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Occupational exposure to diesel engine exhaust and serum cytokine levels

Yufei Dai[1,2], **Dianzhi Ren**[3], **Bryan A. Bassig**[2], **Roel Vermeulen**[4], **Wei Hu**[2], **Yong Niu**[1], **Huawei Duan**[1], **Meng Ye**[1], **Tao Meng**[1], **Jun Xu**[5], **Ping Bin**[1], **Meili Shen**[1], **Jufang Yang**[3], **Wei Fu**[3], **Kees Meliefste**[4], **Debra Silverman**[2], **Nathaniel Rothman**[2], **Qing Lan**[2,*], and **Yuxin Zheng**[1,*]

[1]Key Laboratory of Chemical Safety and Health, National Institute for Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention. Beijing, China [2]Occupational and Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute. Rockville, Maryland, U.S.A. [3]Chaoyang Center for Disease Control and prevention, Chaoyang, China [4]Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands [5]Hong Kong University, Hong Kong, China

Abstract

The International Agency for Research on Cancer has classified diesel engine exhaust (DEE) as a human lung carcinogen. Given that inflammation is suspected to be an important underlying mechanism of lung carcinogenesis, we evaluated the relationship between DEE exposure and the inflammatory response using data from a cross-sectional molecular epidemiology study of 41 diesel engine testing workers and 46 unexposed controls. Repeated personal exposure measurements of $PM_{2.5}$ and other DEE constituents were taken for the diesel engine testing workers before blood collection. Serum levels of six inflammatory biomarkers including interleukin (IL)-1, IL-6, IL-8, tumor necrosis factor (TNF)-α, macrophage inflammatory protein (MIP)-1β, and monocyte chemotactic protein (MCP)-1 were analyzed in all subjects. Compared to unexposed controls, concentrations of MIP-1β were significantly reduced by ~37% in DEE exposed workers ($P < 0.001$) and showed a strong decreasing trend with increasing $PM_{2.5}$ concentrations in all subjects ($P_{trend} < 0.001$) as well as in exposed subjects only ($P_{trend} = 0.001$). Levels of IL-8 and MIP-1β were significantly lower in workers in the highest exposure tertile of $PM_{2.5}$ (>397μg/m^3) compared to unexposed controls. Further, significant inverse exposure-response relationships for IL-8 and MCP-1 were also found in relation to increasing $PM_{2.5}$ levels among the DEE exposed workers. Given that IL-8, MIP-1β, and MCP-1 are chemokines that play important roles in recruitment of immunocompetent cells for immune defense and tumor cell clearance, the observed lower levels of these markers with increasing $PM_{2.5}$ exposure may provide insight into the mechanism by which DEE promotes lung cancer.

*Corresponding author: Yuxin Zheng, MD, PhD, Tel: 86-10-83132593, Fax: 86-10-83132515, yx_zheng@139.com, Qing Lan, Ph.D., Tel: 1-240-276-7171; Fax: 1-240-276-7835; qingl@mail.nih.gov.

Author's contribution

YZ, QL, NR, RV, and DS conceived of and designed the study. YD, WH, and RV conducted the data analyses. YD, BB, QL, NR, and YZ were primarily responsible for drafting the paper. YD, DR, WH, YN, HD, MY, TM, PB, MS, RV, NR, QL, YZ, JX, WF, KM, JY, YZ participated in data collection. All authors reviewed and approved the manuscript.

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Keywords

diesel engine exhaust; occupational exposure; molecular epidemiology; inflammatory biomarkers

Introduction

Diesel engine exhaust (DEE) is generated from incomplete combustion of diesel fuel and is a predominant contributor to urban air pollution [Zheng et al. 2007]. DEE is a complex mixture consisting of gaseous and organic components such as carbon monoxide (CO), nitrogen dioxide (NO_2), sulphur dioxide (SO_2), and polycyclic aromatic hydrocarbons (PAHs), which are absorbed onto carbon core particles. Exposure to DEE has been associated with several adverse health effects in humans, including cardiovascular diseases [Langrish et al. 2012; Lanki et al. 2008; Peters et al. 2004], respiratory diseases such as allergy [Riedl and Diaz-Sanchez 2005], asthma [Jerrett et al. 2008; McCreanor et al. 2007], and chronic obstructive pulmonary disease [Hart et al. 2012; Hart et al. 2009], as well as lung cancer [Pedeli et al. 2011; Silverman et al. 2014; Vermeulen et al. 2014]. Recently, DEE was classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC) based on its association with lung cancer in epidemiological studies [International Agency for Research on Cancer 2013].

