

REVIEW

Cytokines and cytokine network in silicosis and coal workers' pneumoconiosis

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Cytokines and cytokine network in silicosis and coal workers' pneumoconiosis. D. Vanhée, P. Gosset, A. Boitelle, B. Wallaert, A.B. Tonnel. ©ERS Journals Ltd., 1995.

ABSTRACT: The alveolar macrophage (AM) is a critically important cell playing a prominent role in lung inflammation *via* the production of oxygen radicals, enzymes, arachidonic acid metabolites, and also a large panel of cytokines. Among interstitial lung disorders, silicosis and coal workers' pneumoconiosis (CWP) are the most widespread fibrotic lung diseases. Although their pathophysiology remains incompletely understood, several lines of evidence suggest the participation of cytokines produced by AMs at least in the initiation of the alveolitis.

In vitro exposure of AMs (obtained from healthy subjects) to coal dust particles triggered a significant release of tumour necrosis factor (TNF) and interleukin-6, by comparison with titanium dioxide used as a biologically inert control dust. Moreover, it appeared that coal mine dust was more aggressive than similar concentrations of pure silica, suggesting that cytokine secretion induced by coal mine dust was not exclusively related to the presence of silica but resulted from a complex interaction between the different components.

In silicosis and CWP, bronchoalveolar lavage showed a large influx of mononuclear phagocytes, with an increased spontaneous production of oxidants, fibronectin, neutrophil chemotactic factor, and also of interleukin-6 and TNF- α . This spontaneous cytokine release was associated with an increased cytokine messenger ribonucleic acid (mRNA) expression in the lungs of coal miners. Two profibrotic factors, platelet-derived growth factor and insulin-like growth factor-1 (PDGF and IGF-1), were the factors mainly secreted by AMs in patients with progressive massive fibrosis, whereas transforming-growth factor- β (TGF- β) production was predominant in AMs obtained from patients with simple pneumoconiosis, suggesting a potential protective effect of TGF- β on the development of pulmonary fibrosis in coal workers' pneumoconiosis.

Eur Respir J., 1995, 8, 834–842.

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Keywords: Alveolar macrophages
coal workers' pneumoconiosis
cytokines
silicosis

Received: June 27 1994
Accepted after revision January 4 1995

Despite the progressive closing of coal mines in Europe, coal workers' pneumoconiosis (CWP) is still a frequent interstitial pulmonary disease. CWP is usually divided into two stages: simple pneumoconiosis (SP), in which fibrotic lesions remain limited, with radiological opacities smaller than 1 cm, and progressive massive fibrosis (PMF), characterized by the development of a perifocal extensive fibrotic response of the lung and severe alterations in pulmonary function [1–5]. Although pathophysiological mechanisms remain incompletely understood, there is acceptance of the concept implicating chronic inflammatory processes in the development of the pulmonary lesions. Following inorganic coal dust exposure, lung tissue responds by initiating three types of phenomena: 1) an accumulation and activation of inflammatory cells in the lower respiratory tract [6–8]; 2) a fibroblast proliferation [9]; and 3) an enhanced synthesis of extracellular matrix components [10, 11]. The intensity of these inflammatory and fibrotic processes

varies and is exaggerated in PMF. In addition, CWP is also frequently associated with the development of perifocal emphysematous lesions [12–14].

Current concepts in the pathogenesis of pneumoconiosis suggest that alveolar macrophages play a pivotal role because of their ability to release mediators, such as eicosanoid metabolites, destructive proteolytic enzymes, and inflammatory growth and differentiation factors [15–17]. More recently, resident cells (such as endothelial cells, epithelial cells, fibroblasts) have been shown to be effector cells themselves, secreting and expressing various cytokines and molecules involved in inflammatory and fibrotic processes [18–20].

In the chronic phase leading to pulmonary fibrosis in the pneumoconiotic lung, it is clear that alveolar macrophage-derived cytokines may play an important part. This short review will concentrate on the role of cytokines in the pathogenesis of silicosis and CWP with a special emphasis on chronic inflammation and fibrosis.

