

Effects of Mineral Fibers on the Expression of Genes Whose Product May Play a Role in Fiber Pathogenesis

Tohru Tsuda,¹ Yasuo Morimoto,¹ Hiroshi Yamato,¹ Hiromi Nakamura,¹ Hajime Hori,² Nobuhiko Nagata,³ Masamitsu Kido,³ Toshiaki Higashi,¹ and Isamu Tanaka¹

¹Institute of Industrial Ecological Sciences, ²School of Health Sciences, and ³Department of Respiratory Disease, University of Occupational and Environmental Health, Kitakyushu, Japan

To determine which factors are useful for the risk assessment of man-made fibers, we examined the gene expression of proinflammatory cytokines, growth factors, manganese superoxide dismutase (MnSOD), and inducible nitric oxide synthase (iNOS) in mineral fiber-exposed rats by means of reverse transcription–polymerase chain reaction (RT–PCR). Male Wistar rats received a single intratracheal instillation of either saline (control) or two types of fibers (2 mg of Union Internationale Centre le Cancer (UICC) chrysotile or alumina silicate refractory ceramic fiber [RCF]). Expression of interleukin-1 α (IL-1 α), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF- α), platelet-deriving growth factor-A, (PDGF-A), platelet-deriving growth factor-B (PDGF-B), transforming growth factor β 1 (TGF- β 1), basic fibroblast growth factor (bFGF), MnSOD, and iNOS mRNA from lung and lipopolysaccharide (LPS)-stimulated alveolar macrophages (AM) were assessed by RT–PCR. Among these factors, IL-1 α , TNF- α , IL-6, bFGF, and iNOS would be the possible parameters for the risk assessment of fibers. In a follow-up study, we investigated the time course (3 days, 1 week, 1 month, and 3 months) of expression of IL-1 α and TNF- α by LPS-stimulated AM exposed to mineral fibers *in vivo*. Male Wistar rats were instilled intratracheally with saline or fibers (2 mg of Union Internationale Centre le Cancer UICC crocidolite or potassium octatitanate whisker [TW]). The expression of IL-1 α mRNA by fibers was greatest in TW, crocidolite, chrysotile, and RCF-instilled rat AM, in that order. The increase of IL-1 α and TNF- α mRNA in AM peaked at 1 month and 3 days after exposure to crocidolite or TW, respectively. The expression of IL-1 α by fibers (crocidolite, chrysotile, TW, and RCF) may be a good indicator of the pathologic potential of fibers. — *Environ Health Perspect* 105(Suppl 5):1173–1178 (1997)

Key words: man-made fiber, asbestos, risk assessment, RT–PCR, cytokine

Introduction

Recently, various types of man-made fibers (MMF) have been developed as substitutes for asbestos; the demand for these products is increasing. Some of these fibers are thought to possess the same adverse biological effects as asbestos because of their similar physiochemical properties (1).

Asbestos fibers and man-made fibers deposited in the lung lead to an activation of alveolar macrophages (AM). AM can release factors such as tumor necrosis factor α (TNF- α), interleukin-1 α (IL-1 α), interleukin-6 (IL-6), and basic fibroblast growth factor (bFGF) that augment cellular inflammation (2).

Release of oxidants by these cells may lead to lung injury (3). Manganese superoxide dismutase (MnSOD) and inducible nitric oxide synthase (iNOS) are biomarkers for superoxide (4). Growth factors like platelet-derived growth factor A (PDGF-A), platelet-derived growth factor B (PDGF-B), and transforming growth factor β 1 (TGF- β 1) signal interstitial fibroblasts to replicate and modulate their production of connective tissue proteins (5). Fibroblast growth factors modulate potent growth of cells and neovascularization (6).

These internal tissue cells and cytokine cascades could account for the chronic nature of the inflammation. The accumulation of inflammatory cells, fibroblasts, and connective tissue matrices leads to lung remodeling such as thickening of alveolar and bronchiole walls.

Even though the mechanism is not completely understood, evidence suggests that various factors are related to each fibrotic process in the lung (Figure 1). It is important to investigate among these factors parameters useful for the risk assessment of MMF and the kinetics of their expression in the process of the lung remodeling.

