Influence of inflammatory mechanisms on the redox balance in interstitial lung diseases


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Summary This study investigated the hypothesis that inflammatory, regulatory and antioxidant systems control the redox balance in interstitial lung diseases. Spontaneous mRNA expression of inflammatory cytokines and redox-active enzymes was examined in bronchoalveolar lavage (BAL) cells from patients with idiopathic pulmonary fibrosis (IPF) and sarcoidosis (SARC) using RT-PCR analysis. Pulmonary oxidative stress was characterized by carbonyl levels in the soluble BAL-fluid protein.

Protein carbonyls were normal in SARC, but 2.4-fold increased in IPF. Here, the protein carbonyls correlated inversely with glutathione peroxidase mRNA. The message for IL-8 increased 14-fold in IPF and was accompanied by a marked influx of PMN, while these parameters were not altered in SARC. Levels of IL-10 transcripts increased in both diseases, but stronger in SARC (33-fold) than in IPF (22-fold), contributing to a high IL-10/IL-8 mRNA ratio in SARC (0.86) in comparison to IPF (0.07) and controls (0.04). In SARC but not in IPF, IFN-γ mRNA was expressed at high levels and correlated inversely with the carbonyl levels. In both diseases, IL-1/β, TNF-α, and IL-6 mRNA transcripts remained at baseline level.

In summary, a low IL-10/IL-8 mRNA ratio was paralleled with significant oxidative stress in IPF, while a high IL-10/IL-8 ratio and enhanced IFN-γ expression went along with a physiological redox-balance in SARC.

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Introduction

Interstitial lung diseases such as idiopathic pulmonary fibrosis (IPF)1–4 and SARC3,5 have been linked to an excessive formation of reactive oxygen species (ROS) arising from the imbalance between ROS-generating and scavenging systems. These disorders are characterized by the accumulation of leukocytes within the lung, followed by progressive pulmonary inflammation, and subsequent destruction of alveolar-capillary units. While IPF is characterized by an increased number of polymorphonuclear neutrophils (PMN) in the epithelial lining fluid,6 SARC is accompanied by the occurrence of lymphocytes,7 thus indicating different pathophysiological mechanisms. The pulmonary redox imbalance in interstitial lung diseases is reflected in (a) an increased oxidative status of...
bronchoalveolar lavage (BAL)-fluid proteins defined by occurrence of methionine sulfoxide and carbonyl groups in these proteins, (b) a formation of nitrotirosine and nitric oxide synthase (iNOS) in the lungs of IPF patients using immunohistochemistry, and (c) an activation of NFκB in mononuclear cells isolated from the blood of SARC patients.

So far, little is known concerning the influence of redox-active enzymes and inflammatory cytokines on the redox balance in interstitial lung disease. One of the relevant enzymes that contribute to ROS formation is the inducible iNOS. Saleh et al. recently reported that the radical nitric oxide (NO), produced by iNOS, is expressed in macrophages and PMN from patients with early to intermediate stages of IPF. Mn dependent superoxide dismutase (Mn-SOD), glutathione peroxidase (GPx), and γ-glutamyl-cysteine synthetase (γ-GCS) play a central role in defence of ROS. SOD catalyses the dismutation from superoxide anion radical (O$_2^-$) to hydrogen peroxide (H$_2$O$_2$), which is further metabolised by GPx. The latter reaction requires reduced glutathione (GSH). De novo synthesis of GSH is dependent on γ-GCS, which is the rate-limiting enzyme of this pathway.

Among the cytokines, tumour necrosis factor-α (TNF-α) and interleukin (IL-1β) are potent early acting pro-inflammatory cytokines that trigger recruitment of inflammatory cells by inducing the expression of adhesion molecules and chemokines. IL-1β increases proliferation of fibroblasts and may induce the expression of Mn-SOD. IL-8 is a potent chemoattractant for PMN, which represent a major source for ROS. IL-6 induces T-cell proliferation and may be, therefore, particularly relevant to sarcoidosis. On the other hand, IL-6 inhibits the production of IL-1β and TNF-α in mononuclear cells, and is also known as an anti-inflammatory mediator. IL-10 plays a crucial role in down-regulating and terminating inflammation. Interferon-γ (INF-γ) functionally defines the T helper cell (Th1) response. It inhibits IL-1β and TNF-α induced production of IL-8 in vitro and may thus control PMN recruitment. IL-4 counteracts INF-γ and is indicative for the Th2 response.

