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Decreased Expression of Heme Oxygenase-1 by Alveolar Macrophages in Idiopathic Pulmonary Fibrosis

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ABSTRACT

Background: Heme oxygenase (HO)-1 is an oxidative stress responsive protein that may be involved in the pathogenesis of interstitial lung disease.

Methods: We investigated HO-1 expression in alveolar macrophages from bronchoalveolar lavage in 24 patients with idiopathic pulmonary fibrosis (IPF), 16 with sarcoidosis, 14 with hypersensitivity pneumonitis (HP), and 13 controls. Using immunocytochemistry, HO-1 expression in macrophages was scored semi-quantitatively from 0 to 3 according to increasing intensity. The mean score of 100 macrophages was calculated. Macrophages were cultured, IL-12 and IL-18 in the culture supernatants was measured by ELISA.

Results: The score of HO-1 was significantly lower in IPF (mean 67) than in sarcoidosis (105) or HP (106) and in controls (106). There was no significant difference between sarcoidosis, HP and controls. The score of HO-1 correlated positively with the lymphocyte percentage ($r=0.38$, $p<0.05$) in sarcoidosis and HP. Positive correlations were found between the score of HO-1 and the release of IL-12 and IL-18 by macrophages ($r=0.58$, $p<0.05$; $r=0.60$, $p<0.05$) in IPF.

Conclusions: The expression of HO-1, a critical defender against oxidative stress, is decreased in macrophages of IPF compared to granulomatous lung disorders. This supports the hypothesis of an oxidant-antioxidant imbalance in the pathogenesis of IPF.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic and fatal lung disease of unknown cause characterized by progressive fibroblast proliferation, destruction of the alveolar architecture, and a relentless decline in pulmonary function [1]. The precise pathogenetic mechanisms of IPF remain to be determined. A number of studies have suggested that an oxidant-antioxidant imbalance plays a role in the progression of pulmonary fibrosis in animal models and also possibly in human IPF [2]. There is ample evidence suggesting increased oxidative stress in patients with IPF. Alveolar macrophages (AM) and neutrophils which dominate in the lower respiratory tract of patients with IPF are capable of inducing oxidant mediated injury to lung parenchymal cells [3,4]. Superoxide radicals and hydrogen peroxide (H_2O_2) are released spontaneously by bronchoalveolar lavage (BAL) cells of IPF patients [3]. High levels of myeloperoxidase associated with epithelial injury were found in the alveolar epithelial lining fluid (ELF) and BAL fluid of patients with IPF [3]. Oxidative modification of mitochondrial DNA has been observed in lung epithelial cells of IPF patients [5]. Oxidized BAL fluid proteins as characterized by oxidation of methionine residues or carbonylation are increased in patients with IPF [6, 7]. 8-Isoprostane, a biomarker of oxidative stress, is also increased in BAL fluid of IPF patients [8].

On the other hand, there is a deficiency of cellular antioxidants in IPF patients [9-12]. Glutathione, a major component of the lung antioxidant defense system, is decreased in ELF, both in the bleomycin model of lung fibrosis and in IPF patients [9]. Antioxidants and detoxification enzymes including thioredoxin, glutaredoxin, manganese superoxide dismutase (MnSOD), catalase and glutamate cysteine ligase (γ-glutamyl cysteine synthetase, the rate limiting enzyme in glutathione synthesis) show a diminished expression in patients with IPF

[10-12]. These data support the hypothesis of an oxidant-antioxidant imbalance in the pathogenesis of IPF.

Heme oxygenase (HO), the rate-limiting enzyme in the catabolism of heme, is responsible for the degradation of heme to biliverdin, free iron, and carbon monoxide (CO). Biliverdin is subsequently converted to bilirubin through the action of the cytosolic enzyme biliverdin reductase, and free iron is sequestered by ferritin [13]. Presently, three isoforms of HO have been identified as HO-1, HO-2 and HO-3. HO-1, the inducible form of HO, is supposed to be an oxidative stress responsive protein, whereas HO-2 and HO-3 are constitutively expressed [13]. HO-1 has been implicated in a number of conditions or diseases associated with lung injury. HO-1 seems to be up-regulated in smokers, asthma, acute lung injury, cystic fibrosis, lung transplant rejection, whereas it is down-regulated in severe chronic obstructive pulmonary disease (COPD) [14-19]. Since an oxidant-antioxidant imbalance contributes to the pathogenesis of lung fibrosis, HO-1, one of the critical defenders against oxidative stress, may be important in fibrotic lung diseases.

