Applied Occupational and Environmental Hygiene

ISSN: 1047-322X (Print) 1521-0898 (Online) Journal homepage: http://www.tandfonline.com/loi/uaoh20

Role of Cytokines and Mineral Particle Profile in the Development of Coal Workers' Pneumoconiosis as Assessed by Bronchoalveolar Lavage

S. L. Webe , N. L. Lapp , V. Vallyathan , V. Castranova , J. Shumaker & D. Schwegler-Berry

To cite this article: S. L. Webe , N. L. Lapp , V. Vallyathan , V. Castranova , J. Shumaker & D. Schwegler-Berry (1996) Role of Cytokines and Mineral Particle Profile in the Development of Coal Workers' Pneumoconiosis as Assessed by Bronchoalveolar Lavage, Applied Occupational and Environmental Hygiene, 11:7, 923-927, DOI: 10.1080/1047322X.1996.10389991

To link to this article: http://dx.doi.org/10.1080/1047322X.1996.10389991

Role of Cytokines and Mineral Particle Profile in the Development of Coal Workers' Pneumoconiosis as Assessed by Bronchoalveolar Lavage

S.L. Weber,[1] N.L. Lapp,[2] V. Vallyathan,[2] V. Castranova,[2] J. Shumaker, and D. Schwegler-Berry[2]

[1]Section of Pulmonary and Critical Care Medicine, West Virginia University; Division of Respiratory Disease Studies, [2]National Institute for Occupational Safety and Health, Morgantown, West Virginia

The alveolar macrophage plays a number of important roles in the pathogenesis of silicosis and coal workers' pneumoconiosis (CWP). These roles include phagocytosis of dust particles, release of fibrogenic cytokines, and recruitment and activation of inflammatory cells. Three cytokines, fibronectin, tumor necrosis factor alpha (TNF-α), and interleukin-1β (IL-1β) were measured in the bronchoalveolar lavage fluid (BALF) of controls and asymptomatic coal miners. In addition the alveolar macrophages (AM) from these subjects were analyzed using transmission electron microscopy with X-ray diffraction backscatter in order to determine the number and types of intracellular particles present in these macrophages. Alveolar macrophages from miners contained significantly more particles and silica than controls. Coal miners also exhibited lower levels of TNF-α in lavage fluid. Fibronectin and IL-1β measured in the lavage fluid was not significantly different than controls. This lower TNF-α and lack of elevation of fibronectin and IL-1β in asymptomatic coal miners contrasts with reports of elevated TNF-α in miners with CWP. This disparity may be relevant to the lack of disease in the presence of low-level exposure. WEBER, S.L.; LAPP, N.L.; VALLYATHAN, V.; CASTRANOVA, V.; SHUMAKER, J.; SCHWEGLER-BERRY, D.: ROLE OF CYTOKINES AND MINERAL PARTICLE PROFILE IN THE DEVELOPMENT OF COAL WORKERS' PNEUMOCONIOSIS AS ASSESSED BY BRONCHOALVEOLAR LAVAGE. APPL. OCCUP. ENVIRON. HYG. 11(7):923–927; 1996.

Exposure to coal mine dust leads to the development of a wide spectrum of pulmonary disorders such as coal workers' pneumoconiosis (CWP), silicosis, chronic bronchitis, Caplan's syndrome, and focal emphysema. Despite intensive research over several decades, the major factor(s) responsible for the development of this spectrum of diseases remains unclear. Coal mine dust is a complex mixture of coal dust containing different proportions of inorganic minerals, metals, and organics with different types of coal depending on the type of coal seam. Several studies have attributed the role of silica, type of coal, and other immunologic factors in the development of CWP and other pulmonary diseases. The relationship between the type and severity of CWP and the composition of inhaled coal mine dust remains a subject of debate. It has been shown that severe CWP could develop after inhalation of silica free carbon[1] and in bituminous miners exposed to coal containing relatively low levels of silica.[2]

Studies that present evidence on the important role of alveolar macrophages in the initiation of injury leading to the fibrogenic process have been well reviewed.[3,4] The interaction between alveolar macrophages and inhaled dust particles is fundamental to the understanding of early events that lead to fibrogenesis. Inhalation of dust leads to an influx of inflammatory cells, increased release of reactive products that can cause cell and/or tissue damage, and the release of inflammatory or fibrogenic cytokines. This results in a retained particle burden that continues the inflammatory and fibrogenic cascade.[5]