Growing evidence suggests a close relationship between chronic inflammation and cancer [Balkwill and Mantovani 2001; Hanahan and Weinberg 2011]. Circulating levels of inflammatory markers have been associated with lung cancer in recent prospective cohort studies [Pine et al. 2011; Shiels et al. 2013], suggesting that evaluations of the relationship between known or suspected lung carcinogens and inflammatory markers may provide important insight into the biologic plausibility and mechanistic characteristics of these exposure and lung cancer associations. We have previously reported in a cross-sectional molecular epidemiological study of diesel engine testing workers and factory workers unexposed to DEE that DEE exposure is associated with an increase in total lymphocyte counts and three of the four major lymphocyte subsets including $CD4^+$T cells, $CD8^+$ T cells, and B cells, which play a key role in the inflammatory process [Lan et al. 2015]. Cytokines are also suspected to be involved in cancer-related inflammation [Lu et al. 2006]. A few short-term human controlled DEE exposure studies provided evidence that DEE can affect cytokine expression. Healthy volunteers who were exposed to DEE under controlled conditions for 1 or 2 hours had significant increases in expression of interleukin (IL)-13 and IL-8 in the bronchial epithelium cells and lavage fluid [Behndig et al. 2006; Pourazar et al. 2004; Stenfors et al. 2004]. Xu Y et al [Xu et al. 2013] observed a trend towards increased serum IL-6 concentrations in volunteers exposed to DEE for 3h. However, these effects were associated with an acute inflammatory response after short-term DEE exposure under experimental conditions, which are not necessarily generalizable to the general population and to potential immune-related effects from chronic DEE exposure.

Considering the important roles of proinflammatory cytokines in the initiation and maintenance of inflammation, we have previously conducted a study of 137 DEE exposed workers and 106 unexposed controls and found that the exposed workers had decreased

serum levels of IL-1β, IL-6, IL-8, and macrophage inflammatory protein (MIP)-1β compared to the unexposed controls [Dai et al. 2016]. To our knowledge, this is the only human study in a non-experimental setting that evaluated associations between occupational DEE exposure and circulating cytokine levels; but this study did not evaluate exposure-response relationships between the cytokines and DEE exposure levels. In the present study, we aimed to replicate our previous findings in a separate study population with varying DEE exposure levels and to expand on our previous study by evaluating the exposure-response relationships between serum cytokine levels and DEE constituents based on personal exposure assessments.

Methods

Study population and Exposure Assessment

The study design and exposure assessment protocol for this cross-sectional molecular epidemiology study has been described previously [Lan et al. 2015]. Briefly, the study consists of 41 male workers who are responsible for testing the diesel engines in a diesel engine manufacturing company and 46 unexposed male workers selected from 4 facilities included a bottling department of a brewery, a water treatment plant, a meat packing facility, and an administrative facility. Based on detailed walk-through surveys, no DEE sources were identified in any of these workplaces. Four control facilities were selected from the same local region as the diesel engine manufacturing company in China. The exclusion criteria for control workers involved having exposure to DEE, other types of particulates, or any known or suspected genotoxic, hematotoxic or immunotoxic chemicals. Control workers were frequency matched to exposed workers by age (± 5 years) and smoking status. The participation rates for DEE exposed workers and controls were approximately 90% and 80%, respectively. The study was integrated into a regular health exam administered by the local Center for Disease Control and conducted in March 2013. All workers completed a questionnaire that inquired about demographic and lifestyle characteristics and provided a peripheral blood sample. Informed consent was obtained from all subjects and the study was approved by Institutional Review Boards at the US National Cancer Institute and the National Institute of Occupational Health and Poison Control, China CDC.

DEE exposure was assessed by particulate matter $(PM)_{2.5}$, elemental carbon (EC), organic carbon (OC), and soot. The exposure assessment survey in the diesel factory was conducted from October 2012 to March 2013 and included all workers in the testing facility. As described previously [Lan et al. 2015], repeated full –shift personal air samples were collected using a cyclone attached to the lapel near the breathing zone with an aerodynamic cut-off of 2.5μm ($PM_{2.5}$) at a flow rate of 3.5L/min using quartz or Teflon filters. Exposure assessments were also conducted for a subset of the controls from each factory except for the beer factory, where no measurements could be obtained.

Cytokine measurements

Serum concentrations of IL-1, IL-6, IL-8, tumor necrosis factor (TNF)-α, MIP)-1β, and monocyte chemotactic protein (MCP)-1 were measured using BD Cytometric Bead Array Human Soluble Protein Flex Set system (BD Biosciences). These immune markers were

selected in the current study for evaluation of exposure-response associations with DEE constituents to follow-up on our findings from a previous cross-sectional study of DEE in a separate study population [Dai et al. 2016]. Human Soluble Protein Master Buffer Kit, Human IL-1β Flex Set (Bead B4), Human IL-6 Flex Set (Bead A7), Human IL-8 Flex Set (Bead A9), Human MCP-1 Flex Set (Bead D8), Human MIP-1β Flex Set (Bead E4), and Human TNF Flex Set (Bead C4) were used for cytokine detection per the manufacturer's protocol (BD Biosciences). Sample measurements were performed using a BD Canto and BD LSRFortessa flow cytometer, and data was analyzed with FCAP Array Software (Soft Flow Inc., Pecs, Hungary). The detection limits for IL-1, IL-6, IL-8, TNF-α, MIP-1β, and MCP-1 were 2.3, 1.6, 1.2, 1.2, 0.8, and 1.3 pg/ml, respectively. If the concentration of a cytokine was lower than the assay detection limit, one half of the detection limit value was substituted for the cytokine level for that sample. The number of samples below detection for IL-1, IL-6, IL-8, TNF-α, MIP-1β, and MCP-1 was 51 (58%), 1 (1%), 0 (0%), 59 (67%), 0 (0%), and 0 (0%), respectively.