In the present study we were interested in investigating the expression of HO-1 in AM recovered by BAL in patients with IPF, sarcoidosis and HP. We also wanted to explore potential correlations between the expression of HO-1 and the production of proinflammatory cytokines. For this purpose, we chose interleukin (IL)-12 and IL-18 since they are involved in regulating the balance between Th1 and Th2 profiles [20, 21], and IPF is thought to be associated with a Th2 profile [23], whereas sarcoidosis and HP are Th1 diseases [24, 25].

Methods

Patients

Twenty-four consecutive patients with IPF, 16 patients with active pulmonary sarcoidosis, 14 patients with chronic HP, and 13 control subjects were investigated (Table 1). No patient was receiving treatment with corticosteroids and/or immunosuppressants at the time of BAL. Written informed consent was obtained according to institutional guidelines.

Twenty-four IPF patients were diagnosed according to the ATS/ERS criteria [1]. Seven of them had a surgical biopsy with histological evidence of usual interstitial pneumonia (UIP).

Sixteen sarcoidosis patients were diagnosed on the basis of compatible clinical and radiographic features, histologic evidence of noncaseating granuloma and/or an increased CD4/CD8 ratio in BAL, and the exclusion of other granulomatous disorders [24]. All of them showed manifestations of disease activity as evidenced by recently developed or worsening symptoms, and/or chest radiographic evidence of progressive disease, and/or deterioration of lung function tests.

Fourteen HP patients fulfilled the following diagnostic criteria: 1. a history of exposure to organic antigens, 2. clinical signs and symptoms consistent with HP, 3. radiologic features and/or functional abnormalities characteristic of interstitial lung disease, 4. evidence of serum precipitins against one or more organic antigens, and 5. increased lymphocytes in the BAL fluid. All fourteen patients presented with the chronic form of insidious onset. On high-resolution CT, they all showed widespread and dominant ground glass densities, with only minor reticulation and no honeycombing. Late stage cases with extensive fibrosis were not investigated in this study.

Thirteen patients underwent diagnostic bronchoscopy and showed no evidence of lung disease. They had normal BAL cytology, and served as controls.

Bronchoalveolar Lavage Procedure

BAL was performed during fiberoptic bronchoscopy according to established guidelines [26]. Sterile isotonic saline solution was instilled into the right middle or left ligular lobe in 20 mL aliquots to a total volume of 100-200 mL, with immediate aspiration by gentle suction after each aliquot. A volume of greater than 50% was retrieved. The BAL cell differentials of the four study groups are shown in Table 2.

Immunocytochemical Analysis

Immunocytochemistry was used to investigate the expression of HO-1 (Stressgen Biotechnologies Corp., Victoria, B.C., Canada) in AM from BAL. The peroxidase-antiperoxidase method was applied as previously described [27]. To evaluate the percentage of HO-1 positive cells, 500 macrophages were counted under a light microscope. In addition, HO-1 expression by AMs was scored semi-quantitatively. The score for each counted AM ranged from 0 to 3 according to increasing intensity (Table 3). The mean score of 100 AM was calculated for each patient, with the score ranging from 0 to 300. All analyses, including immunocytochemical grading, were performed in a blinded fashion by two investigators. The interobserver correlation was good. The Spearman's r value for the correlation between the two counts was 0.89 ($p<0.01$).

Culture of Alveolar Macrophages

AM cultures were performed in 12 patients with IPF, 8 with sarcoidosis, and 4 with HP, as previously described [21]. The AMs from BAL were incubated for 24 hours with 1 mL RPMI 1640 medium alone, or RPMI 1640 medium with 100 ng/mL lipopolysaccharide (LPS, Sigma). The culture supernatants were centrifuged and stored at – 80°C.

