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Objectives—Previous studies have shown that residential proximity to a roadway is associated with increased cardiovascular disease risk. Yet, the nature of this association remains unclear, and its effect on individual cardiovascular disease risk factors has not been assessed. The objective of this study was to determine whether residential proximity to roadways influences systemic inflammation and the levels of circulating angiogenic cells.

Approach and Results—In a cross-sectional study, cardiovascular disease risk factors, blood levels of C-reactive protein, and 15 antigenically defined circulating angiogenic cell populations were measured in participants (n=316) with moderate-to-high cardiovascular disease risk. Attributes of roadways surrounding residential locations were assessed using geographic information systems. Associations between road proximity and cardiovascular indices were analyzed using generalized linear models. Close proximity (<50 m) to a major roadway was associated with lower income and higher rates of smoking but not C-reactive protein levels. After adjustment for potential confounders, the levels of circulating angiogenic cells in peripheral blood were significantly elevated in people living in close proximity to a major roadway (CD31$^+$/AC133$^+$, AC133$^+$, CD34$^+$/AC133$^+$, and CD34$^+$/45^{dim}/AC133$^+$ cells) and positively associated with road segment distance (CD31$^+$/AC133$^+$, AC133$^+$, and CD34$^+$/AC133$^+$ cells), traffic intensity (CD31$^+$/AC133$^+$ and AC133$^+$ cells), and distance-weighted traffic intensity (CD31$^+$/34$^+$/45$^+$/AC133$^+$ cells).

Conclusions—Living close to a major roadway is associated with elevated levels of circulating cells positive for the early stem marker AC133$^+$. This may reflect an increased need for vascular repair. Levels of these cells in peripheral blood may be a sensitive index of cardiovascular injury because of residential proximity to roadways. **(*Arterioscler Thromb Vasc Biol*. 2015;35:2468-2477. DOI: 10.1161/ATVBAHA.115.305724.)**

Key Words: air pollution ■ cardiovascular diseases ■ endothelial progenitor cells ■ epidemiology ■ risk factors

Several studies suggest that exposure to environmental pollutants increases the risk of developing cardiovascular disease (CVD).[1,2] Chronic exposure to polluted environments is associated with metabolic and inflammatory changes, increased progression of subclinical measures of CVD, and acceleration of atherogenesis.[3] Acute exposure to high levels of ambient pollutants has also been linked to the precipitation of acute cardiovascular events.[4] Although specific pollutants that contribute to cardiovascular risk and injury have not been identified with certainty, cardiovascular injury has been found to be most closely associated with the levels of fine particulate matter with an aerodynamic diameter ≤2.5 μm ($PM_{2.5}$) in the ambient air.[3,5] Specific source apportionment studies suggest that the cardiovascular effects of ambient air pollutants

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Nonstandard Abbreviations and Acronyms	
CVD	cardiovascular disease
$PM_{2.5}$	fine particulate matter with an aerodynamic diameter ≤2.5 μm

could be linked to the pollutants generated by both stationary and mobile sources.[6,7] In most urban environments, mobile sources, such as gasoline and diesel engine exhaust, contribute to a significant portion of ambient air particles and volatile organic compounds.[8,9]

The concept that chronic exposure to traffic-generated pollutants could contribute to CVD is supported by epidemiological studies, showing that living in close proximity to a major roadway is associated with increased CVD risk and CVD mortality. Close proximity to roadways has been associated with increased coronary artery disease mortality,[10–12] myocardial infarction,[13,14] heart failure,[15] deep vein thrombosis,[16] and stroke mortality.[17] C-reactive protein, a clinical CVD risk indicator and marker of inflammation, has also been positively associated with traffic density.[18] In addition, inverse associations have been identified between roadway proximity and subclinical risk predictors, including coronary artery calcification[19] and oxidized low-density lipoprotein.[20] Nevertheless, the mechanisms by which residential proximity to roadways increases CVD risk remain unclear.

This study was designed to investigate how residential proximity to roadways affects systemic inflammation and circulating levels of angiogenic cells. Circulating angiogenic cells have been shown to participate in vascular repair and regeneration.[21–24] These cells are mobilized from the bone marrow into the circulation by cytokines, growth factors, and hormones and have been found to play an important role in maintaining vascular health. Several observational studies show a robust inverse association between circulating angiogenic cell levels and CVD risk[25–27] and severity.[28–31] In a prospective analysis, the levels of these cells were found to be predictive of CVD mortality.[32] We have previously reported that acute exposure to elevated levels of ambient $PM_{2.5}$ decreases the levels of these cells in circulation.[33] However, the effect of exposure to traffic pollution on the levels of circulating angiogenic cells has not been assessed. Thus, the main objective of our study was to determine whether residential proximity to a roadway affects the levels of angiogenic cells in peripheral blood as a measure of CVD risk and whether this effect is related to changes in systemic inflammation because of roadway proximity.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Geographic Distribution

Participant addresses were geocoded using data obtained from the Louisville/Jefferson County Information Consortium composite locator via ArcMap 9.3+ geographic information system software. The geographic distribution of the participants is shown in the Figure (the residences presented are geographically masked). Study participants were concentrated in the northwestern region of Jefferson County also known as West Louisville. The Louisville Metro Department of Public Health and Wellness reports that this area has disproportionately high rates of CVD and high levels of air pollution.[34,35] This area also has a higher concentration of major roadways than other geographic locations in Jefferson County, aside from the central business district.

