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Research Article

Effects of Occupational Exposure to Carbon Black on Peripheral White Blood Cell Counts and Lymphocyte Subsets

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The International Agency for Research on Cancer has classified carbon black (CB) as a possible (Group 2B) human carcinogen. Given that most CB manufacturing processes result in the emission of various types of chemicals, it is uncertain if the adverse health effects that have been observed in CB-exposed workers are related to CB specifically or are due to other exposures. To address this issue, we conducted a cross-sectional molecular epidemiology study in China of 106 male factory workers who were occupationally exposed to pure CB and 112 unexposed male workers frequency-matched by age and smoking status from the same geographic region. Repeated personal exposure measurements were taken in workers before biological sample collection. Peripheral blood from all workers was used for the complete blood cell count and lymphocyte subsets analysis. Compared to unexposed workers, eosinophil counts in workers exposed to CB were increased by 30.8% ($P = 0.07$) after adjusting for potential confounders. When stratified by smoking status, statistically significant differences in eosinophils between CB exposed and unexposed workers were only present among never smokers ($P = 0.040$). Smoking is associated with alterations in various cell counts; however, no significant interaction between CB exposure and smoking status for any cell counts was observed. Given that inflammation, characterized in part by elevated eosinophils in peripheral blood, may be associated with increased cancer risk, our findings provide new biologic insights into the potential relationship between CB exposure and lung carcinogenesis. Environ. Mol. Mutagen. 57:615–622, 2016.

Key words: carbon black; occupational exposure; inflammation; immunotoxicity

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INTRODUCTION

Carbon black (CB) is virtually pure elemental carbon (>97%) in the form of colloidal particles that are produced by the reaction of a hydrocarbon fuel such as oil or gas with a limited supply of combustion air under controlled conditions. The principal uses of CB are as a reinforcing agent in rubber compounds (especially tires) and as a black pigment in printing inks, surface coatings, paper, and plastics. Numerous studies in animals have been conducted to identify the potential toxicity of CB, given the capability of this exposure to penetrate deep into the airways [Seaton et al., 1995]. Results from these studies have indicated that inhalation of CB can induce lung inflammation, fibrosis, epithelial hyperplasia, and lung carcinomas [Nikula et al., 1995; Donaldson and Stone, 2003; Borm et al., 2004; Elder et al., 2005; Carter et al., 2006]. These data from animal models are consistent with limited data in humans, which have suggested that exposure to CB is associated with respiratory symptoms including cough, sputum production, bronchitis, pneumoconiosis, and decrement in lung function [Gardiner et al., 2001; Harber et al., 2003].

The carcinogenicity of CB in humans has been evaluated in a limited number of epidemiologic studies, including among CB production workers in the United Kingdom [Hodgson and Jones, 1985; Sorahan et al., 2001], Germany [Buchte et al., 2006; Wellmann et al., 2006], and the United States [Robertson and Inman, 1996; Dell et al., 2006]. While results from these studies have provided some evidence of an excess of lung cancer among CB-exposed workers, the association is inconclusive. The International Agency for Research on Cancer evaluated the carcinogenicity of CB in 2010, taking into account available epidemiologic and mechanistic evidence, and concluded that CB is a possible carcinogen (Group 2B) in humans [IARC, 2010].

Two manufacturing processes (furnace black and thermal black) produce nearly all of the world's CB, with the furnace black process being the most common and the one that has been the main focus of prior epidemiologic studies that evaluated CB. The furnace black process uses heavy aromatic oils as feedstock and produces emissions including particulate matter, carbon monoxide, organics, nitrogen oxides, sulfur compounds, polycyclic organic matter, and trace elements, some of which might be absorbed to CB [Tsai et al., 2002]. In this case, it is uncertain whether or not the adverse health effects among CB workers are related to those other emissions or are due to CB specifically.

