



# STATEMENT

Synopsis of Research Report 105

HEALTH  
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## Genetic Differences in Induction of Acute Lung Injury and Inflammation in Mice

### INTRODUCTION

Epidemiologic studies have indicated that small, short-term increases in the concentration of particulate matter (the complex and variable mixture of particles in the atmosphere) are associated with short-term increases in morbidity and mortality in the population. Some individuals appear to be more at risk than others. Genetic differences among individuals may be one reason for their differing responses to particulate matter, but this mechanism is currently not well understood. One likely explanation for genetically determined differences in susceptibility is that some genes are present in the population as slightly different variations or alleles of a basic gene; as a result, these genes are expressed at higher or lower levels in some individuals than others. Identifying such genetic polymorphisms in individuals with a particular disease may help to identify those who are at risk of developing the disease, or alternatively who may respond best to treatment. An initial step in understanding the role of genetic control in responses to particulate matter is to study responses in mice because multiple animals of identical genetic composition (strains) can be generated easily.

### APPROACH

Dr George Leikauf and colleagues at the University of Cincinnati Medical Center hypothesized that the mouse response to high concentrations of inhaled nickel particles was under genetic control. Nickel has been shown to cause adverse effects at high concentrations in humans and in other species and is one of the important group of transition metals (which includes iron, copper, and vanadium) found in ambient air. The investigators sought to identify the genes involved in controlling the inflammatory and toxic effects of continuous exposure to nickel particles. The primary endpoint measured was an exposed mouse's survival time, or mean survival time for a group of exposed mice, but other endpoints related to lung inflammation and injury were

also measured. Leikauf and colleagues evaluated responses in different mouse strains; in first-generation offspring that resulted from crosses of different strains (F1 mice); in backcross mice—that is, F1 mice crossed with mice of one of the parental strains; and in mice expressing different levels of the human gene for transforming growth factor- $\alpha$  (TGF- $\alpha$ ), a factor associated with responses to lung injury. In the latter case, the TGF- $\alpha$  gene had been inserted into the mouse genome as a transgene, generating a transgenic mouse.

To identify the genes involved in the response to nickel, Leikauf and colleagues performed several complementary genetic and molecular analyses. The first was quantitative trait locus (QTL) analysis on backcross mice. This approach identified regions of individual chromosomes that were more or less closely associated with the trait or genetic characteristic of interest (namely, survival to nickel exposure). The second was haplotype analysis, which evaluated the contribution of one or several of these genetic regions to survival. The third was to use the novel microarray (or gene chip) technology; with this technique, the levels of expression of thousands of lung genes could be simultaneously evaluated during exposure to toxic levels of nickel.

### RESULTS AND INTERPRETATIONS

Leikauf and colleagues showed convincingly that genetic factors play a key role in determining the acute response of mice to nickel toxicity. Initial experiments indicated that mice could be separated into susceptible or resistant strains according to how long they survived exposure to highly toxic levels of nickel sulfate particles (at the extremes were A strain [50 hours] and B6 strain [130 hours]). The A and B6 mouse strains showed a similar pattern of survival response to 2 other toxic agents, ozone and polytetrafluoroethylene, suggesting that similar mechanisms may govern survival of exposure to all these agents. The investigators also found little correlation between some of the hallmark parameters of lung inflammation (increased protein

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level and percentage of neutrophils) and survival time in response to nickel exposure. This suggests that either lung inflammation per se or the inflammatory parameters evaluated in the study are not linked to survival.

Both QTL and haplotype analysis indicated that genes on 5 or 6 chromosomes, and a region on chromosome 6 in particular, were linked to survival to nickel exposure. The identified region on chromosome 6 contains genes for TGF- $\alpha$ , surfactant-associated protein B (SP-B), and aquaporin-1, genes that play a key protective role in lung responses to injury. Therefore, genes in this region are likely to be important in the response to nickel toxicity. Even after identifying a QTL or QTLs associated with a particular trait, however, definitively identifying the specific gene or genes responsible for the trait is a lengthy and complex task. The results of experiments in transgenic mice expressing human TGF- $\alpha$  also suggested the possible involvement of a mechanism involving both TGF- $\alpha$  and SP-B in survival: mice expressing the highest levels of the human TGF- $\alpha$  gene were the most resistant to nickel toxicity and showed the smallest decline in lung levels of SP-B.

Results from the microarray analysis indicated that a small fraction (about 200) of the more than 8,000 genes (examined in lung cells derived from either nickel-sensitive or nickel-resistant mice) changed their level of expression during exposure to nickel. The expression of some genes changed in both sensitive and resistant mice, whereas the expression of other genes changed only in sensitive mice or only in resistant mice. Genes whose levels of expression changed could be grouped functionally (for example, those involved in cellular metabolism and signal transduction) or temporally (those that showed steady or delayed increases or steady or delayed decreases in expression levels). Some changes in expression were detected in genes of unknown function, indicating that some of these unidentified genes may be important in

the response to nickel toxicity. The investigators enhanced the credibility of the findings from the microarray analysis by showing that selected genes with changed expression level when analyzed by other methods had similar patterns of change.

Generally, though, results from the microarray analysis could not be easily compared with those from the other genetic and molecular approaches because many genes of interest in inflammatory and injury responses were not present on the gene chip used. The use of microarrays has other limitations: It does not provide information about levels of proteins so it is not clear how detected changes in gene expression correlate with protein levels in the cell. In addition, the technique cannot distinguish in which cells, of the many found in the lung, gene expression changes are occurring. At least some of the gene expression changes detected at later times during the response to nickel probably occurred in cells that migrated into the lung as a consequence of the inflammatory response, rather than in lung cells per se.

Overall, Leikauf and colleagues have shown that mice respond to high toxic levels of inhaled nickel particles by altering gene expression. They have also preliminarily identified a small number of genes involved in susceptibility in this response. Similar genes may be involved in human responses to nickel particles in high concentrations. Additional studies are required to determine whether similar genes are involved in responses to low, ambient levels of nickel and other airborne pollutants. Further characterization of the genes involved in these responses will assist in efforts to understand the mechanisms by which pollutants act, characterize similarities and differences in gene expression among individuals' responses to a stimulus, and ultimately identify individuals who may be particularly susceptible to pollutant effects.