Participant Characteristics

Adult participants with moderate-to-high CVD risk were recruited between October 2009 and May 2013 from the University of Louisville Hospital and affiliated clinic system. Participants from this cohort were middle aged (50±10 years), with a slightly higher proportion of men (n=162; 51%) and whites (n=179; 58%; Table 1). A high percentage of the population was comprised of current (n=109; 35%) or former (n=91; 29%) smokers. A majority of the cohort was diagnosed with hypertension (n=220; 71%), hyperlipidemia (n=187; 60%), and obesity (body mass index, ≥30; n=183; 59%). Several participants were being treated with angiotensin-converting enzyme inhibitors (n=155; 50%), β-blockers (n=157; 50%), or statins (n=149; 48%). Of the 345 subjects, 316 (92%) were successfully geocoded. Patients without a valid address could not be geocoded and were not included in the study.

Demographic Comparison

Demographic characteristics of study participants living within 50 m of a major roadway (roadway carrying a mean of at least 5000 vehicles per day) or >50 m from a major roadway are shown in Table 1. These two groups do not differ in age, sex, ethnicity, hypertension, hyperlipidemia, diabetes mellitus, obesity, environmental tobacco smoke exposure, empirical smoke exposure (cotinine), medical history, medication

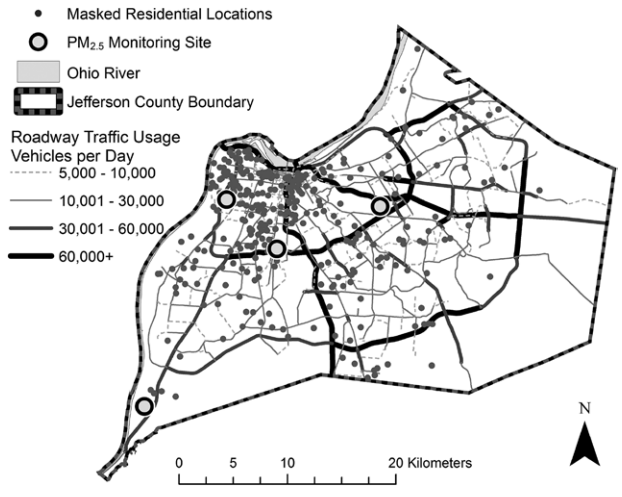

Figure. Patients' residential locations in Jefferson County, KY. Each dot represents the residential location of a single subject. Residential locations are geographically masked to preserve subject confidentiality, although general trends in subject distribution throughout the county are maintained. A major roadway is defined as a roadway with an annual mean traffic volume of >5000 vehicles per day.

Table 1. Demographics and Medical History of the Study Population Stratified by Major Roadway Proximity

	Total, n=316	<50 m, n=57 (%)	≥50 m, n=259 (%)	*P* Value
Categorical variable, n (%)				
Sex				
Female	154 (49)	26 (46)	128 (49)	0.662
Male	162 (51)	31 (54)	131 (51)	
Ethnicity				
White	179 (58)	27 (51)	152 (60)	0.168
Black	121 (40)	26 (49)	95 (37)	
Hispanic	7 (2)	0 (0)	7 (3)	
CVD risk factors				
Hypertension	220 (71)	36 (63)	184 (72)	0.199
Hyperlipidemia	187 (60)	33 (58)	154 (61)	0.765
Diabetes mellitus	99 (32)	17 (30)	82 (32)	0.875
Obese (BMI, ≥30)	183 (59)	34 (61)	149 (59)	0.881
Current smoker (self-report)	109 (35)	27 (47)	82 (32)	0.031
Never smoked (self-report)	114 (36)	14 (25)	100 (39)	0.048
Former smoker (self-report)	91 (29)	16 (28)	75 (29)	1.000
High CVD risk category*	191 (63)	32 (57)	159 (64)	0.360
Medical history				
Myocardial infarction	88 (28)	16 (28)	72 (28)	1.000
Stroke	26 (8)	7 (12)	19 (7)	0.284
CABG/PCI/stents	70 (22)	13 (23)	57 (22)	1.000
Heart failure	46 (15)	6 (11)	40 (16)	0.529
Cancer	6 (2)	2 (4)	4 (2)	0.296
Medication				
Angiotensin-converting enzyme inhibitor	155 (50)	30 (53)	125 (49)	0.662
Angiotensin-receptor blockers	18 (6)	2 (4)	16 (6)	0.544
β-Blocker	157 (50)	29 (51)	128 (50)	1.000
Calcium-channel blockers	65 (21)	12 (21)	53 (21)	1.000
Diuretics	118 (38)	21 (37)	97 (38)	1.000
Statins	149 (48)	24 (42)	125 (49)	0.381
Aspirin	157 (50)	30 (53)	127 (50)	0.770
Continuous variable, mean±SD				
Age, y	50±10	49±9	50±11	0.652
Cotinine, μg/g of creatinine	520±1133	555±985	512±1163	0.374
Creatinine, mg/dL	138±94	137±73	139±99	0.534
Inflammation				
hsCRP, mg/L	4.6±4.7	4.9±5.0	4.5±4.6	0.429
CVD risk				
Framingham Risk Score	6.6±.7.6	6.8±11.6	6.5±.5±	0.150
Sum of CVD risk factors†	3.3±1.4	3.5±1.5	3.3±1.3	0.332
Median household income,‡ $\times 10^3$	33±19	23±14	35±20	<0.001
$PM_{2.5}$, $\mu g/m^3$	13.1±5.6	13.3±5.5	13.0±5.6	0.756

