 mm i.d., 0.25 μm film thickness with initially temperature at 120 °C (stable for 3 min), increased to 310 °C with a rate of 5 °C/min (stable for 1 min) and finally raised to 325 °C (at 10 °C/min, stable for 1 min). The mass spectrometer detector was operated at full scan for screening of the sample using the GC-MS libraries (NIST107.lib, Wiley7.lib) and at the selected ion-monitoring mode for polycyclic aromatic hydrocarbons (PAHs) monitoring and quantification. The inlet temperature, the interface and the ion source temperatures were 300 °C, 310 °C and 230 °C, respectively (Tzatzarakis et al., 2014). The retention times, as well as the used m/z ions for the determination and qualification of PAHs were presented in Table 1.

2.4. In vivo toxicity testing

2.4.1. Survival experiments

The VEPs, at various dose levels were evaluated for their effect on the mice survival rate. Samples of VEPs were sterilized in bactericidal UV box (Liston-U2103, Russia) and then suspended in sterilized saline

Table 1
Retention times and m/z ions used to determine polycyclic aromatic hydrocarbons and internal standard (TCN-IS) by gas chromatography-mass spectrometry.

compound	Rt (min)	Q1 m/z	Q2 m/z
acenaphtylene	10.03	152	76
fluorene	12.96	166	82
anthracene +phenanthrene	17.27	178	76
TCN (IS)	20.77	266	194
pyrene	23.85	202	101
benzo(a)anthracene +chrysene	29.61	228	114
benzo(k)fluoranthene+benzo(a)fluoranthene +benzo(a) pyrene	34.41	252	126
benzo(g,*h*, i)perylene+dibenz(a,h)anthracene	40.18	276	138
indeno(1,2,3-cd)pyrene	40.97	276	138

solution (0.9% NaCl). 102 mice were randomly assigned into 3 groups to be exposed to different kinds of VEPs. Each group was divided into subgroups to be exposed to different doses of the same kind of VEPs. Thus, mice were subcutaneously instilled with either D, G or L vehicle particles of 100, 250 or 500 mg/kg doses. The control group consisted of 11 mice and only received saline solution, subcutaneously. Injections were done with syringe needle gauge 14 and volume of injection did not exceed 150 µl. Mice were monitored for 30 days and their weight were recorded. At the end of 30 days, all survived mice were sacrificed after anesthetizing by Ketamine and Xylazine at doses of 80 mg/kg and 10 mg/kg, respectively.

2.4.2. Evaluation of immunological and hematological parameters

The study group consisted of 27 mice which were randomly assigned into 3 groups. Similar to the survival study, each group divided into subgroups to be exposed to different doses of the same VEP. Thus, 3 mice in each subgroup were subcutaneously instilled with either low dose 100 and 250 or high dose 500 mg/kg of D, G or L VEP that was suspended in 0.9% NaCl. The control group consisted of 4 mice that received only 0.9% NaCl by the same exposure route. Blood samples were collected into individual heparin-coated tubes, 24 h after the treatment. Neutrophil, lymphocyte, eosinophil and basophil percentages were measured with the automatic blood analyzer Sysmex XT-2000i (Sysmex Corporation, Japan). For the immunological study, the samples were prepared with Lympholyte kit (Cedarline, USA) and the clusters of differentiation of cells (CD3+, CD4+, CD8+, CD16+, CD16−, CD25+) were analyzed by flow cytometry and cell sorting BD FACSAria III (BD Biosciences, USA).

2.5. Statistical analysis

Statistical analyzes were performed in STATISTICA 10 (StatSoft, Inc., USA) and statistical package SPSS, version 13.0 (SPSS Inc., Chicago, Illinois, USA). The differences between the groups were analyzed using the Mann-Whitney and Kolmogorov Smirnov tests. The results of the hematological parameters were indicated as Mean ± Standard error of mean (SEM). A value of $p \leq 0.05$ was considered statistically significant.