ELISA Assay for Cytokines

The concentration of IL-12p70 and IL-18 in culture supernatants was quantified using commercially available human enzyme-linked immunosorbent assay (ELISA) kits (R&D systems Inc. USA; MAL, Japan) with a sensitivity of 0.5 pg/mL and 12.5 pg/mL respectively. The concentrations of above-measured cytokines were expressed as picogram per milliliter per 10^6 AMs after correction for the proportion of AMs.

Total Bilirubin and Ferritin Analysis

The supernatants were analysed using ELISA kits for total bilirubin (Beckman Coulter, Fullerton, CA) and for ferritin (Immulite, DPC, USA) with sensitivities of 0.1 mg/mL and 0.4 ng/mL respectively, using the manufacturer' s instructions.

Statistical Analysis

Values are expressed as mean±SEM. To compare the four study populations IPF, sarcoidosis, HP and control subjects, the data were analyzed using Kruskal-Wallis one-way analysis of variance on ranks. Two groups were compared using Student t-test for parametric data. The correlation of different parameters was analysed by Spearman rank. A level of $p<0.05$ was accepted as statistically significant.

Results

Expression of Heme Oxygenase –1

HO-1 immunostaining was mainly observed in BAL AMs, occasionally observed in epithelial cells, but not in other BAL cells including lymphocytes, neutrophils, eosinophils, mast cells and plasma cells.

The score of HO-1 positive AM was significantly lower in patients with IPF (67.4±5.0) than in those with sarcoidosis (105.4±7.1, $p<0.01$), or HP (105.6±5.4, $p<0.01$), or control subjects (105.9±5.2, $p<0.01$) (Figure 1). The percentage of HO-1 positive macrophages was also significantly decreased in patients with IPF (56±3%) compared to those with sarcoidosis (76±3%, $p<0.01$), HP (74±3%, $p<0.01$), and control subjects (86±2%, $p<0.01$). There was no difference in the score or percentage of HO-1 positive macrophages between sarcoidosis, HP and controls.

IL-12 and IL-18 Release from BAL Macrophages in IPF and Granulomatous Lung Disorders

Since the number of BAL samples with a number of cells sufficient for AM culture was small, the patients with sarcoidosis (n=8) and HP (n=4) were grouped together as granulomatous lung disorders to allow statistically meaningful comparisons. After 24 hours' culture, the spontaneous production of IL-12 from BAL macrophages was significantly lower in IPF than in granulomatous lung disorders (0.7±0.07 vs 7.5±2.9 pg/mL per 10^6 AM, $p<0.05$). Similarly, the level of IL-18 was significantly lower in IPF than in granulomatous lung disorders (55.2±10.7 vs 188.7±44.7 pg/mL per 10^6 AM, $p<0.01$) (Figure 2).

The LPS-stimulated production of IL-12 and IL-18 was significantly higher than the spontaneous release in both groups ($p<0.05$ or $p<0.01$). Again, the LPS-stimulated production of IL-12 and IL-18 was significantly lower in IPF than in granulomatous lung disorders (IL-12:

6.5±3.2 vs 33.8±13.7 pg/mL per 10^6AM, $p<0.05$; IL-18: 123.1±24.2 vs 358.8±81.7 pg/mL per 10^6AM, $p<0.01$) (Figure 2).

Correlations between HO-1 Expression and BAL Cell Differentials

In IPF patients, there were only weak correlations between the score of HO-1 positive AM and the percentage of neutrophils ($r=0.40$, $p=0.052$) or eosinophils ($r=0.33$, $p=0.111$) in the BAL fluid, statistically of borderline or of no significance. In sarcoidosis and HP patients, the score of HO-1 positive AMs showed a significant correlation with the percentage of lymphocytes ($r=0.38$, $p<0.05$) in the BAL fluid (Figure 3).

Correlations between HO-1 Expression and Cytokine Release from BAL Macrophages

In IPF patients, positive correlations were found between the score of HO-1 positive AM and the spontaneous production of IL-12 and IL-18 in supernatants of AM cultures ($r=0.58$, $p<0.05$; $r=0.60$, $p<0.05$) (Figure 4). No further correlations were found between HO-1 expression and cytokine production either in IPF or in the granulomatous lung disorders.