It has also been shown that continued presence of tumor necrosis factor (TNFα) and interleukin 1-β (IL-1-β) in a balanced state prevent cell damage.[6] Because the macrophages are the major source of these cytokines, it is hypothesized that the cytokine functions are perturbed or modified by the interaction of toxic and nontoxic dust at variable levels, resulting in prolonged inflammatory response and fibrogenesis. It is also possible a decreased TNF and IL-1β production as a result of toxic dust interactions could result in direct death of macrophages. In experimental animals exposed to crystalline silica or bleomycin, TNF-α and IL1-β secreted by the alveolar macrophages have been shown to have a major role in the development of fibrosis.[7]

Stimulation of TNF-α and IL1-β release from rat alveolar macrophages by exposure to crystalline silica has been shown to be dose dependent and such an effect is not present with titanium dioxide, a nontoxic dust.[8] From these studies, it is evident that TNF-α and IL1-β have important roles in the fibrogenesis process, often exerting synergistic effects including stimulation of fibroblast proliferation.

In this study we postulated that activated alveolar macrophages after phagocytosis of toxic and nontoxic particles are upregulated in the synthesis of cytokines. In an autoregulatory mode an inhibitory cytokine, IL-10, also shown to be produced by human monocytes, downregulates the synthesis and release of inflammatory fibrogenic cytokines, thereby exhibiting only a transient reaction. However, in contrast, an upregulation of the inflammatory cytokines could be expected by the outlined process of cell death, repeated phagocytosis, impaired clearance, or impaired release of IL-10 due to direct, prolonged release of inflammatory cytokines.

Methods

This study was approved by the Institutional Review Board for the Protection of Human Subjects.

Subjects were recruited by word of mouth, advertisements in newspapers, contact with unions, and by posters. Twenty miners and 19 controls were recruited for study. Miners were required to have at least 5 years underground exposure, most of which included jobs with high silica exposure such as surface drilling, roof bolting, or rock drilling. All subjects were required to be lifelong nonsmokers. An explanation of the study was given and consent to participate was obtained in writing. Both miners and controls completed a symptom, occupational, and medical history questionnaire and underwent physical examination of the cardiopulmonary system. Posteroanterior and lateral chest radiographs were obtained and classified on the ILO system for the appearances of pneumoconioses. Screening pulmonary function tests included spirometry and diffusing capacity.

The day following screening studies, the subjects underwent bronchoscopy with bronchoalveolar lavage in a subsegment of the right middle lobe. The procedure was carried out with atropine premedication to reduce secretions and local anesthesia with topical lidocaine. Lavage was performed with warmed saline solution instilled in 50-ml aliquots and removed with gentle suction. The total lavage volume instilled was constant at 200 ml. Variable amounts of lavage fluid were obtained by gentle suction. The mean return volume was approximately 60 to 65 percent of that instilled in both the controls and miners.

Cytokine Analysis

Lavage fluid was spun down at 600 *g* on a Sorvall RT6000B Centrifuge to separate the supernatant from suspended cells or particles. The supernatant was then stored at 10°C and kept overnight to be concentrated 10 times on a Minitan Ultrafiltration System (Millipore Co., Bedford, Massachusetts). The concentrate was aliquoted into microcentrifuge tubes and frozen at −20°C until ready to be used in the immunoassay tests.

On the day of each assay, aliquots were thawed and run undiluted in the human TNF-α and Il-1β ELIZA kits (R&D Systems, Minneapolis, Minnesota). The tests were run according to the standard procedure and read on a Dynatech Microplate Reader (Dynatech Labs, Inc. Alexandria, Virginia). The human Fibronectin Immunoassay (Biomedical Technologies, Inc. Stoughton, Massachusetts) was also run according to procedure and read on a Beckman DU650 Spectrophotometer (Beckman Instruments, Inc. Fullerton, California).

