Eur Respir J 2001; 18: Suppl. 32, 30s–42s
Printed in UK – all rights reserved

European Respiratory Journal
ISSN 0904-1850
ISBN 1-904097-01-4

REPORT OF WORKING GROUP 3

Interstitial lung disease induced by exogenous agents: factors governing susceptibility

B. Nemery*, A. Bast[#], J. Behr[¶], P.J.A. Borm[+], S.J. Bourke[§], Ph. Camus[ƒ], P. De Vuyst**, H.M. Jansen[##], V.L. Kinnula[¶¶], D. Lison[++], O. Pelkonen[§§], C. Saltini[ƒƒ]

Interstitial lung disease induced by exogenous agents: factors governing susceptibility. B. Nemery, A. Bast, J. Behr, P.J.A. Borm, S.J. Bourke, Ph. Camus, P. De Vuyst, H.M. Jansen, V.L. Kinnula, D. Lison, O. Pelkonen, C. Saltini. ©ERS Journals Ltd 2001.

ABSTRACT: The purpose of this review is to describe the present state of knowledge regarding host susceptibility factors that may determine the occurrence, development and severity of interstitial lung disease (ILD) caused by exogenous agents.

First, host susceptibility may pertain to differences in the delivery and/or persistence of the noxious agent in the lung. The deposition and clearance of inhaled particles or fibres may vary depending on innate anatomical or physiological characteristics, and on acquired changes, such as nasal disease or smoking-induced alterations. Genetically- or environmentally-induced interindividual differences in the expression of pulmonary biotransformation enzymes may form the basis for, or contribute to the risk of, drug-induced interstitial lung disease.

Secondly, there are genetic and acquired variations in various enzymatic and nonenzymatic defence systems that protect cells and tissues against oxidative stress, which is often involved in the pathogenesis of interstitial lung disease caused by particles, fibres, metals, organic agents and drugs.

Thirdly, the occurrence of immunological sensitization is dependent on both genetic and environmental factors. This has been demonstrated in chronic beryllium lung disease and in hypersensitivity pneumonitis.

Fourthly, the propensity of individuals to develop particular types of inflammation, such as granulomas, is probably under genetic control. The regulation and resolution of inflammation and fibrogenesis caused by dust particles are also partly determined by genetic factors, involving cytokine networks and growth factors.

In conclusion, although the issue of genetics pervades the entire discussion of host susceptibility, genes are not the only determinants of health and disease. Environmental factors may be equally important in shaping host susceptibility. Therefore, research must be focused on both the genetic bases and the environmental determinants of interstitial lung disease, in order to provide mechanism-based prevention strategies, early detection of, and improved therapy for these conditions.

Eur Respir J 2001; 18: Suppl. 32, 30s–42s.

*Laboratory of Pneumology (Lung Toxicology), Katholieke Universiteit Leuven, Leuven, Belgium. [#]Dept of Pharmacology, University of Maastricht, Maastricht, the Netherlands. [¶]Dept of Internal Medicine I, Division for Pulmonary Diseases, University of Munich, Munich, Germany. [+]Dept of Fibre and Particle Toxicology, Medical Institute for Environmental Hygiene, Düsseldorf, Germany. [§]Dept of Respiratory Medicine, Royal Victoria Infirmary, Newcastle upon Tyne, UK. [ƒ]Division of Pneumology and Critical Care, Centre Hospitalier Universitaire de Dijon, Dijon, France. **Division of Pneumology, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium. [##]Dept of Pulmonology, Academic Medical Centre, Amsterdam, the Netherlands. [¶¶]Dept of Internal Medicine, Division of Pulmonary Medicine, University of Oulu, Oulu, Finland. [++]Unit of Industrial Toxicology and Occupational Medicine, Louvain Catholic University, Belgium. [§§]Dept of Pharmacology and Toxicology, University of Oulu, Oulu, Finland. [ƒƒ]Division of Respiratory Diseases, Università di Roma "Tor Vergata", Ospedale L. Spallanzani-IRCCS, Rome, Italy.

Correspondence: B. Nemery, Laboratorium voor Pneumologie (Longtoxicologie), K.U. Leuven, Herestraat 49, B-3000 Leuven, Belgium.
Fax: 32 16347124

Keywords: Drug-induced lung disease
environment
genetics
hypersensitivity pneumonitis
oxidative stress
pneumoconioses

Although a number of interstitial lung diseases (ILDs), such as hypersensitivity pneumonitis, drug-induced parenchymal reactions, some metal-related lung diseases and mineral pneumoconioses, are associated with exposure to well-defined exogenous agents, little is known about the factors that determine the occurrence and/or severity of these diseases in individual subjects.

For some conditions, such as the mineral pneumoconioses (*e.g.* coal worker's pneumoconiosis (CWP), silicosis, asbestosis) or certain drug-induced reactions (*e.g.* amiodarone, bleomycin), the risk of lung disease is related, by and large, to the amount of material that the individual has been exposed to. However, even for these dose-related conditions, it is often found that people with similar degrees of exposure do not necessarily respond in similar ways. Consequently, the question is: what are the mechanisms that determine why some subjects exhibit high susceptibility, while others appear to be resistant to the development of significant pulmonary disease?

For other diseases, cumulative exposure is less

critical and host susceptibility plays a much more prominent role. This is the case, for instance, in hypersensitivity pneumonitis and berylliosis, where the disease process involves immunological sensitization, although even in these immunologically mediated processes, exposure intensity plays a role in the risk of sensitization and in the maintenance and progression of the disease. Here, the questions are: what are the reasons why some subjects become sensitized and what is the link between immunological sensitization and expression of disease?

The purpose of the present paper is to describe the present state of knowledge, and the gaps in this knowledge, regarding susceptibility factors that modulate the occurrence, development, severity and persistence of ILDs caused by exogenous agents, both those where the total burden is important and those where the relationship with dose is less straightforward. These questions are not only important for ILDs that are caused by known agents, they are also relevant for ILDs caused by hitherto unknown agents. In fact, a lot of the research conducted to unravel the mechanisms of specific types of ILDs might be applicable to the pathogenesis of idiopathic pulmonary fibrosis (IPF) or sarcoidosis.

To approach the issue of individual susceptibility to lung-damaging agents, four main mechanisms are proposed, derived, in part, from a conceptual scheme that is generally applicable to toxicology [1]. The first mechanism concerns the issue of delivery (and persistence) of the toxic agent to its targets; the second and third mechanisms deal with biochemical and immunological responses, respectively, which mainly take place at the cellular level; the fourth mechanism concerns the cascade of inflammatory and other events, including fibrosis, at the tissue and organ level. These successive mechanisms are not mutually exclusive. For some agents and diseases more than one mechanism may apply or interact, and in some individuals, several mechanisms may coexist to produce ILD.

Mechanisms of host susceptibility

Toxicokinetic factors

One mechanism of susceptibility may be broadly defined as pertaining to differences in the delivery and/or persistence of the noxious agent in the lung. Within this category, a distinction may be made between inhaled aerosols and organic chemicals, which reach the lung *via* the airways or blood circulation.

Inhaled agents. Background. Individuals may vary in the way particles or fibres penetrate into and are cleared from their respiratory tracts. Such variations may be due to innate anatomical or physiological characteristics, or to acquired changes, such as nasal disease or smoking-induced alterations.

Modelling studies, as well as experimental observations, have shown that the branching pattern of the bronchial tract and the ventilation pattern determine the overall and regional deposition of inhaled aerosols [2–4]. Although BECKLAKE *et al.* [5] found that subjects with asbestosis were smaller and had shorter intrathoracic tracheal lengths and narrower transthoracic diameters than equally exposed controls, this line of investigation does not appear to have been pursued further and the practical implications of individual variations in anatomical characteristics, in terms of susceptibility to dust-induced lung disease, are not known. What is better established, however, is the general concept that exercise enhances the pollutant dose delivered to the lung because it increases minute ventilation and causes a switch from nasal to oronasal breathing [6]. A well-known application of the notion that exercise increases pollutant dose is to be found in the field of ozone toxicity, where standard exposure protocols include moderate exercise, and where it is recommended that heavy exercise should be avoided during smog alerts.

Some disease states may influence the deposition profile of inhaled particles. Thus, it has been shown experimentally, both in animals and humans, that the pattern of particle deposition in the lung is altered by the presence of chronic bronchitis or emphysema. An almost double deposition of particles was observed in the airways of rats with chronic bronchitis, compared to controls [7]. In a hamster model of emphysema, the deposition of particles in the lung was found to be more heterogeneous than in control animals [8]. The pulmonary deposition rate of fine particles (2 μm) was found to be ~2.5 times greater in chronic obstructive pulmonary disease (COPD) patients, compared to controls, and among COPD patients the deposition rate was increased with the degree of airways obstruction [9]. It has also been reported that the respiratory tract deposition of ultrafine particles is increased in COPD patients [10]. Obstructive airways disease should, therefore, be considered as a condition that modifies the deposition rate of inhaled particles and predisposes to the formation of "hot spots" of concentrated particles in the respiratory tract. In addition, poor clearance of insoluble particles and fibres from the alveoli and interstitium may be caused by defective ciliary motion, inappropriate mucus characteristics, or insufficient lymphatic drainage. Thus, in patients with chronic bronchitis, it has been shown that the clearance of particles in small airways is reduced, compared to controls [11].