Major roadways were defined as roads carrying an annual mean of ≥5000 vehicles per day. Major roadway proximity was calculated as a straight line distance from the residential address of the subject to the nearest major roadway using geographic information system technology. Cotinine was measured in the urine by gas chromatography-mass spectrometry analysis, urinary creatinine was measured using a Cobas Mira Autoanalyzer, and hsCRP was measured using the VITROS kit as described before.[58] BMI indicates body mass index; CABG, coronary artery bypass graft; CVD, cardiovascular disease; hsCRP, high sensitivity C-reactive protein; PCI, percutaneous coronary intervention; and $PM_{2.5}$, fine particulate matter with an aerodynamic diameter ≤2.5 μm.

*Individuals with high CVD risk category had a Framingham Risk Score of ≥20 or had previously experienced a cardiovascular event.

†The sum of CVD risk factors includes the following Framingham risk factors: age ≥ 40 y, male sex, current smoker, hypertension, hyperlipidemia, and diabetes mellitus.

‡Median household income is reported in USD at the US Census block group level.

Table 2. Comparison of Circulating Angiogenic Cell Levels Stratified by Roadway Proximity

Circulating Angiogenic Cell Type*	<50 m, Mean±SD	≥50 m, Mean±SD	*P* Value
Cell type 1 ($CD31^+/34^+/45^{dim}$)	1.07±1.07	0.94±1.07	0.209
Cell type 2 ($CD31^+/34^+/45^+$)	0.11±0.20	0.23±1.82	0.929
Cell type 3 ($CD31^+/34^+/45^{dim}/AC133^+$)	0.63±0.71	0.51±0.64	0.198
Cell type 4 ($CD31^+/34^+/45^+/AC133^+$)	0.02±0.03	0.02±0.06	0.478
Cell type 5 ($CD31^+/AC133^+$)	3.25±3.92	1.98±4.03	0.002
Cell type 6 ($CD31^+/34^+$)	1.50±1.48	1.69±3.83	0.621
Cell type 7 ($CD31^+/34^+/45^{dim}/AC133^-$)	0.44±0.52	0.42±0.71	0.886
Cell type 8 ($CD31^+/34^+/45^+/AC133^-$)	0.09±0.18	0.21±1.80	0.943
Cell type 9 ($CD34^+$)	1.59±1.53	1.87±4.58	0.621
Cell type 10 ($CD31^+$)	280±204	255±231	0.291
Cell type 11 ($AC133^+$)	4.24±4.73	2.86±5.02	0.006
Cell type 12 ($CD45^+$)	695±647	615±633	0.321
Cell type 13 ($CD34^+/AC133^+$)	0.72±0.75	0.56±0.69	0.049
Cell type 14 ($CD34^+/45^+/AC133^+$)	0.02±0.04	0.03±0.10	0.306
Cell type 15 ($CD34^+/45^{dim}/AC133^+$)	0.61±0.69	0.47±0.59	0.109

*Data show the number of circulating angiogenic cells per microliter of blood, determined by flow cytometry as described in Materials and Methods in the online-only Data Supplement.

use, lymphocyte count, inflammation, or the Framingham Risk Score. Participants living within 50 m of a major roadway were more likely to self-report being a current smoker (47% versus 32%; *P*=0.031) and less likely to report having never smoked (25% versus 39%; *P*=0.048) than those living >50 m from a major roadway. People living closer to roadways also lived in areas with significantly lower median household incomes ($23 204 versus $35 494; *P*<0.001). These income levels were substantially lower than the median household income of $46 298 for the entire Jefferson County, KY, from 2007 to 2011.[36] There was no significant association between ambient $PM_{2.5}$ levels and roadway proximity.

