

Review Article

***In Vivo* Studies of Man-Made Mineral Fibers —Fibrosis-Related Factors—**

Yasuo MORIMOTO* and Isamu TANAKA

Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, 1–1 Iseigaoka, Yahatanishi, Kitakyushu 807-8555, Japan

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Abstract: Pneumoconiosis that pursues a chronic course may result from repeated injury and repair caused by dust particles that remain in the lungs, leading to fibrosis. We will introduce *in vivo* studies concerning these processes using animals exposed to man-made mineral fibers and asbestos. We will report on whether there are developmental changes with the mineral fiber and animal model in proinflammatory cytokine, chemokine, free radicals, proteinase and other genes that lead to injury, as well as in genes that effect repair such as growth factor, and we will also report on the effects of surfactant protein and clara cell secretory protein on fibrosis.

Key words: *In vivo*, Man-made mineral fiber, Asbestos, JFM, Ceramic fiber, Potassium octatitanate whiskers

Abbreviation

MMMFs: man-made mineral fibers
JFM: Japan Fibrous Material
RF1: refractory ceramic fibers
PT1: potassium octatitanate whiskers
TNF: tumor necrosis factor
IL-1: interleukin-1
IL-6: interleukin-6
TGF- α : transforming growth factor- α
PDGF: platelet-derived growth factor
IL-1RN: IL-1 receptor antagonist
IL-1R: interleukin-1 receptor.
MIP-2: macrophage inflammatory protein-2
8-OH-dG: 8-Hydroxydeoxyguanosine
iNOS: inducible nitric oxide synthase
MMPs: matrix metalloproteinases
TIMPs: tissue inhibitors of metalloproteinases
IGF: insulin-like growth factor

EGF: epidermal growth factor
CCSP: clara cell secretory protein
SP: surfactant protein
TTF-1: thyroid transcription factor 1

Introduction

Large numbers of the different types of man-made mineral fibers (MMMFs) are being used as substitutes for asbestos. The concern for these materials having similar physiochemical properties to asbestos exists because some fibers can cause pulmonary fibrosis.

Pneumoconiosis which pursues a chronic course, can be considered the result of a series of events comprising repeated inflammation and repair due to the continued presence of dust in the lungs, eventually leading to fibrosis¹⁾. Animal studies of pneumoconiosis induced by exposure to mineral fibers by intratracheal instillation or inhalation have made a major contribution to our understanding of the underlying pathology of pulmonary fibrosis^{2, 3)}. Gene expression of many factors is thought to contribute to pulmonary fibrosis

*To whom correspondence should be addressed.

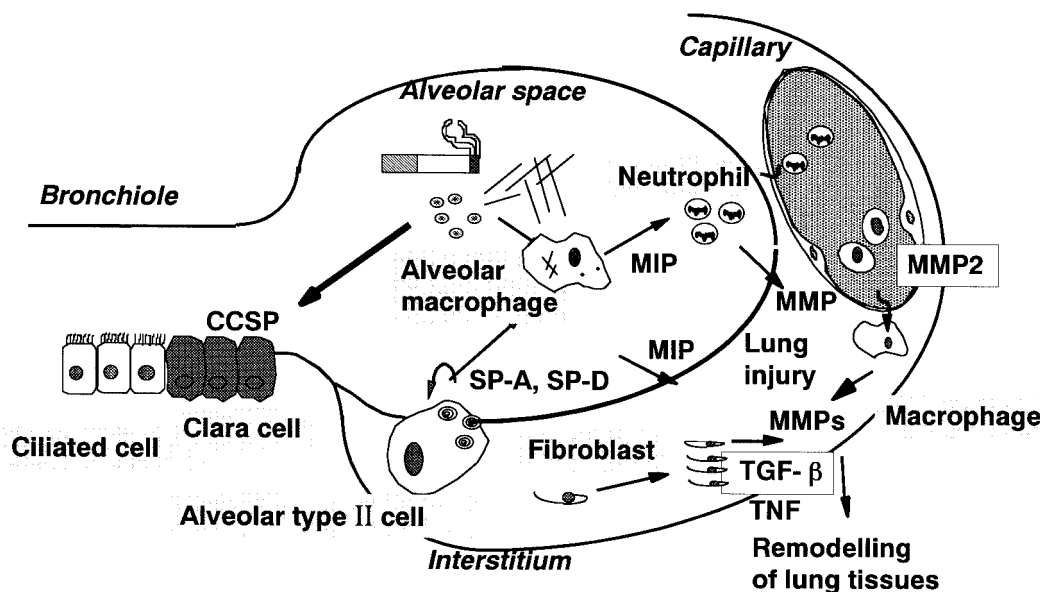


Fig. 1. Lung remodeling exposed to dusts.