Particle Analysis and Characterization

Samples of BAL lavage alveolar macrophages were pelleted and fixed overnight in Karnovsky's fixative at 4°C. They were then post-fixed in osmium tetroxide and embedded in LX-112 resin. Two-micron sections were cut and placed on spectroscopically pure carbon planchets and attached by placing on a hot-plate. The specimens were viewed in an ETEC scanning electron microscope equipped with a solid-state backscatter electron detector and a PG&T energy dispersive spectrometer (EDS). Particles within the macrophages were identified by backscatter electron imaging and analyzed by EDS for 60 seconds at a magnification ×2000. Fifteen random areas in two sample preparations were evaluated for number of macrophages viewed and number of particles identified as silica. Approximately 250 macrophages were counted and evaluated for number of particles and particles identified as silica.

TABLE 1. Characteristics of the Study Population

Parameter	Controls	Miners
Age (y)	39.1 (1.6)*	41.9 (1.3)
Height (cm)	175 (1.5)	176 (1.2)
Years underground	0	16.2 (1.4)
FVC (L)	4.79 (0.19)	4.85 (0.12)
FEV_1 (L)	3.98 (0.14)	4.01 (0.12)
FEV_1/FVC (%)	83 (1.7)	82 (1.2)
FEF25-75% ($L \times sec^{-1}$)	4.51 (0.30)	4.23 (0.25)
DLCO ($ml \times mmHg \times min^{-1}$)	34 (1.6)	36 (1.0)

*Mean ± SEM.

Statistical Methods

Comparisons between the control subjects and miners were made using the Mann-Whitney U statistic for nonparametric data. Significance was determined when $p < 0.05$. The data were expressed as mean ±SEM.

Results

The characteristics of the study populations are summarized in Table 1. The control subjects and miners were well-matched with regard to age and height. The miners had spent an average of 16.2 years underground exposed to coal mine dust, whereas none of the control subjects had significant dust exposures of any type. The chest radiographs of the controls were all normal (category 0/1 or less). Two of the miners' radiographs were classified as category 1/0, four with category 0/1 and the rest were classified as 0/0.

The results of screening spirometry and single breath diffusing capacity are also listed in Table 1. There were no significant differences between the controls and miners with regard to either restriction of lung volumes (FVC) or airflow obstruction (FEV_1, $FEV_1/FVC\%$, or $FEF_{25-75\%}$). Similarly, no significant differences were observed between controls and miners with regard to gas exchange (D_{LCO}).

The results of analysis of TNF-α in the BAL fluid are presented in Figure 1. TNF-α measured in the miner's BAL fluid was significantly lower than in the BAL fluid from controls. In contrast, neither IL-1β (Figure 2) nor fibronectin (Figure 3) were found to be significantly different in the BAL fluid from controls or miners.

The results of the alveolar macrophage particle analysis are presented in Figures 4 and 5. The miners' macrophages had significantly more intracellular particles and many more particles identified as silica than the control subjects. These differences were significant.

Discussion

A growing body of evidence indicates that fibrotic lung disease in humans is associated with elevated release of various cytokines in bronchoalveolar lavage samples and in enhanced production of mediators and reactive oxygen species from harvested alveolar macrophages. Idiopathic pulmonary fibrosis and sarcoidosis are characterized by stimulation of fibronectin

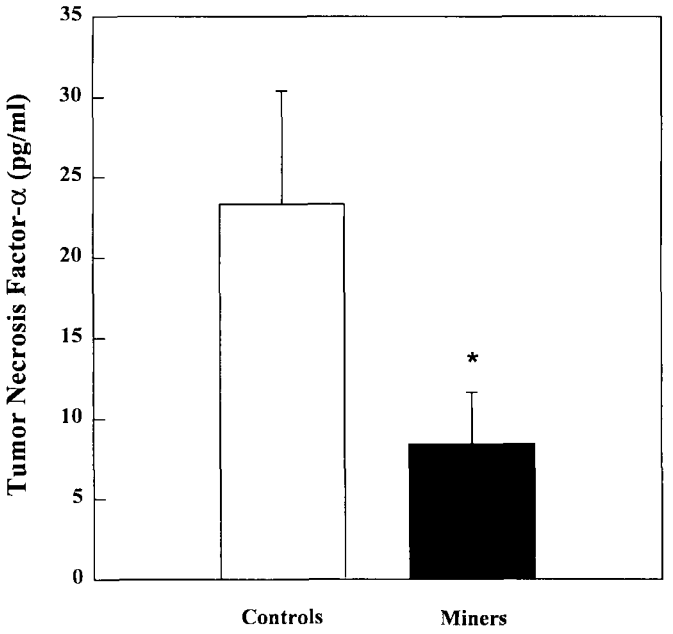

FIGURE 1. Tumor necrosis factor-α measured in the bronchoalveolar lavage fluid of controls and miners. *, $p < 0.05$.