It is intuitively accepted that particle deposition and clearance must be of great importance in determining the final burden of fibrogenic materials in the lung, but to what extent these factors effectively determine human susceptibility to develop pneumoconiosis is still largely unknown. However, it has been hypothesized that the destruction of hilar lymph nodes by "silicotic" fibrosis impairs lung clearance and could thus, play an important role in the development of progressive massive fibrosis (PMF) and, in subjects exposed to very high concentrations of respirable quartz, of rapidly progressive silicosis [12].

Examples in the area of interstitial lung disease. The role of quantitative differences in the deposition and persistence of inhaled fibrogenic agents has

been extensively studied in asbestosis. It is commonly accepted that asbestosis will not develop to produce clinical manifestations below lifetime exposures of 25 fibres·mL^{-1}·yr^{-1} and that the relationship between cumulative exposure and risk of asbestosis is approximately linear [13]. However, this also implies that not all similarly exposed subjects will develop asbestosis. Although many factors may account for this variability in susceptibility, individual differences in fibre retention are thought to be important. Thus, experimental studies in the sheep model by BÉGIN and coworkers [14, 15] have documented a higher degree of fibre retention, as evaluated by bronchoalveolar lavage (BAL), in those animals with asbestosis, compared to those without disease, despite similar exposure. Alveolar dust retention preceded the disease. In males with established asbestosis, mineral analyses of BAL or lung tissue have consistently shown higher burdens of asbestos fibres or asbestos bodies than in exposed males with no apparent disease or disease that is limited to the airways or pleura [15–17]. Asbestosis, or at least its radiological manifestations, also appears to be more prevalent among smokers than nonsmokers, which may be partly due to a higher fibre deposition and poorer clearance in smokers [18].

Several *post mortem* studies have shown that in coal workers, 40–60 g of total dust may be found in the lungs, with an accumulation rate of 0.4–1.7 g of dust retained each year [19]. The retained free silica load is usually a reflection of its content in respirable dust, but is "concentrated" in lymph nodes, compared to lung tissue. Although it has been suggested [20] that there is an increased quartz retention in those with CWP and PMF, most other studies [21] have not reported such a simple difference and have included coal rank as an important factor in quartz retention [22].

Organic chemicals. Background. Chemical-induced toxicity is generally the result of the production of reactive intermediates by biotransformation enzymes and the balance between such activation and other detoxication pathways [23]. Consequently, inter-individual differences in the expression of these enzymes may form the basis for, or contribute to the risk of, adverse drug reactions. This concept has been verified mainly in the field of hepatotoxicity [24] and rarely with regard to drug-induced pneumotoxicity. However, susceptibility to lung cancer has been correlated to some extent, with a high potential for activation of polycyclic aromatic hydrocarbons coupled with a low potential for enzymatic detoxication by glutathione [25]. Similarly, an association between susceptibility to emphysema/COPD and a polymorphism for microsomal epoxide hydrolase has been described [26].

Variations in the rate and pathways of the biotransformation of foreign compounds may be determined by genetic polymorphism (leading to so-called "idiosyncratic" reactions) and/or environmental factors (interactions with pollutants, dietary factors, other drugs, *etc*).

Several different xenobiotic-metabolizing cytochrome P450 (CYP) and conjugation enzymes have been shown to be present in the respiratory tract, including the lung parenchyma [27–29]. An overview of the expression and localization of these drug-metabolizing enzymes in the human lung is presented in the article by HUKKANEN *et al.* [30] in this Supplement. In contrast to the liver, the cellular distribution of biotransformation enzymes in the lung is very heterogeneous, with some cells, such as non-ciliated bronchiolar (Clara) cells and type II pneumocytes, containing a higher activity than others. However, this does not mean that other cells, such as pulmonary endothelial cells, alveolar macrophages or even type I pneumocytes, do not possess any xenobiotic-metabolizing potential [27]. Besides CYP, other enzyme systems, such as flavine-dependent mono-oxygenase or prostaglandin G synthase, may also be involved in drug metabolism in the lung.

In general, the pulmonary biotransformation enzymes do not appear to differ substantially from those found in the liver, but the relative proportion and cellular distribution of isozymes and the ratio of activating to detoxifying enzymes may well be different, thus explaining why some chemicals exert a specific toxicity in the lung, even when they are also metabolized in the liver. Differences in the species' susceptibility to chemical-induced lung toxicity are sometimes quite pronounced, pointing to the possibility that individual differences in biotransformation pathways could also influence susceptibility in humans.

There is a large body of experimental evidence from animal studies that suggests that some lung toxicants need metabolic activation by pulmonary CYP enzymes to be able to cause toxicity (table 1).

Monocrotaline, a pyrrolizidine alkaloid, causes a pulmonary vascular response leading to both acute and more delayed types of pneumotoxicity. However, it is not known whether the critical metabolic activation occurs in the liver or in lungs [36], and furthermore, there is no knowledge about the exact nature of the activating CYP enzymes. Naphthalene causes pneumotoxicity in mice, possibly by activation *via* CYP2F1 [42], but this reaction is highly species-specific and it is not known whether it also occurs in humans [36]. 4-Ipomeanol is activated locally in the lungs by CYP2B enzymes [43], but again there is a possibility that this is a species-specific reaction. 3-Methylindole is a selective toxicant to pulmonary Clara cells [36]. Coumarin causes a selective Clara cell injury in mouse lung, probably *via* activation by CYP2B enzymes [31]. Interestingly, Clara cell CYP2E1 and type II cell CYP1A1 are induced by chronic treatment of Wistar rats with quartz-containing particles [44, 45]. It is not known whether this induction is associated with increased inflammation or with the fibrotic process.

Examples in the area of interstitial lung disease. For certain potentially pneumotoxic drugs, there is some experimental evidence of the role of pharmacokinetic factors in their toxicity. Thus, activities of bleomycin hydrolase, which detoxifies bleomycin, appear to determine, at least in part, the degree of

Table 1. – Examples of pneumotoxic chemicals (in animals) requiring metabolic activation

Chemical	Species/mechanism	Activating enzyme	References
Coumarin	Mouse/Clara cell toxicity	CYP2B?	[31]
Coumarin	Rat/general toxicity		[31]
1,1-Dichloroethylene	Mouse/general?	CYP2E1?	[32, 33]
4-Ipomeanol	Mouse/Clara	CYP2B?	[34]
Methylene chloride	Mouse/Clara	?	[35]
3-Methylindole	Mouse/Clara	?	[36]
Monocrotaline	Various/vascular injury	?	[36]
Naphthalene	Mouse/Clara	CYP2F1	[36–38]
1-Nitronaphthalene	Rodent/general	?	[39]
Paracetamol	Mouse/Clara	CYP2E1?	[40]
Trichloroethylene	Mouse/Clara	?	[41]

?: uncertainty.

toxicity of this drug in mice [46]. However, it is fair to say that the pulmonary metabolism of most drugs that exhibit frequent or occasional pneumotoxicity in humans has hardly been studied [47]. Most studies of systemic lung toxicity have been conducted in experimental animals with model compounds.

It is accepted that xenobiotic-metabolizing enzymes also exhibit considerable variability in the lungs, and consequently, activation reactions, as well as detoxication reactions, would also be highly variable. However, there is very little knowledge about genetic or nongenetic factors in variability. In addition, the specific pattern of enzymes expressed in the lungs seems to show at least some species-specificity.

In conclusion, research is still required to understand even the basal expression and modifying factors of xenobiotic-metabolizing enzymes in human pulmonary tissue and their possible involvement in ILD, particularly those variants that are caused by foreign chemicals, such as therapeutic drugs.

Biochemical defence mechanisms

A second type of susceptibility mechanism relates to the broad area of the pulmonary cellular defence against noxious agents.

Background. An issue that is attracting considerable interest in many areas of biomedical research, including fibrogenesis [48], is the cell′s defence against oxidative attack. Various enzymatic and nonenzymatic defence systems exist to protect cells and tissues from oxidants, and it is possible that genetic and acquired variations in these systems account for interindividual variation in the response to oxidative stress. This is especially relevant for the lung, which is exposed to oxygen and oxidant pollutants, including those present in cigarette smoke. Moreover, lung cells are also a potential target of the endogenous production of oxidants by inflammatory cells.

The enzymes involved in the cellular defence against toxic oxygen species include the superoxide dismutases (SODs), catalase and glutathione peroxidases, and the glutathione system. Eukaryotes contain three different types of SOD: a copper- and zinc-containing form (CuZnSOD) in the cytosol; a manganese-containing form (MnSOD) in the mitochondria; and an extracellular form (ECSOD) in the extracellular space. CuZnSOD is constitutively expressed, especially in bronchial epithelium, and MnSOD is induced by oxidants and cytokines, mainly in alveolar macrophages and alveolar epithelium. Catalase is localized to peroxisomes and constitutively expressed in alveolar pneumocytes and blood neutrophils, and to a lesser degree, in alveolar macrophages and bronchial epithelium [49–51]. ECSOD is a secretory Cu/Zn-containing glycoprotein, synthesized in at least alveolar macrophages and is the major SOD in the extracellular fluids in the human lung [52]. Other important proteins with antioxidant properties have also been identified in the lung, the most important of them being glutathione-S-transferases, haemoxygenase and the thioredoxin reductase system. Moreover, a network of nonenzymatic antioxidants exists in the lung. Comparison of two extracellular fluids, *i.e.* plasma and the lung epithelial lining fluid (ELF), shows a different contribution of the various nonenzymatic antioxidants. The concentration of glutathione is 300-times higher in human ELF than in plasma. Glutathione is synthesized by the rate limiting enzyme gamma-glutamyl cysteine synthetase, but both the distribution and expression of this enzyme in the human lung are largely unknown. A greater understanding of the way in which the relatively high levels of glutathione are achieved in the ELF is needed. Thus far, the changes that occur in the pulmonary antioxidant system upon oxidative stress have not been adequately defined. It is clear, however, that adequate characterization of changes in the antioxidants in various lung compartments is necessary for rational antioxidant supplementation.