in asbestos-induced disease. Figure 1 shows the process of pulmonary fibrosis and the fibrosis-related genes induced by mineral fibers. Explained simply, asbestos fibers deposited in the lung lead to the activation of alveolar macrophages. Alveolar macrophages and parenchymal and epithelial cells release proinflammatory cytokines, such as tumor necrosis factor (TNF), interleukin-1 (IL-1), and interleukin-6 (IL-6), and other cytokines which augment cellular inflammation. Release of oxidants and proteinases by these cells may lead to lung injury. Some growth factors signal interstitial fibroblasts to replicate and modulate production of connective tissue proteins. The accumulation of inflammatory cells, fibroblasts, and connective tissue matrix leads to lung injury and angiogenesis, resulting in fibrosis and carcinoma. In this paper, we review the results of animal studies with asbestos and man-made mineral fibers according to the various fibrosis-related genes.

Standard fibers are required to evaluate the effects of fibers on the human body. Japan Fibrous Material standard reference samples, which include man-made mineral fibers, are now used instead of UICC asbestos. A total of ten standard reference samples of mineral fibers were well-characterized^{5,6} and prepared for use in *in vitro* and *in vivo* biological experiments. This review includes currently reported *in vivo* experiments done in which JFM standard reference samples were used.

Proinflammatory Cytokines

Typical proinflammatory cytokines include TNF, IL-1, and IL-6. TNF has attracted interest for both its role in inflammation and its involvement in collagen deposition and other aspects of the repair process, since it has been shown that TNF antibodies inhibit silica-induced collagen accumulation⁶ and that an interstitial pneumonia-like disease state occurs in transgenic mice with elevated TNF levels⁷. IL-1 has similar effects to TNF, but levels of IL-1 do not increase in the inflammation phase⁸. IL-6 is a multifunctional cytokine which exhibits both inflammatory and anti-inflammatory action⁹. IL-6 appears to have an inflammatory effect, as it has been shown that alveolitis occurs as a result of overexpression of human IL-6 and IL-6 receptors in the lungs of rats following adenovector-mediated transfer of these genes by intratracheal instillation¹⁰, and lymphocyte infiltration of the lung is seen in transgenic mice exhibiting overexpression of these genes.

Tsuda *et al.*³ noted changes in expression of genes involved in pathologic changes and remodeling in the lungs of rats after exposure to refractory ceramic fibers (RF1, one of JFM standard references) or potassium octatitanate whiskers (PT1, one of JFM standard references) by inhalation for 12 months. Mild fibrotic changes were seen in the lungs of the PT1-exposed rats, but no appreciable change was evident after exposure to RF1. The fact that IL-6 and TNF- α gene expression in lung tissue was greater in the PT1-exposed rats than in the RF1-exposed animals suggests that there is

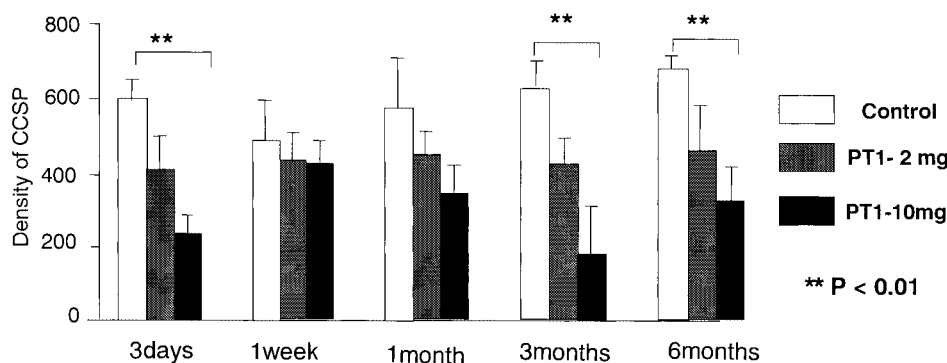


Fig. 2. Gene expression of clara cell secretory protein in rats lungs exposed to potassium octatitanate whiskers.

a correlation between these genes and the fibrotic potential of mineral fibers. Morimoto *et al.*¹¹⁾ investigated gene expression of IL-1, TNF, and IL-6 in lung tissue or alveolar macrophages in rats after exposure to four types of mineral fibers (chrysotile, crocidolite, refractory ceramic fibers (RF1), and potassium octatitanate whiskers) by intratracheal instillation for four weeks. IL-1 and IL-6 gene expression was accelerated in the rats exposed to crocidolite and potassium octatitanate whiskers, which exhibit high fibrotic potential, suggesting that these factors are good biomarkers of pathologic potential.