Correlations between HO-1 Expression and Lung Function

The score of HO-1 failed to show any correlation with the lung function values of the patients with interstitial lung disease.

Total Bilirubin and Ferritin in the Macrophage Culture Supernatants

Total bilirubin was significantly lower in the AM culture supernatants of IPF patients than in those of sarcoidosis and HP patients (3.6±0.4 vs 9.9±2.1 mg/mL per 10^6 AM, $p<0.05$), whereas

the ferritin concentration was not different between IPF and granulomatous lung disorders (170±34 vs 189±35 ng/mL per 10^6 AM, $p>0.05$) (Figure 5).

Discussion

The present study showed that the expression of HO-1 by AMs is reduced in patients with IPF. In these patients, the score of HO-1 expression in macrophages correlated positively with IL-12 and IL-18 release from AM *in vitro*. There was no difference in the AM HO-1 expression between patients with the granulomatous lung disorders (sarcoidosis and HP) and the control subjects. Our results are in agreement with an immunohistochemical study which showed that HO-1 expression was higher in newly diagnosed sarcoidosis than in UIP patients [28]. The limitation of our study lies in the fact that we did not determine the causality of the decreased expression of HO-1 in AM from IPF patients in this report.

HO-1, as an important mediator of cytoprotection against oxidative injury, may be implicated in the pathogenesis of IPF. Basal expression of HO-1 has been described in hamster fibroblasts and was associated with resistance to hyperoxia [29]. In a murine model of pulmonary fibrosis, HO-1 was found to be one of the most differentially expressed genes as revealed by cDNA array analysis, and increased HO-1 protein expression was also observed [30]. A potential salutary effect of HO-1 in the pathogenesis of pulmonary fibrosis has been proposed. Tsuburai and colleagues [31] reported that adenovirus mediated transfer of the HO-1 gene can attenuate the intensity of bleomycin induced pulmonary fibrosis in a murine model. On the other hand, Zn-deuteroporphyrin (Zndtp), a chemical inhibitor of HO activity, has recently been shown to be protective in a bleomycin induced lung fibrosis model by decreasing total lung collagen and the degree of extracellular matrix deposition and increasing glutathione levels [32]. How can the different effect of the two studies be explained, one showing a protection against bleomycin

induced fibrosis by HO-1 overexpression [31], the other a similar protection by HO inhibition [32]? Firstly, the antioxidative properties that are intrinsic to the metalloporphyrin Zndtp itself may act to restore the alveolar antioxidant capacity. Secondly, the role of HO-1 in tissue injury is complex and is determined by its local concentration and temporal prominence [16, 33]. In the study by Tsuburai and colleagues [31] HO-1 protein expression was induced before bleomycin treatment, whereas in the study by Atzori et al. [32] Zndtp was given after the initial inflammatory phase. This may account for the reduced fibrosis in both the overexpression study and the inhibitory study, suggesting that HO may play different roles in the early inflammatory phase and in the later fibroproliferative phase of bleomycin induced lung toxity. At this point it is also important to note that the bleomycin model does not completely correlate with human IPF. The bleomycin model results from acute lung injury leading to early fibrosis, whereas clinically diagnosed IPF patients are mostly in the late fibrotic stage, with little evidence of inflammation.

The role of the observed down-regulation of HO-1 in the pathogenesis of IPF may be explained in two ways: it may either serve as an adaptive mechanism to protect the tissue against harmful effects of heme degradation products, or it may indicate that loss of protection against oxidative stress is involved in the pathogenetic mechanisms of IPF. There is more evidence to support the second assumption. Similarly to HO-1, the expression of manganese superoxide dismutase, an important superoxide radical scavenging antioxidative enzyme, has been shown to be increased in inflammatory and granulomatous lung disorders but to be low in the fibrotic lesions of IPF lungs [12]. Likewise, other antioxidants and detoxification enzymes including catalase, glutamate cysteine ligase, glutaredoxin and thioredoxin show similar changes in opposite directions, that is up-regulation in acute inflammation models or diseases and down-regulation in chronic fibrosis models or diseases [2, 10-12].