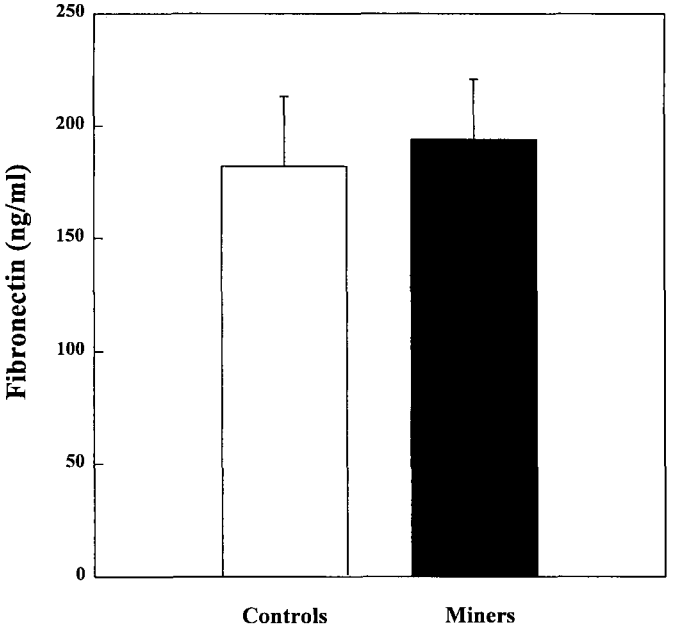

FIGURE 3. Fibronectin measured in the bronchoalveolar lavage fluid of controls and miners.

and insulin-like growth factor (IGF) production by alveolar macrophages.[9,10] Alveolar macrophages harvested from patients with sarcoidosis also release elevated levels of (IL-1β).[11] Further, increased production of reactive oxygen species and enhanced generation of chemiluminescence by alveolar macrophages has been reported in patients with idiopathic pulmonary fibrosis or silicosis.[12,13] A similar profile of elevated mediator production has been reported for CWP with increased production of fibronectin, interferon (IGF), and reactive oxygen species by alveolar macrophages noted.[13,14] In addition, TNF-α release from bronchoalveolar lavage cells is elevated in workers suffering from CWP.[15]

Animal and cellular studies support the role of cytokines and reactive species in the development of pulmonary fibrosis. Reactive oxygen species can cause cell damage and the resulting scarring of lung tissue.[16] Fibronectin acts as a competence

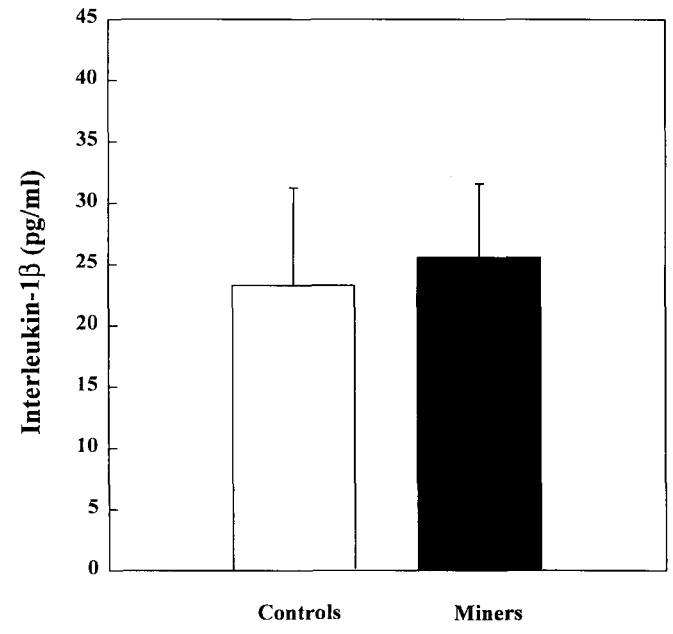

FIGURE 2. Interleukin-1β measured in the bronchoalveolar lavage fluid of controls and miners.