Liu *et al.*¹²⁾ found that mice with genes for both the 55 kDa and 75 kDa receptors for TNF- α knocked out failed to develop fibroproliferative lesions after inhalation of asbestos, suggesting that decreased expression of transforming growth factor- α (TGF- α) and platelet-derived growth factor-A (PDGF-A) plays a role.

Studies of fibrosis and polymorphisms of proinflammatory cytokines have been a focus of interest¹³⁾. In the presence of one of these polymorphisms, the IL-1 receptor antagonist (IL-1RN) which binds to the IL-1 receptor (IL-1R type 1), does not elicit a response. A single base variation of this occurs at position +2018 in the IL-1RN. In addition, a polymorphism in the TNF locus has been identified in the promoter region (-308) of the TNF- α gene. Whyte *et al.*¹³⁾ found that IL-1RN (+2018) allele 2 and TNF- α (-308) allele 2 are significantly more common in patients with fibrosing alveolitis, suggesting that these polymorphisms confer increased risk of developing fibrosing alveolitis and, therefore, that unopposed IL-1 β and/or excessive TNF- α may play a pathophysiologic role in this condition. The TNF (-308) polymorphism is reported to be common in coal miner pneumoconiosis¹⁴⁾, but it is unclear whether this causes overproduction of TNF, since there was no rise in TNF

release. However, further research with asbestos and man-made mineral fibers is required, since genetic mutations caused by environmental exposure are also a possibility.

Chemokines

Factors involved in neutrophil and macrophage migration include chemokines, such as IL-8, macrophage inflammatory protein-2 (MIP-2), and KC¹⁵⁾. These are heparin-binding proteins which belong to the C-X-C cytokine family. Studies conducted in recent years have shown that C-X-C chemokines are also involved in angiogenesis¹⁶⁾, and that the second structural domain determines angiogenic potential.

IL-8 is released by various cells and also plays a role in neutrophil-induced tissue injury by stimulating neutrophil granule release and active oxygen production, as well as by stimulating neutrophil migration¹⁷⁾. Rats and mice^{18,19)} have chemokines such as MIP-2 and KC instead of IL-8 as seen in humans. These chemokines are homologous to the growth-related protein family purified from supernatant from cultured human melanoma cell lines and, like IL-8, have a potent stimulatory effect on neutrophil chemotaxis¹⁷⁾. Driscoll reported that MIP-2¹⁵⁾ played a major role in mediating the neutrophilic inflammatory response of the rodent lung to particles such as quartz, crocidolite asbestos, as well as high doses of other relative innocuous dusts such as titanium dioxide. The sources of MIP-2²⁰⁾ in the rodent lung after particle exposure include macrophages as well as epithelial cells.

Ishihara *et al.*²¹⁾ studied gene expression in lung tissue after intratracheal instillation of mineral fibers in rats. Exposure to fibrous titanium dioxide whiskers or amosite increased in total protein and expression of cytokine-induced neutrophil chemoattractant / growth-regulated gene, but

exposure to nonfibrous titanium dioxide did not. It was concluded from these findings that whisker-induced acute biological effects in the lung may be related to the shape of the whiskers and not to their chemical composition or surface crystal structure, showing biological effects similar to those of UICC amosite. Damage to alveolar and airway epithelial cells, major sources of chemokines, may also be the first step in pulmonary inflammation and fibrosis. In a study by Hagimoto *et al.*²²⁾, bronchiolar epithelial cells underwent apoptosis and also secreted IL-8 in response to Fas ligation following exposure of airway epithelial cells to anti-Fas antibody. Attention has focused on the role played by airway and alveolar epithelial cells in inflammation caused by exposure to mineral fibers, since alveolar and airway epithelial cell damage is also observed in patients with pneumoconiosis and animal models of the condition^{24, 25)}, and IL-8 is produced in response to stimulation of alveolar epithelial cells by asbestos.