A large amount of research, using various *in vivo* and *in vitro* experimental systems, has been carried out to assess the lung′s response to oxidative stress caused by high concentrations of oxygen, ozone, inhaled or circulating foreign compounds, including particles, and oxidants released from inflammatory cells [53]. Interindividual susceptibility in response to ozone has been the subject of considerable research. Highly reproducible, significant, interindividual variations in human pulmonary function responses to ozone support the hypothesis that genetic background is an

important determinant in susceptibility to ozone [54]. Genetic linkage analyses in a variety of mice strains with varying susceptibility to ozone have indicated that the resistant phenotype was linked to chromosome 11 [55]. Mice, genetically deficient in Clara cell protein (CC10 or CC16), were found to be hypersusceptible to ozone exposure, indicating that this protein has a protective role in the defence against oxidant-mediated lung injury [56]. Some research in the field of oxidative stress has also been devoted to acute lung injury (hyperoxia, acute respiratory distress syndrome (ARDS)) and chronic obstructive disease in humans [57, 58]. The importance of proteins with antioxidant properties in the susceptibility to environmental lung diseases is also supported by a recent study that shows an association between a microsatellite polymorphism in the haem oxygenase-1 gene promoter and the development of pulmonary emphysema [59].

In smokers, elevated levels of glutathione in the ELF have been described, which are thought to be a defence against oxidative damage induced by cigarette smoke [60]. Interindividual variability of mechanisms regulating glutathione levels in response to exogenous toxic substances may be involved in disease manifestation. However, knowledge about these pathophysiological links is, as yet, mainly speculative.

Although there is some biochemical and experimental evidence linking oxidative stress and a lack of antioxidant defence to the pathogenesis of diverse interstitial lung diseases, the contribution of these factors to the destruction of pulmonary architecture *in vivo* is not known. Intervention studies in animal models of lung fibrosis using antioxidants such as *N*-acetylcysteine (NAC) or SOD, may give some insight into this important area. From the studies, NAC has been shown to reduce inflammation and formation of fibrosis in a variety of animal models employing hyperbaric oxygen, bleomycin, or amiodarone [61–64]. In parallel to these observations, a positive effect on redox balance and formation of oxidation products has also been shown in patients with fibrosing alveolitis using oral NAC as a glutathione precursor [65, 66]. Moreover, intracellular glutathione levels have been linked to the activation of transcription factors, gene expression, and the release of proinflammatory mediators, making glutathione and its precursors an attractive target for therapeutic interventions [67, 68]. However, it should not be forgotten that the belief that low glutathione levels are harmful, as they increase the individual′s susceptibility to oxidative stress, is simplistic and does not take into account possible beneficial consequences of low glutathione levels, especially with respect to activation of cellular defence mechanisms by redox signalling. There is still a significant lack of knowledge about these mechanisms, which at present precludes specific interventions [69].

Examples in the area of interstitial lung disease. The toxicity of particles such as quartz and fibres such as asbestos, but also ambient air particles (particles with a 50% cut-off aerodynamic diameter of 10 μm (PM_{10}) and ultrafine particles (<100 nm)), have been associated with the production of toxic oxygen species, either indirectly from inflammatory cells activated by particles, or directly from the particles or fibres themselves. Freshly fractured silica particles have been shown to produce more free-radicals and exert a higher cellular toxicity than aged particles [70], and asbestos fibres and PM_{10} have been shown to produce oxygen-derived free radical species, not only by iron-related, but also by nitric oxide (NO)-mediated reactions [71]. In addition, most particles and fibres lead to considerable attraction and activation of macrophages and polymorphonuclear leukocytes, which also produce toxic oxygen species. Both inflammatory response and fibrosis could be reduced in animal models using crystalline silica inhalation and concomitant administration of antioxidant enzymes, such as polyethylene glycol-conjugated (PEG)-catalase [72]. However, induction of endogenous antioxidant enzymes is not enough to overcome the initial inflammation and persistent fibrotic response [73].

A series of studies is available on the antioxidant enzymes (AOEs) of coal miners, with or without CWP [74–77]. These studies show abnormal levels of several AOEs in macrophages, red blood cells or serum from coal miners with CWP, with or without PMF. The studies mainly demonstrate upregulation of glutathione-dependent enzymes in red blood cells at later stages of CWP. In addition, a recent study on hypersensitivity pneumonitis (farmer′s lung) has indicated upregulation of MnSOD, but not CuZnSOD, in alveolar macrophages and granulomas, but not in the fibrotic areas in the human lung [78]. It has also been shown that levels of glutathione are low in the alveolar lining fluid of patients with IPF [79], and recent data from Behr *et al.* [80] indicate that glutathione homeostasis in the lung′s extracellular fluid may be one of the host susceptibility factors that governs the occurrence of acute episodes of farmer′s lung.

Some metals, most notably cobalt, are also capable of causing oxidative stress in the lung [81]. The production of toxic oxygen species increases when cobalt is associated with tungsten carbide in hard metal [82]. However, the exact role of oxidant injury in the pathogenesis of hard metal lung (or in other conditions of added oxidative stress) is still largely speculative, even if the progression of the disease appears to be adversely influenced by the administration of oxygen [83].

Several drugs exert their toxicity *via* oxidative mechanisms. These compounds elicit lung toxicity *via* redox cycling, *i.e.* they shuttle electrons to oxygen. Examples are bleomycin, nitrofurantoin, and paraquat [23]. It is possible that the occurrence of drug-induced pulmonary disease is influenced by, or due to innate deficiencies in, the defence against oxidants. A case report [84] showed pulmonary oedema after administration of a normally nontoxic concentration of oxygen in a child deficient in CuZnSOD; another case report [85] indicated that a hemizygote deficiency in glucose-6-phosphate dehydrogenase (which is necessary for generating NADPH that serves as a donor of reducing equivalents for the reduction of glutathione) may have determined the occurrence

of mefloquine-induced pneumonitis. Alterations in antioxidant defences may also develop as a result of dietary habits (vitamins and enzyme cofactors), life-style factors (smoking) and environmental exposures (which may induce the lung's antioxidant defence) or therapeutic drugs. Thus, the commonly taken drug paracetamol may cause a significant decrease in glutathione concentrations in alveolar macrophages and type II pneumocytes *in vitro*, even at concentrations that correspond to therapeutic plasma levels [86].

Immunological sensitization

A third type of mechanism belongs to the area of immunological sensitization and is of obvious relevance to hypersensitivity pneumonitis, chronic beryllium lung disease and some drug-induced allergic reactions.

Background. The factors that determine the occurrence of immunological sensitization in an individual are still poorly understood. Again, both genetic and environmental factors must be involved in the process of allergic sensitization. This has mainly been studied in the field of bronchial asthma. A number of studies have attempted to identify genes that are linked to atopic asthma or major histocompatibility complex (MHC) class II alleles associated with occupational asthma, due to specific agents such as toluene di-isocyanate (TDI) [87, 88] or trimellitic anhydride (TMA) [89]. The occurrence of atopy and asthma is also influenced by nongenetic factors, collectively labelled "westernized lifestyle".

A particular form of immunological sensitization is related to autoimmunity. Thus, a therapeutic drug, such as halothane, may be hepatotoxic as a result of the covalent binding of a trifluoroacetyl moiety, derived from (oxidative) P450 metabolism, to microsomal proteins, which are then no longer recognized as self-proteins [90]. Recent *in vitro* studies have shown that the capacity to generate trifluoroacetylated antigens is dependent on CYP interindividual variability [91]. Similar mechanisms are presumably applicable to drug-induced lupus-reactions or chemical-associated systemic sclerosis.

Examples in the area of interstitial lung disease. Chronic beryllium lung disease is the pulmonary condition in which genetic susceptibility to immunological sensitization has been best characterized [92]. RICHELDI *et al.* [93] found a strong association between susceptibility to berylliosis and an HLA-DPB1 genetic marker, the amino acid variant HLA-DPB1Glu69. This finding has been confirmed by an independent study by WANG *et al.* [94] who found that HLA-DPB1Glu69-positive alleles were present in 95% of a series of 20 beryllium disease patients. The mechanistic basis of this phenomenon is thought to be related to the presentation of beryllium to T-cells, specifically by the HLA-DP Glu69 molecule [95]. In addition, a preliminary report indicated that the tumour necrosis factor (TNF)-α allelic variant causing a high production of TNF (see later) was also associated with beryllium hypersensitivity. Interestingly, while the TNF-α genetic marker was associated with sensitization to beryllium, the HLA-DP marker was associated with disease progression, suggesting that the genetic and environmental factors may have a variety of interactions in relation to disease mechanisms. Interestingly, the susceptibility to cobalt-related interstitial lung disease also appears to be related to the HLA-DPB1Glu69 allele [96], although evidence of this is less solid than in the case of beryllium.