To address this question, we conducted a cross-sectional molecular epidemiology study to evaluate the independent effects of CB exposure. We enrolled workers who were occupationally exposed to high levels of pure CB, which is produced by thermal decomposition of acetylene at a temperature of 800–1800°C ($C_2H_2 = 2C + H_2$). This reactive process does not generate other organic chemicals [Yang et al., 2007]. A control group of workers not occupationally exposed to CB as the exposed group were enrolled from the same region and were frequency matched on key demographic characteristics. We initially demonstrated that exposure to nanoscale CB particles was associated with reduced pulmonary function and higher levels of pro-inflammatory cytokine secretion among workers who packed CB in the manufacturing factory [Zhang et al., 2014]. The inflammatory response, which is characterized by the production and release of a series of signaling molecules including cytokines and chemokines, and which operates in a complex network between epithelial cells and immune cells [Salvi and Holgate, 1999; Davies and Holgate, 2002], is considered a key step in the development of health effects associated with particulate matter exposure [Salvi and Holgate., 1999; Donaldson and Stone, 2003; Kelly and Fussell, 2011; Ristovski et al., 2012]. However, the effects of CB exposure on the inflammatory response and levels of peripheral blood cells are still unclear. To address this issue, we evaluated whether levels of white blood cells and major lymphocyte subsets, including T cells, B cells, and NK cells, were altered in the peripheral blood of workers exposed to pure CB compared to unexposed controls, and whether these associations differed by smoking status.

MATERIALS AND METHODS

Study Population and Sample Collection

The design of this cross-sectional molecular epidemiology study in Jiaozuo city, China has been described in our previous publication that evaluated lung function and cytokine levels in a subset of the study population [Zhang et al., 2014]. Exposed workers in the current study consisted of 106 males from one manufacturing factory who are responsible for packing CB. The pure CB in this factory was produced by thermal decomposition of acetylene at a temperature of 800–1800°C ($C_2H_2 = 2C + H_2$). In the packing workshops, CB is transported by an entirely open system and is easily dispersed in the workplace, resulting in both dermal and inhalatory exposures. The control group in the current study consisted of 112 healthy unexposed male workers from a drinking water treatment plant located in the same geographic region as the CB manufacturing factory. Unexposed workers were frequency-matched to the exposed workers by age and smoking status (i.e., never, former, current).

Demographic information from each subject, as well as an assessment of lifestyle characteristics including smoking and drinking habits, occupational exposure histories, and a personal medical history was collected by trained interviewers using a detailed questionnaire. Our *a prio*ri exclusion criteria for both CB-exposed and control subjects included having a history of cancer or autoimmune disease (including rheumatoid arthritis, progressive systemic sclerosis, systemic lupus erythematosus, and hemolytic anemia), acute infections (including pharyngitis, pneumonia, an upper respiratory tract infection, diarrhea, tonsillitis, or conjunctivitis) during the past week, taking medicine within the past week, and previous exposure to other occupational chemicals that are known

TABLE I. Air Concentrations of Particulate Matter at the Control Factory and Carbon Black Packing Workshop (mean ± SD)

Exposure	Control factory	Carbon black packing workshop	*P* value[a]
$PM_{2.5}$ ($\mu g\ m^{-3}$)	71 ± 11.4	800 ± 574.5	<0.001
Total carbon ($\mu g\ m^{-3}$)	42 ± 3.4	696 ± 78.1	<0.001
Organic carbon ($\mu g\ m^{-3}$)	38 ± 3.3	39 ± 6.7	0.78
Elemental carbon ($\mu g\ m^{-3}$)	4 ± 0.2	657 ± 73.7	<0.001
Elemental carbon/total carbon (%)	10 ± 0.7	94 ± 0.7	<0.001
Number of particle in the air, particles/cm^3 (% in total particles)			
Particle size <0.523 μm	45 ± 2.5 (48.0)	234 ± 24.3 (50.8)[b]	<0.001
Particle size of 0.523–1.0 μm	46 ± 4.0 (49.2)	211 ± 5.5 (45.9)[b]	<0.001
Particle size of 1.0–2.5 μm	2 ± 0.2 (2.4)	13 ± 0.6 (2.9)[b]	<0.001
Particle size of 2.5–20 μm	0.4 ± 0.01 (0.4)	2.1 ± 0.13 (0.4)[b]	<0.001
Total	94 ± 6.3 (100)	460 ± 28.4 (100)	<0.001