Association Between Circulating Angiogenic Cells and Distance to Roadway

To examine the influence of roadway proximity on circulating angiogenic cells, we first compared the levels of these cells in the peripheral blood of individuals living within 50 m of a major roadway with the levels of these cells in the peripheral blood of those living >50 m from a major roadway estimated using the straight line distance to the nearest major roadway. The results of these unadjusted *t* test analyses are shown in Table 2. Of the 15 types of circulating angiogenic cell subpopulations examined, the levels of cell type 5 ($CD31^+/AC133^+$; *P*=0.002), cell type 11 ($AC133^+$; *P*=0.006), and cell type 13 ($CD34^+/AC133^+$; *P*=0.049) were significantly and inversely associated with distance to roadway, that is, the levels of these circulating angiogenic cells were higher in people living closer to a major roadway. Cell types 5 and 11 remained significantly associated after adjustment for multiple comparisons (*P*=0.026 and *P*=0.039, respectively). No associations were observed with other circulating angiogenic cell subpopulations.

To examine the influence of potential confounders, adjusted regression analyses were completed using generalized linear models. These regressions describe the association between circulating angiogenic cell levels and roadway proximity and were adjusted for potential confounders: age,

Table 3. Association Between Major Roadway Proximity and Circulating Angiogenic Cell Levels

Circulating Angiogenic Cell Type	Change (%)	95% CI	*P* Value
Cell type 4 ($CD31^+/34^+/45^+/AC133^+$)	−13.6	−49.5 to 48.0	0.595
Cell type 5 ($CD31^+/AC133^+$)	−39.5	−58.2 to −12.4	0.008*
Cell type 11 ($AC133^+$)	−40.6	−60.0 to −11.7	0.010*
Cell type 13 ($CD34^+/AC133^+$)	−34.2	−54.7 to −4.33	0.028*
Cell type 14 ($CD34^+/45^+/AC133^+$)	−17.8	−57.4 to 58.9	0.560
Cell type 15 ($CD34^+/45^{dim}/AC133^+$)	−32.5	−54.2 to −0.67	0.046*

Participants were dichotomized by distance into those living <50 m, which was compared with those living >50 m from a major roadway. Associations are corrected for age, sex, ethnicity, body mass index, cigarette smoking, median household income, diabetes mellitus, myocardial infarction, and $PM_{2.5}$. CI indicates confidence interval; and $PM_{2.5}$, fine particulate matter with an aerodynamic diameter ≤2.5 μm.

*$P<0.05$.

Table 4. Association Between Total Distance of Major Road Segments and Circulating Angiogenic Cell Levels

Circulating Angiogenic Cell Type	Change (%)	95% CI	*P* Value
Cell type 4 ($CD31^+/34^+/45^+/AC133^+$)	2.04	–4.16 to 8.28	0.519
Cell type 5 ($CD31^+/AC133^+$)	5.82	1.25 to 10.4	0.013*
Cell type 11 ($AC133^+$)	5.86	0.96 to 10.8	0.019*
Cell type 13 ($CD34^+/AC133^+$)	4.60	0.01 to 9.20	0.049*
Cell type 14 ($CD34^+/45^+/AC133^+$)	2.31	–5.00 to 9.67	0.537
Cell type 15 ($CD34^+/45^{dim}/AC133^+$)	4.25	–0.50 to 9.00	0.079

Percent change in cell populations per 10-m increase in major road distance within a 50-m radius of an individual's residence. Associations are adjusted for age, sex, ethnicity, body mass index, socioeconomic status, cigarette smoking, diabetes mellitus, myocardial infarction, and $PM_{2.5}$. CI indicates confidence interval; and $PM_{2.5}$, fine particulate matter with an aerodynamic diameter ≤2.5 μm.

*$P<0.05$.

†$P<0.05$ for the population with 6-month residential duration.

sex, ethnicity, body mass index, cigarette smoking, median household income, myocardial infarction, diabetes mellitus, and 24-hour $PM_{2.5}$. Patients with cancer (n=6) were excluded from all adjusted regression analyses. After adjustment, the levels of cell type 5 (P=0.008), cell type 11 (P=0.010), cell type 13 (P=0.028), and cell type 15 ($CD34^+/45^{dim}/AC133^+$; P=0.046) were significantly associated with distance to roadway (Table 3). The levels of these cells in peripheral blood were higher in individuals living within 50 m of a major roadway. Specifically, the levels of cell types 5, 11, 13, and 15 were greater by 40%, 41%, 34%, and 32%, respectively, in the population living closer to a major roadway.