For hypersensitivity pneumonitis (HP) also, there is evidence that genetic factors govern the immune response to inhaled antigen. ZABEL and SCHLAAK [97] have studied cytokine gene polymorphisms in HP and found that the genotype for "high responder" TNF-α2 was associated with the occurrence of farmer's lung. SELMAN *et al.* [98] reported similar findings, as well as links with some HLA alleles. Similar cytokine gene polymorphisms have been shown to be important in other immunologically mediated diseases. For example, high-producing TNF-α genotype is associated with acute rejection of transplanted organs [99]. However, environmental factors may also influence the immune response in HP, with smoking being protective, and concomitant viral or *Mycoplasma pneumoniae* infections being possible adjuvants [100, 101]. Smoking is associated with a reduced prevalence of all forms of HP and this is probably related to the complex effects of smoking on several components of the immune response to inhaled antigen. For example, smoking has been shown to reduce cytokine release from macrophages, depress levels of interleukin (IL)-6 and suppress the antibody response to inhaled antigen [102, 103]. Viruses are commonly found in the lungs of patients with acute HP [104] and the onset of farmer's lung has been associated with *M. pneumoniae* infection [100]. In a mouse model of HP, CORMIER *et al.* [104] showed that viral infection enhanced cytokine production and increased cellular responses to antigen challenge. Other animal models of HP suggest that multigenic factors are important in determining the susceptibility of certain strains of mice to the development of HP, and the progression of the disease usually requires the induction of nonspecific lung inflammation by adjuvants such as bacille Calmette-Guérin (BCG) or carrageenan [105]. Thus, evidence from different sources suggests that susceptibility to the development of HP is a complex phenomenon, which probably involves both genetic and environmental factors.

It must be recognized that even when a disease is based on allergy or immunological sensitization, it cannot be assumed that individual susceptibility is the sole determinant in the genesis and maintenance of the disease, and that the intensity of exposure does not play a role. This has been well demonstrated in the field of occupational asthma, where there is a strong relationship between the attack rate of the disease and the level of exposure (with peaks of exposure perhaps more important than the time-integrated level). The concept that exposure intensity is also important in immunologically mediated ILDs is supported by

epidemiological studies in both HP and chronic beryllium disease. Several studies have demonstrated a close relationship between the amount of airborne micro-organisms in the work environment of farmers and the risk of developing farmer's lung [106–108]. This is also reflected in the close relationship between the prevalence of farmer's lung and meteorologic conditions (mainly humidity) [109, 110]; thus, both regional variations (dependent on altitude) and year-to-year variations in rainfall influence the incidence of farmer's lung in the Doubs region [111]. This is outlined in more detail in the Review by BOURKE *et al.* [112] in this Supplement. A recent case-control study [113] confirmed that patients with farmer's lung came from farms where the total amount of airborne micro-organisms was higher, but also found that the fungal flora in these farms differed qualitatively from that in farms without affected subjects, by the presence of *Absydia corymbifera*. The existence of a threshold for the maintenance and progression of HP is also suggested by the fact that wearing respiratory protection devices appears to prevent the recurrence of acute bouts of HP [114–116], even though the intensity of exposure may remain high even with the most effective masks.

With regard to beryllium disease, KREISS *et al.* [117] have shown that the type, or level, of beryllium exposure is an important determinant of disease incidence among exposed workers. Using job descriptions as a marker of exposure types or levels in the course of an individual's occupational history, it has been shown that exposure intensity is associated with increased disease risk, suggesting that a dose-effect relationship may determine disease risk in beryllium-exposed subjects. Interestingly, though, a study of the interaction between exposure and genetic factors in the determination of disease-risk conducted by RICHELDI *et al.* [118], has shown that genetic predisposition plays a major role in risk determination, having an additive/supramultiplicative effect upon exposure intensity [118]. In this study, heavily exposed workers carrying the HLA-DPB1Glu69 marker had a disease risk that was 8–10-times higher than that of the similarly exposed HLA-DPB1Glu69-negative workers, suggesting that exposure may exert dose-related effects that differ by one order of magnitude between the susceptible and the nonsusceptible individuals in an exposed population.

No autoimmune mechanisms appear to have been identified that explain the occurrence of drug-induced pneumotoxicity, but there is no reason why this could not occur in the lung as in other organs, such as the liver, since various lung cells have the capability of activating chemicals to reactive metabolites that can covalently bind to cellular proteins (see Toxicokinetic factors). Moreover, cellular injury by foreign chemicals may lead to immunogenic alterations in structural or other proteins, thereby inducing autoimmune reactions. This may help to develop an understanding of the pathogenesis underlying silica-induced systemic sclerosis. Similarly, the recurrence of giant cell interstitial pneumonitis in a transplanted lung, despite cessation of exposure [119], is also suggestive of an autoimmune process.

Inflammation and fibrogenesis

A fourth type of mechanism conferring susceptibility to ILD and the propensity to develop fibrosis is that of the regulation and resolution of inflammation and fibrogenesis.

Background. Various types of tissue reactions may result from cellular injury or immunological sensitization. In the most favourable case, normal repair processes lead to complete resolution or minimal residual damage. However, if the injurious agent remains present or recurs, or if the normal homeostasis mechanisms are perturbed, a more persistent and damaging process of inflammation and/or fibrosis may be set in motion. The propensity of individuals to develop particular types of inflammation, such as granulomas and/or fibrosis, is probably under genetic and environmental control.

The paradigm that lung fibrosis is always preceded by, and dependent on, the severity of inflammation is probably valid in many situations, but it has been argued more recently, mainly on the basis of pathological observations, that this is not necessarily true for all instances of lung fibrosis. Thus, fibrogenesis (*i.e.* excessive deposition of extracellular matrix and cell proliferation) may represent a more "independent" disease process than was previously believed.

Examples in the area of interstitial lung disease. Inflammation and fibrogenesis have been studied, to a large extent, in the areas of sarcoidosis and IPF, as well as in particle- and fibre-induced lung injury, and models of pneumoconioses. Most animal models that have studied particle-induced lung fibrosis have been able to block fibrosis when initial inflammation was prevented or lowered with co-administration of aluminium [15, 120] or polyvinylpyridine-*N*-oxide (PVNO) [121]. However, instillation of *N*-formyl-L-methionyl-leucyl-phenylalanine (FMLP)-activated polymorphonuclear neutrophils after instillation of silica, led to a reduction in lung load and fibrosis in mice [122]. The degree to which dust particles, such as silica or asbestos, lead to fibrosis has been shown to be under genetic control, probably through the response of cytokine networks and growth factors which: 1) determine the cumulative lung load by attenuating clearance; and 2) regulate the cascade of events leading to fibrogenesis. Thus, experimental animals have varying susceptibility to silica between species (*e.g.* the rat is more sensitive than the mouse or hamster) and within the same species (*e.g.* significant differences of susceptibility to the development of silicosis have been noted among different mouse strains). Some of these differences in susceptibility could be due to variations in the responses to oxidative stress (see previously), but some studies, *e.g.* using knock-out mice, indicate that variations in cytokines or their receptors are critical for inducing fibroproliferative responses in the lung. This is based on the classical mechanism that occurs when alveolar macrophages are stimulated by particle uptake or bacterial lipopolysaccharide (LPS) and secrete cytokines for recruitment of more inflammatory cells

as well as tissue remodelling. Within this concept, the cytokine-phenotypes concerning TNF-α, transforming growth factors (TGF) and IL-6 have been shown to play an important role in the initiation and progression of mineral dust-induced disorders, such as CWP [123, 124]. Moreover, the TNF type 2 polymorph (see previously) has been shown to occur more frequently in coal miners with CWP than in controls [125]. Animal studies have demonstrated that silica-induced lung fibrosis in mice could be ameliorated using a specific anti-TNF antibody [126], and that the infusion of soluble-TNFR75 receptors that complex circulating free TNF could prevent and reduce existing fibrosis [127]. Increased levels of TNF and TNF-receptors have also been found in BAL and tissue specimens of subjects with various ILDs. More specifically, in patients with CWP or PMF, changes of TNF-release from alveolar macrophages, and changes in IL-6, TGF-β, insulin-like growth factor-1 (IGF-1) and platelet-derived growth factor (PDGF) were noted [123]. In addition, both TNF and IL-6 messenger ribonucleic acid (mRNA) have been shown to be increased in lung tissue biopsies from coal miners, especially in areas where coal dust was present [128].

In experimental animals, like chimpanzees, induction of acute alveolar damage with LPS appeared to be the cause of activation of coagulation and fibrinolysis in the alveolar space, which was cytokine-dependent [129]. As a result, the locally produced serine protease thrombin further increases endothelial permeability, stimulates granulocyte influx and serves as a fibroblast mitogen. This thrombin-mediated fibroblast activation appeared to be dependent on IL-6 and TNF-α [129], and upregulates IL-8 intracellularly [130], suggesting that this coagulation protein has an important role in the induction of fibrosis.