Statistical analysis

Unadjusted summary measures are presented for all endpoints. Linear regression using the natural logarithm of each endpoint was used to test for differences in the serum concentration of cytokines between DEE exposed and control workers. To evaluate dose-response trends, linear regression was used to compare cytokine levels across tertiles of $PM_{2.5}$ and EC exposure relative to control workers, as well as for exposed workers only. As we previously reported [Lan et al. 2015], a very high correlation was observed between EC and OC (Spearman $r = 0.86$, $p < 0.0001$) and between EC and soot (Spearman $r = 0.91$, $p < 0.0001$). Therefore, separate analyses were not conducted for OC and soot. All linear regression models were adjusted for age (as a continuous variable), current smoking status (yes or no), current alcohol consumption (yes or no), body mass index (BMI), and recent infection (flu or respiratory infections in the previous month, yes or no). Interaction between DEE exposure and smoking was analyzed by adding cross-product terms to the generalized linear models. For IL-1β and TNF-α, which both had a large percentage of non-detectable values in the overall study population (>50%), differences in concentrations between DEE exposed workers and control workers were evaluated using Spearman correlations and the Wilcoxon rank sum test, rather than linear regression. Multiple comparisons were accounted for by applying a 20% false discovery rate (FDR) criterion to the p-values from the linear regression models, using the Benjamini-Hochberg method [Benjamini and Hochberg 1995]. The statistical analyses were performed using SPSS 19.0.

Results

The demographic characteristics of the study subjects are summarized in Table I. Except for alcohol use, the distributions of age, BMI, and current smoking were not significantly different between DEE exposed workers and controls. The average number of years of employment among DEE-exposed workers was 20.8±6.0 years. The unadjusted exposure levels of $PM_{2.5}$, EC, and OC for exposed workers were $0.37\pm0.07 mg/m^3$, $58.1 \pm 24.0 \mu g/m^3$, and $138.1 \pm 27.2 \mu g/m^3$, respectively (Table II). Based on detailed walk-through surveys, no DEE sources were identified in any of the control factories, and it was therefore assumed

that exposure levels of these DEE constituents for control workers were the result of background outdoor levels in this region of China. The adjusted exposure levels of $PM_{2.5}$, EC, and OC for exposed workers that considered background exposure levels were 0.15±0.07μg/m^3, 47.0±24.0μg/m^3, and 69.4±27.2μg/m^3, respectively (Table II).

Associations between DEE exposure and serum cytokine concentrations are shown in Table III. Concentrations of MIP-1β were 37% lower in DEE exposed workers compared to controls (P<0.001), adjusted for age, smoking status, alcohol use, BMI, and recent infection. After stratifying by smoking status, a statistically significant difference in MIP-1β levels between exposed and control workers was present only among current smokers. The findings for MIP-1β remained statistically significant after adjusting for multiple comparisons. A borderline significant interaction between DEE exposure and smoking was observed with respect to levels of MIP-1β (P=0.06). In addition, a significantly higher level of IL-8 was observed in smokers compared to non-smokers among control workers. There were no statistically significant differences in concentrations of IL-1β, IL-6, IL-8, TNF-α, or MCP-1 between DEE exposed workers and controls.

We further analyzed the exposure-response relationships for cytokines across controls and DEE exposed workers, with concentrations categorized into tertiles of exposure based on personal air monitoring for $PM_{2.5}$ (Table IV). A significant exposure-dependent decline in MIP-1β (P_{trend}< 0.001) and a borderline significant decline in IL-8 (P_{trend} = 0.053) with increasing $PM_{2.5}$ concentrations was observed across all subjects, and the concentrations of MIP-1β and IL-8 were significantly lower in workers exposed to the highest levels of $PM_{2.5}$ (median, range: 408, 397–536 μg/m^3) compared to unexposed controls (reduced by 56.7% and 21.0%, respectively) (Table IV). In analyses conducted among the DEE exposed workers only, a significant exposure- response relationship for MIP-1β and IL-8 as well as MCP-1 was also found in relation to increasing $PM_{2.5}$ concentration, and these associations also remained significant after adjustment for multiple comparisons (Table IV). The correlations between $PM_{2.5}$ concentrations and these three chemokines were weak to moderate in the overall study population (r_{sp} ranging from −0.16 to −0.43), but were stronger in exposed subjects only (r_{sp} ranging from - −0.43 to −0.51; Table IV). There were no associations between air levels of EC or OC and cytokine levels in analyses comparing DEE exposed and control workers, or among exposed workers only (data not shown).