The present study was designed to investigate which of these enzymes and cytokines plays a major role in the modulation of the redox balance in IPF and SARC. We found a strongly enhanced message for IL-8, a normal IL-10/IL-8 ratio, a decreased message for Mn-SOD associated with a marked redox imbalance in IPF. A high level of IL-10 message, a high IL-10/IL-8 ratio, and a strong expression of IFN-γ governed the inflammatory response in SARC.

Materials and methods

Study population

Patients with idiopathic pulmonary fibrosis
In this group nine patients (4 women and 5 men; mean age 59 years, range 37–74 years; 7 non-smoker/2 smoker) were studied. The two smoking patients had more than 24 pack years, respectively. The diagnosis of IPF was based on medical history, clinical and physical examinations, chest radiograph, HR-CT, pulmonary function test, bronchoscopy, BAL, and biopsy evidence. Four out of the nine patients had histological confirmation of usual interstitial pneumonia, in all other patients fibrosis was clinically most likely. Two patients received a steroid therapy with 40 mg prednisone at the time of BAL, respectively.

Patients with sarcoidosis
This group consisted of nine patients (5 women and 4 men; mean age 39 years, range 24–55 years; 7 non-smoker/2 smoker). One of the smoking patients had more than 24 pack years, and the other one had 10 pack years. The diagnosis was based on medical history, clinical and physical examinations, chest radiograph, pulmonary function test, bronchoscopy, BAL, and biopsy evidence. By chest X-ray findings three patients were stage I and six were stage II. No steroids were taken at the time of BAL.

Control group
As control subjects, five healthy non-smoking volunteers (4 women, 1 man; mean age 30 years, range 22–41 years), were studied. All volunteers were free of respiratory symptoms and had normal pulmonary function tests.

BAL

BAL was performed after informed consent was obtained from each subject. Using a flexible bronchoscope 3 × 50 ml of sterile warmed saline was infused into the right middle lobe of the lungs and then was immediately collected by suction. The BAL fluids recovered were centrifuged at 500 × g for 10 min to separate BAL cells from the supernatant. The concentrations of total protein and oxidized protein were determined in the supernatant. The cellular fraction was resuspended in phosphate buffered saline solution and a cell differential was performed on cytospin preparations. Remaining BAL cells (< 5 × 10$^5$) were again centrifuged at 500 × g for 10 min and lysed with
1ml 4M guanidinium thiocyanate, 25 mM sodium acetate, 0.5% sodium laurylsarcosine, and 0.12 M 2-mercaptoethanol, pH of 6.0. The lysates were then sheared with four passages through a 22-gauge syringe.

RT-PCR

Total cellular RNA was pelleted from BAL cell lysates by ultracentrifugation through 5.7 M cesium chloride and 0.1 M EDTA. Complementary DNA (cDNA) was generated from total RNA (0.25 µg/15 µl) with a RT from the First-Strand cDNA Kit and the pD (N6) primer (Pharmacia, Germany). The resultant cDNA was amplified by PCR using 0.01 µl of the RT reaction for GPx and Mn-SOD, 0.1 µl for GAPDH, IL-8, IL-1/β, and γ-GCS, 0.5 µl for TNF-α and INF-γ, 2 µl for IL-6 and IL-10, and 5 µl for iNOS and IL-4. The respective amounts of the RT reaction were added to Ready To Go PCR beads (Pharmacia, Germany) together with the protein-specific 5’ and 3’ primers. Thirty-five cycles were performed with a Hybaid Thermal Cycler (Germany) with settings of 95°C, 1 min at the respective annealing temperature, and 45 s at 72°C. Annealing temperatures were 55°C for GAPDH, IL-8, γ-GCS, TNF-α, INF-γ, IL-4, GPx, and Mn-SOD, 58°C for IL-1/β and IL-6, 59°C for IL-10, and 60°C for iNOS.