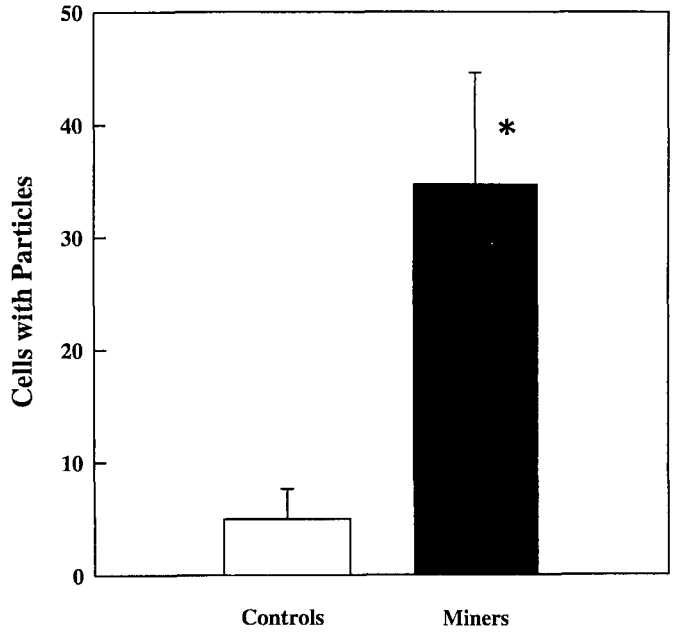

FIGURE 4. Number of alveolar macrophages in bronchoalveolar lavage from controls and miners that contained particles. *, $p < 0.05$.

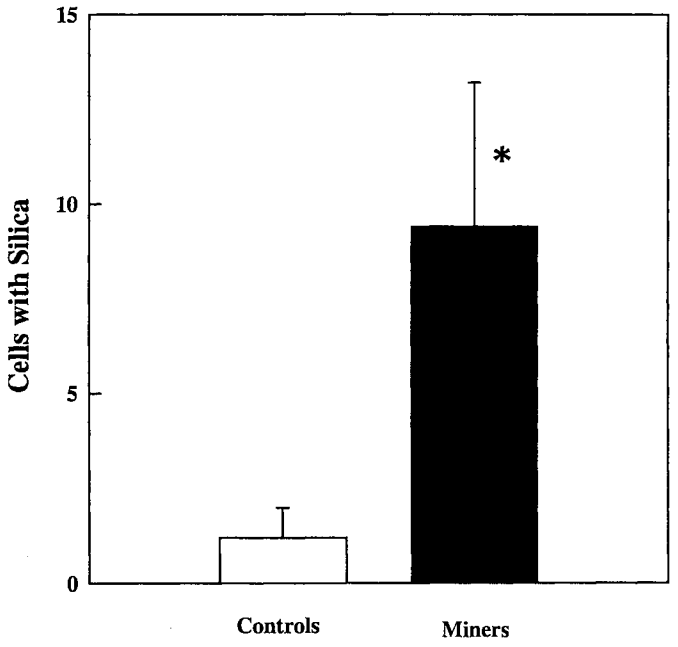

FIGURE 5. Number of alveolar macrophages in bronchoalveolar lavage from controls and miners that contained silica. *, $p < 0.05$.

factor for the proliferation of fibroblasts.[17] TNF-α activates neutrophils,[18] causes pulmonary granulomas,[19] and increases proliferation and collagen synthesis by fibroblasts.[20] The important role of TNF-α in the development of pulmonary fibrosis is emphasized further by a study showing that pretreatment of mice with anti-TNF-α prevents the silica-induced rise in pulmonary collagen levels.[7] IL-1β has been associated with pulmonary granulomas.[21] However, the effect of IL-1β on fibroblasts is controversial with evidence that it both induces and inhibits proliferation.[22,23] Mangan et al.[6] proposed that it is the balance between TNF-α and IL-1β levels that is critical in controlling programmed cell death (apoptosis) and fibrogenesis.