Although the role of inflammation in the pathogenesis of IPF remains controversial, evidence supports an imbalance of the Th1 (IFN-γ, IL-12 and IL-18) and Th2 (IL-4 and IL-5) cytokine profile in favour of the Th2 profile which may contribute to the accumulation of collagen [23]. Sarcoidosis and HP are characterized by a Th1 type immune response which leads to exaggerated inflammation, setting the stage for granuloma formation and only rarely for the development of irreversible remodelling of the lung tissue and fibrosis [24, 25]. IL-12 and IL-18 are key cytokines involved in regulating the balance between Th1 and Th2 profiles. Our results showed that both the spontaneous and LPS stimulated production of IL-12 and IL-18 from BAL macrophages was significantly higher in sarcoidosis and HP patients than in IPF patients. This is consistent with the assumption that sarcoidosis and HP are Th1 diseases and IPF is a Th2 disease [23-25].

Recently IL-12 has been shown to attenuate bleomycin-induced murine lung fibrosis by an IFN-γ dependant manner [22]. In our study, the score of HO-1 expression in AM failed to correlate with the production of IL-12 and IL-18 in patients with sarcoidosis and HP. However, in IPF patients low HO-1 expression in AMs correlated with low spontaneous production of IL-12 and IL-18, indicating that the switch to the Th2 pattern was most profound in those IPF patients with the lowest HO-1 expression. The involvement of HO-1 in the regulation of inflammation has been shown in a number of diseases and animal models. Both HO-1 and CO can decrease the LPS induced expression of the proinflammatory cytokines tumor necrosis factor (TNF)-α, IL-1β, and macrophage inflammatory protein-1β, and augment the expression of the antiinflammatory cytokine IL-10 [34, 35]. IL-10 mediated protection against LPS induced septic shock in mice can be significantly attenuated by co-treatment with the HO inhibitor zinc protoporphyrin [35]. Exogenous administration of CO to mice or macrophages

leads to a decrease in the production of IL-6 [36]. To summarize these findings, HO-1 is involved in the inflammatory modulation of lung diseases, the major effect being down regulation of proinflammatory cytokines. On the other hand, transforming growth factor-β1, an important profibrotic cytokine, is able to down-regulate HO-1 in the rat lung [37]. Diminished expression of HO-1 is likely associated with a cellular microenvironment that promotes fibrosis.

Although the mechanisms underlying the cytoprotective function of HO-1 remain unclear, the heme degradation products including bilirubin, CO and iron induced ferritin seem to be mediators of the effects of HO-1 activation [13]. In asthmatic patients, exhaled CO and sputum bilirubin levels have been shown to be elevated in concert with increased HO-1 staining [15, 38]. Increased ferritin staining in AMs was observed in cystic fibrosis lungs compared to normal lungs, and the number of ferritin-stained macrophages correlated with the amount of HO-1 staining [17]. Inhaled CO had suppressive effects on fibrosis in bleomycin models of lung injury [39]. Bilirubin is also regarded as a powerful antioxidant substance in vitro [40] and a very effective physiologic antioxidant in vivo [41]. Bilirubin is not only an important antioxidant in serum, but may also play an important role in pulmonary fibrosis. This was suggested by a recent study where hyperbilirubinemia was shown to attennate bleomycin-induced pulmonary fibrosis [42]. In the present study, total bilirubin was significantly lower in the AM culture supernatants of IPF patients than in those of sarcoidosis and HP patients, similar to the changes observed for the HO-1 expression in AM.

In conclusion, our study showed that HO-1, an important antioxidant, is depleted in BAL macrophages of IPF patients. Given the fact that murine models have been reported suggesting that overexpression of HO-1 attenuate bleomycin-induced lung injury [30], our study translates

the findings of murine models into the human realm, which is necessary to advance the field and not previously reported. Our findings support the hypothesis of an oxidant-antioxidant imbalance in the pathogenesis of IPF. Targeting HO-1 expression in the lungs of patients with IPF may be a new therapeutic approach, but further research is warranted to elucidate the complex role of HO-1 in interstitial lung disease that may be beneficial or harmful.