Active Oxygen (Reactive Oxygen Species) and Proteases

Active oxygen

Alveolar macrophages and neutrophils phagocytose mineral fibers, resulting in the production of superoxide and other active oxygen species due to the action of NADPH oxidase²⁵⁾. Active oxygen leads to tissue destruction, cell death, and cell proliferation by causing membrane damage due to lipid peroxidation, protein oxidation, and DNA damage. 8-Hydroxydeoxyguanosine (8-OH-dG) is typical of reactive oxygen species which induce mutagenic oxidative DNA damage. Asbestos also induces the release of reactive oxygen species from neutrophils and macrophages²⁶⁾, suggesting that asbestos directly damages DNA through the action of reactive oxygen species. Adachi *et al.*²⁷⁾ compared the potential of asbestos and man-made fibers to attack DNA based on measurement of 8-OH-dG yields, and found that asbestos induced higher 8-OH-dG yields than man-made fibers. Yamaguchi *et al.*²⁸⁾ investigated 8-OH-dG levels and 8-OH-dG repair activity in lung tissue after intratracheal instillation of mineral fibers in hamsters. Crocidolite enhanced 8-OH-dG and its repair activity, but glass fibers did not. These results suggest that not only 8-OH-dG, but also 8-OH-dG repair activity, are useful for evaluating the effect of fibers on oxidative DNA stress. In a study of inducible nitric oxide synthase (iNOS) gene expression in alveolar macrophages after intratracheal instillation of four types of mineral fibers (chrysotile, crocidolite, refractory ceramic fibers (RF1), and potassium octatitanate whiskers)

in rats, Morimoto *et al.*¹¹⁾ found that only exposure to crocidolite increased iNOS gene expression. Further research is needed, since nitric oxide radicals and peroxy nitrite anions are also potent radicals produced via iNOS.

Matrix metalloproteinases and tissue inhibitor of metalloproteinases

Matrix metalloproteinases (MMPs) are a family of zinc and calcium-dependent endopeptidases that play a key role in extracellular matrix remodeling in the lung²⁹⁾. MMPs have the combined ability to degrade the various components of connective tissue matrices, specifically directed to extracellular matrix components³¹⁾. However⁴⁾, the activities of MMPs are controlled at several levels, including their interactions with specific inhibitors, the tissue inhibitors of metalloproteinases (TIMPs)²⁹⁾. MMP-1 and -13 are interstitial collagenases that specifically degrade connective tissue fibrillar collagen (type I collagen), and MMP-2 and -9 are gelatinases that degrade type IV globular basement membrane collagen³⁰⁾. Morimoto *et al.*³¹⁾ examined gene expression of MMPs, TIMPs, and collagen in the rat lung and alveolar macrophages exposed to mineral fibers and/or cigarette smoke. The mineral fibers and cigarette smoke had combined effects on the expression of MMP-2 and -13 and TIMP-1, suggesting that MMPs and TIMPs may be associated with remodeling of lung tissue induced by two agents.

Growth Factor

Growth factors play an important role in pulmonary remodeling by regulating mesenchymal cell proliferation and extracellular matrix production, and are primarily made up of PDGF, insulin-like growth factor (IGF), TGF- β , and epidermal growth factor (EGF). As well as being chemotactic for fibroblasts and smooth muscle cells, PDGF is a competence factor which induces progression from the resting stage to early G1 in the cell cycle. IGF-1 is structurally related to proinsulin and is a progression factor which induces transition through G1, S, and G2 to mitosis³²⁾.

EGF and TGF- α belong to the EGF family and both bind to EGF receptors and are functionally very similar. These factors promote the proliferation of interstitial cells, such as fibroblasts and endothelial cells, as well as epithelial cells, and play an important role in re-epithelialization, including epithelial cell chemotaxis and proliferation³³⁾. Progressive fibrosis also occurs in the lungs of transgenic mice exhibiting overexpression of TGF- α .

TGF- β is a multifunctional cytokine with three isoforms (TGF- β 1-3) in lung tissue. It has a diverse range of functions,

including promoting the production of extracellular matrix components, such as collagen and fibronectin, inhibiting interstitial cell proliferation, and inhibiting inflammatory cell function. The form of expression of these three isoforms varies depending on the cell and disease state, and differences in their expression have also been reported in relation to pulmonary fibrosis^{34, 35}.