The following primers were utilised: GAPDH (product 353 bp): 5’ primer: 5’-CCA TGA GAA GTA TGA CAA CAG CC-3’, 3’ primer, 5’-TGG CAG GTT TTT CTA GAC GG-3’; IL-1/β (product 391 bp): 5’ primer: 5’-AAA CAG ATG AAG TGC TCC TTC CAG G-3’, 3’ primer: 5’-TGG AGA ACA CCA CTT GCT CCA-3’; TNF-α (product 280 bp): 5’ primer, 5’-CCA AAG TAG ACC TGC CCA GA-3’, 3’ primer, 5’-TCT ACT CCC AGG TCC TCT TCA-3’; IL-8 (product 245 bp): 5’ primer, 5’-AGC CTT CCT GAT TTC AGC-3’, 3’ primer, 5’-AAA ACT TCT CCA CAA CCC TCT GC-3’; IL-6 (product 330 bp): 5’ primer, 5’-AGG CAG TGG CAG AAA ACA ACC-3’, 3’ primer, 5’-GAT GAG TTT TCA TGT CCT GCA GC-3’; IL-10 (product 352 bp): 5’ primer, 5’-ATG CCC CAA GCT GAG AAC CAA GAC CCA-3’, 3’ primer, 5’-TCT CAA GGG GGT GGG CAG TCT GCT ATC CCA-3’; IL-4 (product 303 bp): 5’ primer, 5’-CAA GTG CGA TAT CAC CTT ACA AGG GG-3’, 3’ primer, 5’-TTC ACA GGA GCA TTT AAC C-3’, INF-γ (product 166 bp): 5’ primer, 5’-ATG CAG GTG ATT CAG ATG TAG C-3’, 3’ primer, 5’-TCC ACA CTC TTT TGG ATG CTC-3’; Mn-SOD (product 329 bp): 5’ primer, 5’-CCT GGA ACC TCA CAT CAA CG-3’, 3’ primer, 5’-AAC CTG AGC CTT GGA CAC C-3’; GPx (product 362 bp): 5’ primer, 5’-ACT TAT CGA GAA TGT GGC GTC C-3’, 3’ primer, 5’-AGG CAA CAT GTG TGC GAC AC-3’; γ-GCS, heavy subunit (product 263 bp): 5’ primer, 5’-CAG CGA GGA GCT TCA TGA TTG-3’, 3’ primer, 5’-TGG CAG AAT CCA GCT GTG C-3’; iNOS (product 462 bp): 5’ primer, 5’-ccg agg caa aca gca cat tca g-3’, 3’ primer, 5’-GTT TGG GGG G TG TGT GTAT GTC-3’.

Quantification of mRNA

We assumed that the mRNA sequences are proportionally represented in the first-strand cDNAs, so that the relative amount of a cDNA sequence represent the relative amount of the corresponding mRNA sequence in the total RNAs. Because we made no adjustment for RT efficiencies, these estimates reflect relative rather than absolute steady-state mRNA levels.

The amplification products were first separated on a 1.5% agarose gel containing ethidium bromide and then analysed by a video-densitometer (CCD camera, and 1D Scan software, MWG, Germany). For each protein, the integrated densities were normalized to the µl of RT reaction used in the PCR and then normalized to the constitutively expressed enzyme GAPDH (all enzymes and cytokines) to correct for variation in the amount of amplifiable cDNA in each sample. Predominantly macrophage-derived cytokines (IL-1/β, TNF-α, IL-8, IL-6, IL-10) were further normalized to the relative number of AM. The ratio obtained was considered to be the relative protein mRNA abundance.

