Proximity to Traffic, Inflammation, and Immune Function among Women in the Seattle, Washington, Area

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BACKGROUND: Traffic-related air pollution has been associated with adverse health outcomes, and the immune system may be a biologic mediator of health effects.

OBJECTIVES: We analyzed associations between living near major roads and immune status as measured by five immune assays. We hypothesized that living near a freeway, arterial, or truck route would be associated with increased inflammation and decreased immune function.

METHODS: We used a geographic information system (GIS) to determine residential proximity to major roads among 115 postmenopausal, overweight women in the greater Seattle, Washington (USA), area whose immunity was assessed at the baseline visit of an exercise intervention trial. We evaluated three inflammatory markers (C-reactive protein, serum amyloid A, and interleukin-6) and two functional assays of cellular immunity (natural killer (NK) cell cytotoxicity and T-lymphocyte proliferation).

RESULTS: Women living within 150 m of arterial roads had 21% lower NK cytotoxicity compared with women who lived farther than an arterial [mean cytotoxicity, 19.5%; 95% confidence interval (CI), 15.6–23.5%; vs. mean cytotoxicity, 24.8%; 95% CI, 22.0–27.5%], after adjustment for both individual-level and census tract–level demographic characteristics. This association was limited to women who reported exercising near traffic. Fewer women lived near freeways and truck routes. Markers of inflammation and lymphocyte proliferation did not consistently differ according to proximity to major roads.

CONCLUSIONS: If the observed association between residential proximity to traffic and decreased NK cytotoxicity is confirmed in other populations, our results may have implications for local land use policy.

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Previous studies of traffic-related pollution have observed associations between motor vehicle pollution and an array of adverse health outcomes, including respiratory and cardiovascular diseases (White et al. 2005). The immune system is a hypothesized biologic mediator of such health effects (Brook et al. 2004; Devalia et al. 1997). The strongest evidence of an association between air pollution and immune status in adults comes from studies of serum levels of C-reactive protein (CRP)—an acute-phase reactant and well-established marker of inflammation—in which indicators of increased air pollution were associated with higher CRP levels (Delfino et al. 2008; Diez Roux et al. 2006; Dubowsky et al. 2006; Peters et al. 2001; Pope et al. 2004; Riediker et al. 2004; Ruckerl et al. 2006; Seaton et al. 1999; Yue et al. 2007; Zeka et al. 2006). The hypothesis that air pollution may cause immune-suppressive effects is supported by animal studies demonstrating increased susceptibility to infection with diesel exhaust exposure [U.S. Environmental Protection Agency (EPA) 2002] and epidemiologic studies showing increases in hospital admissions for respiratory infections with increases in nitrogen dioxide among the general population (Busco et al. 2001; Lin et al. 2005). None of the previous studies of air pollution and immune function in adults has directly evaluated exposure to traffic.

We studied the association between traffic-related pollution and biomarkers of systemic inflammation and cellular immunity in the Puget Sound region of Washington State. Because traffic-related pollutants, such as nitrogen dioxide and ultrafine particles, are high near major roadways and then decay exponentially over a short distance (Lebret et al. 2000; Roorda-Knape et al. 1999; Zhu et al. 2002), we assessed exposure according to residential proximity to major roads. Our study population consisted of overweight, postmenopausal women, a group that may be particularly vulnerable to air pollution–related health effects, based on results of previous studies showing the strongest air pollution associations with inflammatory markers among obese persons (Dubowsky et al. 2006; Zeka et al. 2006).