Several clinical and experimental data indicate that T-helper 2 (Th2) polarization is not only important in the pathogenesis of lung diseases, such as bronchial asthma, but also in ILD. The Th2 cytokines IL-4, -5, -10 and -13 are all expressed in the lung tissue of patients with IPF, and the severity of the disease is apparently related to the level of expression of these cytokines [131, 132]. *In vitro* studies indicate that IL-4 stimulates the proliferation of fibroblasts and their production of collagen. Conversely, interferon (IFN)-γ inhibits the proliferation of fibroblasts [133, 134]. Injection of specific antibodies directed against IL-4 and IL-10 confirmed the profibrotic activity of these cytokines in a model of liver fibrosis induced by parasite eggs [135]. Similarly, in the lung, the fibrotic response induced by these parasites was accelerated by the overexpression of IL-4 [136]. In experimental models of lung fibrosis induced by ionizing radiation or bleomycin, fibrosis was associated with an increased production of Th2 cytokines [137, 138]. In addition, pulmonary fibrosis induced by bleomycin is significantly reduced by the administration of IFN-γ [139]. HUAUX *et al.* [140] have shown that IL-12 p40, a Th2 cell cytokine, is persistently upregulated in response to the administration of silica particles. Although IL-10, another Th2 cell cytokine, limited the amplitude of the alveolitis induced by silica, it also exerted a profibrotic activity, which is reminiscent of the possible discordance between inflammation and fibrosis [141]. This observation about IL-10 might apply more generally to a large array of cytokines that share both anti-inflammatory and profibrotic activities (TGF-β, IL-13, *etc.*). There is also evidence of multiple routes of polyclonal T-cell activation through several peptides derived from antigens and viruses. Progression of fibrosing alveolitis can occur under circumstances in which mutations in the Fas-FasL (receptor-ligand) interactions on activated cells cause lymphoproliferative disorders with autoimmune manifestations [142]. Fas-receptors, which are members of the TNF-receptor-ligand family, are expressed on bronchiolar and alveolar cells and expression of FasL occurs on infiltrating lymphocytes and granulocytes [143]. The Fas-FasL pathway is involved in normal apoptosis of cell subsets, but in fibrosing alveolitis, induced in experimental animals, the expression of the receptors is strongly upregulated; this can be measured in cells derived from BAL fluid (BALF) and may induce pulmonary toxicity, including deoxyribonucleic acid (DNA) damage, leading to pulmonary fibrosis [144]. Although a normal apoptosis cycle avoids the inflammation associated with necrotic cell death, a pathologically upregulated Fas-FasL pathway can induce pro-inflammatory cytokines, such as IL-8 [143], indicating that the Fas pathway can also lead to inflammation and fibrosis with production of pro-collagen and fibronectin at the alveolar level. Therefore, hyperreactive Fas-FasL pathways, as well as TNF-α, can induce exaggerated IL-8 production and excretion by airway epithelial cells [145], with resulting damage to the extracellular matrix and fibrogenesis.

Differences in the susceptibility to ILD might to some extent depend on: 1) the host′s proinflammatory response and the persistence of inflammation; and 2) the capacity of the host to mount a Th2 immune response. Although well established, the field of inflammatory cytokines has barely been used as a tool to explain differences in susceptibility in ILD. Within the context of the Th2 response, it is interesting to note that there is strong evidence that a "western lifestyle", with its relatively high standard of hygiene, plays a crucial role in the increasing prevalence of allergic diseases. It is possible that the immune system in early life remains rather unstimulated by intense infections that normally elicit Th1 reactions (*e.g.* mycobacteria, vaccinations), leading to a biased Th2 immune polarization, and hence, increased susceptibility to sensitization with otherwise harmless allergens in the environment [146]. Whether this paradigm might also hold for ILDs remains completely unexplored.

Conclusion

Although this paper has dealt with ILDs of "known" aetiology, relatively little is known about the reasons why individuals exposed to these agents develop lung disease.

The issue of genetics pervades the entire discussion of host susceptibility. Genes largely determine the way the respiratory tract is made, how well lungs can be cleared of unwanted deposited materials, which pathways are used to biotransform foreign chemicals, how to defend against oxidative attack, whether individuals are prone to develop particular types of immunological response, and which cytokines and growth factors are activated preferentially. Knowledge in this area is likely to expand rapidly in the future when the entire human genome will be available to be explored. Large follow-up studies of cohorts of people whose genetic make-up can be analysed, thanks to the availability of suitably banked biological samples, should be conducted to try and discover these susceptibility genes. However, the legal and ethical implications in this field should not be forgotten. For instance, it would not be acceptable to institute recruitment policies that systematically exclude carriers of genotypes conferring some susceptibility to occupational agents, without ensuring that exposures to dangerous chemicals are well controlled.

However, although a particular genotype will often predispose individuals to disease, it is clear that genes are not the only determinants of health and disease. Environmental factors may be equally important in shaping host susceptibility. For one thing, diseases do not generally arise "out of the blue" and there must be exogenous factors that cause or at least trigger all diseases, including those termed "cryptogenic", "idiopathic" or "primary". A complete understanding of many of these environmental and lifestyle factors, which are amenable to modulation by interventions at the individual and society level, is still a long way off. Refinement of the assessment of current and past occupational and other environmental exposures in individuals and groups of individuals is greatly needed. Good (bio)markers of exposures must be developed to help disentangle the relationship between susceptibility and exposure.

Clinical and epidemiological research, as well as experimental research, into both the genetic bases and the environmental determinants of interstitial lung diseases should lead us to better mechanism-based prevention strategies, early detection of, and therapy for these conditions.

References

1. Aldridge WN, ed. Mechanisms and concepts in toxicology. London, Taylor & Francis, 1996.
2. Lippmann M, Yeates DB, Albert RE. Deposition, retention, and clearance of inhaled particles. *Br J Ind Med* 1980; 37: 337–362.
3. Stöber W, McClellan RO, Morrow PE. Approaches to modeling disposition of inhaled particles and fibers in the lung. *In*: Gardner DE, Crapo JD, McClellan RO, eds. Toxicology of the Lung. 2nd Edn. New York, Raven Press, 1993; pp. 527–601.
4. Foster WM. Deposition and clearance of inhaled particles. *In*: Holgate ST, Samet JM, Koren HS, Maynard RL, eds. Air pollution and health. London, Academic Press, 1999; pp. 294–324.
5. Becklake MR, Toyota B, Stewart M, Hanson R, Hanley J. Lung structure as a risk factor in adverse pulmonary responses to asbestos exposure. A case-referent study in Quebec chrysotile miners and millers. *Am Rev Respir Dis* 1983; 128: 385–388.
6. Frampton MW, Utell MJ. Clinical studies of airborne pollutants. *In*: Gardner DE, Crapo JD, McClellan RO, eds. Toxicology of the Lung. 3rd Edn. London, Taylor & Francis, 1999; pp. 455–481.
7. Sweeney TD, Skornik WA, Brain JD, Hatch V, Godleski JJ. Chronic bronchitis alters the pattern of aerosol deposition in the lung. *Am J Respir Crit Care Med* 1995; 151: 482–488.
8. Sweeney TD, Brain JD, Leavitt SA, Godleski JJ. Emphysema alters the deposition pattern of inhaled particles in hamsters. *Am J Pathol* 1987; 128: 19–28.
9. Bennett WD, Zeman KL, Kim C, Mascarella J. Enhanced deposition of fine particles in COPD patients spontaneously breathing at rest. *Inhal Toxicol* 1997; 9: 1–14.
10. Anderson PJ, Wilson JD, Hiller FC. Respiratory tract deposition of ultrafine particles in subjects with obstructive or restrictive lung disease. *Chest* 1990; 97: 1115–1120.
11. Svartengren K, Ericsson CH, Svartengren M, Mossberg B, Philipson K, Camner P. Deposition and clearance in large and small airways in chronic bronchitis. *Exp Lung Res* 1996; 22: 555–576.
12. Seaton A, Cherrie JW. Quartz exposures and severe silicosis: a role for the hilar nodes. *Occup Environ Med* 1998; 55: 383–386.
13. Ontario Ministry of the Attorney General. Ontario Royal Commission on matters of health and safety arising from the use of asbestos in Ontario, 1984.
14. Bégin R, Massé S, Sébastien P, *et al.* Asbestos exposure and retention as determinants of airway disease and asbestos alveolitis. *Am Rev Respir Dis* 1986; 134: 1176–1181.
15. Bégin R, Cantin A, Massé S. Recent advances in the pathogenesis and clinical assessment of mineral dust pneumoconioses: asbestosis, silicosis and coal pneumoconiosis. *Eur Respir J* 1989; 2: 988–1001.
16. Gibbs AR, Gardner MJ, Pooley FD, Griffiths DM, Blight B, Wagner JC. Fiber levels and disease in workers from a factory predominantly using amosite. *Environ Health Perspect* 1994; 102: Suppl. 5, 261–263.
17. De Vuyst P, Karjalainen A, Dumortier P, *et al.* Guidelines for mineral fibre analyses in biological samples: report of the ERS Working Group. European Respiratory Society. *Eur Respir J* 1998; 11: 1416–1426.
18. Churg A, Tron V, Wright JL. Effects of cigarette smoke exposure on retention of asbestos fibers in various morphologic compartments of the guinea pig lung. *Am J Pathol* 1987; 129: 385–393.
19. International Agency for Research on Cancer. Silica, some silicates, coal dust and para-aramid fibrils. Lyon, IARC-WHO, 1997.
20. Davis JM, Chapman J, Collings P, *et al.* Variations in the histological patterns of the lesions of coal workers' pneumoconiosis in Britain and their relationship to lung dust content. *Am Rev Respir Dis* 1983; 128: 118–124.
21. Nagelschmidt G, Rivers D, King EJ, Trevella W. Dust and collagen content of lungs of coal workers with progressive massive fibrosis. *Br J Ind Med* 1963; 20: 181–191.