[a]*P* Value of *t* test, comparing the control factory and carbon black packing workshop.
[b]Data were originally presented in our previously published paper [Zhang et al., 2014].

hematotoxins or carcinogens. No workers reported a history of having autoimmune or atopic conditions, cancer, or previous exposure to other hematotoxic chemicals in the workplace. However, 12 workers in the control group and 3 CB-exposed workers were excluded from the study due to having an acute infection. Of the eligible workers, the response rate for the study was 100%.

Venous blood samples were collected from each of the subjects after their work shift for purposes of analyzing the complete blood cell count and lymphocyte subsets. The study was approved by the Research Ethics Committee of the National Institute for Occupational Health and Poison Control, the Chinese Center for Disease Control and Prevention. Informed consent was obtained from each participant.

CB Exposure Assessment

Sixteen personal air samples were collected over two consecutive days from 8 CB packing workers and 5 air samples were collected from 5 workers employed at the water plant on the day before biological sample collection. The sampling for both exposed and unexposed workers was performed during work hours for 4 hr per day. BGI 400S personal air sampling pumps (BGI, USA) were used to collect $PM_{2.5}$ on pre-weighed PTFE filters (37 mm, PALL, USA) and elemental carbon onto quartz fiber filters (37 mm, SKC, USA) using cyclones with a $PM_{2.5}$ aerodynamic cut point. CB exposure was assessed by $PM_{2.5}$, $PM_{2.5}$ related elemental carbon (EC), organic carbon (OC), and total carbon (TC). EC, OC, and TC were determined using a Carbon Analyzer (DRI 2001A, Atmoslytic, USA).

The size and morphology of CB products were measured using transmission electron microscopy (Tecnai G220, USA) and scanning electron microscopy (Sirion 200, Holland). These measurements showed that the produced CB had high carbon purity of more than 99.8% and consisted of globular shaped particles ranging in size from 30 to 50 nm. Agglomerates in the range of 200 to 400 nanometers were formed. The distribution of particles in the atmosphere of the workplace was monitored using an Aerodynamic Particle Sizer Spectrometer 3321(TSI, USA) and the data were analyzed using TSI software (Aerosol Instrument Manager).

Complete Blood Cell Count and Lymphocyte Subset Analysis

Within 4 h after blood sample collection, the complete blood cell count and differential were analyzed using the Sysmex platform (XT-1800i, Sysmex, USA). For the major lymphocyte subset analyses, lymphocytes were stained with monoclonal antibodies conjugated to fluorescein isothiocyanate (FITC), allophycocyanin (APC), phycoerythrin (PE), or peridinin chlorophyll protein (PerCP) (BD Biosciences, San Jose, CA). Briefly, 50 μL of EDTA-preserved whole blood from each sample was added to two individual test tubes containing preadded monoclonal antibodies. The first tube contained an antibody combination of CD45-PerCP, CD3-FITC, CD4-APC, and CD8-PE; the second tube contained an antibody combination of CD45-PerCP, CD3-FITC, CD56 + 16-PE, and CD19-APC. The tubes were incubated for 20 min at room temperature in the dark. After adding 450μL lysing solution, the tubes were vortexed for 30 s and incubated for 15 min. Lymphocyte subset analyses were performed using BD FACSArial II and FACS DIVA software (BD Biosciences).