Materials and Methods

Fiber Preparation

The fibers used in this study were Union Internationale Centre le Cancer (UICC) crocidolite asbestos (crocidolite), UICC chrysotile asbestos ([chrysotile]; potassium octatitanate whisker (TW), and alumina silicate refractory ceramic fibers (RCF) (7). The crocidolite preparation, measured using scanning electron microscopy, had a geometric mean diameter of (0.20 μ m (SD 1.5) and a geometric mean length of 1.3 μ m (SD 2.3). For chrysotile, geometric mean diameter and geometric mean length were 0.085 μ m (SD 1.4) and 0.7 μ m (SD 1.9), respectively. For TW, they were 0.41 μ m (SD 1.5) and 2.8 μ m (SD 2.0), respectively. For RCF, they were 1.2 μ m (SD 1.7) and 9.6 μ m (SD 1.9), respectively.

Intratracheal Instillation Study 1

Ten-week-old male Wistar rats, in groups of five per treatment, were intratracheally instilled with saline or fiber (2 mg chrysotile or RCF). One month after tracheal instillation, bronchoalveolar lavage (BAL) was performed using the left lung. The cells recovered from BAL were plated in tissue culture plates and allowed to attach for 1 hr at

This paper is based on a presentation at The Sixth International Meeting on the Toxicology of Natural and Man-Made Fibrous and Non-Fibrous Particles held 15–18 September 1996 in Lake Placid, New York. Manuscript received at *EHP* 26 March 1997; accepted 23 April 1997.

Address correspondence to Dr. T. Tsuda, Department of Occupational Pneumology, Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, Japan, 1-1 Iseigaoka, Yahata-nishi, Kitakyushu, Japan 807. Telephone: 81 93 691 7466. Fax: 81 93 691 4284. E-mail: tsuda@med.uoeh-u.oc.jp

Abbreviations used: AM, alveolar macrophage(s); BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; bFGF, basic fibroblast growth factor; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MMF, man-made fiber(s); MnSOD, manganese superoxide dismutase; NIH, National Institutes of Health; PDGF-A, platelet-deriving growth factor A; PDGF-B, platelet-deriving growth factor B; RCF, refractory fiber(s); RT–PCR, reverse transcription–polymerase chain reaction; TGF- β 1, transforming growth factor β 1; TNF- α , tumor necrosis factor alpha; TW, potassium octatitanate whisker; UICC, Union Internationale Centre le Cancer; UV, ultraviolet.

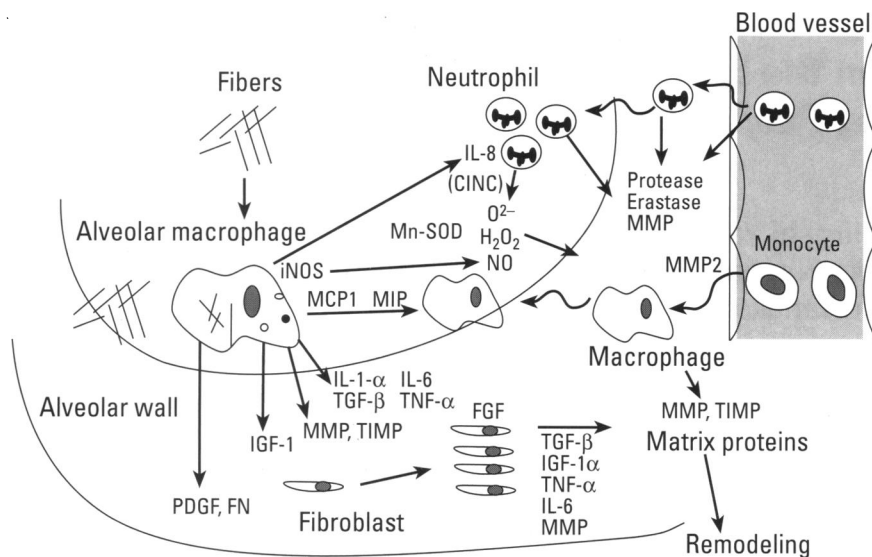


Figure 1. Factors affecting fiber-induced lung remodeling.