In non-smokers, a significant exposure-response relationship for MIP-1β and IL-8 was also found in relation to increasing $PM_{2.5}$ concentrations among the DEE exposed workers only (P_{trend}< 0.001 and 0.01, respectively), but not among all subjects. The correlation coefficients between $PM_{2.5}$ concentrations and the two chemokines in DEE exposed non-smokers were −0.70 (MIP-1β) and −0.71 (IL-8) (both P=0.005).

Discussion

In this cross-sectional molecular epidemiological study, which included workers exposed to DEE for about 20 years and unexposed workers, we found that occupational DEE exposure was associated with a significant decline in serum levels of MIP-1β compared to unexposed controls and that increasing $PM_{2.5}$ concentrations were associated with a strong decreasing

trend in MIP-1β levels in all subjects as well as in exposed workers only. Further, significant exposure- response relationships were also observed for IL-8 and MCP-1 among the DEE exposed workers, and the levels of IL-8 and MIP-1β were significantly lower in workers in the highest exposure tertile of $PM_{2.5}$ (>397μg/m^3) compared to unexposed controls. These findings are consistent with our previous report in a separate study population showing that occupational DEE exposure is associated with a decrease in levels of cytokines [Dai et al. 2016], which provides further indication that DEE exposure can alter immune function.

IL-8, MIP-1β, and MCP-1 belong to the chemokine family that are expressed and released by a wide range of normal cells such as monocytes, neutrophils, macrophages, keratinocytes, fibroblasts, and endotheliocytes. Our previous studies showed a decrease in neutrophils (4146±1164 cells/μl) and monocytes (310±110 cells/μl) in DEE exposed workers compared with controls (4633±1530 cells/μl and 330±150 cells/μl, respectively), although the differences were not statistically significant (P=0.07 and 0.08, respectively) [Lan et al. 2015 and Dai et al. 2016]. An *in vitro* study showed that DEE exposure suppresses the release of cytokines from human and murine alveolar macrophages [Amakawa et al.2003]. Therefore, the suppressed function and decreases in the number of chemokine-producing cells caused by DEE exposure may partly contribute to the decreases in chemokines that we observed in DEE exposed workers in the current study. The chemokines play an important role in eliciting an immune response by recruiting T cells, macrophages, and NK cells to sites of tumor growth or infections. Among these cells, alveolar macrophages are the first line of lung defense and are responsible for eliminating most foreign particulate matter and microorganisms from the distal airways. An *in vivo* study showed that DEE exposure suppresses macrophage function and decreases the pulmonary clearance of *Listeria monocytogenes* in rats [Yang et al. 2001]. Considering the biological function of chemokines on recruiting macrophages, we speculate that the suppressive effect of DEE exposure on these chemokines could decrease the clearance of the particulate matter component of DEE and in turn expand the airway inflammatory process, which is increasingly being recognized as playing an important role in the aetiology of lung cancer [Shiels et al. 2011]. Although an exposure-response relationship was observed for levels of IL-8, MCP-1, and MIP-1β in exposed workers, a statistically significant decline in these three cytokines was only observed among the highest exposed workers compared with controls, suggesting that the immune-related effects of DEE may be most apparent only at higher levels of exposure. Whether an exposure threshold exists for these effects will require evaluation in larger studies.

There are currently a limited number of available epidemiological studies that have evaluated associations between long-term exposure to combustion-related air pollution and the inflammatory response [Brook et al. 2010]. However, controlled human exposure studies have provided evidence that airway inflammation, characterized by increasing neutrophils and B lymphocytes in bronchoalveolar lavage after exposure to diesel exhaust particles, is observed at higher concentrations with a threshold dose approximating 300μg/m^3, which is close to our high level of exposure (>397μg/m^3) [Ghio et al. 2012]. An in-vivo study also reported that chronic inhalation of DEE affects cytokine expression in murine lung depending on the dose, i.e. IL-4 expression levels increased following low-dose DEE exposure (100μg/m^3) but were suppressed by high-doses of DEE exposure (3000μg/m^3)

[Saito et al. 2002]. Ambient DEE levels are typically lower than the range of exposures found in controlled human exposure studies, occupational exposure studies, and in-vivo studies. Therefore, the effects of DEE exposure on the immune system at lower concentrations of exposure that are typically present in the general population will require further study in other populations.

A multi-ethnic study reported that smokers have significantly higher levels of inflammatory markers compared to never smokers [Mcevoy et al. 2015], which is consistent with our finding that smoking is associated with a significant increase in IL-8 levels, suggesting that careful evaluation of smoking status is needed in studies of DEE exposure and the inflammatory response. To control for this potential confounding effect, we adjusted for smoking status in the overall analyses that compared levels of cytokines in DEE exposed workers and unexposed controls, and we also conducted analyses that evaluated interactions between DEE exposure and smoking status. We observed that the inverse association between DEE exposure and MIP-1β levels was present among current smokers, but not among non-smokers. Further larger studies are needed to replicate these findings and explore the mechanism of interaction between DEE exposure and smoking on the inflammatory response.