Association of Circulating Angiogenic Cell Levels and Cumulative Major Road Segments

Although living within 50 m of the nearest major roadway showed significant association with specific angiogenic cell populations, the total exposure to roadways could be affected by the presence of other major roadways in close proximity to the residence. Hence, we examined how exposure to all surrounding major roads near the residence would influence circulating angiogenic cell levels. For this, all major road segments within a circular 50-m radius buffer zone around subjects' residences were combined to obtain cumulative road segments within the buffer zone. The results from the adjusted generalized linear model of the relationship between circulating angiogenic cells and cumulative major road segments are shown in Table 4. After adjustment, cell type 5 (P=0.013), cell type 11 (P=0.019), and cell type 13 (P=0.049) were significantly associated with the cumulative distance of each roadway segment within 50 m of the residence. These results indicate that as the total distance of major road segments increases within a 50-m buffer, the levels of these specific circulating angiogenic cells also increase. Each meter of major roadway within the 50-m buffer was associated with a 0.6% increase in cell types 5 and 11 and a 0.5% increase in cell type 13. Importantly, when expanded to all road segments within a 50-m buffer, none of these cell populations remained associated with cumulative road segment distances. These data support the notion that the levels of specific circulating angiogenic cells are associated with residential distance from a major roadway and not background exposures.

Association of Circulating Angiogenic Cell Levels and Major Road Segment Intensity

To build on the notion that the sum of road segments in close residential proximity is associated with circulating angiogenic cell levels, we investigated whether traffic concentration on

Table 5. Association Between Major Road Segment Intensity and Circulating Angiogenic Cell Levels

Circulating Angiogenic Cell Type	Change (%)	95% CI	*P* Value
Cell type 4 ($CD31^+/34^+/45^+/AC133^+$)	–13.0	–74.7 to 48.6	0.679
Cell type 5 ($CD31^+/AC133^+$)	41.4	3.70 to 79.2	0.032*
Cell type 11 ($AC133^+$)	46.7	6.50 to 86.9	0.023*
Cell type 13 ($CD34^+/AC133^+$)	34.0	–2.90 to 70.9	0.071
Cell type 14 ($CD34^+/45^+/AC133^+$)	–9.10	–78.5 to 60.2	0.796
Cell type 15 ($CD34^+/45^{dim}/AC133^+$)	32.4	–5.10 to 70.0	0.091

Percent change in cell populations per 1-km increase in total vehicle distance traveled within a 50-m radius of individual's residence. Associations are adjusted for age, sex, ethnicity, body mass index, socioeconomic status, cigarette smoking, diabetes mellitus, myocardial infarction, and $PM_{2.5}$. CI indicates confidence interval; and $PM_{2.5}$, fine particulate matter with an aerodynamic diameter ≤2.5 μm.

*$P<0.05$.

†$P<0.05$ for the population with 6-month residential duration.

Table 6. Association Between Circulating Angiogenic Cell Levels and Distance-Weighted Roadway Traffic Intensity

Circulating Angiogenic Cell Type	Change (%)	95% CI	*P* Value
Cell type 4 ($CD31^+/34^+/45^+/AC133^+$)	3.69	0.16 to 7.23	0.040*†
Cell type 5 ($CD31^+/AC133^+$)	0.66	–2.49 to 3.81	0.682
Cell type 11 ($AC133^+$)	0.16	–3.24 to 3.57	0.927
Cell type 13 ($CD34^+/AC133^+$)	0.63	–2.38 to 3.64	0.683
Cell type 14 ($CD34^+/45^+/AC133^+$)	2.66	–1.18 to 6.52	0.175
Cell type 15 ($CD34^+/45^{dim}/AC133^+$)	0.38	–2.71 to 3.49	0.808

Percent change in cell populations per 10-m (weighted by distance to roadway) increase in total vehicle distance travelled within a 300-m radius of individual's residence. Associations are adjusted for age, sex, ethnicity, body mass index, cigarette smoking, median household income, diabetes mellitus, myocardial infarction, and $PM_{2.5}$. CI indicates confidence interval; and $PM_{2.5}$, fine particulate matter with an aerodynamic diameter ≤2.5 μm.

*P<0.05.

†P<0.05 for the population with 6-month residential duration.

these road segments influences this association. For this, we calculated roadway traffic intensity as the daily sum of vehicle distance travelled on the major road segments within 50 m of the participant's address. We found that cell type 5 (P=0.032) and cell type 11 (P=0.023) were positively associated with traffic intensity. These results suggest that as the traffic intensity increases within a 50-m buffer, there is an increase in the levels of cell types 5 and 11(Table 5). Quantitatively, this analysis indicates that for each kilometer traveled within the buffer, there was a 0.04% increase in cell type 5 and a 0.05% increase in cell type 11.