We investigated three markers of systemic inflammation—CRP, serum amyloid A (SAA), and interleukin-6 (IL-6)—and two measures of cellular immunity, natural killer (NK) cell cytotoxicity and T-lymphocyte proliferation. CRP is a recognized predictor of cardiovascular disease, and SAA and IL-6 may also predict inflammation-related diseases (Ershler 1993; Johnson et al. 2004; Kritchevsky et al. 2005; Yeh and Willerson 2003). The NK cytotoxicity assay measures the ability of NK cells to kill cancerous target cells (Albers et al. 2005; Vedhara et al. 1999). Low levels of NK cytotoxicity are believed to reflect a defect in the natural immune response and may predict risk of future adverse health events, including infection and cancer (Imai et al. 2000; Levy et al. 1991; Mizutani et al. 1996; Ogata et al. 2001). Higher levels of T-lymphocyte proliferation are believed to reflect a more effective immune response (Albers et al. 2005; Imai et al. 2000; Levy et al. 1991; Ogata et al. 2001; Vedhara et al. 1999).

Materials and Methods

Study design. We conducted a cross-sectional analysis of the associations between traffic-related pollution and a set of five immune assays using data from the baseline visit of an intervention trial of exercise conducted at the Fred Hutchinson Cancer Research Center (FHCRC) (McTiernan et al. 1999). In our study procedures, we complied with all applicable U.S. requirements (including the FHCRC and University of Washington institutional review boards), and all women gave written informed consent before participation in the study.

Study population. Women were recruited from the greater Seattle area from 1998 to 2000 to participate in the Physical Activity for Address correspondence to A.J. De Roos, Fred Hutchinson Cancer Research Center, Program in Epidemiology, 1100 Fairview Ave. N, M4-B74, Seattle, WA 98109-1024 USA. Telephone: (206) 667-7315. Fax: (206) 667-4787. E-mail: aderoos@fhcrc.org

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Total Health study, a 12-month randomized controlled intervention trial comparing the effects of a moderate-intensity exercise intervention versus a stretching control program on endogenous sex hormones in postmenopausal women (McTiernan et al. 1999). Subjects in a substudy of immune function (n = 115) were women who also met criteria for measurement of immunologic outcomes. Women were 50–75 years of age, were nonsmokers, consumed fewer than two alcohol drinks per day, were sedentary, were overweight or obese [body mass index (BMI) ≥ 25.0; or between 24.0 and 24.9 with percentage body fat > 33%], were postmenopausal and not taking hormone replacement therapy in the preceding 6 months, and had no history of invasive cancer, diabetes, cardiovascular disease, or asthma; additional eligibility criteria have been published previously (McTiernan et al. 1999). Women in the substudy of immune function were eligible if they had no current serious allergies, were not regular (two or more times/week) users of aspirin or other nonsteroidal anti-inflammatory medications, and were not using corticosteroids or other medications known to affect immune function (Shade et al. 2004).

**Questionnaires and interviews.** Information on demographics (age, education, income, employment status, marital status, and race/ethnicity), smoking history, and exercise was collected via a self-administered questionnaire. Body height and weight were measured during a clinical exam using a standard protocol. Use of multivitamins was determined from an in-person interview with each subject where supplement labels were photocopied and data were abstracted.

**Immune measures.** All women came to the University of Washington Department of Laboratory Medicine for blood draws. Twelve-hour fasting blood samples were taken between 0730 and 0830 hours following strict blood-draw criteria described previously (Boynton et al. 2007). Serum and plasma were processed within 1 hr of collection and stored at −70°C. All immune assays were conducted at the University of Washington Clinical Immunology Laboratory in the Department of Laboratory Medicine.

We measured serum CRP and SAA by latex-enhanced nephelometry using high-sensitivity assays on the Behring Nephelometer II analyzer (Dade-Behring Diagnostics, Deerfield, IL) with lower detection limits of 0.2 mg/L for CRP and 0.7 mg/L for SAA. The interassay (between-batch) coefficients of variation (CVs) were 5–9% for CRP and 4–8% for SAA.

For serum IL-6, we added anti-CD3 antibody (BD Biosciences, Franklin Lakes, NJ) with lower detection limits of 3.3–diiodoacetylloxycarbonyl perchlorate (DiO; Live/Dead cytotoxicity kit no. L7010; Molecular Probes, Eugene, OR). We incubated, washed, and resuspended the cells to a concentration of 1 x 10⁶ cells/mL and then filtered them through a 35-μm strainer.