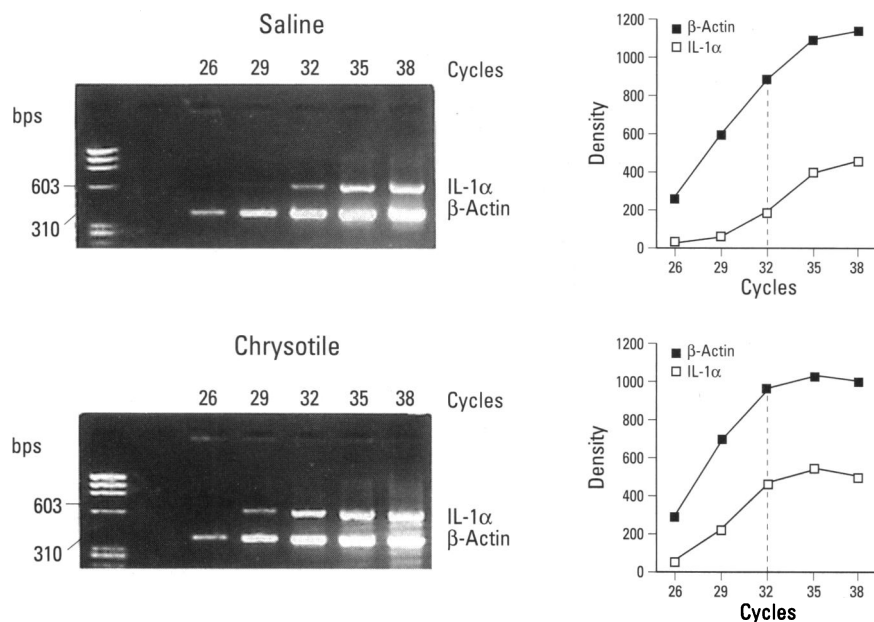


Figure 2. Semiquantification of IL-1 α mRNA and β -actin in serial thermocycle. IL-1 α and β -actin was co-amplified in LPS-stimulated rat AM exposed to saline or chrysotile. The number of thermocycles used allowed quantitation without saturation. IL-1 α and the β -actin PCR products quantitated by densitometry analysis using NIH image 1.56 were plotted against the number of amplification cycles.

37°C with RPMI-1640 medium containing 10% fetal bovine serum (7). Adhered AM were adjusted to a concentration of 1×10^5 /ml, and were stimulated with 10 μ g/ml lipopolysaccharide [LPS]; Sigma Chemical Co., St. Louis, MO) (7). Cells were cultured for 2 and 6 hr on cell culture plates at 37°C in a CO₂ incubator. After incubation, mRNA was extracted using a Quick Prep kit (Pharmacia Biotech,

Uppsala, Sweden). RNA was also extracted from the right lung using the guanidinium thiocyanate-phenol-chloroform method (8).

cDNA Synthesis, Polymerase, and Chain Reaction

RNA was used for the synthesis of single-strand cDNA using Moloney murine leukemia virus-derived reverse transcriptase (Perkin Elmer, Norwalk, CT). Equal

amounts of cDNA from each sample were then used for amplification by specific primers for rat IL1 α , IL-6, TNF- α , PDGF-A, PDGF-B, TGF- β 1, bFGF, MnSOD, and iNOS. The amplification was performed with a Thermocycler (Astech, Fukuoka, Japan) under the following conditions: 94°C for denaturation for 45 sec, 60°C for 45 sec for annealing, and 72°C for 2 min for extension. β -Actin was co-amplified as an internal standard to quantitate polymerase chain reaction (PCR) amplification of mRNA. The number of thermocycles used allowed quantitation without saturation (9) (Figure 2). Four to five cDNAs per treatment group that expressed β -actin uniformly were entered into the study.

Detection of the fragments amplified by the PCR was made by electrophoresis on a 2% agarose gel and visualized by ethidium bromide staining. The gel was photographed with Polaroid Type 665 positive/negative film (Polaroid, Cambridge, MA) over ultraviolet (UV) light at the same exposure and developing time. The bands of the positive film were scanned and the density of each PCR product was measured using National Institutes of Health (NIH) image 1.56 software (written by W. Rasband, NIH, Bethesda, MD). The ratio of specific gene product to β -actin product was used for further analysis.

Statistical Analysis

Data are expressed as mean $\pm$ SEM. Comparisons were performed using the Student's *t* test with a paired test used for paired data. Correlation coefficients in IL-1 α expression by fibers were obtained by Spearman's rank-order method; *p* values less than 0.05 were considered significant.

Intratracheal Instillation Study 2

Wistar rats in groups of five animals per treatment were intratracheally instilled with saline or fibers (2 mg of crocidolite or TW). After exposure for 3 days, 1 week, 1 month, and 3 months, AM were recovered from the left lung and stimulated with 10 μ g/ml LPS for 2 hr. Expression of IL-1 α and TNF- α by LPS-stimulated AM was assessed by reverse transcription (RT)-PCR as in intratracheal instillation study 1.

Results

Gene Expression One Month after Intratracheal Instillation

We examined rat IL1- α , IL-6, TNF- α , PDGF-A, PDGF-B, TGF- β 1, bFGF,

MnSOD, and iNOS from LPS-stimulated AM and right lung. When compared with the control (saline-instilled) group, rats exposed to chrysotile and RCF had significantly increased levels of IL-1 α and TNF- α mRNA in AM (Figure 3). TNF- α mRNA in the lung increased only in chrysotile-exposed rats. TNF- α , iNOS, and bFGF mRNA in the lung increased significantly in chrysotile-exposed rats. IL-6 mRNA in AM was significantly increased in chrysotile-exposed rats.

