The Detrimental Health Effects of Traffic-related Air Pollution

A Role for DNA Methylation?

Exposure to air pollution and particularly that from diesel engines has been known for many years to affect asthma symptoms and hospitalizations (1) as well as all-cause (2) and cardiovascular mortality (3). Indeed, it has been shown that 2 hours of walking in shopping areas with high levels of diesel fumes (Oxford Street, London) containing ultrafine particles and elemental carbon can result in a significant loss of lung function and an increase in inflammatory mediators (4). Such changes were rapid and prolonged with a return to normal lung function occurring between 5 and 22 hours. The effect of chronic exposure was not examined.

Traffic-related air pollution consists of both gaseous and particulate-matter pollutants. The former include nitrogen dioxide, benzene, and sulfur dioxide. The latter consist of particulate matter of varying aerodynamic diameters. Because of their small size, these particles can be inhaled deeply into the lungs and deposited in the alveoli. In this issue of *Journal* (pp. 572–578), Baccarelli and colleagues (5) studied the effects of air pollution on the molecular level and in an epidemiological context by looking at associations between DNA methylation and short-term exposure to particulate air pollution.

Historically, the word "epigenetics" was used to describe events that could not be explained by genetic principles (6). If the information in DNA is likened to the notes of an orchestral score, epigentics is like the conductor who interprets and controls the dynamics of a symphonic performance. In this regard, cellular functions, including the regulation of inflammatory gene expression, DNA repair, and cell proliferation, are regulated by epigenetic changes such as DNA methylation. Methylation of CpG dinucleotides is generally associated with gene silencing and is maintained through cellular division. Multiple CpG sequences frequently occur at the transcriptional start site of active genes, with most clusters of CpGs being hypomethylated (7). These changes in DNA methylation were considered permanent until it was reported that dynamic cyclical alterations in DNA methylation occur in some estrogen-regulated genes (8, 9).

Baccarelli and colleagues' population-based cohort study (Normative Aging Study) (5) addressed several potential confounding factors including age, body mass index, cigarette smoking, statin use, and blood glucose and made statistical adjustments for these factors. Exposure to benzene is of particular interest in relation to DNA methylation because DNA methylation plays a role in benzene-related diseases and also appears to participate in contributing to the early effects of low-level benzene exposure (10). Because of these previous observations, black carbon or particulate matter with aerodynamic diameter less than 2.5 μm ($PM_{2.5}$) might present an epiphenomena or a proxy for exposure to benzene that is also traffic related. Moreover, the observation that only the black carbon effect on DNA methylation was statically significant in two-pollutant models (including both black carbon and $PM_{2.5}$) highlights traffic-related exposure as a main determinant of the observed changes in DNA methylation.

Can we link the present results with previously reported observations on the association between homocysteine and air pollution? Data from the Veterans Affairs Normative Aging Study showed that particles generated from traffic may elevate concentrations of plasma homocysteine (11). At the molecular level, it has been proposed that homocysteine induces protein homocysteinylation and endoplasmic reticulum dysfunction through an oxidative stress–mediated process. An alternative view, however, is that hyperhomocysteinaemia itself is not toxic, but that it inhibits methyl fluxes during transmethylation of methionine (12, 13). In methionine transmethylation, methionine is first transformed into *S*-adenosylmethionine, which is the methyl donor in reactions that yield methylated acceptors such as DNA and *S*-adenosylhomocysteine. *S*-adenosylhomocysteine is hydrolyzed to homocysteine. Hyperhomocysteinaemia will increase the concentration of *S*-adenosylhomocysteine, which is a powerful inhibitor of transmethylation reactions, possibly leading to the observed epigenetic associations reported in Baccarelli's article.