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Table 1. Demographics of the study population

	IPF	Sarcoidosis*	HP†	Controls
n, female:male	24, 4f:20m	16, 9f:7m	14, 6f:8m	13, 5f:8m
Age, yr	67±2‡‡‡	48±4‡,§§§,||	61±4‡‡‡	37±3
Nonsmokers, n	14	16	10	13
Current smokers, n	0	0	0	0
Ex-smokers, n	10	0	4	0
VC, %pred	72±4‡‡	80±6	70±7‡‡	99±4
TLC, %pred	68±3‡‡‡	79±4	78±5	97±4
FEV1/VC, %	77±2	74±2	75±2	85±5
PaO2, mmHg	71±2‡‡‡	77±2	73±4‡‡	90±2
AaDO2, mmHg	30±3‡‡‡	21±2	32±5‡‡	11±1

Definition of abbreviations: IPF = idiopathic pulmonary fibrosis; HP = hypersensitivity pneumonitis; VC = vital capacity; TLC = total lung capacity; FEV_1 = forced expiratory volume in one second.

Data are expressed as mean ± SEM.

* According to chest roentgenographic staging, 6 patients had stage I, 6 patients had stage II, and 4 patients had stage III disease.

† Six were budgerigar fanciers, 7 were pigeon breeders and 1 had humidifier lung.

‡ $p<0.05$; ‡‡ $p<0.01$; ‡‡‡ $p<0.001$ (IPF, sarcoidosis and HP vs controls).

§§§ $p<0.001$ (sarcoidosis or HP vs IPF).

|| $p<0.01$ (sarcoidosis vs HP).

Table 2. Bronchoalveolar Lavage Fluid Cell Differentials

	IPF	Sarcoidosis	HP	Controls
Total cells, $\times 10^6$	15.0±2.4	17.0±2.6*	25.3±3.7$^{***, \dagger}$	6.9±1.0
$\times 10^4$/mL	8.1±1.1	12.0±1.4*	19.3±3.5$^{***, \dagger\dagger}$	5.8±0.9
Macrophages, %	76.3±3.3	52.9±5.4***	23.0±2.4$^{***, \dagger\dagger\dagger}$	92.1±0.9
$\times 10^4$/mL	6.2±1.0	5.8±0.7	4.5±1.0	5.3±0.8
Lymphocytes, %	14.0±2.6	43.7±5.1$^{***, \dagger\dagger}$	64.8±2.7$^{***, \dagger\dagger\dagger}$	6.8±0.9
$\times 10^4$/mL	1.3±0.4	5.8±1.0$^{**, \dagger\dagger}$	12.7±2.6$^{***, \dagger\dagger\dagger}$	0.4±0.09
Granulocytes, %	9.7±1.5***	3.7±0.8‡	11.8±2.2***	1.2±0.3
$\times 10^4$/mL	0.7±0.1	0.5±0.1$^{***, \dagger\dagger}$	2.1±0.5$^{***, \dagger}$	0.07±0.02
Neutrophils, %	7.0±1.2***	2.5±0.6	8.5±2.0***	1.1±0.3
$\times 10^4$/mL	0.5±0.09**	0.3±0.08**,‡	1.6±0.5***	0.07±0.02
Eosinophils, %	2.5±0.5***	0.9±0.3	2.6±0.7***	0.08±0.03
$\times 10^4$/mL	0.2±0.03***	0.1±0.05**	0.4±0.08***	0.001±0.00

Data are expressed as mean ± SEM.

* $p<0.05$; ** $p<0.01$; *** $p<0.001$ (IPF, sarcoidosis or HP vs controls).

† $p<0.05$; †† $p<0.01$; ††† $p<0.001$ (sarcoidosis or HP vs IPF).

‡ $p<0.05$ (sarcoidosis vs HP).

Table 3. Semi-quantitative score of heme oxygenase-1 expression in BAL macrophages

Semi-quantitative score	Staining intensity
0	No staining
1	Weak staining in the cytoplasm
2	Moderate staining in the cytoplasm, nucleus still visible
3	Strong staining in the cytoplasm, nucleus not visible

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Figure Legends.

Figure 1 Semi-quantitative score of heme oxygenase (HO)-1 expression in alveolar macrophages in bronchoalveolar lavage (BAL) fluid in patients with idiopathic pulmonary fibrosis (IPF), sarcoidosis, hypersensitivity pneumonitis (HP) and controls.