3. Results

3.1. VEP sample analyzes results

Samples of VEPs were analyzed using GC-MS to determine the PAH content (Table 2). As the peaks of the anthracene and phenanthrene, benzo(a)anthracene and chrysene, benzo(k)fluoranthene and benzo(a) fluoranthene and benzo(a) pyrene, as well benzo(g,h,i)perylene and dibenz(a,h)anthracene were very close, their amounts were given as totals. The PAH content of the locomotive sample was almost 2 times higher than diesel vehicle and 3.8 times more than gasoline vehicle. The main ingredient of the sample mixtures was “anthracene + phenanthrene” for all the vehicles.

Table 2
Polyaromatic hydrocarbon content of the samples from D, G and L vehicles *(n.d.; non-detectable)*.

Compound	Concentration (ng/mg sample)		
	Sample D	Sample G	Sample L
Acenaphtylene	n.d.	0.06	n.d.
Fluorene	1.13	0.23	1.26
Anthracene+phenanthrene	4.19	2.06	8.64
Pyrene	0.49	0.43	0.92
Benzo(a)anthracene+chrysene	0.03	0.04	0.05
Benzo(k)fluoranthene+benzo(a) fluoranthene+benzo(a) pyrene	0.01	0.01	0.01
Benzo(g,*h*, i)perylene+dibenz(a,h) anthracene	n.d.	n.d.	n.d.
Indeno(1,2,3-cd)pyrene	n.d.	0.04	n.d.
Total polyaromatic hydrocarbons	5.84	2.86	10.88

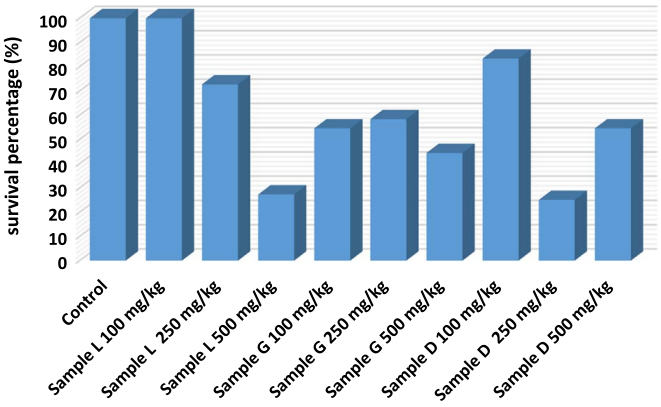

Fig. 1. The survival percentage of mice at 30th day after exposure to different concentrations of VEPs.

3.2. In vivo toxicity testing results

Toxicity test of VEP samples revealed that the vehicle type and the dose of the exposed VEPs effect the survival rates. Even though the PAH content was highest for sample L compared the other vehicle samples, after exposure to 100 mg/kg of L emitted particles, all the mice were alive at the 30th day, but at the highest dose, only around one quarter of the exposed population survived (Fig. 1). Compared to the other vehicles, despite its low PAH content, only 45% of mice were alive after exposure to the low dose, 100 mg/kg of vehicle G sample. As shown in Table 3, VEP exposure led to a decrease in monocyte percentages in the peripheral blood of mice compared to the control group ($p < 0.05$), while the neutrophils significantly increased in response to the VEP exposure ($p < 0.05$) (Table 3).

The alteration in the percentages of the CD3+CD4+, CD3+CD8+, CD16+, and CD25+ cells, CD16+ and CD16- monocytes, eosinophils and basophils of mice that were exposed to three different doses of three different VEPs were compared with the control group (Fig. 2).

Various VEPs differentially effect survival of exposed animals. In our study, overall, the mice survival was inversely associated with exposed L particle dose, while the decrease in the mice viability was not dependent on the dose of exposed G sample. On the other hand, when the mice were treated with L particles, the highest survival rate was at 100 mg/kg dose group, but the lowest was at 250 mg/kg dose. For all the VEPs, despite the decrease in CD3+CD4+ cell numbers, the survival rate of the animals was independent of the CD3+CD4+ cell percentages. The highest CD3+CD4+ cell percentage was in 250 mg/kg L exposed group and the lowest was in 250 mg/kg D exposed mice. Although the survival rates of mice were high at 100 mg/kg of VEP exposure, the CD3+CD8+ cell proliferation was suppressed at all dose levels. This suggested that VEP exposure had toxic effect on CD3+CD8+ cells. The percentage of the CD3+CD8+ cells were not associated with the survival rates of mice.