Determination of protein-bound carbonyl groups

BAL supernatants were kept frozen at −25°C until analysed. Protein-bound carbonyl groups in frozen samples are stable for at least 2 years (unpublished results). Carbonyl groups were determined as described. Briefly, aliquots of BAL supernatant were dialysed against H2O and evaporated to dryness. Carbonyl groups were reduced to alcoholic labels different from those in amino acids, the tritiated proteins were hydrolysed and the amino acids were separated from carbohydrates by chromatography on Dowex 50 (Biorad, Germany).

Protein determination from the acid hydrolysates

We estimated the protein concentration from the acid hydrolysates by determining the amount of amino acids using o-phthalaldehyde as described.
The carbonyl content of proteins (oxidized proteins) was expressed as nmol carbonyl/mg BAL fluid protein.

Statistics

Statistical analysis was performed using Statgraphics plus 4.0. Results were expressed as the arithmetic mean ± SEM. Significant differences between groups were identified using the non-parametric Mann–Whitney test. Correlation coefficients were determined by the Spearman rank test. Probability values of <0.05 were considered as statistically significant.

Results

None of the smoking patients or the patients that were on prednisone treatment showed a pronounced tendency to differ from the non-smoking patients in the parameters that were measured. Therefore for comparison of groups and statistical evaluation data from all patients were included.

BAL cell counts

Compared to the control group total BAL cell numbers obtained from both IPF and SARC patients were elevated 3.5-fold and 5.3-fold, respectively (Table 1). Increases in total cell numbers in IPF patients were largely due to an influx of alveolar macrophages, PMN, and lymphocytes. In SARC patients, the increases could be ascribed to an influx of alveolar macrophages and lymphocytes.

BAL fluid protein and their oxidative status

The total protein content of BAL fluid of the control group was 69 μg/ml (Table 1). This parameter increased 4-fold in the SARC group, but was normal in the IPF-group. The oxidative status of the BAL fluid protein, defined as nmol carbonyl groups/mg protein, increased 2.4-fold in IPF, and remained unchanged in the SARC group.

Analysis of enzyme and cytokine mRNA in BAL cells

Semi-quantitative RT-PCR (Fig. 1) analysis revealed that BAL cells from both IPF and SARC patients expressed lower amounts of specific mRNA for the antioxidant enzymes Mn-SOD (IPF \( P = 0.033 \) and SARC \( P = 0.083 \)) and GPx (IPF \( P = 0.062 \) and SARC \( P = 0.003 \)) than control cells (Fig. 2a). By contrast,
mRNA transcripts of the enzymes iNOS and \( \gamma \)-GCS were neither altered in IPF nor in SARC.

In IPF, mRNA expression for IL-8 and IL-10 markedly increased \((P = 0.004\) and \(0.003\), respectively\) and decreased for TNF-\(\alpha\) \((P = 0.02)\) compared with controls (Figs. 2b and c). In contrast, BAL cells from SARC patients showed normal IL-8 and TNF-\(\alpha\) mRNA levels \((P = 0.14\) and \(0.5\), respectively\), while the levels of IL-10 mRNA were elevated \((P = 0.003)\) to a higher extent as in IPF.

**Figure 1** Patterns of enzyme and cytokine mRNA expression in each subject. Using RT-PCR, total RNA was analyzed for mRNA levels of Mn-SOD, GPx, iNOS, \( \gamma \)-GCS, IL-1/\(\beta\), TNF-\(\alpha\), IL-8, IL-6, IL-10, IL-4, and INF-\(\gamma\) in BAL cells from control subjects, IPF and SARC patients. Representative ethidium-stained amplification products of all proteins analyzed by agarose gel electrophoresis are shown. The numbers represent the individual subject number. The patients with the numbers 9, 24, 31 and 37 were smokers, and the patients with the numbers 7 and 8 received prednisone before BAL.

