

ANNEX 6

Updated chapter from WHO's report (2005) provided by Prof. Stephen Holgate

EFFECTS OF AIR POLLUTION ON THE CHILD'S RESPIRATORY SYSTEM

The child's respiratory system is a primary target for air pollutants. They cause a wide range of acute and chronic effects, either as a single risk factor or, more often, in combination with other external agents and/or the child's susceptibility characteristics. Here we review the role of exposure to air pollution in acute respiratory infections, in the development and manifestation of asthma and allergies, and in the development of lung function. The introductory section provides an overview of mechanisms of injury caused by air pollution on the child's respiratory system, addressing the possible links between pollution, acute infections and chronic respiratory diseases.

Besides the objectively or subjectively recognised symptoms, or objective measures of effects of pollution on lung function, some studies have addressed indirect indicators of ill health in children such as absenteeism from school. Since respiratory symptoms are the most plausible health reason for such absenteeism, a short summary of these studies is provided at the end of this chapter. These studies contribute to the overall evidence on the short-term effects of air pollution on children's health and activities.

MECHANISMS BY WHICH AIR POLLUTION INJURES THE CHILD'S RESPIRATORY SYSTEM

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BACKGROUND

The interaction of air pollutants with the lung represents a good example of interplay between genes and environment in a complex system. An important aspect of this is variation in the genes that protect from or generate a response to air pollutants to create variable susceptibility. The receptors and metabolic pathways involved mature at different rates during lung development and throughout childhood in relation to nutritional and environmental exposures giving rise to the concept of a critical window when vulnerability to the adverse effect(s) of a pollutant may be especially pronounced.

Although epidemiological studies reveal evidence for short-term effects of outdoor air pollutants on children's health and lung growth, few studies have examined the question of whether exposure to pollutants can initiate asthma, as has been shown in the case of passive smoking. However, evidence is accumulating from animal, in vitro and most recently in vivo studies showing that high levels of ambient air pollution increase the risk of children developing lung disease.

The lung is a highly complex heterogeneous structure with the principal function of delivering oxygen to and removing carbon dioxide from the body. On account of the enormous volume of air that passes into the lung during ventilation, it is well equipped to neutralize or break down chemical and biological substances present in the inhaled air. The epithelium that overlies the conducting airways and lines the alveoli has considerable capacity to protect the underlying cells and tissue from inhaled toxicants (1). In the case of outdoor air pollution, it is the oxidant pathways that are especially important, since the majority of the tissue-damaging effects of ozone, nitrogen oxides and particulates result from their direct or indirect actions as oxidants (2;3). There is important cell-cell communication within lung compartments fundamental to understanding how pollutants lead to damage and repair (4;5). More than 40 cell phenotypes have been found in the lungs and all have the

capacity to respond to a greater or lesser extent to toxic stress, even when only one subpopulation is exposed, such as columnar epithelial cells or Clara cells (6).

Age at the time of exposure to inhaled pollutants plays a major role in the pattern of injury and repair. This is especially true for the very young in the early postnatal period, when the respiratory system is completing its growth and maturation (7-9). Infants are more susceptible to injury by lung toxicants than are adults of the same species, even at doses below the no observable-effect level (NOEL) for adults (10;11). This appears to be closely governed by the differentiation of progenitor target cell populations and the induction and maturation of their relevant enzyme systems (12;13). Differential expression of detoxification systems also shows a time-dependant pattern during postnatal lung development, and could suggest mismatches between activation and detoxification potential that could account for the increased susceptibility of infants (14). There appear to be critical points during prenatal and postnatal lung development when this susceptibility is higher than at other times. Another impact of age as it relates to the postnatal development of infant lungs is the failure of acute epithelial injury in the lung to repair properly (15;16).

HOW THE LUNG DEVELOPS

The human lung begins to develop as an outgrowth from the foregut and undergoes complex linear growth and branching that is internally programmed within the primitive epithelial and mesenchymal cells that comprise the lung bud. The development of the human respiratory system begins approximately 24 days after fertilization (17). Branching of the airway system down the terminal bronchioles is complete by 17 weeks in utero, but further growth and cellular differentiation continues at various distinct periods until early adulthood (18). Alveolar development in humans starts at 28 weeks of gestation, but by term between one third and one half (150 million) of the ultimate number of alveoli (300-600 million) are present (19;20), the remainder developing rapidly after birth such that the final number is achieved by about 18 months of age (21).