Time-Course Expression of IL-1 α and TNF- α

Total cell counts in the saline-instilled group were approximately 5×10^5 cells (Figure 2). In the crocidolite- and TW-instilled groups, total cell counts increased approximately 3-fold in 3 days compared with those in the control group. Subsequently, the total cell counts from these groups decreased at 1 month, then increased again 3 months after instillation. Most of the bronchoalveolar lavage fluid (BALF) consisted of AM. Contents of neutrophils in BALF were around 25% at 3 days and at 1 week after crocidolite and TW instillation (Figure 4).

Figure 5A shows the time course of gene expression of IL-1 α by RT-PCR in LPS-stimulated AM after 3 days, 1 week, 1 month, and 3 months of exposure to saline, crocidolite, or TW. From the densitometric analysis, the level of IL-1 α mRNA in AM peaked at 1 month after instillation of crocidolite or TW (Figure 5B). Levels of TNF- α mRNA in AM peaked at 3 days after the instillation of crocidolite or TW (Figure 6).

IL-1 α mRNA Expression by Fibers

As the level of IL-1 α mRNA peaked at 1 month after instillation, we compared the expression of IL-1 α by fibers (crocidolite, chrysotile, TW, and RCF) 1 month after instillation. The expression of IL-1 α mRNA by fibers was greatest in TW-, crocidolite-, chrysotile-, and RCF-instilled rat AM in that order (Figure 7), which was significant ($p < 0.05$) by Spearman's rank correlation coefficient.

Discussion

It has been suggested that MMF with widths and lengths similar to asbestos are most likely associated with the induction of pulmonary fibrosis and lung cancer (10). To assess molecular factors that may be useful for risk assessment of MMF, we examined the gene expression of proinflammatory cytokines, growth factors,

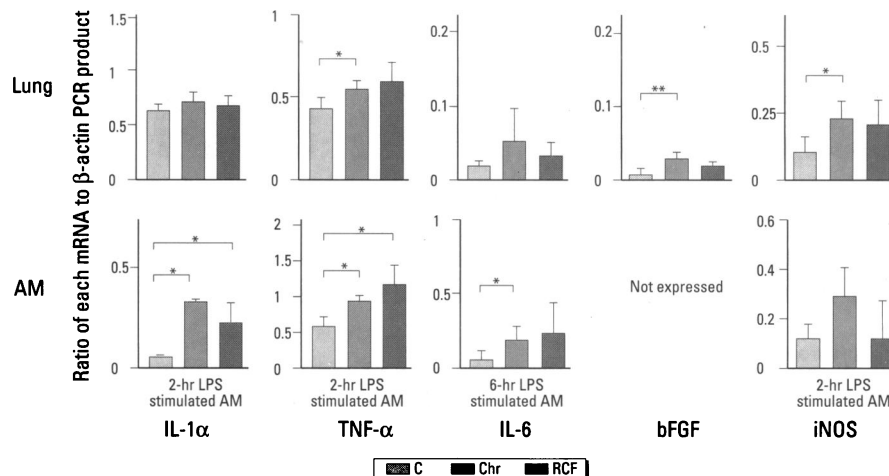


Figure 3. Gene expression 1 month after intratracheal instillation of fibers. Abbreviations: C, saline-instilled rat (control); Chr, chrysotile; RCF, alumina silicate refractory ceramic fibers. IL-1 α , TNF- α , and iNOS mRNA in AM were expressed only in 2-hr LPS stimulation. Steady-state mRNA expression of IL-6 in 6-hr LPS-stimulated AM was higher than in 2-hr LPS-stimulated AM. Mean $\pm$ SEM of four to five rats per category; * $p < 0.05$ compared with saline (control) rats. ** $p < 0.01$ compared with saline (control) rats.

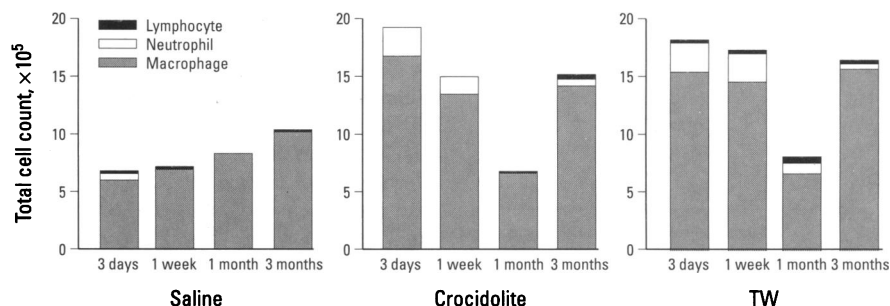


Figure 4. Total and differential cell counts in bronchoalveolar lavage fluid from left lungs after intratracheal instillation.

