STATEMENT

Synopsis of Research Report 134

HEALTH EFFECTS INSTITUTE

Black Material in Airway Cells from Healthy Children: Association with Lung Function and Modeled Levels of Particulate Matter

BACKGROUND

In the 1990s, several epidemiologic and controlled exposure studies suggested an association between exposure to air pollution from traffic-derived particulate matter (PM) — in particular the fraction of PM that is inhalable (i.e., has an aerodynamic diameter ≤ 10 µm [PM_{10}]) — and increases in symptoms of airway diseases, including exacerbations of asthma. Some studies had also suggested that exhaust from diesel engines — which are used in a large fraction of vehicles worldwide and particularly in Europe — contributed to these effects.

To address more fully the possible association between exposure to PM and the exacerbation of asthma and airway allergic conditions, HEI issued a request for applications, RFA 00-2, *Effects of Diesel Exhaust and Other Particles on the Exacerbation of Asthma and Other Allergic Diseases*. In response, Professor Jonathan Grigg, University of Leicester, United Kingdom, submitted an application titled "The relationship between pollutant particles in alveolar macrophages from normal children and proxy markers of PM_{10} exposure." Professor Grigg proposed to evaluate whether the quantification of particles in airway macrophages — the principal cell type that ingests (or *phagocytoses*) agents that enter the airways — could be used as a marker of children's exposure to PM_{10}. Professor Grigg also proposed to collect the airway macrophages by a noninvasive technique, the induction of sputum. HEI's Research Committee recommended Professor Grigg's proposal for funding; although the proposal was not directly responsive to the RFA, committee members thought the possible development of a noninvasive biomarker of exposure to PM would be useful for future studies of air pollutant effects.

AIMS

Grigg and colleagues' primary hypothesis was that the level of particles detectable in the airway macrophages of healthy children correlates with modeled estimates of local, traffic-derived PM_{10} at the children's home addresses. They were specifically interested in carbonaceous particles because carbon is a major component of particles derived from combustion sources such as traffic. A secondary objective was to determine whether the level of carbonaceous particles detected inside airway macrophages could be correlated with markers of airway inflammation. Although not part of their original aims, Grigg and colleagues also evaluated whether the area of carbonaceous particles inside the children's macrophages correlated with several pulmonary function parameters they had measured. Because they did not definitively determine that the particulate material inside the macrophages was carbonaceous, the investigators refer to it as "black-pigmented material" in the Investigators' Report.

Thus the report focuses on two associations: the association between amount of black-pigmented material detected in airway macrophages and modeled estimates of locally derived PM_{10} at the child's home address; and the association between the amount of black-pigmented material detected in airway macrophages and pulmonary function.

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This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Professor Jonathan Grigg of the Institute of Cell and Molecular Science, Barts and the London School of Medicine and Dentistry, London, United Kingdom. The complete report, *Black-Pigmented Material in Airway Macrophages from Healthy Children: Association with Lung Function and Modeled PM_{10}*, can be obtained from HEI or our Web site (see last page). **GRIGG 134**

APPROACH

Grigg and colleagues recruited 116 healthy children aged 8 to 15 years in and around the city of Leicester, United Kingdom. To increase the possibility of detecting a correlation between modeled exposure and particles detected in macrophages, the investigators selected children with widely different mobile-source-derived PM_{10} exposures: the highest-exposure group (>3.82 µg/m^3) and the lowest-exposure group (≤ 2.3 µg/m^3). The investigators measured baseline lung function in each participant: forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), the ratio of FEV_1 to FVC (FEV_1/FVC), and forced expiratory flow between 25% and 75% of the forced vital capacity ($FEF_{25\%–75\%}$). They calculated the percentage of predicted values for each child, taking into account the child's age, sex, ethnic origin, height, and weight. Based on the answers to a questionnaire they gave to the participants about their physical activity over the previous two weeks, the investigators calculated an activity score for each child.

Using nebulized hypertonic saline, Grigg and colleagues induced sputum in the participants and obtained 66 adequate samples for the study of airway macrophages. They excluded data from two children living at the same address because these children had much greater areas of black material in their macrophages than other children in the study. Of the remaining 64 participants, 40 identified themselves as white (i.e., they had parents of European extraction), 22 as Asian (i.e., their parents were from the Indian subcontinent), and 2 as "other."

The investigators prepared slides of the sputum-derived cells and performed a differential cell count for leukocyte subsets. Leukocytes, and macrophages in particular, were further characterized on slides by light microscopy. Grigg and colleagues captured and analyzed two-dimensional images of 100 macrophages, deleting the nucleus from each image because the image-analysis software identified it as a large particle. They then calculated the total area of particles inside each cell in micrometers squared. They also measured levels of the cytokine interleukin-8 (IL-8) — a neutrophil chemoattractant — in the supernatant of the sputum samples.