**Figure 2** Alterations in the relative mRNA abundance. Amplification products from BAL cells of controls, patients with IPF and patients with SARC were quantified by video densitometry. (a) Mn-SOD, GPx, iNOS, and \( \gamma \)-GCS; (b) IL-1/\(\beta\), TNF-\(\alpha\), and IL-8; (c) IL-6, IL-10, IL-4 and INF-\(\gamma\). All values shown were normalized to the amount \((\mu\text{l})\) of the RT reaction used in PCR and to the constitutively expressed mRNA for GAPDH. Data are presented as mean ± SEM; *\(P<0.05\).
The IL-10/IL-8 mRNA ratio was 0.04 ± 0.03 (mean ± SEM) in controls, 0.07 ± 0.02 (P = 0.124) in IPF patients, and 0.86 ± 0.25 (P = 0.003) in SARC patients, suggesting a prominent role of IL-10 in SARC. INF-γ mRNA levels increased 5-fold (P = 0.003) in SARC, but were normal in IPF (Fig. 2c). Expression of IL-4 mRNA increased in both patient groups (P = 0.004 in IPF, and P = 0.007 in SARC) compared to the control group (Fig. 2c). No significant differences were observed in the mRNA expression of IL-1β and IL-6 both in IPF and in SARC (Figs. 2b and c).

Relationships between oxidized proteins, enzymes and cytokines

Spearman correlation analysis (Table 2a/b) revealed an inverse relationship between the carbonyl content of BAL fluid proteins and GPx mRNA levels (r = −0.85, P = 0.036) in IPF and also an inverse relationship between protein carbonyl and INF-γ mRNA levels (r = −0.78, P = 0.027) in SARC. Furthermore, IL-10 correlated positively with TNF-α (r = 0.82; P = 0.044), IL-1β (r = 0.89, P = 0.029), and IL-8 (r = 0.86, P = 0.036) in IPF and with Mn-SOD (r = 0.71; P = 0.044) in SARC.

Discussion

Recent studies have postulated a major role of oxidative stress in the pathogenesis of interstitial lung diseases. The present work was aimed to enlighten the relationship between redox-balance and inflammatory mechanisms in IPF and SARC.

Among the BAL cells, AM exhibit a pivotal role in recruitment and activation of inflammatory cells by releasing different mediators. Major agonists are IL-1β, TNF-α, and IL-8 as pro-inflammatory mediators, the pleiotropic IL-6 which triggers acute phase reactions, and IL-10 as an anti-inflammatory counterpart. Particularly IL-1β and TNF-α influence the expression of various pro-inflammatory cytokines. In this context, cytokines may have a substantial impact on the redox imbalance, which is defined by the net result of radical generating and consuming enzymes. The redox balance was identified in the present study by increased concentrations of protein carbonyls in the epithelial lining fluid.

Cytokine mRNA production in BAL cells was examined as an indication of ongoing cytokine synthesis within the lungs of IPF and SARC patients using RT-PCR analysis. Our data indicate that there is a distinct increase in expression of IL-8 mRNA in IPF coinciding with increased numbers of PMN. By contrast, IL-8 expression in SARC stayed at baseline levels which was consistent with normal numbers of PMN.

Interestingly, increased levels of IL-10 transcripts were observed in IPF and to a higher extent in SARC. Enhanced IL-10 mRNA expression was also described by other authors for IPF and SARC. This cytokine efficiently antagonizes pro-inflammatory responses of alveolar macrophages and other pulmonary cells, e.g. by down-regulating IL-8 expression or inhibition of the respiratory burst activity. The IL-10/IL-8 ratio for specific mRNA expression may provide helpful information on the anti-inflammatory efficiency of IL-10. In IPF, the IL-10/IL-8 ratio was low (0.07) and comparable to the control value (0.04) suggesting the absence of a substantial anti-inflammatory response by IL-10. In SARC, however, the high IL-10/IL-8 ratio of 0.86 implies a strong suppression of IL-8 message, which might abolish the influx of PMN and consequently of PMN-mediated oxidative stress.