We serially diluted the culture-suspended NK cells to four effector-to-target cell (E:T) ratios of 50:1, 25:1, 12.5:1, and 6.25:1 and then pelleted and incubated the cells. We added propidium iodide to a final concentration of 0.03 mg/mL and transferred the cells to a polypropylene tube for flow cytometric analysis to identify dead cells. We used the percentage of dead target cells among a total DiO-identified target cells as the measure of NK cytotoxicity. We performed each assay in duplicate and with appropriate controls. Within-run CVs ranged from 5.9% to 8.9%.

We assessed T-lymphocyte proliferation using cryopreserved PBMCs with two methods: ³H-thymidine incorporation in response to the mitogen phytohemagglutinin (PHA), and the cell-division tracking method in response to anti-CD3 antibody. We have described these assays previously (Boynton et al. 2007). We prepared PBMCs by Ficoll-Hypaque separation and froze cells in 30% fetal calf serum, 60% RPMI medium, and 10% dimethyl sulfoxide (Gibco, Gaithersburg, MD). We included cells from two control subjects in every run.

For the ³H-thymidine incorporation, we incubated PBMCs in microtiter plates with PHA of 0.1 and 0.5 µg/mL in five replicates each. After incubation for 3 days at 37°C, we pulsed the cells for 24 hr with ³H-thymidine and then harvested and counted the cells with a β-counter. We express the PHA-stimulated lymphocyte proliferation index as counts per minute of stimulated cells divided by counts per minute of unstimulated cells.

For the cell-division tracking method, we added carboxy-fluorescein diacetate succinimidyl ester (Molecular Probes), a precursor of carboxy-fluorescein succinimidyl ester suspension at a final concentration of 10 μM. We incubated, washed twice, and resuspended the cells and then pipetted them into 16 wells of a microtiter plate (100,000 cells/well). Next, we added anti-CD3 antibody (BD Biosciences, San Jose, CA) to eight of the wells to specifically stimulate T lymphocytes. We used the remaining eight wells as control unstimulated cells. After incubation for 3 days at 37°C, we pooled identical wells and incubated the cells for 3 more days. On the sixth day, we
Traffic and immune status

Census covariates. We extracted census tract variables from the 2000 Census Summary File 3 (U.S. Census Bureau 2002), including variables describing the racial and ethnic composition, unemployment rate, median income, education level, housing tenure rate, and population and housing density of the residential census tract for each subject.

Statistical analysis. We performed all statistical analysis using SAS version 9.0 (SAS Institute Inc., Cary, NC). Because of skewed distributions, we transformed CRP and SAA data using the natural logarithm, and IL-6 using the natural logarithm of the observed value plus 0.5.

We used linear regression analysis to investigate associations between each of the traffic proxy metrics and each of the immune outcomes, adjusting for potential confounding factors. We considered CRP, SAA, IL-6, and T-lymphocyte proliferation stimulated by PHA or anti-CD3 as independent outcomes. We considered NK cytotoxicity as a nonindependent repeated measure in generalized estimating equations (GEEs) using the two intermediate E:T ratio dilutions: 12.5:1 and 25:1; these two dilutions had the greatest reproducibility and were in the linear range (Shade et al. 2004). For the dichotomous traffic proxy variables for residence within 150 m of a particular road type, we present model-adjusted least squares means and 95% confidence intervals (CIs) for each category, generated from the lsmeans statement of Proc GENMOD in SAS.

We selected variables a priori for adjustment as potential confounders, including age, BMI, education (individual-level), season of enrollment, time spent outdoors, and median income in the census tract of residence. We investigated associations of the traffic proxies with the immune outcomes among several pairs or trios of subgroup strata: a) overweight (BMI < 30) versus obese (BMI ≥ 30) subjects; b) low (< $35,000) versus middle ($35,000 to $75,000) versus high (> $75,000) income; and c) subjects reporting an hour or more of exercise near traffic versus subjects reporting no exercise near traffic in the previous 3 months on the baseline questionnaire.