Statistical Analysis

Comparisons of baseline demographic characteristics between exposed and unexposed workers were conducted using Student's *t* test for continuous variables or Pearson χ^2 for categorical variables. Linear regression was used to test for differences in the levels of each cell count between exposed and control workers. All statistical models were adjusted for age (as a continuous variable), smoking status (never, former, current), current alcohol consumption (yes or no), and body mass index (BMI). Analyses were also conducted separately for each endpoint in never smokers and current smokers. Separate analyses in former smokers were not conducted because of the small number of workers in this category (five former smokers in the exposed group and five former smokers in the control group). Interaction between CB exposure and smoking was analyzed by adding cross-product terms to the generalized linear models. Duration–response relationships were evaluated by categorizing the exposed workers into tertiles of exposure based on their exposed working years. The correlations between blood cells and levels of cytokines in the exposed workers were evaluated by the spearman rank correlation coefficient using the cytokine data reported in our previous paper [Zhang et al., 2014]. Values of $P < 0.05$ were considered significantly different. All statistical analyses were performed using SPSS 19.0.

RESULTS

CB Exposure Levels and Particle Characteristics

A significantly higher average concentration of $PM_{2.5}$, TC, and EC was observed among CB-exposed workers (800, 696, and 657 $\mu g\ m^{-3}$, respectively) compared to workers from the control factory (71, 42, and 4 $\mu g\ m^{-3}$,

respectively), based on the personal exposure measurements (Table I). Conversely, the concentration of OC was very similar in exposed and unexposed workers. EC accounted for 94.4% of the TC in the CB packing workshop (Table I), suggesting that these workers are primarily exposed to pure CB rather than other organic chemicals.

Distributions of particle sizes that were measured in the exposed and control factories are also shown in Table I. The distributions of particle sizes were similar in the CB packing workshop and control factory, with particle sizes <1.0 μm and <0.5 μm accounting for ~97% and ~50% of the total particle distribution in both factories, respectively. However, the number concentrations of total particles were significantly higher in the CB packing workshop (460 ± 28.4 particles/cm^3) compared to that in the control factory (94 ± 6.3 particles/cm^3, $P < 0.01$) (Table I).

TABLE II. Characteristics of Workers in the Control and Carbon Black Exposure Groups

Variables	Control ($N = 112$)	Carbon black exposure ($N = 106$)	P value
Age (years, mean ± SD)	44.4 ± 5.8	45.4 ± 5.2	0.17[a]
BMI (kg m^{-2}, mean ± SD)	24.9 ± 3.3	24.6 ± 3.7	0.46[a]
Smoking status			
Current smoker, n (%)	72 (64.3)	72 (67.9)	0.82[b]
Former smoker, n (%)	5 (4.5)	5 (4.7)	
Never smoker, n (%)	35 (31.2)	29 (27.4)	
Current alcohol use			
Yes, n (%)	63 (56.2)	51 (48.1)	0.37[b]
No, n (%)	47 (43.8)	55 (51.9)	
Working year (years, mean ± SD)	—	11.5 ± 10.5	—

[a] t test for difference between two groups.
[b] χ^2 test for difference between two groups.
BMI, body mass index.

Demographic Characteristics of the Study Population

The demographic characteristics of the men included in the study are summarized in Table II. The distributions of age, BMI, and history of smoking and alcohol use were not significantly different between the two groups. The average number of years of employment among CB-exposed workers was 11.5 ± 10.5 years.

Systemic Effects of CB on the Hematological Indices in the Peripheral Blood

Associations between CB exposure and levels of peripheral blood cell counts and lymphocyte subsets are shown in Table III. Eosinophil counts were increased by 30.8% in CB-exposed workers compared to unexposed workers ($0.17 \pm 0.14 \times 10^9$/L *vs.* $0.13 \pm 0.10 \times 10^9$/L), an effect that was borderline significant after adjusting for age, BMI, smoking status, and alcohol use ($P = 0.072$). There were 3 CB exposed workers and 0 unexposed workers with eosinophilia (defined as $> 0.5 \times 10^9$/L in peripheral blood). No significant differences in other cell counts were observed between CB-exposed workers and unexposed workers (Table III). Analyses based on duration of exposure did not suggest a significant duration–response association for any cell count among all subjects or in exposed workers only (P_{trends} all >0.05; data not shown).