Alveolar macrophage activation and cytokine production after *in vitro* exposure to mineral particles

After exposure to mineral dust particles *in vitro*, alveolar macrophages (AMs) are known to release oxygen radicals and eicosanoids [21, 22]. Macrophages also have the ability to secrete a large panel of cytokines and fibroblast growth and differentiating factors when cultured in the presence of mineral particles [23], such as pure silica and coal mine dust, or inert particles, such as titanium dioxide (TiO₂).

Silica was shown to trigger the secretion of tumour necrosis factor- α (TNF- α) [24], interleukin-1 (IL-1) [25], or interleukin-6 (IL-6) [24] in a density- and time-dependent manner, and at higher levels than those observed after inert particle exposure [26]. DUBOIS *et al.* [27] demonstrated that alveolar macrophages incubated in the presence of silica produced both TNF and leukotriene B₄ (LTB₄), and that endogenous lipoxygenase metabolites could act to amplify TNF production. Silica exposure could also trigger the generation of prostaglandin E₂ and D₂ (PGE₂ and PGD₂) and thromboxane B₂ (TxB₂) [21]. Interestingly, PGE₂ modulates TNF-induced macrophage activation. Conversely, TNF stimulates PGE₂ release [28], which in turn suppresses TNF synthesis in an autocrine manner [29]. Thus, LTB₄ and PGE₂ may represent two important regulatory molecules in the response to inorganic particle exposure.

However, the respective roles of silica and other compounds present in coal mine dust have been debated. GOSSET *et al.* [24] investigated the effects of *in vitro* exposure to coal dust and to its silica content on TNF, IL-1 and IL-6 production by normal human AMs. Coal dust induced the release of significant quantities of TNF and IL-6 compared to TiO₂, whereas IL-1 β secretion was not modified despite an enhanced expression of messenger ribonucleic acid (mRNA) for this cytokine. Moreover, after having investigated the respective roles of silica and coal dust in the stimulation of alveolar macrophages, GOSSET *et al.* [24] suggested that cytokine secretion can be induced by complex interactions of different compounds of coal mine dust and is not exclusively related to the unique presence of silica.

In addition to proinflammatory cytokines, numerous studies have also found factors susceptible to modify fibroblast proliferation and collagen deposition in supernatants of AMs exposed to mineral particles [30, 31]. Both compact and fibrous particles induce AMs to secrete fibronectin [32–34], and large amounts of platelet-derived growth factor (PDGF) [35–37], which is known as a competence factor for fibroblast proliferation [38, 39].

Cytokines and experimental pneumoconiosis

Several recent studies have produced data supporting the implication of alveolar macrophage-derived cytokines in experimental models of pneumoconiosis.

The role of TNF has largely been documented in various animal models. Its central role has been clearly demonstrated by PIGUET and co-workers [40]. After intratracheal instillation of silica, a marked increase in the level of lung TNF mRNA was observed, which persisted beyond the 70th day. More interestingly, the silica-induced collagen production was significantly reduced after treatment with anti-TNF antibodies. In the same model, PIGUET and co-workers [41], suggested the role of IL-1 receptor antagonist (IL-1ra), since treatment of silica-exposed mice with IL-1ra prevented the development of fibrotic lesions.

DRISCOLL *et al.* [42] observed the production of two chemotactic mediators, macrophage inflammatory protein-1 and -2 (MIP-1, MIP-2) by alveolar macrophages, but also by fibroblasts or epithelial cells in rats exposed to silica.