Association of Circulating Angiogenic Cell Levels and Distance-Weighted Traffic Intensity

To examine exposure measures in greater detail, we calculated major roadway vehicle traffic intensity weighted for distance to those roadways. These values were generated on a continuous raster surface at 10-m resolution and extracted by address points. A cutoff value of 300 m from major roads was selected because it is the distance at which most pollutants reach background levels.[37] After adjustment, cell type 4 ($CD31^+/34^+/45^+/AC133^+$; P=0.040) was significantly associated with distance-weighted roadway traffic intensity. For each 10-m increase in the value of weighted roadway intensity, there was a 4% increase in cell type 4 (Table 6). This association remained consistent within the population with 6-month residential duration (P=0.011), corresponding to a 0.6% increase in cell type 4 for each unit increase in roadway density.

Adjusted Association of Circulating Angiogenic Cell Levels and $PM_{2.5}$

Ambient levels of $PM_{2.5}$ were estimated by calculating the 24-hour average of all regional environmental protection agency–validated monitoring stations within 30 km of Jefferson County, KY, that report daily $PM_{2.5}$ levels.[38] Our analysis identified a significant association between circulating angiogenic cells and ambient $PM_{2.5}$ in the 24-hour proceeding enrollment in the total population, where the levels of cell type 3 ($CD31^+/34^+/45^{dim}/AC133^+$; P=0.037), cell type 4 (P=0.001), cell type 14 ($CD34^+/45^+/AC133^+$; P<0.001), and cell type 15 (P=0.032) were inversely associated with ambient $PM_{2.5}$ after adjusting for age, sex, ethnicity, body mass index, cigarette smoking, median household income, myocardial infarction, and diabetes mellitus (Table 7). Cell types 4 and 14 remained significantly associated after adjustment for multiple comparisons in the total population (P=0.007 and P=0.002, respectively). These observations indicate that circulating angiogenic cell levels are inversely related to the levels of ambient 24-hour $PM_{2.5}$ levels and that each 10-μg/m^3 increase of $PM_{2.5}$ was associated with a 62% decrease in cell type 4 and an 81% decrease in cell type 14. The levels of cell types 5, 11, and 13, however, were not associated with $PM_{2.5}$ levels. Similar associations were observed when the dichotomous distance to major roadway variable was included in the model (data not shown). No significant association was observed between $PM_{2.5}$ levels and roadway proximity. Cell types 4 and 14 also remained significantly associated in the population with 6-month residential duration (P=0.007 and P<0.001, respectively) and after adjustment for multiple comparisons within that population (P=0.049 and P=0.005, respectively). Collectively, these data suggest that exposure to increased ambient $PM_{2.5}$ is associated with a decrease in the levels of circulating angiogenic cell levels. Although roadway proximity and $PM_{2.5}$ affect similar circulating angiogenic cell subpopulations, their effects are opposite to one another.

Discussion

The major finding of this study is that residential proximity to major roadways is associated with a selective increase in the levels of circulating angiogenic cells that are positive for AC133, an antigen that indicates an immature cell, early in the process of differentiation. The results obtained were similar when exposure was estimated using either as a straight line distance to major roadway, the sum of roadways in a 50-m buffer, traffic intensity within a 50-m buffer, or the distance-weighted roadway traffic density within a 300-m buffer. However, no association was observed between residential proximity to major roadways and the inflammatory marker high-sensitivity C-reactive protein, suggesting that changes in circulating angiogenic cell levels are unlikely to be driven

Table 7. Association Between 24-h $PM_{2.5}$ and Circulating Angiogenic Cell Levels

Circulating Angiogenic Cell Type	Change (%)	95% CI	*P* Value
Cell type 3 ($CD31^+/34^+/45^{dim}/AC133^+$)	−27.6	−52.1 to −1.68	0.037*
Cell type 4 ($CD31^+/34^+/45^+/AC133^+$)	−62.0	−95.5 to −26.5	0.001*†
Cell type 5 ($CD31^+/AC133^+$)	20.2	−4.91 to 45.5	0.117
Cell type 11 ($AC133^+$)	−2.00	−27.7 to 24.2	0.875
Cell type 13 ($CD34^+/AC133^+$)	−23.7	−48.1 to 0.84	0.058
Cell type 14 ($CD34^+/45^+/AC133^+$)	−81.5	−122 to −40.0	<0.001*†
Cell type 15 ($CD34^+/45^{dim}/AC133^+$)	−27.6	−52.8 to −2.44	0.032*

Percent change in cell populations per 10-μg/m^3 increase in regional $PM_{2.5}$ on the day before enrollment. CI indicates confidence interval; and $PM_{2.5}$, fine particulate matter with an aerodynamic diameter ≤2.5 μm.

*P<0.05.

†P<0.05 for population with 6-month residential duration.

significantly by an increase in systemic inflammation. Thus, regardless of other concurrent changes, our results suggest that the levels of angiogenic cells in peripheral blood may be useful biomarkers of cardiovascular injury associated with residential proximity to roadways or traffic exposure.