Results

Study population. The 115 women in the study were highly educated, mostly non-Hispanic white, with high BMI; almost half the study population was classified as obese (BMI ≥ 30 kg/m²) (Table 1); 27% (n = 31) of women in the study lived near (within 150 m) major arterials, 3% (n = 3) lived near freeways,

Table 1. Study population characteristics.

| Characteristic                        | All subjects (n = 115) | Not near arterial* (n = 84) | Near arterial* (n = 31) | P Valuea |
|-------------------------------------|------------------------|-----------------------------|-------------------------|----------|
| Age, years [no. (%)]                |                        |                             |                         |          |
| 50–55                               | 33 (29)                | 23 (27)                     | 10 (32)                 | 0.38     |
| 56–60                               | 33 (29)                | 24 (29)                     | 9 (29)                  |          |
| 61–65                               | 16 (14)                | 13 (15)                     | 3 (10)                  |          |
| 66–70                               | 16 (14)                | 14 (17)                     | 2 (6)                   |          |
| 71–75                               | 17 (15)                | 10 (12)                     | 7 (23)                  |          |
| Mean ± SD                           | 60.7 ± 6.8             | 60.6 ± 6.7                  | 61.1 ± 7.3              | 0.55     |
| BMI (no. [%])                       |                        |                             |                         |          |
| ≤ 24 to < 30                        | 63 (55)                | 46 (55)                     | 17 (55)                 | 0.99     |
| ≥ 30 to < 35                        | 37 (32)                | 27 (32)                     | 10 (32)                 |          |
| ≥ 35                                | 15 (13)                | 11 (13)                     | 4 (13)                  |          |
| Mean ± SD                           | 30.3 ± 3.9             | 30.3 ± 3.9                  | 30.2 ± 3.9              | 0.95     |
| Race [no. (%)]                      |                        |                             |                         |          |
| White (not of Hispanic origin)      | 101 (89)               | 75 (90)                     | 26 (84)                 | 0.33     |
| Nonwhite                            | 13 (11)                | 8 (10)                      | 5 (16)                  |          |
| Education [no. [%]]                 |                        |                             |                         |          |
| High school or less                 | 17 (15)                | 13 (15)                     | 4 (13)                  | 0.54     |
| Some college or college degree      | 57 (50)                | 39 (46)                     | 18 (58)                 |          |
| Graduate degree                     | 41 (36)                | 32 (39)                     | 9 (29)                  |          |
| Season of enrollment [no. [%]]      |                        |                             |                         |          |
| Winter                              | 23 (20)                | 18 (21)                     | 6 (19)                  | 0.96     |
| Spring                              | 44 (38)                | 31 (37)                     | 12 (39)                 |          |
| Summer                              | 23 (20)                | 16 (19)                     | 7 (23)                  |          |
| Fall                                | 26 (22)                | 19 (23)                     | 6 (19)                  |          |
| Time spent outdoors [no. [%]]       |                        |                             |                         |          |
| 0–1 hr/week                         | 17 (15)                | 13 (15)                     | 4 (13)                  | 0.97     |
| 2 hr/week                           | 24 (21)                | 18 (21)                     | 6 (19)                  |          |
| 3–5 hr/week                         | 29 (25)                | 21 (25)                     | 8 (26)                  |          |
| 6 or more hr/week                   | 45 (39)                | 32 (38)                     | 13 (42)                 |          |
| Median income of the census tract of residence (mean ± SD)(1,000) | $58 ± $18 | $62 ± $19 | $50 ± $14 | 0.04 |

*Near arterial*: an arterial road was located within 150 m of a woman’s residence. For continuous variables, the P value is from a t-test (assuming equal variances) comparing the variable values between those living near arterials and those not living near arterials; for categorical variables, the P value is from a Pearson chi-square test of independence. Numbers not equal to total because of missing data.
and 15% (n = 9) of the 61 residents of Seattle, where truck routes were assessed, lived near designated truck routes. Women who lived near arterials were more likely to be younger, nonwhite, and less educated than those who did not and were more likely to live in census tracts in which the median income was lower; however, only the difference in census tract–level income was statistically significant (Table 1). Women who lived near arterials reported similar amounts of time spent outdoors compared with women who did not live near arterials. Table 2 shows the distribution of each immune outcome and the number of subjects with data for inclusion in analyses. We did not have adequate power to assess the effect of living near freeways, because only three subjects lived within 150 m of a freeway (data not shown).