MnSOD, and nitric oxide synthase (NOS) in mineral fiber-exposed rats using RT-PCR. RT-PCR has multiple advantages (9): *a*) its exquisite sensitivity allows the detection of extremely rare mRNA in small numbers of cells in a semiquantitative manner; *b*) it differs from the assay system of proteins, and the same methodology can be applied in analyzing the expression of many genes; and *c*) cDNA can be used for future studies. However, RT-PCR has several limitations. It can give useful information about transcription but does not provide information about translation, posttranscriptional processes, or cytokine exportation. In addition, because of exponential amplification over repeated cycles and differences in reaction efficiencies, RT-PCR is useful for detecting relative differences in the amount of a given mRNA. A number of studies describe semiquantitative RT-PCR techniques

whereby relative mRNA production was estimated to be between samples; comparison of amplified target molecules co-amplified constitutively expressed mRNA (11). Housekeeping genes such as β -actin or D-glyceraldehyde-3-phosphate dehydrogenase (G3PDH) often are selected as endogenous internal standard in such studies (11). This housekeeping gene may be increased under some conditions. In our experiment, β -actin expression was not altered significantly in the samples from different groups, based on AM cell numbers (Figure 2). Since the endogenous internal standard is usually a housekeeping gene, it frequently is found in higher concentration than the target message. This limitation was avoided by using different PCR cycles for both products. Because limitations of the endogenous internal standard, many investigators have used exogenous internal standards such as

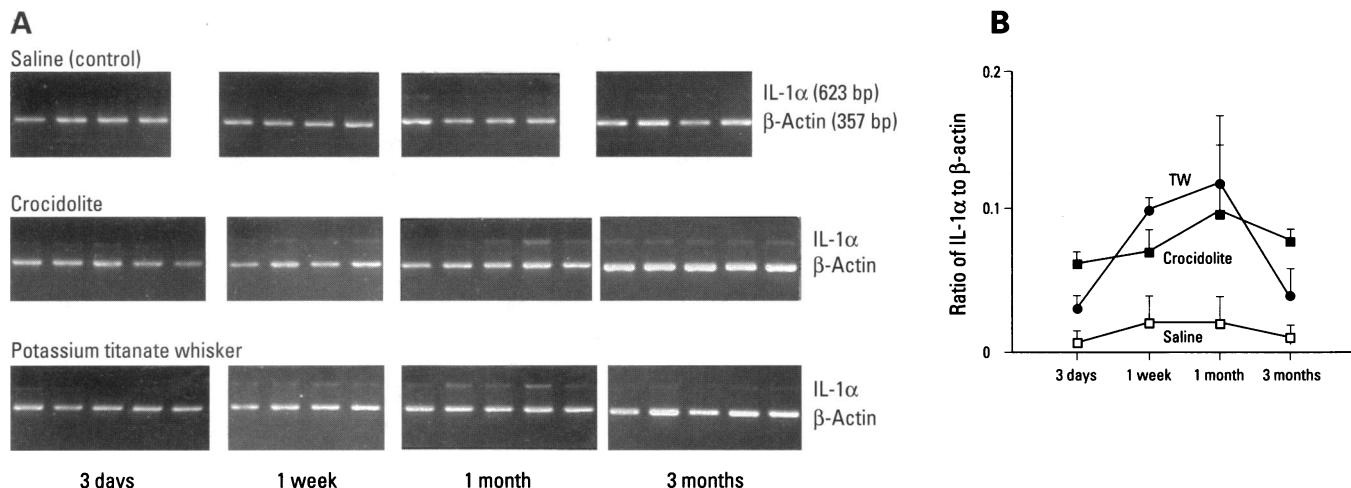


Figure 5. Time-course of expression of IL-1 α mRNA in LPS-stimulated rat AM exposed to mineral fibers. (A) Ethidium bromide staining of PCR products separated in 2% agarose gel. The 623-bp products for IL-1 α and the 357-bp products for β -actin are indicated. LPS-stimulated AM from four to five rats per treatment per time point were examined. (B) Levels of IL-1 α mRNA in AM exposed to mineral fibers. Results are expressed as the ratio of IL-1 α to β -actin (mean $\pm$ SEM).