In addition, the expression of proliferative activities for different cell types has been described. An epithelial type II cell growth factor (with biochemical characteristics consistent with platelet-derived growth factor (PDGF) or fibroblast growth-factor (FGF)-like molecules) was observed in bronchoalveolar lavage fluid from silica-exposed sheep [43]. In the same model, the expression of different proliferative activities for fibroblasts was observed. The implication of PDGF and its receptor, of transforming growth factor- β (TGF- β), or of fibronectin have been suggested in different animal models of pneumoconiosis [34, 44, 45]. In addition to its modulatory role in the production of collagen [46], TGF- β may act as a potent inhibitory mediator for the proliferation of type II cells, as shown in a murine model of silicosis, and thus stabilize the lung structure and the behaviour of the alveolar cell population [47].

Another potential participant in the cellular network involved in pneumoconiosis is the lymphocyte. In a murine model of silicosis, RAKESH *et al.* [48] suggested the implication of lymphocyte-derived interferon- γ (IFN- γ) in the production of fibroblast growth factors by macrophages obtained from silica-exposed animals, but not from TiO₂-exposed animals. However, a recent study seems to contradict these conclusions. BRODY *et al.* [49] demonstrated that recombinant IFN- γ , although stimulating the production of PDGF by AMs, was a potent inhibitor of fibroblast proliferation. Moreover, IFN- γ might also be able to inhibit the secretory functions of fibroblasts [46].

Cytokines and alveolar macrophage activation in silicosis and coal workers' pneumoconiosis

On the basis of results obtained *in vitro* and in animal models, a list of mediators, potentially implicated in the development of silicosis and coal workers' pneumoconiosis have been defined. To determine the clinical relevance of these mediators more precisely, studies have been carried out in coal miners. In some studies, AMs, recovered by bronchoalveolar lavage (BAL) in mineral dust-exposed patients and unexposed controls [50], were tested for their spontaneous ability to secrete these factors.

TNF, IL-1 and IL-6

In silicosis, blood monocytes secrete exaggerated quantities of TNF and IL-1 when compared to controls [51]. In contrast, AMs from patients with coal workers' pneumoconiosis released increased amounts of TNF and IL-6, but not of IL-1 [24, 52, 53]. Moreover, when patients were divided into two groups, simple pneumoconiosis (SP) and progressive massive fibrosis (PMF), an increased secretion of TNF and IL-6 was observed in PMF patients compared to SP [52]. In our laboratory, we recently confirmed the presence of these proinflammatory cytokines, TNF and IL-6, in the lung of pneumoconiotic patients [54, 55, 56]. The anatomical relationship between the expression of the specific mRNA and the presence of particles was also confirmed, since a tight correlation was found between the expression of mRNA and the macrophage load of particles. In addition to the demonstration of AM activation in human lung exposed to mineral dust, this study also showed evidence for an endothelial cell activation, demonstrated by enhanced expression of IL-6 mRNA in vascular sections. The increased expression of TNF and IL-6 mRNA by AMs has been confirmed in other models of occupational lung disease, such as chronic beryllium disease [56] or asbestos [57].

These cytokines play a central role in inflammatory and fibrotic processes, as suggested by several lines of evidence. TNF induces the recruitment of inflammatory cells: lymphocytes, eosinophils and neutrophils, all of which are involved in interstitial lung disease. TNF is able to induce the production of chemotactic factors [18, 19, 58] or enhance the expression of adhesion molecules by resident cells (epithelial or endothelial cells) or AMs [59, 60]. *In vivo* TNF infusion induces pulmonary hyperpermeability and oedema, and elicits a rapid neutropenia and lymphocytopenia [61–63]. In the development of fibrosis following mineral dust exposure, TNF is known to stimulate fibroblast chemotaxis [64] or growth *in vitro* [65], either directly or by induction of growth and differentiating factors potentially active on fibroblast behaviour [66–68]. Although the role of TNF in the matrix deposition remains incompletely understood, there is evidence for an inhibition of collagen synthesis by TNF [69, 70], whilst collagenase gene expression is enhanced [28].