Reciprocal signalling between the overlying epithelium and underlying mesenchymal stem cells, which occurs during lung development, results in alternating linear growth and branching (22;23). At different stages during branching morphogenesis and alveolar maturation, a series of growth factors and their receptors are engaged in the epithelium and underlying mesenchymal cells to produce a pre-programmed pattern of alveolar growth and branching (24;25) (Fig 1). Linear growth of the airways and accompanying blood vessels is promoted by growth factors, especially fibroblast growth factor 2 (FGF-2), bone morphogenic protein-4, (BMP-4) and vascular endothelial growth factor-A (VEGF-A), (26-29). FGF-2 is intimately involved in the developments of the subepithelial basement membrane, whose function is to integrate communication between the epithelium and the underlying mesenchyme (30). FGF-2 (31), as well as other FGFs (FGF-9 and FGF-10) and proteoglycans (32-34) and the cell adhesion molecule laminin- α 5 (35), are encrypted within the subepithelial basement membrane, enabling their biological functions to be finely controlled. At the level of the mesenchymal stem cells, proliferation and differentiation is regulated by *Sonic hedgehog* (*Shh*) protein and its target receptor *Patched* (*Ptc*) (36). *Shh* increases the expression of the *Ptc* as well as a set of epithelial and mesenchymal cell differentiating factors related to transforming growth factor- β (TGF- β) (TGF- β itself, BMP-4 and VEGF-A and *Noggin* (37)). Other molecules that contribute to lung development through their interaction with mesenchymal stem cells include proteoglycans and metalloproteases (MMP3 (38) and MMP9 (39)) that mediate remodelling responses within the tissues. Together, the opposing layers of epithelial and mesenchymal cells in the developing lung comprise the epithelial mesenchymal trophic unit (40-42). The area between the two layers of cells, the basement membrane zone, contains an extracellular matrix and a network of nerve fibres. Recognition of the attenuated fibroblast sheath as a distinct layer of resident fibroblasts is not only key to understanding branching morphogenesis in the developing fetal lung but also provides a basis for alterations in structure and function that follow lung injury, either in the fetus through placental transfer of toxicants or during the first few years of infant life by environmental factors that impinge

upon the epithelium (11;43), (Figure 2). Creation of adhesion plaques and gap junctions provides a means of communication, since the fibroblast sheath is an anatomical unit that is continuous throughout the interstitial space, including the alveoli. The concept of the epithelial mesenchymal trophic unit in establishing the trajectory and pattern of lung development in utero and during the first few years of postnatal life is fundamental to understanding how maternal diet and exposure to environmental chemicals might influence lung development and maturation in relation to the origin of diseases such as asthma (44;45). This includes alveolar development in the first three to five years of life (46) and the response of the airways and alveoli to environmental insults associated with chronic lung disease (47-50).

INFLUENCE OF POLLUTANTS ON LUNG DEVELOPMENT

With the differentiation and maturation of any organ, toxic substances that cross the placenta may influence development. It has long been known that tobacco smoking by the mother is one of the strongest environmental risk factors for developing asthma. This may possibly occur through its effects on lung morphogenesis linked to altered mesenchymal function and abnormal airway alveolar attachment points (51-53); it may also enhance the risk of the offspring being atopic (54) although this is controversial (55;56).

At present it is not known with certainty whether maternal exposure to high ambient air pollutant levels influence intrauterine lung development, although profound effects have been observed both in ferrets and in non-human primates over the postnatal period. Rasmussen & McClure (53) have described effects of NO₂ (0.5 ppm and 10 ppm) on postnatal lung development in ferrets. Over an exposure period of 14 weeks, these concentrations of NO₂ resulted in thickening of the alveolar walls, increased cellularity and collagen deposition indicative of oxidant damage. It remains possible that both the developing fetal lung and the postnatal lung during alveolar growth and maturation are especially sensitive periods, when air pollutant exposure impairs responses as revealed in epidemiological studies. NO₂ exposure can attenuate cigarette smoke-induced cytokine production in mice (57) but can promote allergic sensitisation (58). These interactions

further emphasise the complexity of NO₂ and its role in altering lung and immune development.