The CD16+ cells and mice viability decreased in parallel to the L particle exposure, while the G and D samples did not have the same effect in dose dependent manner. On the other hand, neither CD16+, nor CD16- cell number alteration was associated with animal survival, in any VEP exposed groups. L particles dose dependently and inversely alter CD25+ cell numbers and survival rates, while D and G did not have similar effects. D and G showed an irregular dose-survival relation. None of the VEPs did alter the basophil numbers in concordance with the survival. However, in our study, the basophils

Table 3
Hematological parameters of control and vehicle emission particle exposed mice groups.

	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)
Control	20.75 ± 2.64	55.50 ± 2.32	20.13 ± 1.78	3.23 ± 0.72
Sample L 100	58.74 ± 1.37*	12.44 ± 0.41	12.44 ± 0.30	14.44 ± 0.30
Sample L 250	63.64 ± 0.33	11.44 ± 0.28	15.94 ± 0.56	9.14 ± 0.53
Sample L 500	44.84 ± 0.68	36.84 ± 0.73	2.64 ± 0.10	15.84 ± 0.53
Sample G 100	57.90 ± 1.10	13.70 ± 0.62	6.90 ± 0.17	12.70 ± 0.13
Sample G 250	54.84 ± 0.94	17.54 ± 0.70	11.34 ± 0.39	8.24 ± 0.16
Sample G 500	45.04 ± 2.18	13.34 ± 0.79	15.04 ± 0.42	26.74 ± 0.86
Sample D 100	55.60 ± 0.40	9.20 ± 0.04	5.00 ± 0.19	25.20 ± 1.10
Sample D 250	67.04 ± 1.02	17.54 ± 0.76	5.24 ± 0.23	6.24 ± 0.04
Sample D 500	77.24 ± 0.44	12.74 ± 1.02	6.34 ± 0.31	2.54 ± 0.32

* $p < 0.05$. statistically significant. control vs. vehicle emission particle exposed groups.

of mice were significantly increased when they were exposed to low dose (100 mg/kg) of any VEP. The increase in basophil percentages was also significant for 250 mg/kg doses of D and G but not for L particle treatment groups. At 100 mg/kg DEP exposure, despite the increase in the basophils, the CD3+CD4+ cells did not proliferate. The CD3+CD4+ cell percentage increased when the mice exposed to 250 mg/kg doses of sample L, only, while basophil numbers were decreased. This suggested that the proliferated CD3+CD4+ cells may not be Th2 type, but Th1. While, 100 mg/kg of G and L particles suppressed the CD3+CD4+ cell proliferation, the circulating levels of CD16+ monocytes were elevated in the peripheral blood. At 500 mg/kg doses of G and L particle exposure, considering the decrease in the basophil numbers, the CD3+CD4+ cells percentage was increased. Despite the decrease in basophil numbers, this elevation was more pronounced by locomotive emitted particle exposure. However, the most significant disappearance of CD16+ monocytes from the peripheral circulation was at the 250 and 500 mg/kg L sample treatment groups. Compared to controls, sharp decreases in CD16+ cells, CD16+ monocytes and basophil percentages were observed following 500 mg/kg L or G particles exposure, whereas D particles did not cause a significant decrease. At 100 mg/kg, the CD16+ cells percentage was increased more than 2-fold when compared to the controls, however at 250 and 500 mg/kg doses of L and 500 mg/kg of G, the CD16+ cell population in the exposed mice was half of the controls. The proliferation of CD3+CD4+ cells into Th2 type and decrease in the CD16+ monocytes in the blood was lowest in DEP exposure.

