Plasma levels of soluble tumour necrosis factor receptors are increased in coal miners with pneumoconiosis

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Plasma levels of soluble tumour necrosis factor receptors are increased in coal miners with pneumoconiosis. R.P.F. Schins, P.J.A. Borm, ©ERS Journals Ltd 1995.

ABSTRACT: Among other cytokines, tumour necrosis factor (TNF-α) is considered to play a key role in the development of mineral dust related fibrosis. Previously, we showed that ex-vivo release of TNF by peripheral blood monocytes is a marker for progression of coal workers’ pneumoconiosis (CWP).

Since soluble TNF receptors (sTNF-Rs) are believed to play an important regulatory role in systemic effects of TNF, we measured plasma levels of sTNF-R55 and sTNF-R75 in coal miners with (n=28) or without (n=76) CWP and in nonexposed controls (n=29).

sTNF-R75 levels were significantly increased in miners with CWP (2.09±0.44 ng·mL⁻¹) versus the nonexposed controls (1.86±0.23 ng·mL⁻¹). Neither sTNF-R55 nor sTNF-R75 were related to exposure, stage of pneumoconiosis, smoking, or (spontaneous or ex-vivo induced) monocyte TNF-release. sTNF-R55 was increased in subjects with medication (especially those using cardiovascular drugs); upon exclusion of these subjects, sTNF-R55 was found also to be significantly increased in CWP.

In conclusion, bearing in mind a confounding effect of medication, soluble TNF receptors are elevated in plasma of retired miners with coal workers’ pneumoconiosis. These observations further support the important role of TNF-mediated pathways in the pathogenesis of mineral dust related fibrosis.


Occupational exposure to mineral dusts can lead to pulmonary fibrosis [1]. The pathogenesis of lung fibrosis involves highly complicated processes of intercellular communication by peptides released from and to various immune cells and lung target cells in a phenomenon appropriately referred to as “cytokine network” [2]. Previously, we and others have shown that silica, as well as coal mine dust, can stimulate the release of monocyte/macrophage-derived proinflammatory cytokines, such as tumour necrosis factor (TNF)-α [3–5], interleukin-1β (IL-1β) [4, 6, 7], interleukin-6 (IL-6) [5], and the macrophage inflammatory proteins la and 2 [8]. With regard to dust exposure in man, we found that monocyte TNF release upon ex-vivo stimulation with coal dust and silica (and endotoxin) was increased in (retired) miners with or without radiological evidence for simple coal workers pneumoconiosis (CWP) compared to subjects never exposed to mineral dust [3, 9]. In line with our observations in monocytes of coal workers, higher TNF secretion was also observed in macrophages of patients with progressive massive fibrosis (PMF) compared to simple pneumoconiosis patients and control subjects [7]. More recently, elevated messenger ribonucleic acid (mRNA) levels of TNF (and IL-6) have been observed in lungs of pneumoconiosis patients [10]. Crucial evidence for the importance of TNF in silica-induced lung fibrosis was demonstrated by Piguet et al. [11] who showed a down-regulatory effect on fibrosis by TNF-antibody treatment.

Two distinct receptors of TNF, i.e. a 55 kDa type (TNF-R55) and a 75 kDa type (TNF-R75) have been identified, and both receptors are expressed on many different cell types and tissues [12–15]. Shedding of the extracellular parts of these TNF receptors from white blood cells and probably also other cell types leads to two soluble TNF receptor types, known as sTNF-R55 and sTNF-R75 [16, 17]. Since naturally occurring soluble TNF receptor levels are believed to play an important regulatory role in systemic effects of TNF [16, 18], plasma TNF receptor levels may represent a feedback mechanism to the proinflammatory systemic action of TNF, or an (in)direct reflection of TNF-related pathological mechanisms within the lung. In the present study, we determined soluble TNF receptors in plasma of coal miners with or without CWP and in controls never occupationally exposed to dust. The purpose of the study was to evaluate the relationship of soluble TNF receptors with cumulative (silica-) dust exposure and the biological effects of pneumoconiosis disease [19]. Secondly it was designed to evaluate the relationship between soluble TNF receptor levels and monocyte TNF release [9, 19] in nonexposed subjects and in coal workers either in the presence or absence of lung fibrosis.
Materials and methods

As a part of a prospective cohort study among coal workers [20], soluble receptor levels were determined in the plasma of coal miners (n=104) and non-dust-exposed controls (n=29), after written informed consent. Prior to the blood sampling, a questionnaire, including informed consent was obtained from each participant. The study was conducted according to the Helsinki declaration of 1975. On the day of blood sampling, a chest radiograph was made of each miner. Confirmation of questionnaires and data from medical files and job history were obtained by personal interviews. Subjects were classified as smokers, nonsmokers, former smokers or lifetime nonsmokers; the amount smoked was expressed in pack years (packs-week\(^{-1}\times\)years smoked). Smoking habits were verified using a personal interview and smoking data from previous cross-sectional studies [21, 22] to minimize reporting bias. The same was done for medical history. All subjects were divided into two categories, i.e. those who used any kind of medication and those who did not use medication for at least 3 days (or in the case of antibiotics for 1 week) prior to blood sampling.

