Alterations in lung structure can perpetuate inflammation leading to chronic respiratory disease

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The role of lung structure in perpetuating the inflammatory response to respiratory pathogens is poorly understood. Mucociliary clearance is a key component of the defence system against inhaled pathogens and relies on the balance between the quantity and chemical composition of the surface liquid and the physical capabilities of the cilia. A defect in any of these components can perpetuate the inflammatory response and lead to the establishment of chronic infection. For example, impaired ciliary motility, excessive mucous production, altered airway structure or altered mucous composition may all contribute to a deficit in mucociliary clearance. This is typified by cystic fibrosis (CF) where a defect in the CFTR gene leads to increased mucous viscosity which reduces mucociliary clearance resulting in chronic bacterial colonisation of the lower airways, chronic inflammation and bronchiectasis. While CF bronchiectasis is clearly the result of a specific genetic defect, the risk factors that predispose to chronic infection and bronchiectasis in non-CF patients are poorly understood. One of the most striking associations between bronchiectasis and a specific risk factor that may hint at mechanism/s comes from an exposure event in Antofagasta in Chile. During the late 1950s a new water source was tapped in order to supply a growing population. Unfortunately this new water source contained levels of arsenic approaching 1000 μg/L which is 100 times the limit considered to be safe by the World Health Organisation. Twenty years later this problem was discovered and the water supply was remediated. While arsenic is a well known carcinogen the highest standardised mortality ratio for all cause deaths in individuals aged 30-49 years who were exposed to arsenic in this population was bronchiectasis (SMR 46.2; 95% CI 21.1-87.7; $p < 0.001$) (Smith *et al.* 2006).

In order to explore the link between arsenic exposure *via* drinking water and non-malignant lung disease we exposed pregnant BALB/c, C3H/HeARC and C57BL/6 mice to water containing 0 or 100 μg/L arsenic ($NaAsO_2$) from day 8 gestation until birth of their young. At 2 weeks of age the offspring were anaesthetized (i.p.; ketamine 200 mg/kg, xylazine 10 mg/kg), tracheostomized and mechanically ventilated (10 ml/kg, 400 breaths/min, 2 cm H_2O PEEP). We measured lung volume and lung mechanics using plethysmography and a modification of the forced oscillation technique respectively. Groups of mice were euthanazed and had lung tissue harvested and stored in RNAlater for analysis of gene expression by microarray. Other groups of mice had their lungs fixed for stereological analysis of lung structure, quantification of goblet cells and expression of Clca3. We found that lung responses to arsenic exposure by drinking water *in utero* were genetically determined with no evidence of an effect on lung function in BALB/c mice, impaired airway mechanics in C3H/HeARC mice and impaired tissue mechanics in C57BL/6 mice. Analysis of gene expression by microarray (confirmed by PCR) showed a strong association between lung function outcomes and the pattern of differential gene expression with a gene related to cancer upregulated in arsenic exposed BALB/c mice (Ssxb), genes related to airway morphogenesis and cilial function (Sox2, Dynlrb2) upregulated in arsenic exposed C3H/HeARC mice and several genes related to mucous secretion and properties (Clca3, Lplunc1, Reg3γ, Scgb3a1, Tff2, Muc5b) upregulated in arsenic exposed C57BL/6 mice. In arsenic exposed C57BL/6 mice there was also evidence of higher numbers of goblet cells and increased expression of Clca3 in the airways. Thus, arsenic exposure altered genes related to airway branching (corresponding to impaired airway function), cilial function and mucous production. All of these factors can contribute to impaired mucociliary clearance and setup the environment necessary for the establishment of chronic respiratory infection and the development of bronchiectasis. These data demonstrate how early life insults can alter lung structure which may perpetuate inflammation leading to chronic lung disease.

Smith AH, Marshall G, Yuan Y, Ferreccio C, Liaw J, von Ehrenstein O, Steinmaus C, Bates MN & Selvin S (2006). Increase mortality from lung cancer and bronchiectasis in young adults after exposure to arsenic *in utero* and in early childhood. *Environmental Health Perspectives* **114:** 1293-1296.