The alteration in eosinophil percentages displayed biphasic response to all VEPs exposures. G and L particles showed a significantly higher response compared to controls at all dose groups ($p < 0.05$). By contrast, compared to the controls, eosinophil numbers were significantly higher at 100 mg/kg and 250 mg/kg D exposure ($p < 0.05$), while at 500 mg/kg the cell numbers were significantly lower ($p < 0.05$).

4. Discussion

Actually, the DEP challenge causes an increase in the numbers of total cells, neutrophils, and mononuclear cells (Inoue et al., 2005; Provoost et al., 2010). In our study, significant decrease in lymphocytes and monocytes might be related to the effect of the unusual high doses of VEP exposure. Inflammation after DEP exposure is evident at higher concentrations only. This inflammatory response has involved neutro-

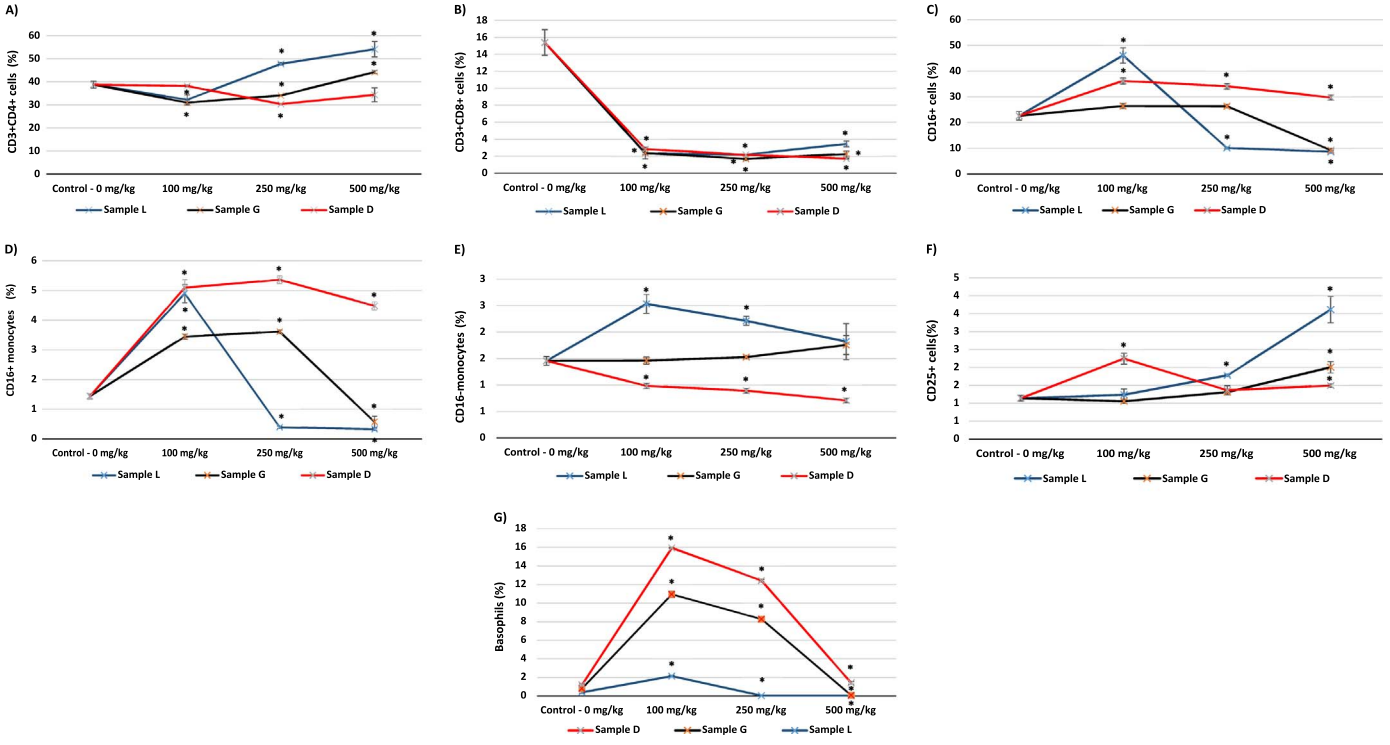

Fig. 2. Effect of different concentrations of various vehicle exhaust particles on the immune cell population frequencies. Percentages of A) CD3+CD4+ cells, B) CD3+CD8+ cells, C) CD16+ cells, D) CD16+ monocytes, E) CD16- monocytes, F) CD25+ cells, G) Basophils (*$p < 0.05$, statistically significant, control vs. vehicle emission particle exposed groups).