Severity of pneumoconiosis was obtained by classification of chest radiographs by three occupational physicians [20], according to the standard protocol of the International Labour Organization (ILO) [23]. High resolution computed tomography (HRCT) was performed in a subgroup of 46 miners on a voluntary basis as described previously [24]. The cumulative dust exposure was determined from job-exposure matrices as described previously [25]. Blood (10 mL ethylenediamine tetra-acetic acid (EDTA)-tube) was sampled, transferred to the laboratory at 4°C, and subsequently centrifuged. Plasma was stored at -70°C. Soluble receptor levels of R-55 and R-75 were determined by sandwich enzyme-linked immunosorbent assay (ELISA) as described by LEEUWENBERG [26]. Briefly, immunoassay plates (Nunc Maxisorp, Roskilde, Denmark) were coated overnight at 4°C with monoclonal anti-TNF-R55 or anti-TNF-R75. Plates were saturated with 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS) (w/v) for 1 h at room temperature. Test samples were added to the plates and incubated for 2 h at room temperature. Plates were washed and subsequently incubated with biotin-labelled rabbit anti-sTNF-R antiserum for 1 h, followed by washing of the plates and addition of peroxidase labelled streptavidin. Peroxidase activity was determined spectrophotometrically after addition of 3,3',5,5'-tetramethylbenzidine substrate at 450 nm by ELISA-reader. Present ELISA methods show no interference between sTNF-R and TNF, as reported previously [26]. In the same subjects, monocyte TNF release was determined as reported previously [3, 9].

Statistical analysis

Study group characteristics were evaluated by the Student’s t-test. Relations between the soluble TNF receptors R-55 and R-75 and age, pack-years, and dust exposure (i.e. miners only) were evaluated by linear regression. Multiple comparisons were evaluated by stepwise regression analysis. All statistical evaluations were made using Statgraphics version 6 (Manugistics Inc., Rockville, MA, USA).

Results

In the plasma of all individuals, levels of both soluble receptors were readily detectable (i.e. >200 pg·mL\(^{-1}\)). Mean and standard deviations were 1.44±0.44 ng·mL\(^{-1}\) (sTNF-R55) and 1.97±0.46 ng·mL\(^{-1}\) (sTNF-R75). Plasma levels of sTNF-R75, but not sTNF-R55, were significantly elevated in miners with pneumoconiosis compared to subjects never exposed to dust (table 1). Age in the nonexposed subjects was not different from miners with CWP, but was higher than in miners without radiological signs of pneumoconiosis (table 1). Cumulative exposure was higher in miners with CWP compared to the reference miners. Plasma levels of sTNF-R55 and sTNF-R75 were not different between current, former or lifetime nonsmokers in any of the subgroups, and not related to the severity of pneumoconiosis as defined by conventional chest radiograph or by HRCT (data not shown).

With the exception of age, no linear correlations were present between the soluble TNF receptors and other variables such as smoking, dust exposure and monocyte TNF release (table 2). A stepwise multiple regression analysis on both soluble TNF receptors using age, cumulative dust exposure (miners only), pack-years smoked, medication, spontaneous- and coal dust-stimulated monocyte TNF release as independent variables was performed in each study group. In the reference miners, both age (t=2.39; p<0.025) and medication (t=-2.32; p<0.025) gave a significant fit in the model to sTNF-R55, but in the nonexposed controls only medication (t=-3.12; p<0.005) was significantly related to sTNF-R55. Subjects using medication had increased plasma levels of sTNF-R55 compared to subjects without medication (table 3). Among the reference miners (but not in other groups), age was significantly higher in those having medication (table 3), thus explaining the correlation between age and plasma sTNF-R55 levels in this subgroup. None of the variables was related to sTNF-R75 levels in any of the subgroups. Moreover, no relation was found between plasma levels of sTNF-R55 or sTNF-R75 and (spontaneous or ex-vivo induced) monocyte TNF release (table 2).

Because of the significant effects of medication on sTNF-R55 levels, statistical analysis was repeated after exclusion of all subjects using medication. The difference in sTNF-R75 between CWP miners and nonexposed controls remained significant (fig 1b), but now an increase of sTNF-R55 was also observed in miners with CWP (p<0.06 vs reference miners). This confounding effect of medication on sTNF-R55 and the increased tendency in sTNF-R55 in pneumoconiosis is illustrated in figure 1a. Again, in this analysis no significant correlations were
present between the soluble receptors and smoking or exposure, nor was a correlation observed with age. The previously significant correlation with age in the reference miners (n=76) was not present in the reference miners without medication (n=55, p>0.25). Again, no relation between soluble receptors and monocyte TNF release was...
Discussion

To the best of our knowledge, this study is the first to describe a significant increase in plasma soluble TNF receptors in (simple) coal workers pneumoconiosis. sTNF-R75 levels were significantly increased in miners with CWP compared to the nonexposed controls. Controls (nonexposed), as well as reference miners, who used medication at the time of blood sampling also had increased levels of sTNF-R55, but upon statistical exclusion of these subjects, sTNF-R55 was also increased in CWP. No effect of smoking was seen and a relation of sTNF-R55 with age could be attributed to the effect of medication.