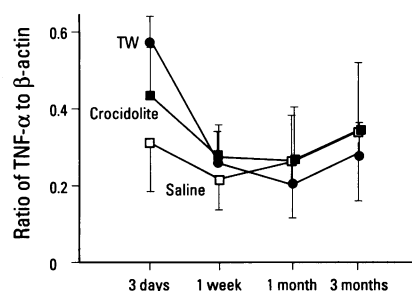


Figure 6. Levels of TNF α mRNA in exposed to mineral fibers. Results are expressed in ratio of TNF- α to β -actin (mean $\pm$ SEM).

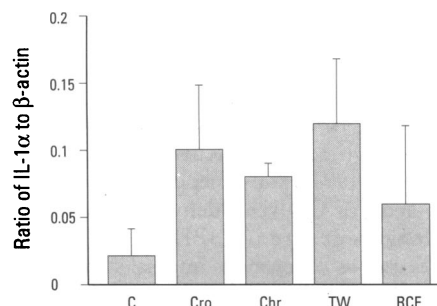


Figure 7. Levels of IL-1 α mRNA by fiber. Abbreviations: C, control; Cro, crocidolite; Chr, chrysotile; TW, potassium octatitanate whisker; RCF, alumina silicate ceramic fibers. Results are expressed in ratio of IL-1 α to β -actin (mean $\pm$ SEM).

synthetic RNA sequence or a synthetic DNA sequence that is not present in the target sample (12). Nevertheless, the house-keeping gene is very useful in controlling for differences in RNA loading and for assessing differences in the quality of RNA (12).

To find more sensitive and specific parameters of lung remodeling by fibers, many factors that may contribute to the process must be considered. RT-PCR may be a suitable method for surveying possible parameters of fiber-induced lung remodeling.

We chose chrysotile and RCF for intratracheal instillation study 1. Though chrysotile can induce lung cancer (fibrosis and mesothelioma), it is one of the least toxic asbestos fibers. There is considerable evidence for the fibrogenicity and carcinogenicity of RCF in laboratory animals, compared to that for other MMF. (13). For intratracheal study 2, we chose more fibrogenic fibers, UICC crocidolite and TW, to detect time-course change of gene expression. TW produces marked pulmonary fibrosis in rats with long-term exposure (14).

In the previous study, unstimulated AM obtained from rats treated with chrysotile did not significantly enhance steady-state levels of IL-1 α mRNA (data not shown). AM harvested from rat lung did not express IL-1 protein, and LPS treatment of quiescent cells (after 24-hr *in vitro* culture) induced low-level expression of IL-1 α and IL-1 β (15). Short-term inhalation of RCF resulted in markedly increased IL-1 β protein expression after stimulation with LPS (15). *In vivo* exposure of AM to LPS increased proinflammatory cytokine mRNA, although the kinetics of upregulation varied (16). For these reasons, we examined mRNA expression in 2 and 6 hr LPS-stimulated AM. As a cautionary note, results from *ex vivo* LPS-stimulated AM may not necessarily indicate a role for a stimulated cytokine in the pathogenesis of

inflammation associated with exposure to fibers *in vivo*.

In intratracheal study 1, AM exposed to chrysotile or RCF were found to have upregulated IL-1 α , TNF- α and IL-6 mRNA transcripts in response to LPS. These are proinflammatory cytokines with both inflammatory and fibrogenic activities such as attraction of inflammatory cells, production of superoxide and collagenases, and proliferation of fibroblasts (17). TNF- α mRNA and protein have been detected in the lung from patients with idiopathic pulmonary fibrosis (18) and in lungs from mice with pulmonary fibrosis elicited by exposure to bleomycin or silica (19). Increased release of IL-1 from AM has been reported after asbestos exposure by inhalation or intratracheal instillation (20). In inflammatory reactions, IL-6 could act as not only a proinflammatory cytokine because of its ability to induce the expression of cellular adhesion molecules on monocytes and the facilitation of their infiltration into the lung, but also as an antiinflammatory cytokine that inhibits the production of TNF and IL-1 (21). An increase has been reported in IL-6 released by bronchoalveolar cells from rats treated with asbestos or coal mineral dust (22).

Oxidants produced by inflammatory cells are thought to lead to lung injury in pulmonary fibrosis. Nitric oxide synthase (NOS) produces reactive species such as nitric oxide (NO *) and peroxynitrite anion. NO * and peroxynitrate are also cytotoxic to host parenchymal cells (4). In our model, levels of iNOS mRNA increased in lungs exposed to chrysotile.