Furthermore, the strongly increased expression of IFN-γ mRNA in SARC may have regulatory consequences on the inflammatory outcome. Similar changes in IFN-γ mRNA production in BAL cells from SARC patients have been described. Robson et al. reported that IFN-γ inhibits IL-1β- and TNF-α-induced production of IL-8 in human peritoneal mesothelial cells and thus controls PMN migration across the mesothelium in vitro and in vivo. We propose a similar mechanism for control of PMN migration across the alveolar-capillary barrier in SARC, which supports the anti-inflammatory function of IL-10, e.g. in down-regulating the expression of IL-8. The positive correlation between IL-10 and Mn-SOD mRNA levels in SARC further contributes to the coordination of anti-inflammatory and anti-oxidant mechanisms.

We found no significant changes in the transcript levels for IL-1β, TNF-α and IL-6 in BAL-cells of individuals with both diseases. Our data suggest that the BAL cells were not an important source for these cytokines at the time at which they were sampled in the stage of the diseases. Since we have found at the same time distinctly increased levels of IL-10, more pronounced in SARC than in IPF, the expression of IL-1β, TNF-α, and IL-6 may have been suppressed by IL-10. In contrast to our finding, several studies have reported increased mRNA expression of these three cytokines in IPF and in SARC while others confirm our results. Ziegenhagen et al. showed that in progressing but not in stable IPF and SARC the mRNA levels of TNF-α
were increased, and in active SARC increased mRNA levels of TNF-α and IL-6 were found.\textsuperscript{20,25} Thus, apart from technical differences in methodology and detection of mRNA, most likely differences in the patient populations may explain the apparent discrepancies.

### Table 2 Spearman rank correlation analysis between the level of each mRNA in patients with (a) IPF and (b) SARC.

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<th>Mn-SOD</th>
<th>GPx</th>
<th>iNOS</th>
<th>GCS</th>
<th>IL-1β</th>
<th>TNF-α</th>
<th>IL-8</th>
<th>IL-6</th>
<th>IL-10</th>
<th>IL-4</th>
<th>INF-γ</th>
<th>Oxidized protein</th>
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<td><strong>(a) In patients with IPF</strong></td>
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<tr>
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|                  |        |      |      |      |       |       |      |      |       |       |        |                 
| **(b) In patients with SARC** |        |      |      |      |       |       |      |      |       |       |        |                 
| Mn-SOD           | NS     | 0.67 | 0.058| NS   | NS    | 0.60  | NS   | 0.71 | 0.088 | 0.059 | NS     | NS              |
| GPx              | NS     | NS   | NS   | NS   | NS    | NS    | NS   | NS   | NS    | NS    | NS     | NS              |
| iNOS             | 0.67   | NS   | NS   | NS   | NS    | NS    | 0.60 | NS   | 0.60  | 0.090 | NS     | NS              |
| GCS              | NS     | NS   | NS   | NS   | NS    | NS    | 0.67 | NS   | 0.67  | 0.059 | NS     | NS              |
| IL-1β            | NS     | NS   | NS   | NS   | 0.60  | NS   | NS   | NS   | 0.09  | NS    | −0.67 | NS              |
| TNF-α            | 0.60   | NS   | NS   | NS   | 0.60  | 0.090 | NS   | NS   | 0.62  | 0.081 | NS     | NS              |
| IL-8             | 0.67   | NS   | NS   | NS   | 0.67  | 0.090 | NS   | NS   | 0.63  | 0.081 | NS     | NS              |
| IL-6             | NS     | NS   | NS   | NS   | 0.83  | 0.018 | NS   | NS   | 0.63  | 0.073 | NS     | NS              |
| IL-10            | 0.71   | NS   | NS   | NS   | 0.83  | 0.018 | NS   | NS   | 0.63  | 0.073 | NS     | NS              |
| IL-4             | NS     | NS   | NS   | NS   | 0.67  | 0.059 | NS   | NS   | 0.62  | 0.081 | NS     | −0.75           |
| INF-γ            | NS     | NS   | NS   | NS   | 0.88  | 0.013 | NS   | NS   | 0.62  | 0.081 | NS     | −0.78           |
| Oxidized protein | NS     | NS   | NS   | NS   | 0.67  | 0.059 | NS   | NS   | 0.62  | 0.081 | NS     | −0.75           |