To evaluate the consistency of these findings by smoking status, we conducted analyses in never smokers and current smokers separately, the results of which are shown in Figure 1. A statistically significant difference in eosinophil counts was observed between CB-exposed workers and unexposed workers only among never smokers ($P = 0.040$), not among current smokers (Figs. 1A and 1B). Consistent with the overall findings, no other cell counts were significantly altered in CB-exposed workers

TABLE III. Peripheral Blood Cell Counts and Lymphocyte Subsets in the Control and Carbon Black Exposure Groups (mean ± SD)

	Control ($N = 112$)	Carbon black exposure ($N = 106$)	Difference in mean levels (%)	P_{crude}[a]	P_{adjust}[b]
White blood cells (10^9/L)	6.60 ± 1.72	6.82 ± 1.74	3.3	0.34	0.24
Neutrophils (10^9/L)	3.99 ± 1.45	4.10 ± 1.23	2.8	0.55	0.42
Eosinophils (10^9/L)	0.13 ± 0.10	0.17 ± 0.14	30.8	0.05	0.07
Lymphocytes (10^9/L)	2.15 ± 0.53	2.16 ± 0.59	0.5	0.82	0.69
T cell (10^9/L)	1.48 ± 0.44	1.50 ± 0.46	1.4	0.65	0.59
CD4$^+$ (10^9/L)	0.79 ± 0.24	0.82 ± 0.27	3.8	0.44	0.49
CD8$^+$ (10^9/L)	0.62 ± 0.30	0.60 ± 0.30	−3.2	0.56	0.68
B cells (10^9/L)	0.24 ± 0.11	0.24 ± 0.12	0.0	0.63	0.52
NK cells (10^9/L)	0.42 ± 0.21	0.38 ± 0.19	−9.5	0.19	0.25
Monocytes (10^9/L)	0.34 ± 0.13	0.31 ± 0.13	−8.8	0.15	0.15

[a] t test for difference between two groups.
[b] Adjusted for age, smoking, alcohol status, and BMI (body mass index).
Basophil counts are not shown in this table, because the counts were too low in the majority of subjects to be analyzable with regard to group differences.