Among the FGFs, bFGF stimulated the replication of endothelial cells *in vitro* and new microvessel growth *in vivo* (23). Asbestos exposure induced lavaged cells to secrete a fibroblast growth factor from 1 to 24 weeks after exposure in rats (24). In lungs exposed to chrysotile, we found increased levels of bFGF mRNA. Based on these results, IL-1 α in the AM, TNF- α in the AM and the lung, IL-6 in the AM, and iNOS and bFGF in the lung would be the possible parameters of risk assessment of man-made fibers in this model. Accordingly, we set out to further investigate the time course of expression of IL-1 α and TNF- α mRNA from exposed to crocidolite and TW.

As previously reported for rats (24), total cell count of BALF at 1 month after chrysotile or TW instillation was almost the same as the control group and increased at 3 months in the present study. Crocidolite or TW instillation resulted in pulmonary inflammation as evidenced by increased

numbers of BALF neutrophils and macrophages at 3 days and 1 week after the exposure. Consistent with the acute inflammation, levels of TNF- α mRNA were greatest at 3 days after the exposure and decreased thereafter. In contrast to TNF- α , levels of IL-1 α mRNA peaked at 1 month after crocidolite or TW exposure.

There have been many attempts to predict the toxicity of mineral fibers based on cytotoxic potentials of fibers using a variety of cell types *in vitro*. In an *in vitro* study using AM, TW caused the highest level of TNF- α production among fibers (1). This is consistent with the present study on TNF- α mRNA expression 3 days after instillation. TNF- α mRNA expression in TW-instilled animals was higher than that in crocidolite-instilled animals. Lee et al. (25) reported that crocidolite was the most potent fibrogenic agent and was 10 times more fibrogenic than potassium octatitanate (Fybex) in terms of exposure concentration. In our

study, the expression of IL-1 α by fiber challenge (TW > crocidolite > chrysotile > RCF) may correlate with the reported pathologic potential of fibers (25,26). Accordingly, our approach may be useful for evaluating the potential toxicity of newly developed man-made fiber. Further investigations using other fibers are necessary to confirm the general applicability of the method.

Both TNF- α and IL-1 α are proinflammatory cytokines. It is necessary to demonstrate that acute inflammation completely predicts the chronic change induced by fibers. As TNF- α plays a key role in lung remodeling (27), further investigations on the correlation between proinflammatory cytokines and the order of the toxicity of fibrous materials are also required. Along this line, correlations between gene expression and pathologic changes induced by fibrous materials may prove to be a powerful approach for assessing health risks due to fiber exposure.