phils, eosinophils, mast cells, and lymphocytes (Ghio et al., 2012a). Thereby, rising in neutrophils in the peripheral blood is an indicator of systemic inflammation (Ghio et al., 2012b). In our study, increases in neutrophil, eosinophil and basophil counts were in accordance with these previous findings.

On the other hand, particulate matter in the emitted exhaust gas is associated with certain PAH derivatives (Li et al., 2014). Evidences suggest that survival was not only related to the PAH content of the VEPs. Collectively, the animal survival may have been altered by residues other than PAH. Additionally, the amount of VEP that is suspended on air may vary daily on a large scale (Reliene et al., 2005). In this context, we aimed to analyze the alteration in the immunological responses at high doses of VEP exposure. PAHs alone significantly stimulate IL-8 secretion from sensitized basophils, whereas benzo[a] pyrene or phenanthrene, two major particles of PAHs, with and without allergen, significantly induce IL-4 secretion. IL-4 is a key factor for Th2 development, from purified sensitized basophils even in the absence of antigen. In addition, PAHs display an adjuvant effect in allergic sensitization. Consequently, DEP-PAHs exert pro-allergic effects on sensitized basophils in an allergen independent fashion (Lubitz et al., 2010). In this respect, eosinophils and basophils contribute to the development of immunological disorders, including allergy or autoimmunity (Mukai et al., 2005; Tsujimura et al., 2008). Thus, the decreasing amounts of PAH were inversely proportional with both the basophil percentage and survival rate at low dose of VEP exposure for L, D and G samples. Despite the increase in basophils, decrease in the survival at 100 and 250 mg/kg suggested that basophil accumulation and sensitization may be primarily responsible of the defective immune surveillance in VEP exposure. Min et al. claimed that basophils induce a Th2 immune response to foreign antigen exposure. Moreover, depletion of circulating basophils can significantly inhibit the Th2 immune response. However, the role of basophils in the development and progression of Th2 immune responses against different allergens is still debated (Min et al., 2012). Basophils promote the development of IL-4-producing CD4+ T cells. It is well accepted that naive CD4+ T-cell differentiation into IL-4-producing Th2 effector cells occurs through IL-4, when present in the microenvironment (Zhu et al., 2010). Moreover, it was shown that basophils produce greater amount of IL-4 compared with differentiated Th2 CD4+ T cells per cell basis. IL-4 production from basophils seems to be primarily responsible for the development of Th2 type responses. Eventually, appearance of the IL-4–producing CD4+ T cells showed a close correlation to the level of basophil accumulation in vivo (Oh et al., 2007). In our study, L and G samples showed a dose-dependent increase in CD3+CD4+ cells at high doses, subsequent to a mild decrease at 100 mg/kg. However, D samples reduced the CD3+CD4+ cells at all doses of VEP. Despite the L samples treatment resulted in the lowest basophil count, the CD3+CD4+ cells were higher. This suggested that basophil sensitization is an important factor rather than cell count.

Indeed, in our study, flow cytometric analysis also proved PAH-enhanced increase in basophil percentage at 100 and 250 mg/kg (Fig. 2). Actually, basophils are effective for the development of IgE-mediated chronic allergic inflammation that is independent of T cells and mast cells. In this respect, high-affinity IgE receptor FcRI-expressing basophils are essential for the development of delayed-type allergic inflammation (Mukai et al., 2005). Moreover, it has been established that basophils can present antigens to CD4+ or CD8+ T cells, thus taking part in immunoregulatory functions, as well as T cell polarization (Schneider et al., 2010; Sokol et al., 2008). Actually, DEP injection may affect the functions of both CD4+ and CD8+ T-cell subsets to modulate the synthesis of pro-inflammatory cytokines (Fujimaki et al., 2001). In this respect, complete CD8+ (suppressor/cytotoxic) lymphocytes inhibition was observed at all doses of VEP exposure. These results were in accordance with the lymphocyte counts.