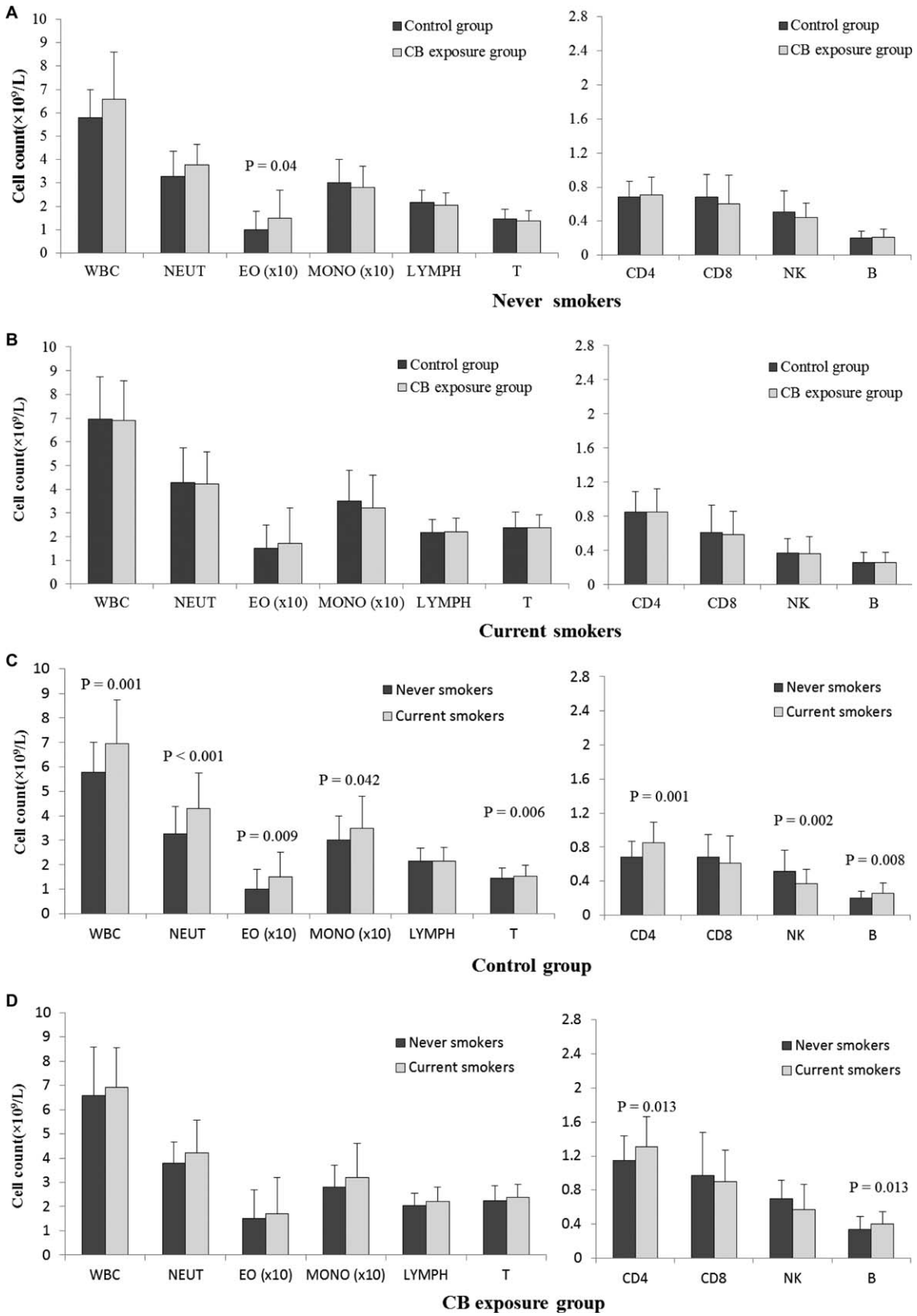

Fig. 1. (A) Comparison of peripheral blood cell counts between control and carbon black exposed groups among never smokers. (B) Comparison of peripheral blood cell counts between control and carbon black exposed groups among current smokers. (C) Comparison of peripheral blood cell counts among never smokers and current smokers in control group. (D) Comparison of peripheral blood cell counts among never smokers and current smokers in carbon black exposure group. All cell counts are displayed as mean ± standard deviation. *P* values were calculated using linear regression models by adjusting for age, alcohol use, and BMI (body mass index). Abbreviations: WBC, white blood cell; LYMPH, lymphocyte; NEUT, neutrophil; T, T lymphocyte; CD4, $CD4^+$ T cell; CD8, $CD8^+$ T cell; NK, natural killer cell; B, B lymphocyte; MONO, monocyte; EO, eosinophil; CB, carbon black.

compared to unexposed workers in either never or current smokers. We then evaluated cell counts in relation to smoking status among CB-exposed and unexposed workers separately (Figs. 1C and 1D). In the unexposed workers, significantly higher levels of white blood cell counts (20.4% increase, $P = 0.001$), neutrophils (31.2% increase, $P < 0.001$), monocytes (16.7% increase, $P = 0.042$), eosinophils (50.0% increase, $P = 0.009$), $CD4^+$ T cells (25.0% increase, $P = 0.001$), and B cells (30.0% increase, $P = 0.008$) were observed among current smokers, whereas levels of NK cells (27.5% decrease, $P = 0.002$) were significantly reduced in current compared to never smokers among the control group (Fig. 1C). On the other hand, only levels of $CD4^+$ T-cells (19.7% increase, $P = 0.013$) and B cells (23.8% increase, $P = 0.013$) were significantly higher in current compared to never smokers among CB-exposed workers (Fig. 1D).

Relationship Between Blood Cell Counts and Levels of Cytokines in Serum of Exposed Workers

NK cells were positively correlated with levels of IL-1β, IL-6, and MIP-1β ($r = 0.27$, 0.26, and 0.20; $P = 0.005$, 0.007, and 0.043, respectively). In addition, we observed a positive correlation between white blood cells and the levels of IL-1β and MIP-1β ($r = 0.24$ and 0.21; $P = 0.014$ and 0.029, respectively), as well as between neutrophils and the levels of MIP-1β ($r = 0.26$, $P = 0.009$) and MCP-1 ($r = 0.20$, $P = 0.043$) (Supporting Information Table I).