IL-6 has been implicated in the pathogenesis of pneumoconiotic disorders, following the observation of its secretion by AMs exposed to coal mine dust [24]. IL-6 is important in inflammatory processes, due to its ability to induce cellular adhesion molecules on monocytes, which facilitates their infiltration into the lung [71]. *In vivo*, IL-6 seems to be implicated in autoimmune processes in association with TNF and IL-1 [72]. The demonstration of IL-6 secretion in the pneumoconiotic lung might explain the frequent association with autoimmune diseases, as well as the hypergammaglobulinaemia observed in coal workers' pneumoconiosis [73, 74]. IL-6 could also be implicated in the fibrotic response due to its ability to induce collagen synthesis *in vivo* [75]. However, IL-6 has also been reported to exert some anti-

inflammatory properties. IL-6 has been shown to have a protective role in a mouse model of hypersensitivity pneumonitis and of septic shock [76, 77]. It also suppressed the acute neutrophil exudation caused by intratracheal instillation of endotoxin in rats [78]. Earlier studies showed that IL-6 inhibits lipopolysaccharide (LPS)-induced TNF and IL-1 production in cultured human monocytes, U937 cells, and in mice [79, 80].

PDGF, IGF-1 and TGF- β

In addition to TNF with its intrinsic capacity to promote fibroblast recruitment and replication [64, 65], other macrophage-derived mediators have been proposed as major mediators in the fibrotic process [9, 57, 81–83]. The development of fibrosis, namely of fibroblast proliferation, needs the interaction of two signals: a competence signal and a progression signal [84]. In this respect, AMs exert both types of activities through their capacities to release PDGF, a competence factor [38, 39], and insulin-like growth factor-1 (TGF-1) a progression factor [85]. In addition to these two well-defined growth-factors, other studies have described the production by AMs [24] of fibronectin, which is known to be a chemotactic factor for fibroblasts and to prime or facilitate fibroblast proliferation [32]. Another cytokine, TGF- β , appears to play an important, although controversial, role in lung fibrosis. The role of this cytokine has not been definitely established: it is thought to act as a mediator regulating chemotaxis and proliferation of fibroblasts [81]. In addition, TGF- β may suppress inflammatory reactions [86]. KALTER and BRODY [87] demonstrated the effective production of this cytokine by AMs exposed to mineral dust *in vitro*.

In order to determine the capacities of AMs, exposed *in vivo* to mineral dust, to modulate fibroblast growth, the secretion of these three mediators (PDGF, IGF-1, TGF- β) has recently been analysed in a population of patients with coal workers' pneumoconiosis [8, 88]. PDGF, IGF-1 and TGF- β were detected at higher levels in the epithelial lining fluid (ELF) of pneumoconiotic patients. The levels were different according to the degree of severity of the disease. PDGF and IGF-1 concentrations were elevated in ELF of patients with PMF, whilst TGF- β was found to predominate in ELF recovered from patients with SP. Although there has been much debate about the cellular origin of these profibrotic mediators, AMs represent one of the major sources [89], at least in pneumoconiosis. Indeed, the profile of the levels of these mediators in ELF mirrored their levels in AM supernatants (fig. 1). The TGF- β present in BAL fluid was almost entirely represented by the active form, which suggests a possible *in vivo* cleavage by a proteolytic enzyme, such as plasmin or cathepsin D [90, 91]. Moreover in CWP, AM supernatants from patients with PMF were able to promote proliferation of fibroblasts. By contrast, AMs from patients with SP did not induce fibroblast growth, but, on the contrary, inhibited ^3H -thymidine incorporation [88]. Also, the inhibition of ^3H -thymidine incorporation induced by AM supernatants