We observed a statistically significant association, in our hypothesized direction of effect, between residence near arterials and NK cytotoxicity, within our a priori hypothesized at-risk group of women living within 150 m of major roads (Table 3). Those who lived near arterials had, on average, NK cytotoxicity (expressed as the percentage of cells killed) of 19.5% (95% CI, 15.6–23.4%), compared with 24.8% (95% CI, 22.0–27.5%) among those who did not live near arterials. NK cytotoxicity was also lower among those living near truck routes than among those who did not, although this was not significant at a two-sided α level of 0.05. These associations were not sensitive to our a priori choice of covariates for adjustment of potential confounding; for example, models without inclusion of the covariates generated similar estimates for the mean NK cytotoxicity among those living near arterials (19.3%; 95% CI, 15.5–23.0%) and those living farther away (24.8%; 95% CI, 22.1–27.6%). The association between NK cytotoxicity and living near arterials was limited to those women who reported exercising near traffic (Table 4) or among women in the middle group of individual income ($35,000 to $75,000) (data not shown).

We observed significant associations of NK cytotoxicity with living near arterials when using alternate cut points of either 100 or 200 m for our definition of proximity to traffic, and the magnitudes of the associations were similar to our main analysis, indicating that this result was not excessively sensitive to our a priori cut point of 150 m. Residential proximity to truck routes was not significantly associated with NK cytotoxicity in the analysis using 100 m as the alternate cut point, and the association was strongest when using the cut point of 200 m. Estimated NK cytotoxicity was 15.9% among women living within 200 m of truck routes compared with 26.2% among women living at greater distances, and this difference was highly significant (p = 0.0009; data not shown).

There were no trends of association with increasing proximity to major roads for either road type. In models including indicator variables for categories of distance from a major road compared with a reference category of >500 m [Supplemental Material, Table 1 (http://www.chonline.org/members/2008/11580/suppl.pdf)].

We observed no associations between the markers of inflammation (CRP, SAA, IL-6) or PHA-stimulated lymphocyte proliferation and the traffic proxy variables (Table 5). We noted a significantly lower average upper generational proliferation index—one of the four measures of anti-CD3–stimulated lymphocyte proliferation—associated with residence near major arterials (Table 5). The other three measures of anti-CD3–stimulated lymphocyte proliferation were not associated with residence near arterials (Table 5), nor was residence near truck routes associated with any of the anti-CD3–stimulated lymphocyte proliferation measures (data not shown).

**Discussion**

Ours is the first study, to our knowledge, of the association of traffic proxy measures and functional assays of cellular immunity. We observed an internally consistent association between residence within 150 m of major arterials or truck routes and lower NK cytotoxicity. The association was limited to those who reported exercising outdoors near traffic—individuals who presumably had higher exposure to traffic-related pollutants. We also found an association of living near arterials with lower than average lymphocyte proliferation; however, this result was not internally consistent among the various traffic proxy variables or lymphocyte proliferation measures, and we therefore consider the association tenuous. We observed no association between the traffic proxies and inflammation markers. In our modest-sized study, we analyzed five assays of immune status and several traffic proxy variables; therefore, our statistically significant results should be cautiously interpreted with respect to the possibility of chance findings and according to the consistency and robustness of the results.