REFERENCES

- Fujino A, Hori H, Higashi T, Morimoto Y, Tanaka I, Kaji H. *In-vitro* biological study evaluates the toxic potentials of fibrous materials. *Int J Occup Environ Health* 1:21-28 (1995).
- Driscoll KE, Maurern JK, Hassenbein D, Carter J, Jansen MWY, Mossman BT, Osier M, Oberdorster G. Contribution of macrophage-derived cytokines and cytokine networks to mineral dust-induced lung inflammation. In: *Toxic and Carcinogenic Effects of Solid Particles in Respiratory Tract* (Mohr U, Dungworth DL, Mauderly JL, Oberdorster G, eds). Washington: ILSI Press, 1994;170-190.
- Ward PA, Mulligan MS. New insights into mechanisms of oxyradical and neutrophil mediated lung injury. *Klin Wochenschr* 69:1009-1011 (1991).
- Warner RL, Paine R 3rd, Christensen PJ, Marletta MA, Richards MK, Wilcoxon SE, Ward PA. Lung sources and cytokine requirements for *in vivo* expression of inducible nitric oxide synthase. *Am J Respir Cell Mol Biol* 12(6):649-661 (1995).
- Vanhee 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 1, and transforming growth factor and relationship to disease severity. *Am J Respir Crit Care Med* 150:1049-1055 (1994).
- Folkman J, Klagsbrun M. Angiogenic factors. *Science* 235:442-447 (1987).
- Jordana M, Richards C, Irving LB, Gauldie J. Spontaneous *in vitro* release of alveolar-macrophage cytokines after the intratracheal instillation of bleomycin in rats. *Am Rev Respir Dis* 137:1135-1140 (1988).
- Chomczynski P, Sacchi N. Single step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162:156-159 (1987).
- Weisner RJ, Zac R. Quantitative approaches for studying gene expression. *Am J Physiol* 260:L179-L188 (1991).
- Pott F. Testing the carcinogenicity of fibers in laboratory animals: results and conclusions. In: *Fiber Toxicology* (Warheit DB, ed.). San Diego: Academic Press, 1993; 395-424.
- Mohler KM, Butler LD. Quantification of cytokine mRNA levels utilizing the reverse transcriptase-polymerase chain reaction following primary antigen specific sensitization *in vivo*. *Molec Immunol* 28:437-447 (1991).
- Zamorano PL, Mahesh VB, Brann DW. Quantative RT-PCR for neuroendocrine studies. *Neuroendocrinology* 63:397-407 (1996).
- Hesterberg TW, Miiller WC, Thevenaz P, Anderson R. Chronic inhalation studies of man-made vitreous fibres: characterization of fibres in the exposure aerosol and lungs. *Ann Occup Hyg* 5:637-653 (1995).
- Lee KP, Barras CE, Griffith FD, Waritz RS. Pulmonary response and transmigration of inorganic fibers by inhalation exposure. *Am J Pathol* 102:314-323 (1981).
- Miller K, Hudpith BN, Meredith C. Secretory and accessory cell function of alveolar macrophage. *Environ Health Perspect* 97:85-89 (1992).
- Xing Z, Jordana M, Kirpalani H, Driscoll KE, Schall TJ, Gauldie J. Cytokine expression by neutrophil and macrophages *in vivo*: endotoxin induces tumor necrosis factor- α , macrophage inflammatory protein-2, interleukin-1 β , and interleukin-6 but not RANTES or transforming growth factor- β 1 mRNA expression in acute lung inflammation. *Am J Respir Cell Mol Biol* 10:148-153 (1994).
- Strieter RM, Phan SH, Showell HJ, Remick DG, Lynch JP, Genord M, Raiford C, Eskandari M, Marks RM, Kunkel SL. Monokine-induced neutrophil chemotactic factor gene expression in human fibroblasts. *J Biol Chem* 264(18):10621-10626 (1989).
- Zhang Y, Lee TC, Guillemin B, Yu MC, Rom WN. Enhanced IL-1 β and tumor necrosis factor- α release and messenger RNA expression in macrophages from idiopathic pulmonary fibrosis or after asbestos exposure. *J Immunol* 150(9):4188-4196 (1993).
- Piguet PF, Vesin C. Treatment by human recombinant soluble TNF receptor of pulmonary fibrosis induced by bleomycin or silica in mice. *Eur Respir J* 7(3):515-518 (1994).
- Lemaire I, Beaudoin H, Masse S, Grondin C. Alveolar macrophage stimulation of lung fibroblast growth in asbestos-

- induced pulmonary fibrosis. *Am J Pathol* 122:205–211 (1986).
21. Schindler R, Mancilla J, Endres S, Ghorbani R, Clark SC, Dinarello CA. Correlations and interactions in the production of interleukin-6 (IL-6), IL-1, and tumor necrosis factor (TNF) in human blood mononuclear cells: IL-6 suppresses IL-1 and TNF. *Blood* 75(1):40–47 (1990).
22. Gosset P, Lassalle P, Vanhee D, Wallaert B, Aerts C, Voisin C, Tonnel AB. Production of tumor necrosis factor- α and interleukin-6 by human alveolar macrophages exposed *in vitro* to coal mine dust. *Am J Respir Cell Mol Biol* 5(5):431–436 (1991).
23. Shing Y, Folkman J, Haudenschild C, Lund D, Crum R, Klagsbrun M. Angiogenesis is stimulated by a tumor-derived endothelial cell growth factor. *J Cell Biochem* 29:275–287 (1985).
24. Lemaire I, Beaudoin H, Dubois C. Cytokene regulation of lung fibroblast proliferation. *Am Rev Respir Dis* 134:653–658 (1986).
25. Lee KP, Barras CE, Griffith FD, Waritz RS, Lapin CA. Comparative pulmonary response to inhaled inorganic fibers with asbestos and fiberglass. *Environ Res* 24:167–191 (1981).
26. Hesterberg T, Chase G, Versen R, Anderson R. Studies to assess the carcinogenic potential of man-made vitreous fibers. In: *Toxicology of Industrial Compounds* (Thomas H, Hess R, Waechter F, eds). Bristol, PA:Taylor and Francis, 1995;93–117.
27. Miyazaki Y, Araki K, Vesin C, Garcia I, Kapanci Y, Whitsett JA. Expression of a tumor necrosis factor- α transgene in murine lung causes lymphocytic and fibrosing alveolitis. A mouse model of progressive pulmonary fibrosis. *J Clin Invest* 96:250–259 (1995).