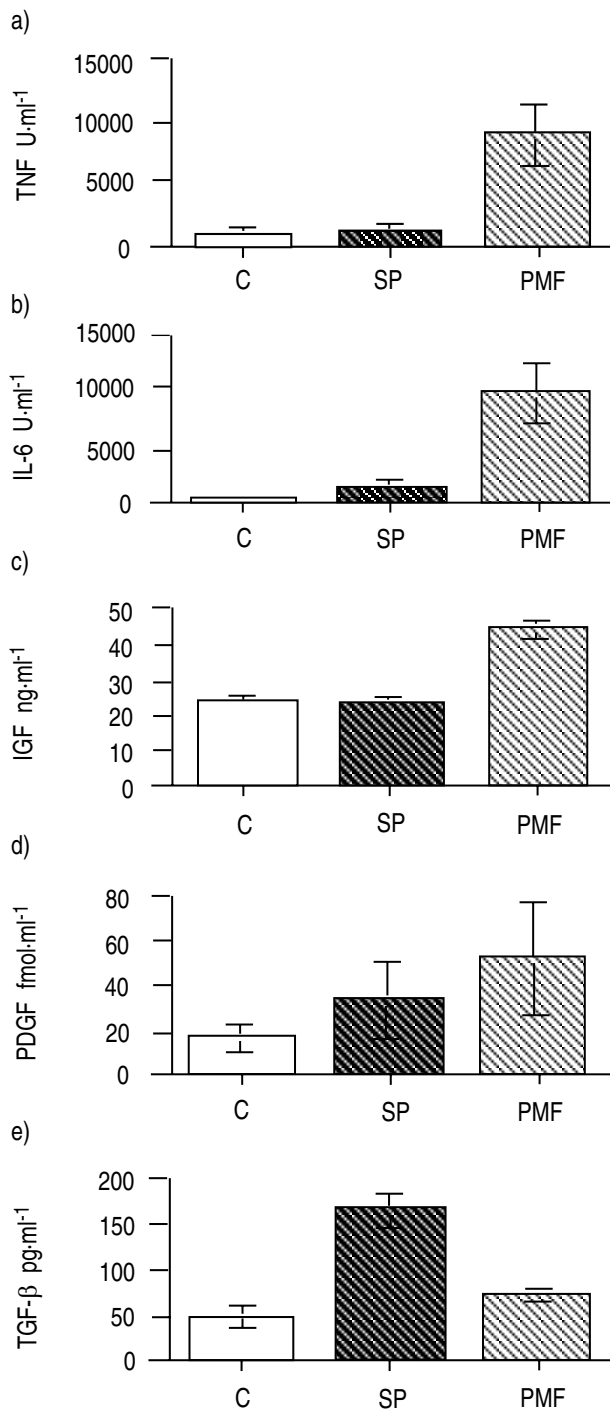


Fig. 1. — Profiles of secretion of: a) TNF; b) IL-6; c) IGF-1; d) PDGF; and e) TGF-β by alveolar macrophages from pneumoconiotic patients and control subjects. Supernatants were obtained from a 3 h culture of 3×10^6 macrophages·ml⁻¹ and then treated by acidification to release the active form of transforming growth factor-β (TGF-β). TNF and IL-6 were biologically assayed [54]. PDGF and IGF-1 levels were determined by radio-immunoassays, TGF-β was assayed by ELISA. Values are mean ± SEM. TNF: tumour necrosis factor; IL-6 interleukin-6; IGF-1: insulin-like growth factor-1; PDGF: platelet-derived growth factor; C: control n=14; SP: simple pneumoconiosis n=18; PMF: progressive massive fibrosis n=9; ELISA: enzyme-linked immunosorbant assay.

from patients with SP or by purified TGF-β was abolished by the addition of anti-TGF-β antibodies (fig. 2), suggesting that TGF-β present in AM supernatants of