In contrast to the human studies cited above, results from the present investigation of asymptomatic, nonsmoking coal miners show no increase in either IL-1β or fibronectin levels in bronchoalveolar lavage samples, and a decrease in lavage TNF-α compared to controls. Similarly, previous studies from our lab indicate that basal and PNA-stimulated chemiluminescence by alveolar macrophages obtained from asymptomatic coal miners were not significantly different from controls.[24]

Even though cytokines and reactive oxygen species were not elevated in asymptomatic miners, indications of particulate exposure were evident. In a randomly selected microscopic evaluation of lavage cell populations, 68 percent of the miners' alveolar macrophages were found to contain dust. The mean particle load in the cells was 9.36 $\pm$ 1.19 of which 1.65 $\pm$ 0.29 showed birefringence. In contrast to this, only 8 percent of control alveolar macrophages were found to have dust. The mean particle count in these cells was 2.46 $\pm$ 0.53 of which only 0.033 $\pm$ 0.033 showed birefringence. In addition, we have shown previously that pulmonary macrophages from asymptomatic miners exhibited more surface ruffling than controls suggesting activation in its early stages.[24] Differences in cytokine and reactive oxygen levels may vary dramatically over the course of disease process. Thus, miners such as ours, without symptoms and radiographic and functional evidence of disease may exhibit normal levels, while patients with frank disease show significant elevations in the levels of these inflammatory and fibrotic mediators.

While it is expected that cytokine levels in the BALF may not accurately reflect the levels found in pulmonary phagocytes at their source, they do seem to correlate with disease progression as assessed by independent means (i.e., radiographic changes). Thus, an explanation for our findings may be that our subjects were not experiencing fibrosis and may not in the future.

Our findings are not explained by differences in BALF recovery. The volume of saline instilled was constant for each subject and the recovery volumes were not significantly different between the miners and controls.

At what point in disease development and progression significant changes in these mediators become discernible is a question for further investigation.

Acknowledgment

This research has been supported by the Department of the Interior's Mineral Institute Program administered by the U.S. Bureau of Mines through the Generic Mineral Technology Center for Respirable Dust under grant number G-1115142.

References

1. Collis, E.L.; Gilchrist, J.C.: Effects of Dusts on Coal Trimmers. J. Ind. Hyg. Toxic. 10:101–109 (1928).
2. Gough, J.: Pneumoconiosis of Coal Trimmers. J. Path. Bact. 51:277–285 (1940).
3. Davis, G.S.: Pathogenesis of Silicosis: Current Concepts and Hypotheses. Lung 164:139–154 (1986).
4. Ziskind, M.; Jones, R.N.; Weil, H.: Silicosis. Am. Rev. Respir. Dis. 113:643–645 (1976).
5. Lapp, N.L.; Castranova, V.: How Silicosis and Coal Workers' Pneumoconiosis Develop—A Cellular Assessment. Occup. Med: State of the Art Reviews. 8:35–56 (1993).
6. Mangan, D.F.; Welch, G.R.; Wahl, S.M.: Lipopolysacharide, Tumor Necrosis Factor Alpha, and IL-1 Beta Prevent Programmed Cell Death in Human Peripheral Blood Monocytes. J. Immunol. 146(5):1541–1546 (1991).
7. Piguet, P.F.; Collart, M.A.; Grau, G.E.; et al.: Requirement for Tumor Necrosis Factor for Development of Silica-Induced Pulmonary Fibrosis. Nature 344:245–251 (1990).
8. Driscoll, K.E.; Mauer, J.K.; Lindenschmidt, R.C.; et al.: Respiratory Tract Responses to Dust: Relationships Between Dust Burden, Lung Injury, Alveolar Macrophage Fibronectin Release, and the Development of Pulmonary Fibrosis. Toxicol. Appl. Pharmacol. 106:88–101 (1990).
9. Rennard, I.; Hunninghake, G.W.; Bitterman, P.B.; Crystal, R.G.: Production of Fibronectin by the Human Alveolar Macrophage: Mechanisms for the Recruitment of Fibroblasts to Sites of Tissue Injury in Interstitial Lung Disease. Proc. Natl. Acad. Sci. USA 78:7147–7151 (1981).
10. Bitterman, P.B.; Adelberg, S.; Crystal, R.G.: Mechanism of Pulmonary Fibrosis: Spontaneous Release of the Alveolar Macrophage-Derived Growth Factor in the Interstitial Lung Disorders. J. Clin. Invest. 72:1801–1813 (1983).
11. Hunninghake, G.W.: Release of Interleukin-1 by Alveolar Mac-

rophages of Patients with Active Pulmonary Sarcoidosis. Am. Rev. Respir. Dis. 129:569–572 (1984).