22. Bergman I, Casswell C. Lung dust and lung iron contents of coal workers in different coal fields in Great Britain. *Br J Ind Med* 1972; 29: 160–168.
23. Smith LL, Nemery B. The lung as a target organ for toxicity. *In*: Cohen GM, ed. Target Organ Toxicity. Volume II. Boca Raton, Florida, CRC Press, 1986; pp. 45–80.
24. Ehrenpreis ED, Ehrenpreis S. Cytochrome P450. Role in drug-induced hepatotoxicity. *Clin Liver Dis* 1998; 2: 457–470.
25. Nakachi K, Imai K, Hayashi S, Kawajiri K. Polymorphisms of the CYP1A1 and glutathione S-transferase genes associated with susceptibility to lung cancer in relation to cigarette dose in a Japanese population. *Cancer Research* 1993; 53: 2994–2999.
26. Smith CI, Harrison DJ. Association between polymorphism in gene for microsomal epoxide hydrolase and susceptibility to emphysema. *Lancet* 1999; 350: 630–633.
27. Bond JA. Metabolism of xenobiotics by the respiratory tract. *In*: Gardner DE, Crapo JD, McClellan RO, eds. Toxicology of the Lung. 2nd Edn. New York, Raven Press, 1993; pp. 187–215.
28. Dahl AR, Lewis JL. Respiratory tract uptake of inhalants and metabolism of xenobiotics. *Annu Rev Pharmacol Toxicol* 1993; 33: 383–407.
29. Raunio H, Hakkola J, Hukkanen J, *et al.* Expression of xenobiotic-metabolizing CYPs in human pulmonary tissue. *Exp Toxicol Pathol* 1999; 51: 412–417.
30. Hukkanen J, Pelkonen O, Raunio H. Expression of xenobiotic-metabolizing enzymes in human pulmonary tissue: possible role in susceptibility for ILD. *Eur Respir J* 2001; 18: Suppl. 32, 122s–126s.
31. Born SL, Fix AS, Caudill D, Lehman-McKeeman LD. Selective Clara cell injury in mouse lung following acute administration of coumarin. *Toxicol Appl Pharmacol* 1998; 151: 45–56.
32. Forkert PG, Stringer V, Racz WJ. Effects of administration of metabolic inducers and inhibitors on pulmonary toxicity and covalent binding by 1,1-dichloroethylene in CD-1 mice. *Exp Mol Pathol* 1986; 45: 44–58.
33. Dowsley TF, Reid K, Petsikas D, Ulreich JB, Fisher RL, Forkert PG. Cytochrome P-450-dependent bioactivation of 1,1-dichloroethylene to a reactive epoxide in human lung and liver microsomes. *J Pharmacol Exp Ther* 1999; 289: 641–648.
34. Boyd MR. Role of metabolic activation in the pathogenesis of chemically induced pulmonary disease: mechanism of action of the lung-toxic furan, 4-ipomeanol. *Environ Health Perspect* 1976; 16: 127–138.
35. Foster JR, Green T, Smith LL, Tittensor S, Wyatt I. Methylene chloride: an inhalation study to investigate toxicity in the mouse lung using morphological, biochemical and Clara cell culture techniques. *Toxicology* 1994; 91: 221–234.
36. Yost GS, Buckpitt AR, Roth RA, McLemore TL. Mechanisms of lung injury by systemically administered chemicals. *Toxicol Appl Pharmacol* 1989; 101: 179–195.
37. Mahvi D, Bank H, Harley R. Morphology of a naphthalene-induced bronchiolar lesion. *Am J Pathol* 1977; 86: 558–572.
38. Tong SS, Lowe MC, Trush MA, *et al.* Bronchiolar epithelial damage and impairment of pulmonary microsomal monooxygenase activity in mice by naphthalene. *Exp Mol Pathol* 1982; 37: 358–369.
39. Johnson DE, Riley MG, Cornish HH. Acute target organ toxicity of 1-nitronaphthalene in the rat. *J Appl Toxicol* 1984; 4: 253–257.
40. Jeffery EH, Haschek WM. Protection by dimethylsulfoxide against acetaminophen-induced hepatic, but not respiratory toxicity in the mouse. *Toxicol Appl Pharmacol* 1988; 93: 452–461.
41. Odum J, Foster JR, Green T. A mechanism for the development of Clara cell lesions in the mouse lung after exposure to trichloroethylene. *Chem Biol Interact* 1992; 83: 135–153.
42. Ritter JK, Owens IS, Negishi M, *et al.* Mouse pulmonary cytochrome P-450 naphthalene hydroxylase: cDNA cloning, sequence, and expression in Saccharomyces cerevisiae. *Biochemistry* 1991; 30: 11430–11437.
43. Czerwinski M, McLemore TL, Philpot RM, *et al.* Metabolic activation of 4-ipomeanol by complementary DNA-expressed human cytochromes P-450: evidence for species-specific metabolism. *Cancer Res* 1991; 51: 4636–4638.
44. Albrecht C, Adolf B, Borm PJA. Cytochrome P450 isozymes in rat lung epithelial cells after instillation of poorly soluble particles. *Am J Respir Crit Care Med* 2000; 161: A171.
45. Miles PR, Bowman L, Miller MR. Alterations in the pulmonary microsomal cytochrome P-450 system after exposure of rats to silica. *Am J Respir Cell Mol Biol* 1993; 8: 597–604.
46. Lazo JS, Hoyt DG, Sebti SM, Pitt BR. Bleomycin: A pharmacologic tool in the study of the pathogenesis of interstitial pulmonary fibrosis. *Pharmacol Ther* 1990; 47: 347–358.
47. Camus Ph. Respiratory disease induced by drugs. *Eur Respir J* 1997; 10: 260–264.
48. Poli G, Parola M. Oxidative damage and fibrogenesis. *Free Radic Biol Med* 1997; 22: 287–305.
49. Kinnula VL, Crapo JD, Raivio KO. Generation and disposal of reactive oxygen metabolites in the lung. *Lab Invest* 1995; 73: 3–19.
50. Lakari E, Paakko P, Pietarinen-Runtti P, Kinnula VL. Manganese superoxide dismutase and catalase are coordinately expressed in the alveolar region in chronic interstitial pneumonias and granulomatous diseases of the lung. *Am J Respir Crit Care Med* 2000; 161: 615–621.
51. Pietarinen-Runtti P, Lakari E, Raivio KO, Kinnula VL. Expression of antioxidant enzymes in human inflammatory cells. *Am J Physiol Cell Physiol* 2000; 278: C118–C125.
52. Oury TD, Day BJ, Crapo JD. Extracellular superoxide dismutase: a regulator of nitric oxide bioavailability. *Lab Invest* 1996; 75: 617–636.
53. Janssen YM, Van Houten B, Borm PJ, Mossman BT. Cell and tissue responses to oxidative damage. *Lab Invest* 1993; 69: 261–274.
54. Kleeberger SR. Genetic susceptibility to ozone exposure. *Toxicol Lett* 1995; 82–83: 295–300.
55. Prows DR, Daly MJ, Shertzer HG, Leikauf GD. Ozone-induced acute lung injury: genetic analysis of F(2) mice generated from A/J and C57BL/6J strains. *Am J Physiol* 1999; 277: L372–L380.
56. Mango GW, Johnston CJ, Reynolds SD, Finkelstein JN, Plopper CG, Stripp BR. Clara cell secretory protein deficiency increases oxidant stress response in conducting airways. *Am J Physiol* 1998; 275: L348–L356.