DISCUSSION

In the present study, we demonstrated that CB exposure is associated with an increase in eosinophil counts. The major contribution of our study is the evaluation of the independent effect of pure CB on levels of immune cells, which provides new insight for understanding the adverse health outcomes associated with CB. CB is characterized by the size distribution of the primary particles and by the degree of their aggregation and agglomeration. The average aggregate particle diameter of CB production ranges from 50 to 600 nm, and some agglomerates can reach up to many micrometers in diameter [Baan, 2007]. Very limited research has been done to evaluate the size distribution of the CB agglomerates in occupational environments. In the present study, the primary particles of CB that the packing workers were exposed to consisted of a globular shape with diameters ranging from 30 to 50 nm, and from 200 to 400 nm for the aggregate form. However, only 50% of the CB particles in the occupational environment were <0.5 μm, and about 49% of the CB particles were from 0.5 to 2.5μm, suggesting that agglomerates of CB are formed in the atmosphere. After inhalation into the lungs, larger particles are phagocytized by alveolar and airway macrophages, but the fine and ultrafine CB remain in the lungs for a longer period of time [Gehr et al., 2006]. Ultrafine particles can enter macrophages and epithelial cells and even penetrate into the circulation. Therefore, ultrafine particles could induce systemic toxic effects in addition to triggering local inflammatory reactions in the lungs [Oberdorster et al., 2005].

Eosinophils are bone marrow-derived cells that are implicated in the pathogenesis of numerous inflammatory processes [Moshkovits et al., 2014]. Eosinophils circulate only a short time in the peripheral blood before they migrate to the thymus or gastrointestinal tract, where they reside under homeostatic conditions. However, in the presence of inflammatory stimuli, eosinophils develop from bone marrow progenitors, and then migrate into the blood and subsequently are recruited to peripheral tissues where their survival is prolonged. Thus, an increased number of eosinophils in the blood and inflamed tissue is often associated with inflammatory diseases such as asthma and eosinophilic esophagitis, but may also be indicative of a subclinical state of inflammation that does not manifest as overt eosinophilia [Hogan et al., 2008; Blanchard and Rothenberg, 2009; Rosenberg et al., 2013]. Increased eosinophils in peripheral blood among CB exposed workers in our present study, together with our previous finding that CB was associated with higher serum levels of pro-inflammatory cytokines including interleukin (IL)−1β, IL-6, IL-8, macrophage inflammatory protein-1β and tumor necrosis factor-α [Zhang et al., 2014], suggests that CB exposure may be associated with inflammation. Many epidemiologic and clinical studies have shown that chronic inflammation is associated with an increased risk of cancer and cardiovascular disease [Libby, 2006; Lu et al., 2006]. In the context of the respiratory system, it was also suggested that cancer risk is positively associated with the severity and duration of inflammatory diseases [Borm and Driscoll, 1996; Keeley and Rees, 1997]. Thus, our findings that suggest an association between CB exposure and indices of chronic inflammation provide some biologic plausibility for the potential role of CB exposure in promoting the development of lung cancer and cardiovascular disease.

A limited number of studies have investigated the relationship between CB exposure and white blood cell counts. Two experimental exposure studies reported conflicting results. One study consisting of 20 healthy volunteers who were exposed to pure carbon particles at a dose of 50 μg m^{-3} ($PM_{0.3}$) for 1h showed no effects on circulating blood cell counts after exposure [Routledge et al., 2006]; another study reported that CB exposure increased neutrophils and decreased monocytes and basophils among healthy adults after exposure to 25 μg m^{-3} of ultrafine carbon particles ($PM_{0.25}$) for 2 h [Frampton et al., 2004]. However, because of the short-term exposure, it is uncertain whether the observed changes in blood cell counts constitute adverse health effects or

reflect a normal immune response to these environmental stimuli in healthy individuals. In the present study, which included workers exposed to CB for an average of 11.5 years, we observed an increase in eosinophils but no significant differences in neutrophils or monocytes between two groups. Additional studies of workers with chronic exposure to CB that have high-quality exposure data would be informative to replicate our findings and to evaluate whether long-term exposure to CB might be associated with immune pathological injury, which includes direct immunotoxicity (immunosuppression or immuostimulation), hypersensitivity, and autoimmunity [Descotes et al., 2000].