The main strength of the current study was the selection of a study population that was employed in specific occupational settings where the potential exposure misclassification was well controlled and the uncertainties were less than those studies conducted in the general population. We also conducted detailed individual exposure assessments, which included repeated personal air samples, to elucidate the exposure-response relationships. A limitation of the current study is the relatively small number of workers included, particularly in analyses stratified by smoking status and by exposure level. As such, these findings should be viewed as exploratory and will require further exploration in larger studies. Moreover, the subjects in our study were generally healthy male workers, and so the results may not be generalizable to other populations with different baseline characteristics or to females.

In summary, we observed a statistically significant exposure-dependent decline in the serum levels of MIP-1β with increasing air levels of $PM_{2.5}$ among DEE exposed workers. Further, levels of IL-8 and MIP-1β were significantly decreased in workers with the highest levels of DEE exposure compared to controls. Our findings suggest that DEE exposure is associated with declines in chemokines that are responsible for eliciting an immune response. These changes may reflect a process of immune dysregulation that contributes to the initiation of carcinogenesis in individuals occupationally exposed to DEE. However, our findings require replication in larger populations with a wider range of DEE exposure levels.

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References:

Amakawa K, Terashima T, Matsuzaki T, Matsumaru A, Sagai M, Yamaguchi K. 2003 Suppressive effects of diesel exhaust particles on cytokine release from human and murine alveolar macrophages. Experimental lung research 29 (3):149–64. [PubMed: 12637227]

Balkwill F, Mantovani A. 2001 Inflammation and cancer: back to Virchow? Lancet 357(9255):539–45. [PubMed: 11229684]

Behndig AF, Mudway IS, Brown JL, Stenfors N, Helleday R, Duggan ST, Wilson SJ, Boman C, Cassee FR, Frew AJ. 2006 Airway antioxidant and inflammatory responses to diesel exhaust exposure in healthy humans. European Respiratory Journal 27(2):359–65. [PubMed: 16452593]

Benjamini Y, Hochberg Y. Controlling the false discovery rate—a practical and powerful approach to multiple testing. 1995 J R Stat Soc Series B Stat Methodol, 57: 289–300.

Brook RD, Rajagopalan S, Pope CA, Brook JR, Bhatnagar A, Diez-Roux AV, Holguin F, Hong Y, Luepker RV, Mittleman MA and et al. 2010 Particulate Matter Air Pollution and Cardiovascular Disease An Update to the Scientific Statement From the American Heart Association. Circulation 121(21):2331–2378. [PubMed: 20458016]

Dai Y, Zhang X, Zhang R, Zhao X, Duan H, Niu Y, Huang C, Meng T, Ye M, Ping B. 2016 Long-term exposure to diesel engine exhaust affects cytokine expression among occupational population. Toxicology Research 5(2):674–681. [PubMed: 30090380]

Ghio AJ, Smith CB, Madden MC. 2012 Diesel exhaust particles and airway inflammation. Curr Opin Pulm Med 18(2):144–50. [PubMed: 22234273]

Hanahan D, Weinberg RA. 2011 Hallmarks of cancer: the next generation. Cell 144(5):646–674. [PubMed: 21376230]

Hart JE, Eisen EA, Laden F. 2012 Occupational diesel exhaust exposure as a risk factor for chronic obstructive pulmonary disease. Curr Opin Pulm Med 18(2):151–4. [PubMed: 22234274]

Hart JE, Laden F, Eisen EA, Smith TJ, Garshick E. 2009 Chronic obstructive pulmonary disease mortality in railroad workers. Occup Environ Med 66(4):221–6. [PubMed: 19039098]

International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Vol 105. Diesel and Gasoline Engine Exhausts and Some Nitroarenes. http://monographs.iarc.fr/ENG/Monographs/vol105/mono105.pdf. Accessed 3/23/2017

Jerrett M, Shankardass K, Berhane K, Gauderman WJ, Kunzli N, Avol E, Gilliland F, Lurmann F, Molitor JN, Molitor JT and et al. 2008 Traffic-related air pollution and asthma onset in children: a prospective cohort study with individual exposure measurement. Environ Health Perspect 116(10): 1433–8. [PubMed: 18941591]

Lan Q, Vermeulen R, Dai Y, Ren D, Hu W, Duan H, Niu Y, Xu J, Fu W, Meliefste K. 2015 Occupational exposure to diesel engine exhaust and alterations in lymphocyte subsets. Occupational & Environmental Medicine 72(5):354–9. [PubMed: 25673341]

Langrish JP, Bosson J, Unosson J, Muala A, Newby DE, Mills NL, Blomberg A, Sandstrom T. 2012 Cardiovascular effects of particulate air pollution exposure: time course and underlying mechanisms. J Intern Med 272(3):224–39. [PubMed: 22724512]