In the present study, no relation was found between monocyte TNF release and plasma levels of both soluble TNF receptors. Several reasons can account for this lack of correlation. Firstly, it is unlikely that monocytes are the main source of sTNF-R in vivo. Secondly, monocyte TNF release was determined ex-vivo in standardized culture conditions, where cells were seeded at equal concentrations in culture dishes, while plasma sTNF-R levels might be related to individual variations in white blood cell concentrations. Unfortunately, no differential or total cell counts of the blood were available.

Since all miners in our study were retired, acute effects of coal dust exposure can be excluded. Moreover, plasma receptor levels were not related to cumulative dust exposure. In the miners with CWP (n=28), a clear relation with disease severity, as determined by conventional chest radiograph or HRCT could not be established (data not shown). From these observations, one might conclude that levels of sTNF-R55 and sTNF-R75 could play a role in the pathogenesis of CWP.

Although many cytokines and growth factors are known to be involved in pulmonary fibrosis, the role of the "early" proinflammatory cytokine TNF-α as a central mediator in mineral dust related fibrosis is underscored by several in vitro and in vivo data [3–5, 7, 10, 27, 28]. The recent discovery of soluble cytokine antagonists - and particularly the soluble TNF receptors R55 and R75 - have put things in a new perspective: soluble cytokine receptors could, on the one hand, act as powerful antagonists of various cytokine actions, but, on the other hand, increase cytokine related effects by a systemic carrier function of shortlived cytokines [17, 29]. PIGUET and VESIN [30] demonstrated a downregulatory effect of TNF-antibodies [11], and more recently of sTNF-R55 in mineral dust related fibrosis. Doing so, they not only proved the crucial role of TNF in this disease, but they also elucidated a possible (therapeutic) role of soluble
TNF receptors in mineral dust related fibrosis. Increased levels of soluble TNF receptors have been observed in several chronic and acute diseases [31–35]. In chronic lung disease sTNF-R55 and sTNF-R75 were found to be elevated in the bronchoalveolar lavage fluid of patients with idiopathic pulmonary fibrosis and sarcoidosis [36]. In line with our observations on monocyte TNF release in coal miners [3, 9, 19], sTNF-R55 and sTNF-R75, therefore, also may play a role in the pathogenesis of coal workers' pneumoconiosis.

Unlike sTNF-R55, plasma levels of sTNF-R75 in the present study were clearly elevated in the plasma of miners with simple coal workers pneumoconiosis. Moreover, sTNF-R75 levels were not affected by medication. This might be in line with recent observations that TNF induced receptor shedding from peripheral blood neutrophils is specific to TNF-R75 [37]. Similarly, the (slower) release of TNF receptors form mononuclear cells in response to lipopolysaccharide (LPS) is a process also predominantly observed for TNF-R75 compared to TNF-R55 [38].

From the above data one might consider the applicability of plasma determination of soluble TNF receptors with regard to monitoring, as previously suggested for high ex-vivo monocyte TNF release as a positive risk factor for disease progression of CWP [9, 19]. However, one should be aware of confounding factors on soluble TNF receptor measurements. Since the kidney is believed to be the main clearance route of soluble TNF receptors, any abnormality in renal function would result in altered plasma levels of both receptors [39]. Shapiro et al. [34] have also stressed the importance of appropriate blood processing for sTNF-R determinations. In the present study, we observed an effect of medication on plasma levels of sTNF-R55. Due to a limited number of subjects using medication, we can only speculate on the action of specific types of medication, although higher plasma levels of sTNF-R55 were found in those subjects using cardiovascular drugs compared to subjects without medication (p<0.05 n=6). Whether (indirect) action of the drugs, or, on the other hand, the (history of) cardiovascular pathogenesis in these medicated subjects alters plasma sTNF-R55 kinetics (e.g. transcription, internalization, shedding, renal clearance) remains to be elucidated. However, it should be realized that in this small subgroup other effects may also account for increased sTNF-R55 levels.

In conclusion, both sTNF-R55 and sTNF-R75 are increased in coal workers pneumoconiosis, although differences in receptor levels with exposed or nonexposed control groups may be confounded by the effect of medication used at the time of blood sampling. The increased levels of soluble TNF receptors further support the important role of TNF in pneumoconiosis in coal workers. Interactions between TNF release and the antagonistic actions of soluble TNF receptors could be important in the development or progression of CWP. If so, measurement of these receptors may be of additional value in routine screening of dust exposed individuals. Support for this should be derived from a prospective study among coal workers, allowing a concurrent evaluation of TNF and its soluble receptors as risk markers.

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