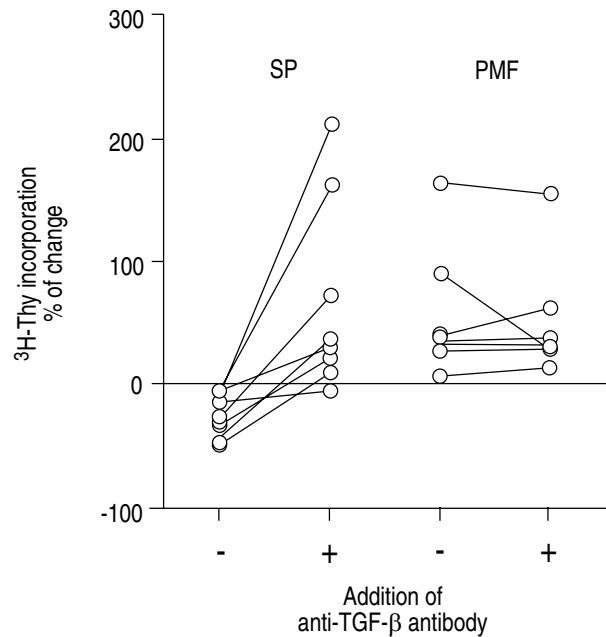


Fig. 2. — Proliferation of a human lung fibroblast cell line (MRC5) induced by alveolar macrophage supernatants from patients with coal workers' pneumoconiosis. Supernatants were obtained from a 3 h culture of 3×10^6 macrophages·ml⁻¹ and then treated by acidification to release the active form of transforming growth factor-β (TGF-β). Results are expressed as the percentage of change of ³H-thymidine (³H-Thy) incorporation between patients, and the mean incorporation obtained with a pool of control subjects ($1,253 \text{ cpm} \pm 115$). Anti-TGF-β antibodies were added to analyse the effect of TGF-β on the fibroblast proliferation. Antibodies were added at the same time as alveolar macrophage supernatants to the fibroblast culture. For abbreviations see legend to fig. 1.

patients with CWP inhibits fibroblast proliferation [88]. TGF-β has recently been described as a potent inhibitor of fibroblast growth [92, 94] due to modulation of PDGF receptor expression [95, 96]. At low concentrations, TGF-β was shown to induce the production of PDGF, which promotes fibroblast growth; at higher concentrations TGF-β down-regulated the PDGF receptor expression, which blocks the autocrine PDGF loop, or possibly directly inhibits fibroblast proliferation. However, other studies have provided contradictory data and are rather in favour of a profibrotic action of TGF-β [82]. Thus, in animals treated with bleomycin, TGF-β was increased and was thought to mediate bleomycin-induced fibrosis [89, 97]. Another possible impact of TGF-β is its ability to promote extracellular matrix protein synthesis [46, 81, 86]. TGF-β is known to induce collagen synthesis at similar levels for both type I and type III collagen [46]. As an increased ratio type I/type III collagen is observed in the lung of PMF [100, 101], this might suggest that TGF-β would not be the only cytokine involved in the development of collagen accumulation in pneumoconiotic lesions.

Additionally, TGF-β is known for its anti-inflammatory properties [86]. TGF-β can act at different points in the development of the inflammatory reaction. GAMBLE and co-workers [100–102] demonstrated that TGF-β could inhibit endothelial cell adhesiveness to leucocytes *via* an inhibition of the cellular adhesion molecule E-selectin. TGF-β deficient mice have been observed to

Table 1. – Characteristics of alveolar macrophage-derived mediators involved in the pathogenesis of coal workers' pneumoconiosis

Family	Mediators	Main functions
Cytokines	TNF	Initiation and regulation of inflammatory reaction Regulation of fibrotic reaction (proliferation of fibroblast and secretion of extracellular matrix components)
Growth factors	IL-6	Chemotaxis and activation of lymphocytes
	PDGF	Chemotaxis and activation of fibroblasts
	IGF-1	Proliferation of fibroblasts
	TGF- β	Regulation of inflammatory reaction Regulation of fibrotic reaction (modulation of fibroblast proliferation, expression of extracellular matrix components)
Chemokines	Fibronectin	Adhesion and proliferation of fibroblasts
	IL-8	Chemotaxis of inflammatory cells
	MIP-1 α	
	MIP-1 β	
Arachidonic acid derivatives	MIP-2	
	LTB $_4$	Regulation of macrophage activation Chemotaxis and activation of neutrophils
	PGE $_2$	Regulation of macrophage activation Regulation of fibroblast proliferation