**NK Cells are involved in the innate immune response and can destroy virally infected and transformed cells (Janeway et al. 2005). Although the relationship between in vivo NK function and NK cytotoxicity is unclear, low NK cytotoxicity has been associated with increased risk of infection, increased cancer risk, and increased risk of death among cancer survivors and institutionalized elderly (Albers et al. 2005; Imai et al. 2000; Kondo et al. 2003; Levy et al. 1991; Ogata et al. 2001). Researchers have found some**

**Table 2. Immune biomarker distributions in the study population.**

| Biomarker                             | No. | Mean ± SD | Minimum | Maximum |
|---------------------------------------|-----|-----------|---------|---------|
| **Inflammation markers**              |     |           |         |         |
| CRP (mg/L)                            | 114 | 3.7 ± 3.5 | 0.2     | 23.3    |
| SAA (mg/L)                            | 114 | 6.2 ± 5.5 | 1.4     | 43.0    |
| IL-6 (pg/mL)                          | 115 | 3.3 ± 3.1 | 0       | 20.0    |
| **NK cytotoxicity (%)**               |     |           |         |         |
| 25:1 E:T ratio                        | 114 | 26.9 ± 13.3| 4.35    | 68.9    |
| 12.5:1 E:T ratio                      | 114 | 19.8 ± 12.0| 3.15    | 59.6    |
| **PHA-stimulated lymphocyte proliferation** |     |           |         |         |
| Proliferation index—PHA 0.1 µg/mL    | 110 | 76.8 ± 47.5| 2.1     | 208.5   |
| Proliferation index—PHA 0.5 µg/mL    | 110 | 207.3 ± 95.9| 2.4     | 457.4   |
| **Anti-CD3–stimulated lymphocyte proliferation** |     |           |         |         |
| Proliferation index                   | 93  | 4.3 ± 2.0 | 1.3     | 11.8    |
| Precursor frequency                   | 93  | 0.3 ± 0.1 | 0.02    | 0.5     |
| Parent percent (%)                    | 93  | 15.0 ± 11.7| 4.4     | 71.9    |
| Upper generation proliferation index  | 93  | 15.3 ± 5.2| 7.3     | 31.7    |

*The number of women with nonmissing data for each assay is shown. *Counts per minute of stimulated cells/counts per minute of unstimulated cells. *Total number of cells (parent and newly proliferated) divided by the number of back-calculated original parent cells. *Fraction of cells from the source population (i.e., parent cells) that divided three or more times. *Percent of cells in the source cell population that did not divide. Total number of cells (parent and newly proliferated from generations three and above) divided by the number of back-calculated original parent cells.

**Table 3. NK cytotoxicity by residence near (within 150 m) different major road types.**

| Residence                        | No. | NK cytotoxicity (%) | | | |
|----------------------------------|-----|---------------------|------------------|
|                                  |     | [mean (95% CI)]     |                   |
| **Residence near arterial**      |     |                     |                   |
| No                               | 84  | 24.8 (22.0–27.5)    | | | |
| Yes                              | 30  | 19.5 (15.6–22.4)    | | | |
| Difference between means         |     | 0.047               |                   |
| **Residence near truck route**   |     |                     |                   |
| No                               | 51  | 25.5 (22.1–28.9)    | | | |
| Yes                              | 9   | 17.0 (10.9–23.1)    | | | |
| Difference between means         |     | 0.06                |                   |