Dai *et al.*³⁶ exposed rat tracheal explants to amosite asbestos, iron oxide, or titanium dioxide and maintained the explants in air organ culture. Asbestos produced significant increases in gene expression of PDGF-A, TGF- β_1 , TGF- α , and procollagen, and caused a small increase in hydroxyproline. It was concluded that mineral dusts can induce airway wall fibrosis by directly upregulating proliferative and fibrogenic mediators as well as matrix components, and that neither airspace nor circulating inflammatory cells are required for these effects. Titanium dioxide increased PDGF-B and TGF- α expression, but there were no findings indicative of collagen proliferation³⁷. Lee *et al.* used immunohistologic staining to study expression of TGF- β and IGF in lung tissue in rats after intratracheal instillation of crocidolite. Their findings demonstrated different immunostaining patterns for IGF-1 and TGF- β in asbestosis, with IGF-1 in the cellular periphery and three TGF- β isoforms in the extracellular matrix consistent with a complementary role in stimulating interstitial fibroblast proliferation and new collagen deposition in areas of active fibrosis.

Brass *et al.*³⁸ reported less marked asbestos deposition and inflammatory cell infiltration at bronchiolar-alveolar duct junctions in the 129 inbred mouse strain than in the C57BL/6 inbred strain after inhalation exposure to asbestos, and suggested that a reduction in TNF- α and TGF- β_1 expression was involved in these responses. Recent studies³⁹ have also shown that the expression of renin-angiotensin system components and the elevation of angiotensin-converting enzyme levels also stimulate fibroblast proliferation. Marshall suggests³⁹ that angiotensin II induces human lung fibroblast proliferation via activation of the angiotensin type 1 receptor and this involves the autocrine action of TGF- β .

Surfactant Protein and Clara Cell Secretory Protein

Surfactant protein produced mainly by alveolar type II epithelial cells and Clara cell secretory protein (CCSP) produced by Clara cells are believed to be closely involved in inflammation or fibrosis⁴⁰. These factors are also

commonly used as biomarkers of lesion activity in pulmonary fibrosis. Surfactant protein (SP) is a complex of tissue-specific protein and lipoprotein. Four types, from A to D, have been analyzed to date. Attention has focused on the hydrophilic proteins SP-A and SP-D. SP-A⁴⁰ acts as a control tower governing the production and recovery of phospholipids which reduce surface tension. This helps to inhibit the progression of fibrosis by preventing alveolar collapse. SP-D⁴⁰ is a glycoprotein with a collagen-like structure similar to that of SP-A and is reported to play a role in phospholipid metabolism and local immune defence⁴¹.

CCSP is isolated from human bronchoalveolar lavage fluid (BALF) as a protein (CC10) with a molecular weight of 10 kDa by SDS-PAGE, and is one of the proteins produced and secreted specifically by Clara cells⁴⁰. It exhibits anti-inflammatory and antithrombotic activity, since its functions are homologous to those of rabbit uteroglobin⁴². Johnson *et al.*⁴³ observed an increase in IL-1 and IL-6 expression in the lung tissue of CCSP knockout mice exposed to high concentrations of oxygen, and concluded that CCSP is involved in the inhibition of inflammatory cytokines. The fact that CCSP also inhibited the migration of fibroblasts in lungs in which PDGF was activated *in vitro* suggests that the protein also has an inhibitory effect on fibrosis⁴⁴.

SP-A and CCSP concentrations are decreased in the BALF of patients with idiopathic pulmonary fibrosis, suggesting some impairment of fibrosis-inhibiting mechanisms⁴⁵. However, unlike in idiopathic pulmonary fibrosis, levels of SP-A and CCSP are increased in the BALF of patients with silicosis and asbestosis⁴⁶. This difference appears to be attributable in part to differences in the severity of peripheral epithelial damage. We studied SP-A, CCSP, and thyroid transcription factor 1 (TTF-1) gene expression in lung tissue after intratracheal instillation of potassium octatitanate whiskers (PT1), which is believed to cause pulmonary fibrosis, for between three days and six months⁴⁷. Gene expression of these factors decreased not only in the acute stage, but in the chronic stage as well, suggesting this decrease may play a role in pulmonary fibrosis. A reduction in SP-A, CCSP, and TTF1 gene expression in the acute and chronic stages was also noted in a study of intratracheal instillation of silicon carbide (data not shown). These results differ from those for silicosis and asbestosis, but the exact reason is unclear. It is possible that in animal studies, intratracheal instillation causes extensive peripheral airway damage in the acute stage, and the results of long-term inhalation studies are therefore awaited with interest.