TNF: tumour necrosis factor; IL: interleukin; PDGF: platelet-derived growth factor; IGF: insulin-like growth factor; TGF: transforming growth factor; MIP: macrophage inflammatory protein; LTB $_4$: leukotriene B $_4$; PGE $_2$: prostaglandin E $_2$

spontaneously develop a multifocal inflammatory disease [103–104]. DUBOIS *et al.* [105] showed that TGF- β is a potent inhibitor of IL-1 receptor expression [105]. TGF- β also induced monocyte production of IL-1ra [106, 107], a molecule that is known for its ability to prevent the development of silica-induced pulmonary fibrosis in mice [41]. Moreover, TSUNAWAKI *et al.* [108] described the deactivation of alveolar macrophages by TGF- β , leading to a possible decrease of TNF synthesis. Other recent observations support the hypothesis that TGF- β could be a protective agent for fibroblast proliferation and inflammatory reaction. MORELAND *et al.* [109] described a significantly higher concentration of TGF- β in normal lung compared to fibrotic scleroderma lung. YAMAUCHI *et al.* [110] discussed a possible role for TGF- β in stabilizing the lung structure and behaviour of cell populations present in the normal lung.

Silicosis and coal workers' pneumoconiosis: pathogenetic hypothesis

Activation of alveolar macrophages by coal mine dust leads to the production of a large panel of inflammatory and fibrotic mediators, possibly implicated in the development of pneumoconiotic lesions (table 1). Various factors, including TNF, IL-6, MIP-1, MIP-2, LTB $_4$, PGE $_2$, preferentially participate in the inflammatory reaction, whereas other mediators, such as TNF, TGF- β , PDGF, IGF-1, seem to be implicated in fibrotic processes.

Among these mediators, two cytokines, TNF and TGF- β , appear to play a key role in the control of the inflammatory and fibrotic response of the lung to coal mine dust. The central role of TNF has been demonstrated by several authors, supporting the concept that the overexpression of TNF must be implicated in the development of the extensive inflammatory and fibrotic reaction observed in patients with progressive massive fibrosis [40, 54, 55, 111].

Recent evidence, however, strengthens a new hypothesis based on the dual biological properties of TGF- β and on the opposite activities of TNF and TGF- β in the development of the inflammatory and fibrotic reaction [66]. TNF, which is produced in large amounts in the lung of patients with progressive massive fibrosis, would be responsible for the initiation and perpetuation of the inflammatory reaction observed in the lung of patients with progressive massive fibrosis. In addition, TNF, which can directly induce fibroblast proliferation, could also trigger the production of mediators, such as PDGF and IGF-1, which are more relevant for fibrosis. By contrast, the anti-inflammatory capacities of TGF- β , which is produced in large amounts in the lungs of patients with simple pneumoconiosis, together with lower amounts of TNF secretion (possibly due to TGF- β activities), could explain the relatively limited development of the inflammatory process in these patients. Moreover, by its ability to inhibit fibroblast growth, the secretion of TGF- β in large amounts could explain the limitation of the fibrotic process observed in simple pneumoconiosis.

In conclusion, alveolar macrophages are present in increased numbers in the lower respiratory tract of patients with coal workers' pneumoconiosis and produce exaggerated amounts of a large panel of mediators and of cytokines. Whilst the load of inhaled particles is often similar in mining workers, quantitative and qualitative differences in the release of macrophage mediators might represent an interesting explanation for differences in outcome observed between similarly exposed subjects [2, 4, 5, 8]. Through a different profile of cytokine production, some coal miners might develop more severe pulmonary lesions and pulmonary function impairment despite similar exposure levels. Therefore, evaluation of the cytokine profile, and in particular of TGF- β , in parallel with TNF, could open new insights in the understanding of pneumoconiosis, and probably also of other interstitial pulmonary disorders.

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