In the cohort examined, we found that the CVD risk in the individuals living closer than 50 m to major roadways was not higher than that in those living >50 m from a major roadway; therefore, the relationship between roadway proximity and circulating angiogenic cell levels could not be attributed to increased CVD risk. Moreover, it has been previously shown that higher CVD risk in individuals with stable CVD is associated with a decrease rather than an increase in circulating angiogenic cell levels.[39] Thus, higher circulating angiogenic cell levels in individuals living next to major roadways seem to contribute to CVD risk not reflected by traditional CVD risk factors. Also, the effect of roadway proximity on circulating angiogenic cells could not be attributed to the effects of ambient $PM_{2.5}$ exposure because the relationship was not affected after adjusting for ambient $PM_{2.5}$ levels. That the effects of roadway proximity are distinct from those of ambient fine PM is further supported by the observation that, despite a decrease in circulating angiogenic cell levels related to ambient $PM_{2.5}$ exposure, residential roadway proximity was associated with an increase in $AC133^+$ progenitor cell levels. Moreover, the effects of $PM_{2.5}$ were predominant on cell types 4 and 14, whereas roadway proximity affected cell types 5, 11, 13, and 15. Although both these populations include $CD34^+$ and $AC133^+$ cells, cell populations affected by $PM_{2.5}$ were $CD45^+$, whereas roadway proximity affected the entire population of $AC133^+$ or $CD34^+$ cells that were either $CD45^+$ or $CD45^-$ or those that were $CD45^{dim}$ (cell type 15). These findings suggest that roadway proximity and ambient $PM_{2.5}$ levels affect different cell populations and that $PM_{2.5}$ selectively affects cells retaining hematopoietic characteristics, whereas roadway proximity has greater effects on immature $AC133^+$ progenitor cells.

Previous work has shown that the reduced number of circulating angiogenic cells is associated with increased CVD risk and that the lower levels of these cells in peripheral blood predict future cardiovascular events.[40] Likewise, chronic exposures to environmental pollutants, such as $PM_{2.5}$ (Table 7) or tobacco smoke, are also associated with a decrease in the circulating levels of these cells.[41] In contrast to these findings, we found higher levels of these cells in individuals living close to a major roadway. Reasons for the anomalous increase in the levels of these cells because of roadway exposure are not clear but may be related to the milder nature of the injury induced by roadway pollutants compared with other insults. Increased levels of angiogenic cells in response to roadway pollutant exposure may be reflective of continuous mobilization of these cells from the bone marrow to peripheral blood, without the suppressive effects of stronger insults. In our previous work, we have found that exposure to the highly toxic pollutant acrolein leads to a 3- to 4-fold increase in the population of angiogenic cells in the bone marrow in mice, but the levels of these cells in circulation are decreased (by 40%) because mobilization of these cells is prevented due to a concurrent defect in vascular endothelial growth factor-1 and stromal cell–derived factor-1 signaling.[42] Our studies also show that exposure to concentrated $PM_{2.5}$ increases the bone marrow abundance of angiogenic cells in mice, although the circulating levels of these cells are decreased because of a selective defect in their mobilization by vascular endothelial growth factor and stromal cell–derived factor-1 but not stem cell factor. Indeed, in response to stem cell factor, more cells are recruited in the blood in $PM_{2.5}$-exposed mice than in mice exposed to filtered air.[43] On the basis of these observations, we speculate that, like acrolein and $PM_{2.5}$, roadway pollutant exposure increases the production of angiogenic cells in the bone marrow, but because there are no additional suppressive effects on mobilization, the levels of these cells are increased in the peripheral blood as well. Although further studies are required to test this hypothesis, elevated levels of angiogenic cells in the blood of individuals living close to a major roadway are consistent with the presence of mild and persistent vascular injury in these individuals.

Vascular injury secondary to burns or coronary artery bypass or myocardial infraction has been shown to be associated with an acute increase in circulating levels of angiogenic cells.[44,45] Exposure to secondhand smoke is also associated with increased levels of angiogenic cells in the peripheral blood 24 hours post exposure.[46] Thus, acute vascular injury seems to be a potent signal for the proliferation and mobilization of these cells particularly in individuals, such as those in our cohort with preexisting CVD and high CVD risk. In

addition, chronic insults, such as persistent tissue hypoxia, inflammation, or demand for tissue repair, could also lead to a persistent increase in the circulating levels of these cells. Several clinical studies have shown that the levels of these cells are chronically elevated in patients with cancer and that higher levels of these cells correlate with angiogenesis, metastases, and reduced patient survival.[47,48] Although all known cases of cancer were excluded from our analysis, chronically elevated levels of angiogenic cells in the blood of individuals living next to major roadways could be a symptom of incipient tumors, inflammation or tissue hypoxia, or ongoing vascular injury, conditions that lead to a persistent increase in the circulating levels of angiogenic cells, especially when the insult is mild and does not overwhelm mobilization. We found that in individuals living <50 m of a major roadway, the levels of these cells were 48% to 65% higher when compared with those living >50 m from a major roadway. In comparison, individuals exposed to secondhand smoke show a 100% to 310% increase in these cells, whereas myocardial infarction is associated with a 213% to 900%; and coronary artery bypass grafting with a 26- to 50-fold increase in the levels of circulating angiogenic cells.[44,46,49] The levels of these cells are chronically elevated 2- to 16-fold in patients with cancer in comparison with healthy controls.[48] Thus, in comparison with other insults, the effects of roadway pollutant seem to be less severe and are likely to be reflective of subclinical vascular injury resulting in increased angiogenic cell mobilization from the bone marrow.