In the absence of strong Th1-polarizing signals, Th2 responses are predominant, and it is proposed that non-classical CD14+CD16+ monocytes colonize in tissues (Sánchez-Torres et al., 2001; Stumbles, 1999). The CD14+CD16− classical and CD14+CD16+ inflammatory monocytes mainly synthesize pro-inflammatory cytokines and, especially the CD14+CD16+ monocytes, express the highest levels of activation markers (Antonelli et al., 2014). CD14++CD16+ subset has an attenuated capacity to promote both naive CD4+ T cell proliferation and polarization into a Th1 phenotype. Furthermore, CD14++CD16+ cells inhibit CD4+ T cell proliferation induced by other monocyte subsets (Liu et al., 2015). In the present study, although there was an increase in CD3+CD4+ cells, a sharp decrease in basophil counts at 500 mg/kg VEP exposure confirms the attenuated inhibitory effect of CD16+ monocyte subsets on CD4+ T cell proliferation and diminished polarization into a Th2 phenotype. Actually, a subset of blood monocytes that are characterized by low density expression of the monocyte-specific CD14 antigen and by the co-expression of the CD16 antigen, represents a specific type of Fc-receptor in human peripheral blood (Passlick et al., 1989). This subpopulation of monocytes is predisposed to become migratory dendritic cells (DCs) and represents approximately 15% of circulating monocytes in a normal individual (Randolph et al., 2002). CD16+ monocytes are phenotypically related to pulmonary alveolar macrophages and some authors have speculated on the commitment of these blood monocytes to go into the lung (Ziegler-Heitbrock, 1996). Moreover, CD4+ T cells stimulated with CD16+ monocyte-derived DCs secrete increased amounts of IL-4 compared to those stimulated by CD16− monocyte-derived DCs (Sánchez-Torres et al., 2001). In our study, a simultaneous alteration in the opposed functional cells, CD16+ monocytes and basophils was observed after VEP exposure. CD3+CD4+ cells were proliferated in accordance with the cell counts of CD16+ monocytes and basophils.

CD3+CD4+ cells were increased at the highest doses of G and L exposure and, while the basophils were significantly decreased. It is proposed that the decreased percentage of the basophils promotes the increased polarization of these cells into Th1 type via the synthesis of Th1 favoring cytokine secretion in G and L emitted particle exposure at higher doses. Besides, the CD16- monocyte levels were effected in an opposite pattern compared to CD16+ monocytes in DEP treatment. The greatest disappearance rate of CD16- monocytes from the peripheral blood was in DEP exposure followed by G and least was in L particle treated groups. These results suggested that DEP exposure did promote neither CD3+CD4+, nor CD3+CD8+ Th cell proliferation in mice. Thus, it has been previously found that the proportion of CD14+CD16+ monocytes was inversely related to the proportion of CD4+ T cells but positively related to the proportion of neutrophil granulocytes (Jiang et al., 2015). Indeed, in our study, the neutrophils of mice in each VEP exposed group for all doses were significantly higher than the controls.

As IL-2 is an important inducer of lymphocyte proliferation, the absence of highly sensitive IL-2 receptors may also significantly hinder activation and clonal expansion of CD8+ and CD4+ lymphocytes and NK cells (Sakaguchi et al., 2008). The higher doses of both VEPs increased the CD3+CD4+ cell proliferation leading to a probable increase in cytokine release. This pattern was consistent with the percentage of the CD25+ cell populations in the VEP exposed mice. Once Th cells are activated, they proliferate by releasing a T cell growth factor, IL-2 which further stimulates itself in an autocrine fashion. Activated T cells also express IL-2 receptor (IL-2R) that binds IL-2 and subsequently activates the T cell's proliferation pathways. On the other hand, while CD25+ cells increased in parallel to CD3+CD4+ cell proliferation, frequency of CD3+CD8+ cells dramatically decreased at all dose levels of all VEPs. Thus, L, G and D emitted particles inhibited cytotoxic T cell proliferation, but may increase the synthesis of IgE via the activation of basophils at low doses of D and G. Subsequently 500 mg/kg causes a sharp decrease in basophil percentages as well as CD3+CD8+ cells for all VEP particles. Independent of allergen