For each major road type, we present model-adjusted least squares means of NK cytotoxicity at 12.5:1 and 25:1 E:T ratios generated from the lmeans statement in proc GENMOD using a generalized estimating equation regression, while accounting for within-person correlation. The p-values represent an analysis of variance test of the difference between means. Estimates are adjusted for covariates selected a priori age, BMI, education, blood draw season, time spent outdoors, and median income of residential census tract. *Truck routes were classified only for the City of Seattle, where 81 women lived, and 80 of these women had nonmissing data for NK cytotoxicity.
Our exposure metric reflects an average exposure level during a very recent time period. Higher CRP levels were associated with air pollution, and associations between air pollution and NK cytotoxicity would strengthen the evidence for the importance of CRP as a marker of cellular immunity. The strength of this study includes the objective outcome assessment using biomarkers, the strict criteria for blood draws, and the use of state-of-the-art assays for characterizing immune function. In the previous studies, there were several differences between our study and those of others (Albers et al. 2005; Frampton et al. 2004; Ghio et al. 2003; Gong et al. 2003; Seaton et al. 1999). We would expect that a human lymphocyte proliferation assay corresponds to a more effective physiologic immune response (Vedhara et al. 1999). Measures from lymphocyte proliferation assays have proven to be less sensitive than NK cytotoxicity assays in nutrition intervention studies, indicating the potential for lower sensitivity of lymphocyte proliferation to detect in vitro effects from external exposures (Albers et al. 2005). On the basis of previous studies, we hypothesized an association between proximity to traffic and increased levels of CRP. About half of the previous studies of air pollution and CRP have observed associations between CRP and at least one measure of air pollution (usually a specific pollutant, e.g., fine or coarse PM) (Delfino et al. 2008; Diez Roux et al. 2006; Dubowsky et al. 2006; Peters et al. 2001; Pope et al. 2004; Riediker et al. 2004; Ruckerl et al. 2006; Seaton et al. 1999; Yue et al. 2007; Zeke et al. 2006); the prior evidence for associations of specific air pollutants with other inflammatory markers, such as SAA or IL-6, is limited and conflicting (Delfino et al. 2008; Diez Roux et al. 2006; Frampton et al. 2004; Ghio et al. 2003; Gong et al. 2003; Seaton et al. 1999). There are several differences between our exposure metric and the studies that found an association with CRP. In the previous studies, higher CRP levels were associated with air pollution levels during a very recent time period such as the day before or preceding 1–9 days. Our exposure metric reflects an average exposure to local traffic-related pollutants in which short-term fluctuations due to background urban air pollution or personal exposures are not captured. It is possible that CRP levels (and other inflammatory markers) change in response to recent exposures in the preceding days or weeks, in which case our metric of average exposure would not be appropriate for modeling the association. Second, proximity to major roads represents the potential for mixed pollutant exposure from traffic. If the inflammatory response is specific to a certain pollutant such as PM, then the nonspecificity of our exposure metric may have obscured any association.

The strengths of this study include the objective outcome assessment using biomarkers, the strict criteria for blood draws, use of state-of-the-art assays for characterizing immune function, objective exposure assessment using a geographic information system (GIS), and the homogeneity of the study population. Our study also had several limitations. Although assigning exposure based on proximity to major roads is a straightforward approach to evaluating exposure to a pollution source, it is not as sophisticated as other approaches such as dispersion modeling. Measurement campaigns have indicated that although proximity measures are correlated with traffic pollutants, misclassification occurs due to varying terrain and meteorologic conditions (Jerrett et al. 2005); therefore, our traffic proxy measures are subject to misclassification that may have caused spurious results. The proximity metric we employed may also be more vulnerable to confounding by socioeconomic factors or traffic-related noise than would more specific air pollutant exposure models. Nevertheless, adjustment for multiple individual-level and census tract–level socioeconomic variables did not change the associations we observed.

In this article, we report an association between residential proximity to traffic and an important in vitro marker of cellular immunity.

### Table 4. NK cytotoxicity by residence near (within 150 m) different major road types and exercise near traffic.

| Residence | No exercise near traffic | Any exercise near traffic |
|-----------|--------------------------|--------------------------|
|           | Mean (95% CI)            | Mean (95% CI)            |
| Near arterial at road* |                          |                          |
| No        | 51 (21.4 (18.6–24.1)     | 26 (28.9 (23.9–33.8)     |
| Yes       | 11 (21.0 (13.6–28.5)     | 15 (20.7 (14.9–26.4)     |
| Difference between means | p = 0.93            | p = 0.08               |
| Near truck route at road* |                          |                          |
| No        | 23 (21.1 (17.7–24.4)     | 22 (29.7 (26.0–33.4)     |
| Yes       | 3 (24.6 (13.1–36.1)      | 6 (10.2 (2.9–17.5)       |
| Difference between means | p = 0.58            | p < 0.01               |

*For each major road type, we present model-adjusted least squares means of NK cytotoxicity at 12.51 and 25.1 E.T ratios generated from the Imsen means in proc GENMOD using a generalized estimating equation regression, while accounting for within-person correlation. The p-values represent an analysis of variance test of the difference between means. Estimates are adjusted for covariates selected a priori: age, BMI, education, blood draw season, time spent outdoors, and median income of residential census tract.