Ozone is a more powerful oxidant agent than NO₂ with a clearly defined effect in causing acute exacerbations of asthma, impairing lung growth and resulting in a greater decline in lung function over time, especially in children of low birth weight (59). Mild sequential exposures to ozone activate a range of signalling pathways (dependant upon the animal's age (60)). Acute inhalation of ozone damages both proximal and distal airway epithelium, initiating a cascade of inflammatory and functional responses that subside as the airway epithelium undergoes repair (61). In adult rhesus monkeys, repeated episodic exposure to ozone at high ambient concentrations, as occurs during photochemical pollution episodes, causes an altered response to ozone-induced epithelial damage resulting in diminution of inflammation and reduced epithelial cell proliferation. This diminished response to ozone-induced injury is associated with progressive airway remodelling, characterized by epithelial cell hypertrophy, hyperplasia and interstitial fibrosis (as well as hyperinnervation of the epithelium (62;63)). The possibility that ozone may alter the normal postnatal development of the lung is indicated by the identification of growth factors important in lung repair following injury, many of which are also involved in fetal lung morphogenesis. Thus, remodelling of the lung by environmental agents in many ways recapitulates the cellular and molecular pathways of lung development (64). As a result, infants repeatedly exposed to ozone would be expected to demonstrate alterations in the regional distribution and relative amounts of individual growth factors within the lung, which might compromise morphogenesis and lung maturation. Moreover, once these structural changes have taken place they are persistent (65).

Environmental factors frequently interact. In the rhesus monkey, episodic exposure to ozone and house dust mite antigen deplete the basement membrane zone of the proteoglycan perican and cause atypical development of this subepithelial zone (66;67). When studied in more detail, this dual insult resulted in altered regulation of fibroblast growth factors (e.g. FGF-2) in the airway epithelial mesenchymal trophic unit. The authors suggested that alterations in FGF-2 regulation are associated with atypical development of

the lung observed in rhesus monkeys after exposure to ozone. In fact monkeys sensitised to house dust mites, a combination of allergen and ozone exposure resulted in a greater inflammatory and mediator response as well as evidence of substantially greater airway wall remodelling than with either of these stimuli given alone (68-70). If these non-human primate studies translate to humans, this would suggest that atopic children exposed to cyclical high ambient ozone concentrations, as reported in cities such as Mexico City (59;71), might be at greater risk of developing asthma or disease of greater severity than would those exposed to clean air. Whether a similar effect could occur with other pollutants such as nitrogen dioxide or particulates, alone or in combination, requires further study. Preliminary evidence with diesel particulates in non-human primates suggests that similar responses to ozone occur, although the mechanisms have yet to be defined (72). The dramatic effect of high ambient ozone concentrations on the epithelial mesenchymal trophic unit in the developing primate lung, in disorganising the basement membrane and altering its interaction with growth factors and cytokines, has clear implications for the epidemiological studies that report adverse effects of air pollutants on lung growth (73-76).

ENHANCEMENT OF ALLERGIC INFLAMMATION

Asthma and rhinitis are characterized by polarisation of the immune response to a subset of T helper lymphocytes, designated Th2, with release of a range of pro-allergic cytokines encoded in a cluster on chromosome 5q31-34. There is subsequent recruitment of mast cells, eosinophils and basophils. Further, B lymphocytes tend to release IgE, the allergic antibody instead of IgG or IgM (77). Recently, there has been an increased focus on the role of vehicle-related air pollutants, specifically diesel exhaust particles (DEPs), in exacerbating allergic airways inflammation (78). In rodents, DEPs have been shown to exert a mucosal adjuvant effect to enhance existing allergic inflammation, including IgE production (79), the hallmark of atopy. Repeated exposure to low-dose diesel exhaust after allergen challenge also accentuates the Th2 type inflammatory response (80). Simultaneous exposure of DEPs with allergen in the human upper respiratory tract markedly increases IgE levels specific to the allergen while deviating the cytokine

repertoire towards a Th2 like pattern (81;82) DEPs have been shown to interact with ragweed allergen in the nasal mucosa, to drive in vivo isotype switching to IgE and to induce sensitisation to a new allergen in people who otherwise would not become sensitised (83). DEPs have also been shown to directly activate both mast cells (84) and basophils (85) for inflammatory mediator independent of IgE signalling. Diesel exhaust particles also embrace T cell activation in severe asthma (86). Taken together, these studies provide a basis whereby exposure to one form of particulate pollution may induce allergic sensitisation. The intra-individual reproducibility of nasal allergic responses to diesel or exhaust particles indicates a susceptible phenotype (87). It is not known whether exposure to ambient air pollutants can enhance allergic sensitisation in children, although there is evidence in non-human primates of a positive interaction between ozone and house dust mite exposures in enhancing both the immunological inflammatory airway responses in sensitised animals in parallel with airway remodelling (61;88). In vitro, interleukin-4 and interleukin-13, two important Th2 cytokine produced in allergic inflammatory responses, are able to interact with the epithelial mesenchymal trophic unit to enhance TGF- β and related growth factor production, thereby providing a mechanism for driving airway remodelling (89).