Lanki T, Hoek G, Timonen KL, Peters A, Tiittanen P, Vanninen E, Pekkanen J. 2008 Hourly variation in fine particle exposure is associated with transiently increased risk of ST segment depression. Occup Environ Med 65(11):782–6. [PubMed: 18524840]

Lu H, Ouyang W, Huang C. 2006 Inflammation, a Key Event in Cancer Development. Molecular Cancer Research 4(4):221. [PubMed: 16603636]

McCreanor J, Cullinan P, Nieuwenhuijsen MJ, Stewart-Evans J, Malliarou E, Jarup L, Harrington R, Svartengren M, Han IK, Ohman-Strickland P and et al. 2007 Respiratory effects of exposure to diesel traffic in persons with asthma. N Engl J Med 357(23):2348–58. [PubMed: 18057337]

McEvoy JW, Nasir K, DeFilippis AP, Lima JA, Bluemke DA, Hundley WG, Barr RG, Budoff MJ, Szklo M, Navas-Acien A and et al. 2015 Relationship of cigarette smoking with inflammation and subclinical vascular disease: the Multi-Ethnic Study of Atherosclerosis. Arterioscler Thromb Vasc Biol 35(4):1002–10. [PubMed: 25745060]

Pedeli X, Hoek G, Katsouyanni K. 2011 Risk assessment of diesel exhaust and lung cancer: combining human and animal studies after adjustment for biases in epidemiological studies. Environ Health 10:30. [PubMed: 21481231]

Peters A, von Klot S, Heier M, Trentinaglia I, Hormann A, Wichmann HE, Lowel H. 2004 Exposure to traffic and the onset of myocardial infarction. N Engl J Med 351(17):1721–30. [PubMed: 15496621]

Pine SR, Mechanic LE, Enewold L, Chaturvedi AK, Katki HA, Zheng YL, Bowman ED, Engels EA, Caporaso NE, Harris CC. 2011 Increased Levels of Circulating Interleukin 6, Interleukin 8, C-Reactive Protein, and Risk of Lung Cancer. Journal of the National Cancer Institute 103(14):1112–22. [PubMed: 21685357]

Pourazar J, Frew AJ, Blomberg A, Helleday R, Kelly FJ, Wilson S, Sandström T. 2004 Diesel exhaust exposure enhances the expression of IL-13 in the bronchial epithelium of healthy subjects. Respiratory Medicine 98(9):821–25. [PubMed: 15338792]

Riedl M, Diaz-Sanchez D. 2005 Biology of diesel exhaust effects on respiratory function. J Allergy Clin Immunol 115(2):221–8; quiz 229. [PubMed: 15696072]

Saito Y, Azuma A, Kudo S, Takizawa H, Sugawara I. 2002 Long-term inhalation of diesel exhaust affects cytokine expression in murine lung tissues: comparison between low- and high-dose diesel exhaust exposure. Experimental Lung Research 28(6):493–506. [PubMed: 12217215]

Shiels MS, Chaturvedi AK, Katki HA, Gochuico BR, Caporaso NE, Engels EA. 2011 Circulating markers of interstitial lung disease and subsequent risk of lung cancer. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 20:2262–72.

Shiels MS, Pfeiffer RM, Hildesheim A, Engels EA, Kemp TJ, Park JH, Katki HA, Koshiol J, Shelton G, Caporaso NE. 2013 Circulating Inflammation Markers and Prospective Risk for Lung Cancer. Cancerspectrum Knowledge Environment 105(24):1871–80.

Silverman DT, Samanic CM, Lubin JH, Blair AE, Stewart PA, Vermeulen R, Coble JB, Rothman N, Schleiff PL, Travis WD and et al. 2012 The Diesel Exhaust in Miners study: a nested case-control study of lung cancer and diesel exhaust. J Natl Cancer Inst 104(11):855–68. [PubMed: 22393209]

Stenfors N, Nordenhäll C, Salvi SS, Mudway I, Söderberg M, Blomberg A, Helleday R, Levin JO, Holgate ST, Kelly FJ. 2004 Different airway inflammatory responses in asthmatic and healthy humans exposed to diesel. European Respiratory Journal 23(1):82–6. [PubMed: 14738236]

Vermeulen R, Silverman DT, Garshick E, Vlaanderen J, Portengen L, Steenland K. 2014 Exposure-response estimates for diesel engine exhaust and lung cancer mortality based on data from three occupational cohorts. Environ Health Perspect 122(2):172–7. [PubMed: 24273233]

Xu Y, Barregard L, Nielsen J, Gudmundsson A, Wierzbicka A, Axmon A, Bo AJ, Kåredal M, Albin M. 2013 Effects of diesel exposure on lung function and inflammation biomarkers from airway and peripheral blood of healthy volunteers in a chamber study. 10(1):60.