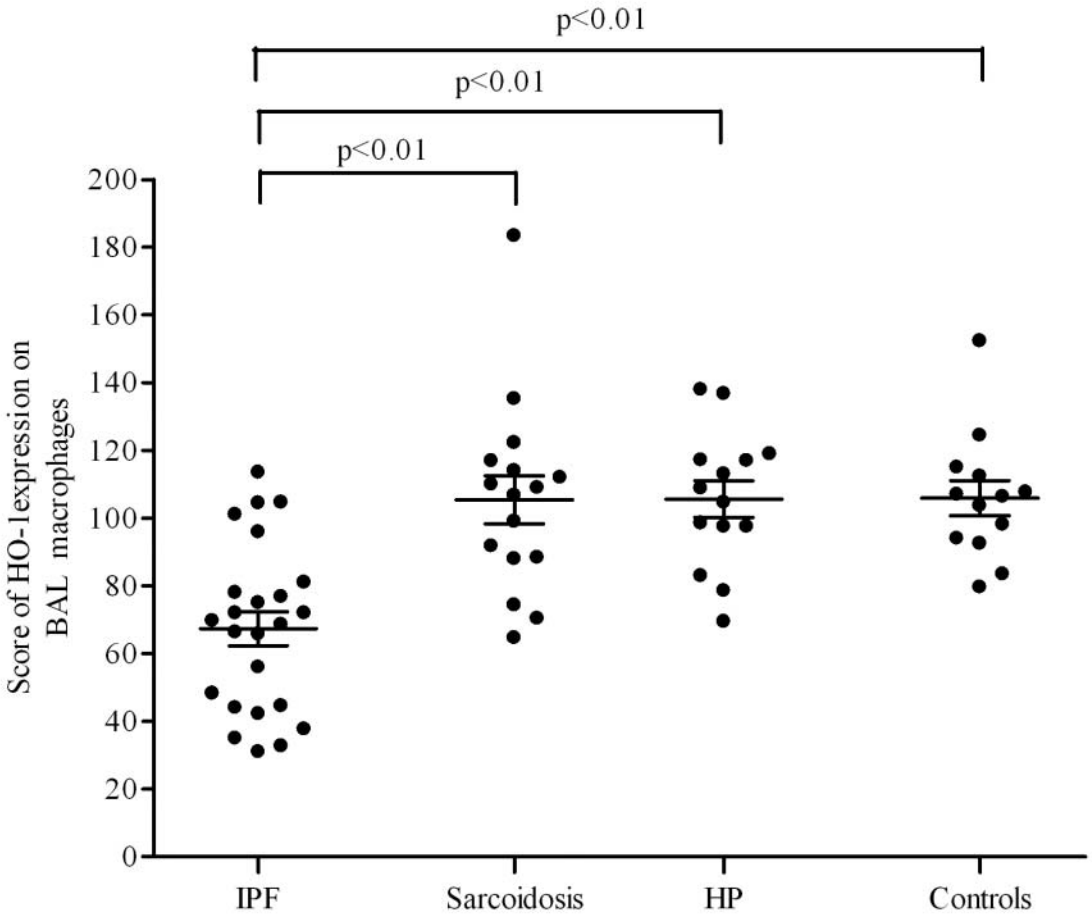

Figure 2 Spontaneous and lipopolysaccharide (LPS)-stimulated production of IL-12 (panel A) and IL-18 (panel B) from bronchoalveolar lavage (BAL) macrophages in patients with idiopathic pulmonary fibrosis (IPF) (n=12) and the granulomatous lung disorders (n=12).