Most investigations of the effects of fibers on the human body have exposed animals to asbestos and made-made

mineral fibers, but few have compared man-made fibers with asbestos. Since it will be difficult in future to use standard asbestos, such as UICC asbestos, studies using substitutes with already-known physicochemical properties will be important for evaluating the pathogenicity of newly developed mineral fibers and should provide additional experimental data on new standard fibers.

References

- 1) Mossman BT, Churg A (1998) Mechanisms in the pathogenesis of asbestosis and silicosis. *Am J Respir Crit Care Med* **157**, 1666–80.
- 2) Tsuda T, Morimoto Y, Yamato H, Nakamura H, Hori H, Nagata N, Kido M, Higashi T, Tanaka I (1997) Effects of mineral fibers on the expression of genes whose product may play a role in fiber pathogenesis. *Environ Health Perspect* **5**, 1173–8.
- 3) Tsuda T, Yamato H, Morimoto Y, Oyabu T, Ishimatsu S, Hori H, Kasai T, Kido M, Higashi T, Tanaka I (1998) One year inhalation study of man-made fibers. In: *Advances in the prevention of occupational respiratory diseases*. eds. by Chiyotani K, Hosoda Y, Aizawa Y. Elsevier Science B.V. Press: 596–600.
- 4) Kohyama N, Tanaka I, Tomita M, Kudo M, Shinohara Y (1997) Preparation and characteristics of standard reference samples of fibrous minerals for biological experiments. *Ind Health* **35**, 415–32.
- 5) Kohyama N, Shinohara Y, Suzuki Y (1996) Mineral phases and some reexamined characteristics of the International Union Against Cancer standard asbestos samples. *Am J Ind Med* **30**, 515–28.
- 6) Miyazaki Y, Araki K, Vesin C, Garcia I, Kapanci Y, Whitsett JA, Pigué PF, Vassalli P (1995) Expression of a tumor necrosis factor-alpha transgene in murine lung causes lymphocytic and fibrosing alveolitis. A mouse model of progressive pulmonary fibrosis. *J Clin Invest* **96**, 250–9.
- 7) Pigué PF, Collart MA, Grau GE, Sappino AP, Vassalli P (1990) Requirement of tumour necrosis factor for development of silica-induced pulmonary fibrosis. *Nature* **344**, 245–7.
- 8) Ohtsuka Y, Munakata M, Ukita H, Takahashi T, Satoh A, Homma Y, Kawakami Y (1995) Increased susceptibility to silicosis and TNF-alpha production in C57BL/6J mice. *Am J Respir Crit Care Med* **152**, 2144–9.
- 9) Jordan M, Otterness IG, Ng R, Gessner A, Rollinghoff M, Beuscher HU (1995) Neutralization of endogenous IL-6 suppresses induction of IL-1 receptor antagonist. *J Immunol* **154**, 4081–90.
- 10) Yoshida M, Sakuma J, Hayashi S, Abe K, Saito I, Harada S, Sakatani M, Yamamoto S, Matsumoto N, Kaneda Y (1995) A histologically distinctive interstitial pneumonia induced by overexpression of the interleukin 6, transforming growth factor beta 1, or platelet-derived growth factor B gene. *Proc Natl Acad Sci U S A* **92**, 9570–4.
- 11) Morimoto Y, Tsuda T, Hirohashi M, Yamato H, Hori H, Ohgami A, Yatera K, Kim HN, Ding L, Kido M, Higashi T, Tanaka I (1999) Effects of mineral fibers on the gene expression of proinflammatory cytokines and inducible nitric-oxide synthase in alveolar macrophages. *Ind Health* **37**, 329–34.
- 12) Liu JY, Brass DM, Hoyle GW, Brody AR (1998) TNF-alpha receptor knockout mice are protected from the fibroproliferative effects of inhaled asbestos fibers. *Am J Pathol* **153**, 1839–47.
- 13) Whyte M, Hubbard R, Meliconi R, Whidborne M, Eaton V, Bingle C, Timms J, Duff G, Facchini A, Pacilli A, Fabbri M, Hall I, Britton J, Johnston I, Di-Giovine F (2000) Increased risk of fibrosing alveolitis associated with interleukin-1 receptor antagonist and tumor necrosis factor-alpha gene polymorphisms. *Am J Respir Crit Care Med* **162**, 755–8.
- 14) Zhai R, Jetten M, Schins RP, Franssen H, Borm PJ (1998) Polymorphisms in the promoter of the tumor necrosis factor-alpha gene in coal miners. *Am J Ind Med* **34**, 318–24.
- 15) Driscoll KE (1994) Macrophage inflammatory proteins: biology and role in pulmonary inflammation. *Exp Lung Res* **20**, 473–90.
- 16) Belperio JA, Keane MP, Arenberg DA, Addison CL, Ehlert JE, Burdick MD, Strieter RM (2000) CXC chemokines in angiogenesis. *J Leukoc Biol* **68**, 1–8.
- 17) Richmond A, Thomas HG (1988) Melanoma growth stimulatory activity: isolation from human melanoma tumors and characterization of tissue distribution. *J Cell Biochem* **36**, 185–98.
- 18) Wolpe SD, Sherry B, Juers D, Davatelis G, Yurt RW, Cerami A (1989) Identification and characterization of macrophage inflammatory protein 2. *Proc Natl Acad Sci USA* **86**, 612–6.
- 19) Frevert CW, Huang S, Danaee H, Paulauskis JD, Kobzik L (1995) Functional characterization of the rat chemokine KC and its importance in neutrophil recruitment in a rat model of pulmonary inflammation. *J Immunol* **154**, 335–44.