Yang HM, Antonini JM, Barger MW, Butterworth L, Roberts BR, Ma JK, Castranova V, Ma JYC. 2001 Diesel exhaust particles suppress macrophage function and slow the pulmonary clearance of listeria monocytogenes in rats. Environmental health perspectives 109 (5):515–21. [PubMed: 11401764]

Zheng M, Cass GR, Ke L, Wang F, Schauer JJ, Edgerton ES, Russell AG. 2007 Source apportionment of daily fine particulate matter at Jefferson Street, Atlanta, GA, during summer and winter. J Air Waste Manag Assoc 57(2):228–42. [PubMed: 17355084]

Table I.

Characteristics of workers in the control and diesel engine exhaust exposure groups in a cross-sectional molecular epidemiology study in China

Variables	Control (n=46)	Diesel engine exhaust exposure (n=41)	*P*
Age (years, mean±SD)	42.4±7.3	43.0±6.1	0.70[a]
BMI (kg/m^2, mean±SD)	25.3±3.9	24.6±2.9	0.31[a]
Current smoking status			0.95[b]
Yes, n (%)	30 (65.2)	27 (65.9)	
No, n (%)	16(34.8)	14 (34.1)	
Current alcohol use			0.047[b]
Yes, n (%)	42 (91.3)	31 (75.6)	
No, n (%)	4 (8.7)	10 (24.4)	
Current infection			0.47[b]
Yes, n (%)	26 (56.5)	20 (48.8)	
No, n (%)	20 (43.5)	21 (51.2)	
Working years (years, mean±SD)	-	20.8±6.0	-

[a] *t*-test for difference between two groups

[b] χ^2 test for difference between two groups

BMI, body mass index

Table II.

Air concentrations of diesel engine exhaust components among exposed workers and controls

Exposure	Control (n=46)	Exposed worker (n=41)
$PM_{2.5}$ (mg/m^3, mean±SD)		
Background adjusted	0	0.15±0.07
Unadjusted	0.20±0.07	0.37±0.07
Elemental carbon ($\mu g/m^3$, mean±SD)		
Background adjusted	0	47.0±24.0
Unadjusted	11.1±1.3	58.1±24.0
Organic carbon ($\mu g/m^3$, mean±SD)		
Background adjusted	0	69.4±27.2
Unadjusted	68.7±4.1	138.1±27.2

Unadjusted values are original measurements. Adjusted values reflect background outdoor levels in this region.

Table III.

Serum levels of cytokines stratified by smoking status in the control and diesel engine exhaust exposure groups (Median, 10%−90%)

Cytokines	Control group n	Control group Median (10%−90%)	Control group P^a	Diesel engine exhaust exposure group n	Diesel engine exhaust exposure group Median (10%−90%)	Diesel engine exhaust exposure group P^a	Difference in median levels (%)	$P_{crude}{}^b$	$P_{adjust}{}^c$	FDR^d	$P_{int.}{}^e$
IL-1β (pg/ml)											
Non-smokers	16	1.2 (1.2–16.0)		14	1.2(1.2–5.9)		0.0	0.45	-	-	
Current smokers	30	1.2(1.2–7.6)	0.92	27	1.2(1.2–25.4)	0.61	0.0	0.71	-	-	
Total	46	1.2(1.2–7.5)		41	1.2(1.2–6.7)		0.0	0.47	-	-	-
IL-6 (pg/ml)											
Non-smokers	16	7.4(5.2–15.2)		14	7.2(3.5–22.9)		−2.7	0.67	0.56	0.90	
Current smokers	30	7.0 (5.2–12.7)	0.38	27	7.4(5.6–19.7)	0.94	5.7	0.81	0.81	0.96	
Total	46	7.2(5.2–12.8)		41	7.4(5.8–21.3)		2.8	0.96	0.86	0.96	0.51
IL-8 (pg/ml)											
Non-smokers	16	10.1(8.0–15.8)		14	9.9(8.8–16.5)		−2.0	0.84	0.84	0.96	
Current smokers	30	12.2 (9.3–18.5)	0.031	27	11.6(9.0–17.5)	0.16	−4.9	0.72	0.81	0.96	
Total	46	11.9(8.5–18.0)		41	10.9(9.0–16.9)		−8.4	0.86	0.63	0.90	0.89
MIP-1β (pg/ml)											
Non-smokers	16	64.0(26.2–135.9)		14	53.4(28.6–239.2)		−16.6	0.98	0.97	0.98	
Current smokers	30	75.7(38.7–131.2)	0.22	27	36.3(10.7–110.8)	0.048	−52.0	<0.001	<0.001	0.001	
Total	46	71.1(31.4–130.8)		41	44.7(15.6–186.6)		−37.1	0.002	<0.001	0.003	0.06
MCP-1 (pg/ml)											
Non-smokers	16	95.6(39.4–254.8)		14	101.9(26.8–167.0)		6.6	0.93	0.98	0.98	
Current smokers	30	126.8(65.9–200.3)	0.53	27	97.2(55.4–236.2)	0.25	−23.3	0.33	0.43	0.78	
Total	46	106.7(59.3–203.9)		41	99.9(54.6–187.6)		−6.4	0.64	0.63	0.90	0.82
TNF-α (pg/ml)											
Non-smokers	16	0.6(0.6–12.7)		14	0.6(0.6–4.3)		0	0.45	-	-	
Current smokers	30	0.6(0.6–5.9)	0.56	27	0.6(0.6–20.3)	0.29	0	0.88	-	-	
Total	46	0.6(0.6–5.1)		41	0.6(0.6–5.0)		0	0.59	-	-	-