INTERACTIONS BETWEEN AIR POLLUTANTS AND INFECTIONS

There is an emerging literature indicating that innate immunity plays a key role in setting the direction of immune responses early in life (90). Antigen-presenting cells such as dendritic cells in the lung, as well as the epithelium itself, express a range of pattern (pathogen) recognition or toll-like receptors (TLR) that can be activated by a large range of biological pollutants in the environment. Examples of such interaction include double-strand viral RNA and TLR3, bacterial endotoxin (lipopolysaccharide) with TLR4 and unmethylated bacterial DNA (CpG) with TLR9 (91). Activation of these receptors serves as a “danger signal” and redirects the behaviour of an antigen-presenting cell if stimulated at the same time as contact with allergen occurs. Since chemical air pollutants are often encountered in the same environment as infectious agents or components of them, it is highly likely that at least some interaction occurs. One example of this is the influence of endotoxin exposure in reducing allergen sensitisation in children and associated rhinitis and asthma (92;93). This, in part, might explain why children raised in urban environments have in general a higher incidence of allergy than those raised in the countryside and on livestock farms (94). Since diesel particulates have been shown to augment the pro-inflammatory activity of microbial components acting through toll receptors (95;96), it is possible that this will have consequences if a child comes into contact with an infectious agent at the same time. Clearly, this is an important area for future research.

GENETIC SUSCEPTIBILITY TO AIR POLLUTANT-INDUCED LUNG INJURY AND REPAIR

It is now becoming clear that gene-environment interactions are pivotal in determining the susceptibility of individuals to the injurious effects of air pollutants and their long-term effects. The first line of antioxidant defence resides in the fluids lining the airways and alveoli, which are rich in a range of enzymatic and low-molecular-weight non-enzymatic antioxidants such as vitamins C and E (97). Epithelial cells in the airways and alveoli are protected against oxidative stress by a wide range of defences, including members of the glutathione S-transferase (GST) superfamily (GSTM1, GSTT1 and GSTP1). The GST enzymes use a wide variety of products of oxidative stress as substrates and have an important role in neutralizing reactive oxygen species. Common genetic variants of the GST genes exist, and some of these are associated with severe inflammatory disorders, including asthma. For example, Gilliland et al. (98) have reported that GSTM1-null children exposed to tobacco smoke in utero have an increased prevalence of early-onset asthma and a range of other respiratory conditions, and that the GSTP1 genotype increases both the risk and severity of respiratory infection in school-age children. Further studies by the same group have shown that the GSTM1-null or GSTP1 Ile105 genotypes exhibit enhanced nasal allergic responses to diesel exhaust particles (99). The GSTM1-null children showed a larger increase in IgE and histamine in nasal lavage fluid after exposure with DEPs or allergen than children with a functional GSTM1 allele. Because DEPs comprise approximately 40% of the PM₁₀ in major cities, these findings have implications for health consequences of ambient air pollution. The ability of DEPs to enhance allergic responses is highly repeatable within individuals but variable between individuals (100) and supports the view that genetic factors are important in determining individual sensitivity to air pollution. At an epidemiological level, a further study has shown that asthmatic children in Mexico city with a genetic deficiency of GSTM1 may be more susceptible to the deleterious effects of ozone on their small airways (101). Supplementation of the diet with vitamin C (250 mg/day) compensates for this genetic susceptibility (102-104). It remains possible that the association

between the antioxidant status of the diet and the clinical manifestations of asthma are mediated through this mechanism.

More recent epidemiological and chamber studies have also demonstrated that the –308-promoter polymorphism of TNF α increases the sensitivity of the airways to the bronchoconstrictor response to inhaled sulphur dioxide (105) and ozone (106).