When first mobilized from the bone marrow, the circulating angiogenic cells are mostly AC133$^+$, an indicator of their immature, early state in the process of differentiation. As these cells mature and differentiate, however, these cells lose AC133$^+$ expression.[23,24] The early AC133$^+$ cells also express the inhibitor of DNA binding, which is a robust indicator of the endothelial progenitor phenotype.[50–52] Thus, the selective increase in AC133$^+$ cells observed in our study cohort is consistent with a scenario wherein bone marrow activation leads to increased mobilization of immature angiogenic cells into peripheral blood to promote endothelial repair or regeneration. In contrast, we found no significant association between proximity to a major roadway and high-sensitivity C-reactive protein, suggesting that this was not because of generalized systemic inflammation in this cohort. Nevertheless, further work is required to assess any contribution of inflammation to cardiovascular injury induced by proximity to roadways and how this might be related to the overall increase in disease risk and mortality.

A major strength of our investigation is the relatively large study population combined with simultaneous measurements of conventional and novel CVD risk factors. Although, in comparison with environmental epidemiological studies, this size of the study population may seem small, most epidemiological studies use population level data, whereas our study is primarily based on individual level data. To the best of our knowledge, our study includes the largest number of circulating angiogenic cell phenotypes assessed to date. The range of CVD risk factors within our study population makes it a diverse group in which to investigate susceptibility to such roadway exposures, which may have had a lesser effect on a young healthy population. In addition, we measured a large number of phenotypically distinct circulating angiogenic cell populations to understand which specific populations were sensitive to residential roadway proximity. Accounting for potential major confounders, such as the levels of cotinine, a urine nicotine metabolite, in addition to collecting data on self-reported smoking status, did not alter the relationship with roadway proximity. Patients with cancer were excluded from the final regression analyses because circulating angiogenic cells are recruited in tumor angiogenesis,[50–52] which may disproportionally increase circulating angiogenic cell levels. Multiple indices of roadway exposure were included in this analysis to obtain a better assessment of traffic-related exposure, including variables of dichotomous 50-m roadway proximity, continuous sum of road segments in a 50-m buffer, continuous traffic intensity, and continuous distance-weighted roadway intensity in a 300-m buffer. Although it is a strength that we adjusted for multiple comparisons, results from this adjustment, however, should be interpreted with caution because multiple correction adjustments are not recommended for highly correlated variables,[53] as is the case with the circulating angiogenic cell populations in the current investigation.

Our study has several limitations. An important limitation is that we did not measure traffic noise, which has been associated with higher blood pressure[54] and increased risk of adverse cardiovascular outcomes.[55,56] Noise is associated with distance to roadway, and thus, it could be related to our outcomes. In addition, land use and tree cover, factors that can mediate or exacerbate the effects of traffic pollution exposure, were not measured in this study. Also, the use of road proximity as an indicator of exposure to traffic pollutants assumes that the study participants spend much of their time at home, and therefore, it does not account for the duration of time individuals spent outside their home, the proximity to roadways during other activities, or indoor exposures in the home. There was also no accounting for time spent in vehicles or in traffic, which has been associated with increased CVD risk.[57] Because our cohort comprised of individuals with high CVD risk, results obtained from this cohort may not be readily extrapolated to a general population of healthy individuals. Finally, because of the cross-sectional design of the study, causality could not be established. Long-term prospective studies are required to examine how recurrent exposure to traffic pollution affects circulating angiogenic cell levels and whether changes in their levels correspond to greater progression of CVD in individuals who live near major roadways.

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Disclosures

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Significance

The results of this study show that residential proximity to major roadways is associated with an increase in the levels of $AC133^+$ circulating angiogenic cell levels. This finding suggest that recurrent exposure to traffic could induce cardiac injury resulting in greater recruitment of premature angiogenic cells into the circulation. We found that the relationship between residential proximity to roadways and $AC133^+$ cells was not confounded by smoking, sex, or socioeconomic status and was not associated with concurrent changes in thrombosis, fibrinogen, or the levels of the inflammatory marker high-sensitivity C-reactive protein. The observed increase in these cells likely reflects an important mechanism that imparts excessive cardiovascular disease risk (perhaps independent of traditional risk factors) in individuals repeatedly exposed to traffic pollutants (eg, volatile organic compounds, particulate matter, and noise).