- 20) Rosenthal GJ, Germolec DR, Blazka ME, Corsini E, Simeonova P, Pollock P, Kong LY, Kwon J, Luster MI (1994) Asbestos stimulates IL-8 production from human lung epithelial cells. *J Immunol* **153**, 3237–44.
- 21) Ishihara Y, Kyono H, Kohyama N, Otaki N, Serita F, Toya T, Kagawa J (1999) Acute biological effects of intratracheally instilled titanium dioxide whiskers compared with nonfibrous titanium dioxide and amosite in rats [In Process Citation]. *Inhal Toxicol* **11**, 131–49.
- 22) Hagimoto N, Kuwano K, Kawasaki M, Yoshimi M, Kaneko Y, Kunitake R, Maeyama T, Tanaka T, Hara N (1999) Induction of interleukin-8 secretion and apoptosis in bronchiolar epithelial cells by Fas ligation. *Am J Respir Cell Mol Biol* **21**, 436–45.
- 23) Schins RP, Borm PJ (1999) Mechanisms and mediators in coal dust induced toxicity: a review. *Ann Occup Hyg* **43**, 7–33.
- 24) Kamp DW, Israbian VA, Preusen SE, Zhang CX, Weitzman SA (1995) Asbestos causes DNA strand breaks in cultured pulmonary epithelial cells: role of iron-catalyzed free radicals. *Am J Physiol* **268**, L471–80.
- 25) DiMatteo M, Antonini JM, Van-Dyke K, Reasor MJ (1996) Characteristics of the acute-phase pulmonary response to silica in rats. *J Toxicol Environ Health* **47**, 93–108.
- 26) Kinnula VL (1999) Oxidant and antioxidant mechanisms of lung disease caused by asbestos fibres. *Eur Respir J* **14**, 706–16.
- 27) Adachi S, Kawamura K, Yoshida S, Takemoto K (1992) Oxidative damage on DNA induced by asbestos and man-made fibers in vitro. *Int Arch Occup Environ Health* **63**, 553–7.
- 28) Yamaguchi R, Hirano T, Ootsuyama Y, Asami S, Tsurudome Y, Fukada S, Yamato H, Tsuda T, Tanaka I, Kasai H (1999) Increased 8-hydroxyguanine in DNA and its repair activity in hamster and rat lung after intratracheal instillation of crocidolite asbestos. *Jpn J Cancer Res* **90**, 505–9.
- 29) Warheit DB, Gavett SH (1993) Current concepts in the pathogenesis of particulate-induced lung injury. In: *Fibre toxicology*. ed. by Warheit DB, 305–22, Academic Press: San Diego.
- 30) Weiss W (1984) Cigarette smoke, asbestos, and small irregular opacities. *Am Rev Respir Dis* **130**, 293–301.
- 31) Morimoto Y, Tsuda T, Hori H, Yamato H, Ohgami A, Higashi T, Nagata N, Kido M, Tanaka I (1999) Combined effect of cigarette smoke and mineral fibers on the gene expression of cytokine mRNA. *Environ Health Perspect* **107**, 495–500.
- 32) Valentinis B, Porcu PL, Quinn K, Baserga R (1994) The role of the insulin-like growth factor I receptor in the transformation by simian virus 40 T antigen. *Oncogene* **9**, 825–31.
- 33) Kheradmand F, Folkesson HG, Shum L, Derynk R, Pytela R, Matthay MA (1994) Transforming growth factor-alpha enhances alveolar epithelial cell repair in a new in vitro model. *Am J Physiol* **267**, L728–38.