Smoking is an important determinant of blood cell counts. In addition to increased eosinophils, we also observed that smoking in the control workers was associated with increases in total white blood cells, neutrophils, eosinophils, monocytes, B lymphocytes, and $CD4^+$ T cells, and a decrease in NK cells. These findings have also been reported in other studies that included healthy adults [Moszczynski et al., 2001; Haswell et al., 2014; S et al., 2014]. Cigarette smoke contains more than 45,000 components ranging from particulate matter to organic and inorganic chemicals and gaseous components, which have various toxic, mutagenic and carcinogenic effects. The immunologic changes in smokers may reflect the combined effects of various compounds [Mehta et al., 2008]. For example, studies have showed that cigarette smoke containing high levels of tar and nicotine induce greater immunologic changes than cigarette smoke that contains lower levels of these compounds [Sopori and Kozak, 1998]. It is also possible that specific constituents of tobacco smoke may impact different types of blood cell counts, which could result in more widespread hematological alterations given the numerous chemical components. In contrast, the CB that workers in the present study were exposed to is pure elemental carbon in the form of colloidal particles rather than a complex mixture of different constituents. However, the combined effects of CB exposure and smoking showed that the effect of CB exposure on eosinophils is diminished among smokers. Specifically, there was no difference in eosinophils in the CB exposed workers compared with unexposed workers among current smokers. The absence of a significant effect for CB among current smokers may be due to the higher baseline eosinophil counts among current smokers in the unexposed group, which would make it more difficult to detect significant differences according to CB exposure status particularly if the effect of smoking on eosinophil counts is stronger than that of CB. These findings and the dramatic effect of smoking on blood cell profiles emphasize the need to conduct stratified analyses by smoking status or restriction to non-smokers in future studies.

A previous nested case-control study observed a weakening of the diesel exhaust effect on lung cancer risk among heavy smokers of at least two packs per day compared with smokers of less than two packs per day [Silverman et al., 2012]. However, little is known about the mechanism underlying the effect modification of CB on the immune system by smoking. One possible explanation might be that CB particulate deposition may be reduced in the lungs of smokers by coughing up sputum, which in turn may reduce the effect of CB on the immune system. Another possible reason might be that the adaption of the immune system to cigarette smoking may cause its non-response to stimulation of CB particulate, since many of the adverse health effects of cigarette smoking could be related to its ability to compromise the immune system.

In addition to the evaluation of pure CB exposure, which is a major strength of our study, further strengths include exposed and unexposed workers that were well-matched by age and smoking status, and that were selected from the same geographic region with similar environmental air pollution levels, which minimizes the impact of confounding on our findings with respect to these variables. Further, we collected medical history data for all subjects in the study using a questionnaire and excluded individuals with a recent acute infection, as this could have influenced levels of the evaluated cell counts. In summary, this study suggests that pure CB may increase the number of eosinophils, which may be reflecting an inflammatory response among CB exposed workers. Additional studies, particularly those with high-quality exposure data, are needed to confirm and extend these findings.

AUTHOR CONTRIBUTIONS

YZ, SY designed the study. YZ obtained the financial support and conducted the study. YD, YN, HD, MY, XZ, TM, PB, XJ, MS, RZ, SY, XY recruited subjects and collected biological samples. YD, WH, XZ, DS, RV conducted the analyses and were primarily responsible for drafting the paper. YZ, QL, BB, RV, NR revised the draft. All authors contributed to the final manuscript.

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