CONCLUSIONS

The intrauterine, prenatal and early childhood periods, during which the lung is developing and maturing, constitute a particularly vulnerable time during which air pollutants may exert deleterious effects. With the knowledge that air pollutants can also enhance pro-allergic pathways in those genetically at risk, additional plausibility is provided for the potentially injurious effects of ambient air pollutants in the causation of paediatric lung disease, including asthma. The interaction between Th2-mediated inflammation and the epithelial mesenchymal trophic unit provides a basis for the origins of asthma, one set of environmental and genetic factors being responsible for predisposing to atopy and the other towards structural changes of tissue remodelling (Fig 3). One intriguing possibility is that the structural changes in the airways characteristic of asthma occur very early in life (e.g. before or shortly after birth) and are necessary to provide the microenvironment for Th2-mediated inflammation associated with atopy to take up long-term residence in the airways, a characteristic feature of chronic asthma. Thus, what has previously been termed “remodelling” sequential to inflammation may need to be renamed “premodelling” when applied to the onset of asthma. The genetic basis of lung growth and asthma (as opposed to atopy) may reside in the structural elements as well as involve immune or inflammatory cells (107;108). The importance of air pollutants alone or in concert with other environmental insults such as respiratory virus infections (109;110) allergen exposure (111-113) and diet in driving the epithelial mesenchymal trophic unit towards a chronic asthma phenotype (Fig 4) will only be recognised once careful monitoring of the environment and genetic susceptibility of the host are taken into account in relation to lung development over time.

SUMMARY

- The developing fetal lung, as well as the infant lung, is more susceptible to injury by lung toxicants that include air pollutants at doses below the no-effect level for adults.
- Detoxification systems exhibit a time-dependant pattern during pre- and post-natal lung development that in part accounts for the increased susceptibility of young children to pollutants, with critical points when susceptibility is higher than at other times.
- Animal studies indicate that intrauterine as well as postnatal exposure to pollutants can lead to impaired lung growth, a feature that has also been described in population-based longitudinal birth cohorts.
- Exposure to diesel particulates, both in vitro and in vivo in animals and humans, enhances the generation of the allergic antibody IgE and sensitisation to aeroallergens.
- Polymorphic variation in susceptibility genes involved in protecting against or driving tissue injury and repair explains some of the variation in individual susceptibility to the adverse health effects of pollutants.
- Based on current knowledge, air pollutants interact with other environmental exposures such as allergens, viruses and diet, that influence the overall impact of air pollutants on children's health.

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Figure Legends.

1) Development of the fetal lung.

In the fetus the lung develops as an outgrowth of the foregut. By differentially regulating the release and actions of growth factors secreted by the epithelium and underlying mesenchymal cells, the airway undergoes branching morphogenesis in which some factors promote linear growth other branching. Linear growth is driven by epidermal- and fibroblast-growth factors that induce the synthesis and release of metalloprotease enzymes. (MMP's) and degradation of extracellular matrix. Growth arrest and branching is promoted by members of the TGF- β family that inhibitors epithelial mesenchymal cell proliferation and reduces MMP-induced matrix degradation.

2) Effects of environmental agents on airway epithelium.

In conditions such as asthma and transplant rejection, damage to the airway epithelium and reduced capacity to efficiently repair leads to the production of pro-fibrogenetic growth factors, with the capacity to "remodel" the airways and cause thickening. In this way, the aberrant epithelial-mesenchymal communication in response to injury recapitulates some of the events in lung morphogenesis shown in figure 1.

3) Early life interactions in the development of asthma.

Important genetic and intrauterine environmental interactions are implicated in determining lung development, such as tobacco smoking and diet that influence both lung growth and maturation, as well as development of a competent immune response. In the case of allergic disease in the offspring, reduced Th-1type and increased Th-2 type immune responses extend into early childhood to increase the risk of allergy developing. For this to manifest itself in a specific organ such as the lungs, morphogenetic genes are involved that also have complex interactions with environmental factors. When these two components come together, clinical atopic disease emerges on further exposure to environmental insults.

4) The interaction between airway inflammation and tissue remodelling in the pathogenesis of asthma.

A disease such as chronic asthma necessitates both Th-2 type inflammation and an abnormal tissue response, with important interactions between the two. The interactive nature of the epithelium and associated mesenchyme has led to the term "epithelial mesenchymal trophic unit" to capture the concept that these cells are also involved in lung development and will be susceptible to environmental insults, including air pollutants.