sensitization, a significant increase in eosinophil number occurred only after antigen challenge combined with diesel exhaust exposure. (Miyabara et al., 1998). Actually, massive infiltration of eosinophils happens after the immediate- and late-phase upon antigen challenge (Gaga et al., 1991). They are also abundant in the IgE-mediated chronic inflammation (Mukai et al., 2005). It has previously been described that DEP may cause an eosinophil-mediated inflammation at high levels of exposure at the second stimuli (Hosseini et al., 2016; Ichinose et al., 2002). However, in our study, the highest mean percentages of eosinophils were obtained at 100 mg/kg doses for all VEPs, meanwhile 250 mg/kg treatment caused a sharp decrease in the cell number which was still significantly higher than the controls. G and L sample exposure caused a biphasic proliferative response, despite the steady decline in the eosinophil counts at the highest dose of DEP. It was suggested that the cytotoxicity potential of NK cells exposed to DEPs is heavily impaired (Noah et al., 2012). While exposure to DEPs decreased the cytotoxic NK cells, the particles also functionally suppressed cell-mediated cytotoxicity (Müller et al., 2013). Müller et al. showed that the suppression of cytokine production of NK cells by DEP could result in an overall reduction or modification of immune response (Müller et al., 2013). Similar to our findings, considering the significant increase in the CD16+ cells at DEP exposure, number of NK cells or ability to produce IFN-gamma was not impaired by DEP, but may reduce the clearance of particles by macrophages because of their low responsiveness to IFN-gamma (Müller et al., 2013). Furthermore, CD16 expressing cells were biphasically effected by sample L.

5. Conclusions

Today, the main part of the investigations is concentrated on the size of VEPs and their chemical composition (Gillies, 2014; Hochmuth et al., 2013; Kalberer et al., 2014; Wichmann and Shapiro, 2006). This is the first study that demonstrates the association between VEP exposure and the alteration in CD16+ and CD16- monocytes and CD25+ expressing cells in the peripheral blood, therefore, there are very limited data in the literature regarding the effects of either gasoline vehicle or locomotive emitted particles on immune system cells. According to our findings, during VEP exposure several immune parameters were altered, neutrophil numbers were increased which indicated a systemic inflammation. VEPs are emitted into the environment combined with PAH. In our study, at low doses of VEP exposure, there was an inverse association between PAH content and both basophil percentage and survival rates of mice which was independent of CD4+ T lymphocytes. However, in order to induce an effective immune response, basophil sensitization was found to be more important than cell counts. In this context, VEP-PAHs provoked the activation of basophils and the resultant immune response in an allergen independent fashion. While CD3+CD4+ cells proliferated in accordance with CD16+ monocytes and basophils, the sharp decrease in basophil counts at the highest dose of VEP exposure indicated the attenuated inhibitory effect of CD16+monocytes on CD4+T cell proliferation and concomitant mitigation into Th2 type cell polarization. Additionally, cytotoxic T cells were inhibited by all VEPs. Eosinophil recruitment, the effector arm of the Th2 response was also skewed. G and L sample exposure caused a biphasic proliferative response whereas, DEP dose dependently decreased the eosinophil counts.

As a result, these destructive immunological modifications of gasoline, locomotive and diesel engine emitted particles may lead to more pronounced health consequences which associate with increases in morbidity and mortality. Therefore, although the restrictions for vehicles emissions differ between countries, globally implementation of more stringent emission standards are needed.

Author disclosure statement

Authors declare no conflict of interest.

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