12. Strausz, J.; Muller-Querheim, J.; Steppling, H.; Ferlinz, R.: Oxygen Radical Production by Alveolar Inflammatory Cells in Idiopathic Pulmonary Fibrosis. Am. Rev. Respir. Dis. 141:124–128 (1990).
13. Rom, W.M.; Bitterman, P.B.; Rennard, S.I.; et al.: Characterization of the Lower Respiratory Tract Inflammation of Non-Smoking Individuals with Interstitial Lung Disease Associated with Chronic Inhalation of Inorganic Dusts. Am. Rev. Respir. Dis. 136:1429–1434 (1987).
14. Wallaert, B.; Lassalle, P.; Fortin, F.; et al.: Superoxide Anion Generation by Alveolar Inflammatory Cells in Simple Pneumoconiosis and in Progressive Massive Fibrosis of Non-Smoking Coal Workers. Am. Rev. Respir. Dis. 141:129–133 (1990).
15. Lasssale, P.; Gosset, P.; Aerts, C.; et al.: Alveolar Macrophage Secretory Dysfunctions in Coal Workers' Pneumoconiosis. Comparison Between Simple Pneumoconiosis and Progressive Massive Fibrosis. In: Effects of Mineral Dusts on Cells, H30:65–71. B.T. Mossman and R.O. Begin, Eds. Springer-Verlag, Berlin (1989).
16. Weiss, S.J.; LoBuglio, A.F.: Biology of Disease: Phagocyte-Generated Oxygen Metabolites and Cellular Injury. Lab. Invest. 47:5–18 (1982).
17. Bitterman, P.B.; Wewers, M.D., Rennard, S.I.; et al.: Modulation of Alveolar Macrophage-Derived Fibroblast Proliferation by Alternative Macrophage Mediators. J. Clin. Invest. 77:700–708 (1986).
18. Tsujimoto, M.; Yokota, S.; Vilcek, J.; Weissman, G.: Tumor Necrosis Factor Provokes Superoxide Anion Generation from Neutrophils. Biochem. Biophys. Res. Commun. 137:1094–1100 (1986).
19. Kasahara, K.; Kobayashi, K.; Shikama, Y.; et al.: The Role of Monokines in Granuloma Formation in Mice: The Ability of Interleukin-1 and Tumor Necrosis Factor-α to Induce Lung Granulomas. Clin. Immunol. Immunopathol. 51:419–426 (1989).
20. Vilcek, J.; Palombella, V.J.; Henriksen-DeStefano, D.; et al.: Fibroblast Growth Enhancing Activity of Tumor Necrosis Factor and Its Relationship to Other Polypeptide Growth Factors. J. Exp. Med. 163:632–643 (1981).
21. Kasahara, K.; Kobayashi, K.; Shikama, Y.; et al.: Direct Evidence for Granuloma-Inducing Activity of Interleukin-1. Am. J. Pathol. 130:629–634 (1988).
22. Prostlethwaite, A.E.; Lachman, L.B.; Kang, A.H.: Induction of Fibroblast Proliferation by Interleukin-1 Derived from Human Monocytic Leukemia Cells. Arthritis Rheum. 27:995–1001 (1984).
23. Hori, T.; Yamunake, Y.; Hayukuwa, M.; et al.: Prostaglandins Antagonize Fibroblast Proliferation Stimulated by Tumor Necrosis Factor. Biochem. Biophys. Res. Commun. 174:758–766 (1991).
24. Lapp, N.L.; Lewis, D.; Schwegler-Berry, D.; et al.: Bronchoalveolar Lavage in Asymptomatic Underground Coal Miners, pp. 159–169. In: Proc. Respir. Dust in the Mine Indus. R.V. Ramani, Ed. Society of Mining Engineering (1991).