For each child at his or her home address, Grigg and colleagues estimated an annual exposure to PM_{10} derived from mobile sources. Estimates were obtained using the Airviro dispersion model, version 2.21, a geographic-information-system-based software that integrates meteorologic data and data on emissions of pollutants from different types of sources — specifically, *point* (such as industrial or commercial facilities), *line* (roads), and *area* (such as residential or large industrial estates) sources. For this study, only concentrations of PM_{10} from roads were estimated.

Grigg and colleagues used linear regression to investigate the relationships between the variables of interest. The response variable for most regressions was either modeled exposure or a measure of lung function, such as FEV_1, and the predictor variable of interest was the median area of black material. They also assessed results using a nonparametric test — the Spearman rank correlation test.

RESULTS AND INTERPRETATIONS

The investigators' main findings regarding their hypotheses were as follows:

- A weak correlation was found between particles detected in sputum-derived macrophages and annual PM_{10} exposure at the child's home; and
- No correlation was found between particles detected in sputum-derived macrophages and any marker of airway inflammation (the level of IL-8 and the percentage of neutrophils and eosinophils measured in sputum).

An additional and potentially important finding of the study was a negative correlation between the area of particles in children's sputum-derived macrophages and both FEV_1 and $FEF_{25\%–75\%}$. A 1-µm^2 increase in the area of black material inside macrophages was associated with a decrease of 17% in predicted FEV_1 and 35% in $FEF_{25\%–75\%}$.

DISCUSSION AND CONCLUSIONS

In this study, Grigg and colleagues attempted to establish whether carbon particles found in airway macrophages of healthy children in Leicester, England

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— a city with little or no heavy industrial sources of pollution — could be used as a biomarker of exposure to traffic-related PM_{10}.

Using light microscopy to identify black areas, presumably particles, in airway macrophages obtained by sputum induction, Grigg and colleagues found a weak correlation between the area of particles and estimates of annual PM_{10} exposure at the child's home address. At face value, these findings suggest that particles detected inside airway macrophages have the potential to be a useful marker of exposure to PM. However, several issues of study design and interpretation of data suggest that the study's main findings should be interpreted cautiously: one important uncertainty is the accuracy of the estimates of individual PM_{10} exposures obtained by using the Airviro dispersion model without validating this approach. A second important issue is that the findings may be confounded by ethnic origin, that is, that children of Asian origin — one of the two major subgroups of study participants, who as a group may have had different lung function — may have had higher modeled exposure to PM and levels of particles in the macrophages than white children, the other major subgroup of participants. In addition, although obtaining sputum-derived macrophages is noninvasive and these macrophages may be more easily obtained than cells deeper in the lungs, they may not be the cells that most accurately reflect an individual's exposure to PM_{10}. Particles detected in macrophages obtained from lower in the airways, particularly in the alveolar region, may be a more appropriate reflection of particle load. Furthermore, the investigators did not establish that the particles found inside the macrophages were carbonaceous or derived from traffic or, indeed, any other outdoor combustion source.

The investigators' attempt to determine whether particles detected in airway macrophages correlated with markers of airway inflammation was worthy. The fact that the investigators did not find associations between the area of particles in airway macrophages and any marker of airway inflammation, however, was perhaps not surprising, because one-time measures of these markers are likely to be more variable within individuals than measures of pulmonary function, such as FEV. In addition to the possibility that there is no correlation with the markers assessed, other possible explanations include that the sampling methods were too insensitive or inadequate to detect changes in the levels of markers or that the level of exposure to pollution of the children in this study was too low for any inflammatory effects of particles to be observed in the airways.

The investigators' finding of a negative correlation between the area of particles in the children's macrophages and the pulmonary function parameters FEV_1 and $FEF_{25\%–75\%}$ is potentially important. At face value, it suggests that greater exposure to PM may lead to impairment of children's lung function. Although the investigators did not specifically address the issue, particles identified in airway macrophages are likely to reflect long-term exposure to air pollution. Thus, Grigg and colleagues' finding is consistent with other data suggesting that long-term exposure to air pollutants affects the development of children's lung function. However, the magnitude of the changes in pulmonary function associated with increased particle area that Grigg and colleagues reported appears surprisingly large, casting doubt on the results of their regression modeling.

The main question explored by Grigg and colleagues — whether particles inside airway macrophages may be used as a biomarker of exposure to PM_{10} (and, in particular, traffic-derived PM_{10}) — remains interesting and important. However, given the caveats regarding the study design and the interpretation of the results discussed here, this study has not answered the question. Nevertheless, the potential importance of the study's main finding — that there are associations between particles detected in airway macrophages and a reduction in key lung function parameters — suggests that further studies are needed to investigate the reported associations.

Black-Pigmented Material in Airway Macrophages from Healthy Children: Association with Lung Function and Modeled PM_{10}

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INVESTIGATORS' REPORT

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