57. van Klaveren RJ, Demedts M, Nemery B. Cellular glutathione turnover *in vitro*, with emphasis on type II pneumocytes. *Eur Respir J* 1997; 10: 1392–1400.
58. van Klaveren RJ, Nemery B. Role of reactive oxygen species in obstructive occupational and environmental pulmonary diseases. *Curr Opinion Pulmon Med* 1999; 5: 118–123.
59. Yamada N, Yamaya M, Okinaga S, *et al.* Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema. *Am J Hum Genet* 2000; 66: 187–195.
60. Cantin AM, North SL, Hubbard RC, Crystal RG. Normal alveolar epithelial lining fluid contains high levels of glutathione. *J Appl Physiol* 1987; 63: 152–157.
61. Wagner PD, Mathieu-Costello O, Bebout DE, Gray AT, Natterson PD, Glennow C. Protection against pulmonary O_2 toxicity by *N*-acetylcysteine. *Eur Respir J* 1989; 2: 116–126.
62. Langley SC, Kelly FJ. *N*-acetylcysteine ameliorates hyperoxic lung injury in the preterm guinea pig. *Biochem Pharmacol* 1993; 45: 841–846.
63. Giri SN, Hyde DM, Schiedt MJ. Effects of repeated administration of *N*-acetyl-L-cysteine on sulfhydryl levels of different tissues and bleomycin-induced lung fibrosis in hamsters. *J Lab Clin Med* 1988; 111: 715–724.
64. Kennedy TP, Gordon GB, Paky A, *et al.* Amiodarone causes acute oxidant lung injury in ventilated and perfused rabbit lungs. *J Cardiovasc Pharmacol* 1988; 12: 23–36.
65. Meyer A, Buhl R, Magnussen H. The effect of oral *N*-acetylcysteine on lung glutathione levels in idiopathic pulmonary fibrosis. *Eur Respir J* 1994; 7: 431–436.
66. Behr J, Maier K, Degenkolb B, Krombach F, Vogelmeier C. Antioxidative and clinical effects of high-dose *N*-acetylcysteine in fibrosing alveolitis. Adjunctive therapy to maintenance immunosuppression. *Am J Respir Crit Care Med* 1997; 156: 1897–1901.
67. Blackwell TS, Blackwell TR, Holden EP, Christman BW, Christman JW. *In vivo* antioxidant treatment suppresses nuclear factor-kappa B activation and neutrophilic lung inflammation. *J Immunol* 1996; 157: 1630–1637.
68. Gosset P, Wallaert B, Tonnel AB, Fourneau C. Thiol regulation of the production of TNF-alpha, IL-6 and IL-8 by human alveolar macrophages. *Eur Respir J* 1999; 14: 98–105.
69. Halliwell B. The antioxidant paradox. *Lancet* 2000; 355: 1179–1180.
70. Vallyathan V, Shi X, Dalal NS, Irr W, Castranova V. Generation of free radicals from freshly fractured silica dust. Potential role in acute silica-induced lung injury. *Am Rev Respir Dis* 1988; 138: 1213–1219.
71. Kinnula VL. Oxidant and antioxidant mechanisms of lung disease caused by asbestos fibres. *Eur Respir J* 1999; 14: 706–716.
72. Mossman BT, Marsh JP, Sesko A, *et al.* Inhibition of lung injury, inflammation, and interstitial pulmonary fibrosis by polyethylene glycol-conjugated catalase in a rapid inhalation model of asbestosis. *Am Rev Respir Dis* 1990; 141: 1266–1271.
73. Janssen YM, Marsh JP, Absher MP, *et al.* Expression of antioxidant enzymes in rat lungs after inhalation of asbestos or silica. *J Biol Chem* 1992; 267: 10625–10630.
74. Engelen JJ, Borm PJ, van Sprundel M, Leenaerts L. Blood anti-oxidant parameters at different stages of pneumoconiosis in coal workers. *Environ Health Perspect* 1990; 84: 165–172.
75. Schins RPF, Keman S, Borm PJA. Blood antioxidant status in coal dust induced respiratory disorders: a longitudinal evaluation of multiple biomarkers. *Biomarkers* 1997; 2: 45–50.
76. Voisin C, Wallaert B, Aerts C, *et al.* Bronchoalveolar lavage in coal worker's pneumoconiosis: oxidant and antioxidant activity of alveolar macrophages. *In*: Beck EG, Bignon J, eds. *In vitro* effects of mineral dusts. Berlin, Springer-Verlag, 1985; pp. 93–100.
77. Nadif R, Bourgkard E, Dusch M, *et al.* Relations between occupational exposure to coal mine dusts, erythrocyte catalase and Cu++/Zn++ superoxide dismutase activities, and the severity of coal workers' pneumoconiosis. *Occup Environ Med* 1998; 55: 533–540.
78. Lakari E, Paakko P, Kinnula VL. Manganese superoxide dismutase, but not CuZn superoxide dismutase, is highly expressed in the granulomas of pulmonary sarcoidosis and extrinsic allergic alveolitis. *Am J Respir Crit Care Med* 1998; 158: 589–596.
79. Cantin AM, North SL, Fells GA, Hubbard RC, Crystal RG. Oxidant-mediated epithelial cell injury in idiopathic pulmonary fibrosis. *J Clin Invest* 1987; 79: 1665–1673.
80. Behr J, Degenkolb B, Beinert T, Krombach F, Vogelmeier C. Pulmonary glutathione levels in acute episodes of Farmer's lung. *Am J Respir Crit Care Med* 2000; 161: 1968–1971.
81. Lewis CPL, Demedts M, Nemery B. Indices of oxidative stress in hamster lung following exposure to cobalt(II) ions: *in vivo* and *in vitro* studies. *Am J Respir Cell Mol Biol* 1991; 5: 163–169.
82. Lison D, Carbonnelle P, Mollo L, Lauwerys R, Fubini B. Physicochemical mechanism of the interaction between cobalt metal and carbide particles to generate toxic activated oxygen species. *Chem Res Toxicol* 1995; 8: 600–606.
83. Nemery B, Nagels J, Verbeken E, Dinsdale D, Demedts M. Rapidly fatal progression of cobalt-lung in a diamond polisher. *Am Rev Respir Dis* 1990; 141: 1373–1378.
84. Ackerman AD, Fackler JC, Tuck-Muller CM, Tarpey MM, Freeman BA, Rogers MC. Partial monosomy 21, diminished activity of superoxide dismutase, and pulmonary oxygen toxicity. *N Engl J Med* 1988; 318: 1666–1669.
85. Drent M. Drug-induced pneumonia associated with hemizygote glucose-6-phosphate- dehydrogenase deficiency. *Eur J Haematol* 1998; 61: 218–220.
86. Dimova S, Hoet PH, Nemery B. Paracetamol (acetaminophen) cytotoxicity in rat type II pneumocytes and alveolar macrophages *in vitro. Biochem Pharmacol* 2000; 59: 1467–1475.
87. Bignon JS, Aron Y, Ju LY, *et al.* HLA class II alleles in isocyanate-induced asthma. *Am J Respir Crit Care Med* 1994; 149: 71–75.
88. Balboni A, Baricordi OR, Fabbri LM, Gandini E, Ciaccia A, Mapp CE. Association between toluene diisocyanate-induced asthma and DQB1 markers: a positive role for aspartic acid at position 57. *Eur Respir J* 1996; 9: 207–210.
89. Young RP, Barker RD, Pile KD, Cookson WOCM, Newman Taylor AJ. The association of HLA-DR3

with specific IgE to inhaled acid anhydrides. *Am J Respir Crit Care Med* 1995; 151: 219–221.
90. Bourdi M, Chen W, Peter RM, *et al.* Human cytochrome P450 2E1 is a major autoantigen associated with halothane hepatitis. *Chem Res Toxicol* 1996; 9: 1159–1166.
91. Eliasson E, Gardner I, Hume-Smith H, de Waziers I, Beaune P, Kenna JG. Interindividual variability in P450-dependent generation of neoantigens in halothane hepatitis. *Chem Biol Interact* 1998; 116: 123–141.
92. Saltini C, Winestock K, Kirby M, Pinkston P, Crystal RG. Maintenance of alveolitis in patients with chronic beryllium disease by beryllium-specific helper T cells. *N Engl J Med* 1989; 320: 1103–1109.
93. Richeldi L, Sorrentino R, Saltini C. HLA-DPB1 glutamate 69: a genetic marker of beryllium disease. *Science* 1993; 262: 242–244.
94. Wang Z, White PS, Petrovic M, *et al.* Differential susceptibilities to chronic beryllium disease contributed by different Glu69 HLA-DPB1 and -DPA1 alleles. *J Immunol* 1999; 163: 1647–1653.
95. Lombardi G, Germain C, Uren J, *et al.* HLA-DP-restricted recognition of beryllium by T cells in chronic beryllium disease accounts for DP-associated susceptibility. *J Immunol* 2001; 166: 3549–3555.
96. Potolicchio I, Mosconi G, Forni A, Nemery B, Seghizzi P, Sorrentino R. Susceptibility to hard metal lung disease is strongly associated with the presence of glutamate 69 in HLA-DPbeta chain. *Eur J Immunol* 1997; 27: 2741–2743.
97. Zabel P, Schlaak M. Tumor Nekrose Faktor alpha bei Lungenerkrankungen. [Tumor necrosis factor alpha in lung diseases]. *Pneumologie* 1993; 47: 49–52.
98. Selman M, Teran L, Mendoza A, *et al.* Increase of HLA-DR7 in pigeon breeder's lung in a Mexican population. *Clin Immunol Immunopathol* 1987; 44: 63–70.
99. Hutchinson IV, Pravica V, Perrey C, Sinnott P. Cytokine gene polymorphisms and relevance to forms of rejection. *Transplant Proc* 1999; 31: 734–736.
100. McGavin C. Farmer's lung after *Mycoplasma pneumoniae* infection. *Thorax* 1986; 41: 68–69.
101. Dakhama A, Hegele RG, Laflamme G, Israel-Assayag E, Cormier Y. Common respiratory viruses in lower airways of patients with acute hypersensitivity pneumonitis. *Am J Respir Crit Care Med* 1999; 159: 1316–1322.
102. McSharry C, Banham SW, Boyd G. Effect of cigarette smoking on the antibody response to inhaled antigens and the prevalence of extrinsic allergic alveolitis among pigeon breeders. *Clin Allergy* 1985; 15: 487–494.
103. McCrea KA, Ensor JE, Nall K, Bleecker ER, Hasday JD. Altered cytokine regulation in the lungs of cigarette smokers. *Am J Respir Crit Care Med* 1994; 150: 696–703.
104. Cormier Y, Assayag E, Tremblay G. Viral infection enhances lung response to *Micropolyspora faeni*. *Am J Ind Med* 1994; 25: 79–80.
105. Peterson LB, Thrall RS, Moore VL, Stevens JO, Abramoff P. An animal model of hypersensitivity pneumonitis in the rabbit. Induction of cellular hypersensitivy to inhaled antigens using carrageenan and BCG. *Am Rev Respir Dis* 1977; 116: 1007–1012.
106. Malmberg P, Rask-Andersen A, Rosenhall L. Exposure to microorganisms associated with allergic alveolitis and febrile reactions to mold dust in farmers. *Chest* 1993; 103: 1202–1209.
107. Kotimaa MH, Husman KH, Terho EO, Mustonen MH. Airborne molds and actinomycetes in the work environment of farmer's lung patients in Finland. *Scand J Work Environ Health* 1984; 10: 115–119.
108. Dalphin JC, Pernet D, Reboux G, *et al.* Influence of mode of storage and drying of fodder on thermophilic actinomycete aerocontamination in dairy farms of the Doubs region of France. *Thorax* 1991; 46: 619–623.
109. Terho EO, Heinonen OP, Lammi S. Incidence of farmer's lung leading to hospitalization and its relation to meteorological observations in Finland. *Acta Medica Scand* 1983; 213: 295–298.
110. Dalphin JC, Debieuvre D, Pernet D, *et al.* Prevalence and risk factors for chronic bronchitis and farmer's lung in French dairy farmers. *Br J Ind Med* 1993; 50: 941–944.
111. Dalphin JC, Pernet D, Dubiez A, Debieuvre D, Allemand H, Depierre A. Etiologic factors of chronic bronchitis in dairy farmers. Case control study in the Doubs region of France. *Chest* 1993; 103: 417–421.
112. Bourke SJ, Dalphin JC, Boyd G, McSharry C, Baldwin CI, Calvert JE. Hypersensitivity pneumonitis: current concepts. *Eur Respir J* 2001; 18: Suppl. 32, 81s–92s.
113. Dalphin J-C, Maheu MF, Dussaucy A, *et al.* Six year longitudinal study of respiratory function in dairy farmers in the Doubs province. *Eur Respir J* 1998; 11: 1287–1293.
114. Nuutinen J, Terho EO, Husman K, Kotimaa M, Harkonen R, Nousiainen H. Protective value of powered dust respirator helmet for farmers with farmer's lung. *Eur J Respir Dis Suppl* 1987; 152: 212–220.
115. Muller-Wening D, Repp H. Investigation on the protective value of breathing masks in farmer's lung using an inhalation provocation test. *Chest* 1989; 95: 100–105.
116. Kusaka H, Ogasawara H, Munakata M, *et al.* Two-year follow up on the protective value of dust masks against farmer's lung disease. *Intern Med* 1993; 32: 106–111.
117. Kreiss K, Mroz MM, Newman LS, Martyny J, Zhen B. Machining risk of beryllium disease and sensitization with median exposures below 2 $\mu g/m^3$. *Am J Ind Med* 1996; 30: 16–25.
118. Richeldi L, Kreiss K, Mroz MM, Zhen B, Tartoni P, Saltini C. Interaction of genetic and exposure factors in the prevalence of berylliosis. *Am J Ind Med* 1997; 32: 337–340.
119. Frost AE, Keller CA, Brown RW, *et al.* Giant cell interstitial pneumonitis. Disease recurrence in the transplanted lung. *Am Rev Respir Dis* 1993; 148: 1401–1404.
120. Brown GM, Donaldson K, Brown DM. Broncho-alveolar leukocyte response in experimental silicosis: modulation by a soluble aluminum compound. *Toxicol Appl Pharmacol* 1989; 101: 95–105.
121. Pott F, Dungworth DL, Heinrich U, *et al.* Lung tumours after intratracheal instillation of dusts. *Ann Occup Hyg* 1994; 38: 357–363.
122. Adamson IY, Prieditis H, Bowden DH. Instillation of chemotactic factor to silica-injected lungs lowers interstitial particle content and reduces pulmonary fibrosis. *Am J Pathol* 1992; 141: 319–326.
123. Vanhée D, Gosset P, Wallaert B, Voisin C, Tonnel AB. Mechanisms of fibrosis in coal workers' pneumoconiosis. Increased production of platelet-derived