**Nonmissing data on both NK cytotoxicity and exercise near traffic were available for 103 women in the study area, and for 54 women in the City of Seattle where truck routes were assessed.**

### Table 5. Inflammation and lymphocyte proliferation measures by residence near (within 150 m) arterial road (estimated mean and 95% CIs).

| Measure | Near arterial | Yes (n = 31) | Difference between means |
|---------|--------------|--------------|--------------------------|
| CRP (mg/mL) | 2.5 (2.1–3.0) | 3.1 (1.8–3.1) | p = 0.62 |
| IL-6 (pg/mL) | 2.0 (2.0–2.5) | 2.1 (1.1–2.9) | p = 0.51 |
| SAA (mg/mL) | 4.9 (4.3–5.5) | 5.3 (4.3–6.6) | p = 0.52 |
| PHA-stimulated lymphocyte proliferation | n = 80 | n = 30 | 
| Proliferation index<sup>a</sup>—PHA 0.1 µg/mL | 75.2 (64.7–85.8) | 81.2 (63.6–98.7) | p = 0.57 |
| Proliferation index<sup>a</sup>—PHA 0.5 µg/mL | 206.9 (185.0–228.8) | 208.4 (172.0–244.9) | p = 0.94 |
| Anti-COD<sup>b</sup>—stimulated lymphocyte proliferation | n = 66 | n = 27 | 
| Proliferation index<sup>a</sup> | 4.9 (4.4–5.4) | 4.7 (4.0–5.5) | p = 0.68 |
| Parent percent<sup>c</sup> | 17.3 (14.3–20.3) | 16.2 (11.5–21.0) | p = 0.71 |
| Precursor frequency<sup>d</sup> | 0.26 (0.23–0.28) | 0.28 (0.24–0.32) | p = 0.32 |
| Upper generation proliferation index<sup>e</sup> | 16.0 (14.8–17.2) | 13.5 (11.7–15.5) | p = 0.04 |

*For each major road type, we present model-adjusted least squares means of each immune measure generated from the Imsen means in proc GENMOD. The p-values represent an analysis of variance test of the difference between means. Estimates are adjusted for covariates selected a priori: age, BMI, education, blood draw season, time spent outdoors, and median income of residential census tract.*

**Numbers reflect the distribution of the complete study population of 115 women; the number of women with nonmissing data for each assay varies, as shown.**

*There were no missing data for the IL-6 assay; n = 115. Counts per minute of stimulated cells/counts per minute of unstimulated cells. **Total number of cells (parent and newly proliferated) divided by the number of back-calculated original parent cells. ***Percentage of cells in the source cell population that did not divide. **Fraction of cells from the source population (i.e., parent cells) that divided three or more times. **Total number of cells (parent and newly proliferated from generations three and above) divided by the number of back-calculated original parent cells.**
immunity. Our study population was selected to be overweight or obese, but otherwise healthy. Approximately two-thirds of U.S. women in the age group we studied fall into this category (Mokdad et al. 2003; Ogden et al. 2006), and if the associations we observed are real, they would be relevant for a large proportion of the population. Additional studies of clinically relevant immune events such as infections and colds in relation to traffic-related pollution are needed to clarify the impact of traffic on the immune system and inform local land use policy.

Correction

The values for the two rows “Proliferation index—PHA” in Table 5 were incorrect in the manuscript originally published online. They have been corrected here.

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