- 34) Khalil N, O'Connor RN, Flanders KC, Unruh H (1996) TGF-beta 1, but not TGF-beta 2 or TGF-beta 3, is differentially present in epithelial cells of advanced pulmonary fibrosis: an immunohistochemical study. *Am J Respir Cell Mol Biol* **14**, 131–8.
- 35) Santana A, Saxena B, Noble NA, Gold LI, Marshall BC (1995) Increased expression of transforming growth factor beta isoforms (beta 1, beta 2, beta 3) in bleomycin-induced pulmonary fibrosis. *Am J Respir Cell Mol Biol* **13**, 34–44.
- 36) Dai J, Gilks B, Price K, Churg A (1998) Mineral dusts directly induce epithelial and interstitial fibrogenic mediators and matrix components in the airway wall. *Am J Respir Crit Care Med* **158**, 1907–13.
- 37) Jagirdar J, Lee TC, Reibman J, Gold LI, Aston C, Begin R, Rom WN (1997) Immunohistochemical localization of transforming growth factor beta isoforms in asbestos-related diseases. *Environ Health Perspect* **5**, 1197–203.
- 38) Brass DM, Hoyle GW, Poovey HG, Liu JY, Brody AR (1999) Reduced tumor necrosis factor-alpha and transforming growth factor-beta1 expression in the lungs of inbred mice that fail to develop fibroproliferative lesions consequent to asbestos exposure. *Am J Pathol* **154**, 853–62.
- 39) Marshall RP, McAnulty RJ, Laurent GJ (1999) Angiotensin II is mitogenic for human lung fibroblasts via activation of the type 1 receptor. *Am J Respir Crit Care Med* **161**, 1999–2004.
- 40) Hermans C, Bernard A (1999) Lung epithelium-specific proteins: characteristics and potential applications as markers. *Am J Respir Crit Care Med* **159**, 646–78.
- 41) Kuroki Y, Takahashi H, Chiba H, Akino T (1998) Surfactant proteins A and D: disease markers. *Biochim Biophys Acta* **1408**, 334–45.
- 42) Stripp BR, Huffman JA, Bohinski RJ (1994) Structure and regulation of the murine Clara cell secretory protein gene. *Genomics* **20**, 27–35.
- 43) Johnston CJ, Mango GW, Finkelstein JN, Stripp BR (1997) Altered pulmonary response to hyperoxia in Clara cell secretory protein deficient mice. *Am J Respir*

- Cell Mol Biol **17**, 147–55.
- 44) Lesur O, Bernard A, Arsalane K, Lauwerys R, Begin R, Cantin ALane D (1995) Clara cell protein (CC-16) induces a phospholipase A2-mediated inhibition of fibroblast migration in vitro. *Am J Respir Crit Care Med* **152**, 290–7.
- 45) McCormack FX, King T Jr., Bucher BL, Nielsen L, Mason RJ (1995) Surfactant protein A predicts survival in idiopathic pulmonary fibrosis [see comments] [published erratum appears in *Am J Respir Crit Care Med* 1995 Oct;152(4 Pt 1):1425]. *Am J Respir Crit Care Med* **152**, 751–9.
- 46) Lesur O, Bernard AM, Begin RO (1996) Clara cell protein (CC-16) and surfactant-associated protein A (SP-A) in asbestos-exposed workers. *Chest* **109**, 467–74.
- 47) Ding L, Morimoto Y, Oyabu T, Kim HN, Yatera K, Hirohashi M, Yamato H, Ohgami A, Hori H, Higashi T, Tanaka I. Gene expression of CCSP, SP-A and TTF-1 in lungs of rats exposed to potassium octatitanate whisker in vivo. *J Occup Med Submitted*.