Data show the correlation coefficient (in italic) and the significance level. NS, \( P>0.1; \) *\( P<0.05\).
In IPF, we found a significant increase of the carbonyl content in the soluble BAL-fluid protein fraction, demonstrating a pronounced redox imbalance. This kind of oxidative stress is presumably based on Fenton-like reactions, which requires \( \text{H}_2\text{O}_2 \) and transition metal ions.\(^{27}\) On the contrary, the level of oxidized BAL-fluid proteins in SARC was normal. Transcripts of the antioxidant enzyme Mn-SOD were markedly reduced in IPF, which might contribute to increased steady-state levels of \( \text{O}_2\). \( \text{O}_2\) triggers reduction of \( \text{Fe}^{3+} \) to Fenton-reactive \( \text{Fe}^{2+} \),\(^{28}\) which is essentially involved in metal-catalyzed oxidation of proteins like formation of protein carbonyls.\(^{27}\) There is evidence for increased levels of free iron in the blood of IPF patients.\(^{29}\) Assuming that iron is also increased in their alveolar space the proposed mechanism is very likely. Though the expression of GPx was not significantly altered in IPF, its inverse correlation with protein carbonyls points at a role of this antioxidant enzyme for \( \text{H}_2\text{O}_2 \) detoxification. In SARC, the absence of an oxidative stress response in terms of carbonyl formation might be explained by normal Mn-SOD expression thus providing a lower \( \text{O}_2 \) steady-state concentration than in IPF. As a consequence, the potential for reduction of free \( \text{Fe}^{3+} \) to \( \text{Fe}^{2+} \) by \( \text{O}_2 \) might not be sufficient to support metal-catalyzed protein oxidation via the Fenton-mechanism even at the significantly lowered expression of GPx. Comparison of the specific carbonyl content in plasma and BAL-fluid in SARC-patients revealed comparable carbonyl levels (data not shown). This observation also suggests the absence of significant oxidative stress in SARC. However, the marked influx of serum protein into the alveolar space might obscure marginally altered redox-processes in the alveolar space.

The absence of a substantial induction of iNOS implies that NO does not contribute markedly to oxidative stress in IPF. This result is in contrast to the findings by Saleh et al.\(^{4}\) who reported expression of iNOS in macrophages and PMN from patients with early to intermediate stages of IPF. The discrepancy between these findings may originate from different disease stages of the patient groups and/or from different analytical procedures used, namely RT-PCR in BAL cell extracts versus immunohistochemistry in lung sections.\(^{4}\)

GSH represents an essential antioxidant in the lungs.\(^{3}\) Previous studies indicated reduced levels of GSH in the epithelial lining fluid of IPF patients,\(^{30}\) which has been suggested as a relevant feature in pathophysiological mechanism of IPF. In SARC GSH levels were normal.\(^{31}\) Analysis of the specific mRNA for the heavy subunit of \( \gamma \)-GCS as key enzyme in GSH biosynthesis indicated no altered expression of this enzyme in IPF or SARC.

### General interpretation

In contrast to IPF, distinct anti-inflammatory mechanisms appear to be activated in SARC, as indicated by the high expression of IL-10 and INF-\( \gamma \) transcripts. While high levels of IL-8 mRNA are expressed in IPF, this cytokine does not play a significant role in SARC. The anti-inflammatory IL-10 and presumably INF-\( \gamma \) seem to prevent an increased IL-8 expression in SARC thus lowering the risk of oxidant injury by PMN and maintaining the redox balance. The level of oxidized BAL-fluid proteins is markedly increased in IPF compared to SARC patients, whereas the expression profiles of the antioxidant enzymes Mn-SOD and GPx do not show pronounced differences.

Taken together, the redox balance in IPF is strongly shifted in favour of oxidants, and we postulate the explicit involvement of an IL-8 dependent mechanism.

### References