a, *t*-test for difference between non-smokers and current smokers

b, *t*-test for difference between control and diesel engine exhaust exposure groups for IL-6, IL-8, MIP-1β, and MCP-1. For IL-1β and TNF-α, the Mann-Whitney U test was used to compare exposure groups given the large percentage of samples below detection (58% and 67%, respectively)

[c] Adjusted for age, alcohol status, infection status, body mass index, and smoking status.

[d] Multiple comparisons were adjusted for by applying the false discovery rate (FDR) using the Benjamini-Hochberg method

[e] *p* value for interaction between DEE exposure and smoking.

Table IV.

Exposure-response relationship between air concentrations of $PM_{2.5}$ and cytokine levels

Cytokines[a]	Diesel engine exhaust exposure category[b]									
		Diesel engine exhaust exposed workers								
	Control workers (n=46)	1st tertile (n=14)	2nd tertile (n=12)	3rd tertile (n=15)	Correlation (all subjects)[c]		Correlation (exposed only)[c]		P_{trend} (FDR) (all subjects)[d]	P_{trend} (FDR) (exposed only)[d]
					r	*P*	*r*	*P*		
IL-1β[e] (pg/ml)	1.2 (1.2–7.5)	2.8 (1.2–72.5)	1.15 (1.2–6.6)	1.15 (1.2–6.2)	−0.13	0.23	−0.24	0.14	-	-
IL-6 (pg/ml)	7.2 (5.2–12.8)	7.4 (6.3–36.3)	7.0 (2.4–23.1)	7.4 (5.3–9.2)	0.04	0.73	−0.20	0.20	0.31 (0.62)	0.12 (0.30)
IL-8 (pg/ml)	11.9 (8.5–18.1)	12.2 (8.9–26.7)	11.3 (9.9–16.6)	9.4* (8.4–11.9)	−0.16	0.14	−0.51	0.001	0.053 (0.15)	0.0023 (0.009)
MIP-1β (pg/ml)	71.1 (31.4–130.8)	67.8 (23.0–236.4)	50.0* (12.9–180.4)	30.8* (8.9–59.4)	−0.43	<0.001	−0.43	0.005	<0.001 (<0.001)	0.001 (0.005)
MCP-1 (pg/ml)	106.7 (59.3–203.9)	118.7 (83.1–328.5)	101.9 (43.3–156.1)	90.0 (27.9–138.4)	−0.21	0.047	−0.43	0.005	0.15 (0.33)	0.009 (0.03)
TNF-α[e] (pg/ml)	0.6 (0.6–5.1)	0.6 (0.6–59.0)	0.6 (0.6–4.1)	0.6 (0.6–4.5)	−0.12	0.28	−0.27	0.09	-	-

[a] Data are shown as unadjusted medians (10^{th}-90^{th} percentiles).

[b] Unadjusted concentrations are presented to characterize $PM_{2.5}$ tertile levels for DEE exposed workers. The median (range) $PM_{2.5}$ (μg/m^3) levels for the three categories: 1^{st} tertile, (296, 203–338 μg/m^3), 2^{nd} tertile, (385, 346–396 μg/m^3), 3^{rd} tertile, (408, 397–536 μg/m^3).

[c] Spearman correlation coefficients for cytokines and $PM_{2.5}$ exposure categorized as 0 (controls), 1 (1^{st} tertile), 2 (2^{nd} tertile), and 3 (3^{rd} tertile) in analyses including all subjects, and 1 (1^{st} tertile), 2 (2^{nd} tertile), and 3 (3^{rd} tertile) in analyses including exposed subjects only.

[d] *P* value using the ln transformation of the dependent variable and category of $PM_{2.5}$ as an ordinal variable taking on values of 0, 1, 2, and 3 (0: controls, 1: 1^{st} tertile, 2: 2^{nd} tertile, 3: 3^{rd} tertile) in analyses including all subjects and 1, 2, and 3 (1: 1^{st} tertile, 2: 2^{nd} tertile, 3: 3^{rd} tertile) in analyses including exposed subjects only, adjusted for age, BMI, smoking status, current alcohol use and recent infection. *FDR*: Multiple comparisons were adjusted for by applying the false discovery rate (FDR) using the Benjamini-Hochberg method.

[e] P_{trend} for IL-1β and TNF-α not analyzed using linear regression models, because the concentrations of IL-1β from 51 samples (58%) and concentrations of TNF-α from 59 samples (67%) were lower than the limit of detection.

* *P* value <0.05 compared to controls, adjusted for age, BMI, smoking status, current alcohol use and current infection.