If homocysteine is an intermediate factor, then folic acid might be able to prevent the epigenetic changes induced by air pollution. Earlier, Baccarelli and colleagues reported (14) a repeated-measures study on heart rate variability in association with genetic polymorphisms (C677T methylenetetrahydrofolate reductase [*MTHFR*] and C1420T cytoplasmic serine hydroxymethyltransferase [*cSHMT*]) and dietary intakes of methyl nutrients that participate in the methionine cycle and contribute to biological processes such as methyl group transfers, homocysteine synthesis, and redox states that are potentially affected by $PM_{2.5}$ exposure. Both genetic and nutritional variations contribute to decreased methionine cycle function and affect heart rate variability either independently or by enhancing the negative effects of PM. In particular, carriers of [CT/TT] *MTHFR* genotypes exhibited lower heart rate variability, which was decreased further in the presence of higher ambient $PM_{2.5}$ in the 48 hours before the examination. In addition, the negative effects of $PM_{2.5}$ on heart rate variability were not observed in subjects with higher intakes (above the median) of vitamin B_6, vitamin B_{12}, or methionine.

The findings of Baccarelli and colleagues provide novel hypotheses aimed at investigating the mechanisms of action of air particles at the epigenetic level. If homocysteine is a causal intermediate in the link between air-pollution effects on epigenetic alterations, supplementation with folic acid may reduce the interactions of air pollution induced with methylation. These data begin to provide a molecular mechanism that begins to meet Hill's criteria for causality (15) and that would link air pollution with asthma and with other endpoints. If this mechanism is correct, this would put an onus on regulators, as polluters can no longer use the lack of understanding of fundamental mechanisms as an excuse to postpone the undertaking of preventive measures. Indeed, epidemiology reflects the experiment of nature, but, with inclusion of modern molecular tools, the observations become more fundamental. The data presented by Baccarelli and colleagues indicates that further efforts

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must be made to reduce air pollution by appropriate legislation and its enforcement.

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Fibrocytes as Potential Biomarkers in Idiopathic Pulmonary Fibrosis

Idiopathic pulmonary fibrosis (IPF) is characterized by myofibroblast accumulation and progressive lung scarring. Mesenchymal cells are responsible for deposition of extracellular matrix proteins such as collagen and fibronectin that fill the lung and compromise gas exchange (1). These mesenchymal cells may arise from three distinct sources. First, resident lung fibroblasts proliferate and differentiate into myofibroblasts, prodigious producers of collagen and contractile contributors to alveolar collapse and traction bronchiectasis (2). The second source may involve epithelial to mesenchymal transition, a process whereby epithelial cells release from basement membrane and undergo reprogramming that allows them to acquire a mesenchymal phenotype (3). The third source involves recruitment of circulating bone marrow–derived precursors, known as fibrocytes, which share mesenchymal and leukocyte markers (4). Animal models have indicated that circulating progenitors can augment airway and interstitial lung fibrosis (5–9). However, verification in human IPF is limited to two studies. One study analyzed four patients with the usual interstitial pneumonia form of IPF as well as one patient with nonspecific interstitial pneumonia and found that fibrocytes comprised between 6 and 10% of the leukocytes in the buffy coat compared with approximately 0.5% in normal controls (10). A histological study demonstrated the presence of cells that coexpressed mesenchymal and leukocyte markers in IPF lung (11).

In this issue of the *Journal*, Moeller and colleagues (pp. 588–594) report on a cohort of fifty-eight patients with IPF (12). Within this cohort, seven patients were identified as experiencing an acute exacerbation, whereas fifty-one were characterized as stable. Significant increases in percentages of circulating fibrocytes were found in both stable and acute cohorts with IPF when compared with healthy volunteers, but the numbers were most striking in patients with IPF acute exacerbations. One surprise in this study was that significantly increased percentages of circulating fibrocytes were not present in patients with acute respiratory distress syndrome. These results may reflect the fact that none of the ten patients with acute respiratory distress that were examined went on to develop fibroproliferative lung disease. These studies suggest that the percentage of fibrocytes in circulation may serve as a biomarker for the presence of fibrotic lung disease and may be a useful biomarker for acute exacerbations. Additionally, the current study indicates that fibrocyte percentages greater than 5% are independent predictors of early mortality in IPF. The fact that circulating fibrocytes are increased in the setting of IPF acute exacerbation, but not in the setting of acute respiratory distress syndrome, also suggests that the biological processes occurring in the setting of IPF acute exacerbations are fundamentally different from those occurring with diffuse alveolar damage during acute lung injury.

Now that the study by Moeller and colleagues (12) provides evidence for increased fibrocyte numbers on a robust cohort of patients with IPF, what are we to do with this information? I believe that these data beg for new areas of both basic science