growth factor, insulin-like growth factor type I, and transforming growth factor beta and relationship to disease severity. *Am J Respir Crit Care Med* 1994; 150: 1049–1055.
124. Schins RP, Borm PJ. Epidemiological evaluation of release of monocyte TNF-alpha as an exposure and effect marker in pneumoconiosis: a five year follow up study of coal workers. *Occup Environ Med* 1995; 52: 441–450.
125. Zhai R, Jetten M, Schins RP, Franssen H, Borm PJ. Polymorphisms in the promoter of the tumor necrosis factor-alpha gene in coal miners. *Am J Ind Med* 1998; 34: 318–324.
126. Piguet PF, Collart MA, Grau GE, Sappino AP, Vassalli P. Requirement of tumour necrosis factor for development of silica-induced pulmonary fibrosis. *Nature* 1990; 344: 245–247.
127. Piguet PF, Vesin C. Treatment by human recombinant soluble TNF receptor of pulmonary fibrosis induced by bleomycin or silica in mice. *Eur Respir J* 1994; 7: 515–518.
128. Vanhée D, Gosset P, Marquette CH, *et al.* Secretion and mRNA expression of TNF alpha and IL-6 in the lungs of pneumoconiosis patients. *Am J Respir Crit Care Med* 1995; 152: 298–306.
129. Levi M, van der Poll T, ten Cate H, *et al.* Differential effects of anti-cytokine treatment on bronchoalveolar hemostasis in endotoxemic chimpanzees. *Am J Respir Crit Care Med* 1998; 158: 92–98.
130. Ludwicka-Bradley A, Tourkina E, Suzuki S, *et al.* Thrombin upregulates interleukin-8 in lung fibroblasts *via* cleavage of proteolytically activated receptor-I and protein kinase C-gamma activation. *Am J Respir Cell Mol Biol* 2000; 22: 235–243.
131. Wallace WA, Ramage EA, Lamb D, Howie SE. A type 2 (Th2-like) pattern of immune response predominates in the pulmonary interstitium of patients with cryptogenic fibrosing alveolitis (CFA). *Clin Exp Immunol* 1995; 101: 436–441.
132. Wallace WA, Howie SE. Immunoreactive interleukin 4 and interferon-gamma expression by type II alveolar epithelial cells in interstitial lung disease. *J Pathol* 1999; 187: 475–480.
133. Sempowski GD, Derdak S, Phipps RP. Interleukin-4 and interferon-gamma discordantly regulate collagen biosynthesis by functionally distinct lung fibroblast subsets. *J Cell Physiol* 1996; 167: 290–296.
134. Serpier H, Gillery P, Salmon-Ehr V, *et al.* Antagonistic effects of interferon-gamma and interleukin-4 on fibroblast cultures. *J Invest Dermatol* 1997; 109: 158–162.
135. Wahl SM, Frazier-Jessen M, Jin WW, Kopp JB, Sher A, Cheever AW. Cytokine regulation of schistosome-induced granuloma and fibrosis. *Kidney Int* 1997; 51: 1370–1375.
136. Lukacs NW, Addison CL, Gauldie J, *et al.* Transgene-induced production of IL-4 alters the development and collagen expression of T helper cell 1-type pulmonary granulomas. *J Immunol* 1997; 158: 4478–4484.
137. Buttner C, Skupin A, Reimann T, *et al.* Local production of interleukin-4 during radiation-induced pneumonitis and pulmonary fibrosis in rats: macrophages as a prominent source of interleukin-4. *Am J Respir Cell Mol Biol* 1997; 17: 315–325.
138. Gharaee-Kermani M, Phan SH. Lung interleukin-5 expression in murine bleomycin-induced pulmonary fibrosis. *Am J Respir Cell Mol Biol* 1997; 16: 438447.
139. Gurujeyalakshmi G, Giri SN. Molecular mechanisms of antifibrotic effect of interferon gamma in bleomycin-mouse model of lung fibrosis: downregulation of TGF-beta and procollagen I and III gene expression. *Exp Lung Res* 1995; 21: 791–808.
140. Huaux F, Lardot C, Arras M, *et al.* Lung fibrosis induced by silica particles in NMRI mice is associated with an upregulation of the p40 subunit of interleukin-12 and Th-2 manifestations. *Am J Respir Cell Mol Biol* 1999; 20: 561–572.
141. Huaux F, Arras M, Vink A, Renauld JC, Lison D. Soluble tumor necrosis factor (TNF) receptors p55 and p75 and interleukin-10 downregulate TNF-alpha activity during the lung response to silica particles in NMRI mice. *Am J Respir Cell Mol Biol* 1999; 21: 137–145.
142. Siegel RM, Fleisher TA. The role of Fas and related death receptors in autoimmune and other disease states. *J Allergy Clin Immunol* 1999; 103: 729–738.
143. Kuwano K, Miyazaki H, Hagimoto N, *et al.* The involvement of Fas-Fas ligand pathway in fibrosing lung diseases. *Am J Respir Cell Mol Biol* 1999; 20: 53–60.
144. Van den Blink B, Jansen HM, Peppelenbosch MP. Idiopathic pulmonary fibrosis: molecular mechanisms and possible therapeutic strategies. *Archivum Immunologiae et Therapiae Experimentalis* 2000; 48: 539545.
145. Roger T, Out T, Mukaida N, Matsushima K, Jansen H, Lutter R. Enhanced AP-1 and NF-kappaB activities and stability of interleukin 8 (IL-8) transcripts are implicated in IL-8 mRNA superinduction in lung epithelial H292 cells. *Biochem J* 1998; 330: 429–435.
146. von Hertzen LC, Haahtela T. Could the risk of asthma and atopy be reduced by a vaccine that induces a strong T-helper type 1 response? *Am J Respir Cell Mol Biol* 2000; 22: 139–142.