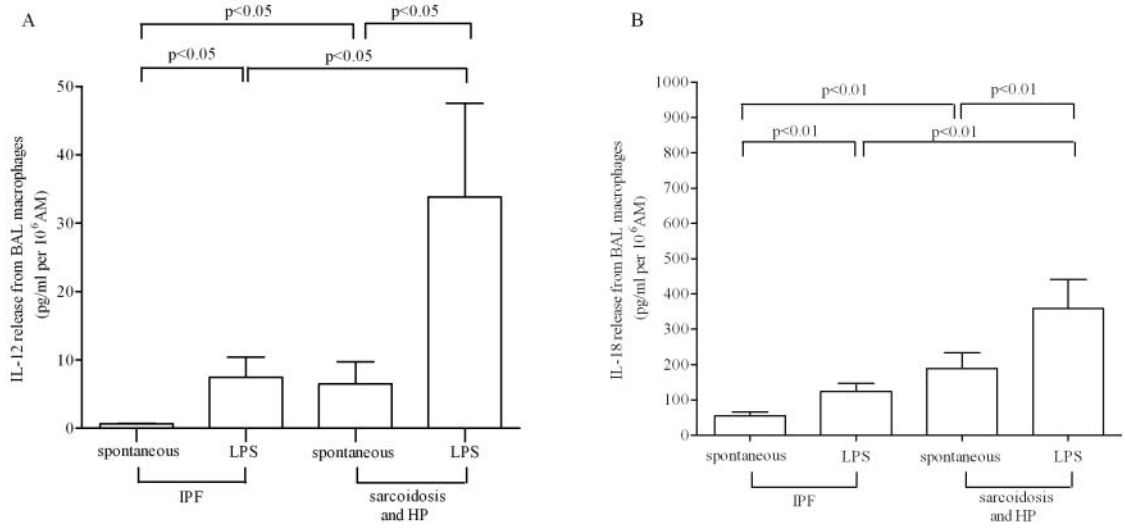

Figure 3 Correlation of scores of heme oxygenase (HO)-1 expression in macrophages with percentage of lymphocytes from bronchoalveolar lavage (BAL) in sarcoidosis (n=16) and hypersensitivity pneumonitis (HP) (n=14) patients (r=0.38, p<0.05).

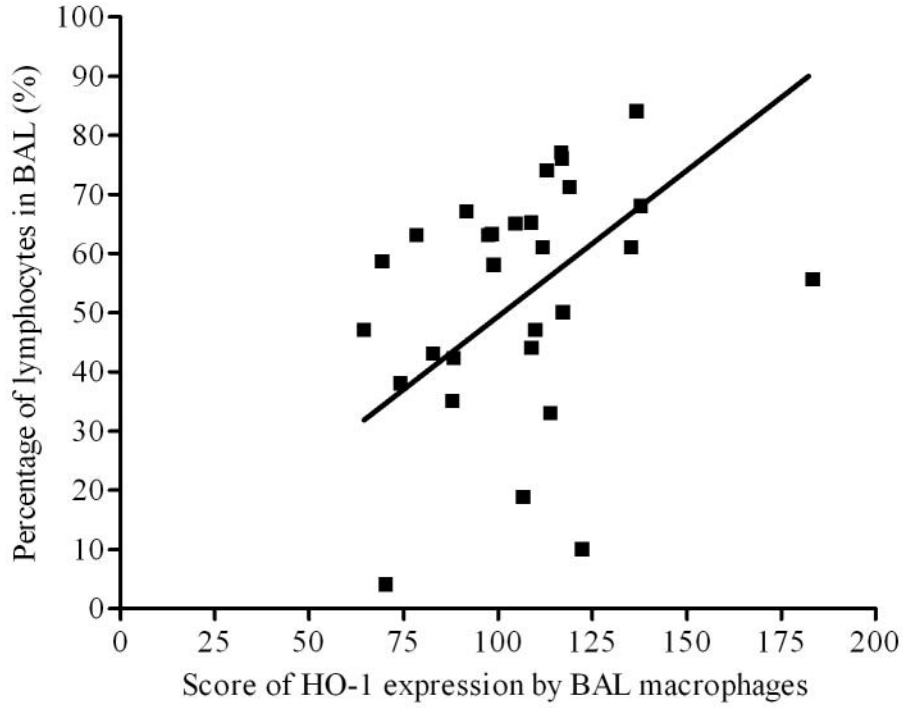

Figure 4 Correlations of IL-12 (panel A) and IL-18 (panel B) with score of heme oxygenase (HO)-1 expression by alveolar macrophages in bronchoalveolar lavage (BAL) in patients with idiopathic pulmonary fibrosis (IPF) (r=0.58, p<0.05; r=0.60, p<0.05, respectively).

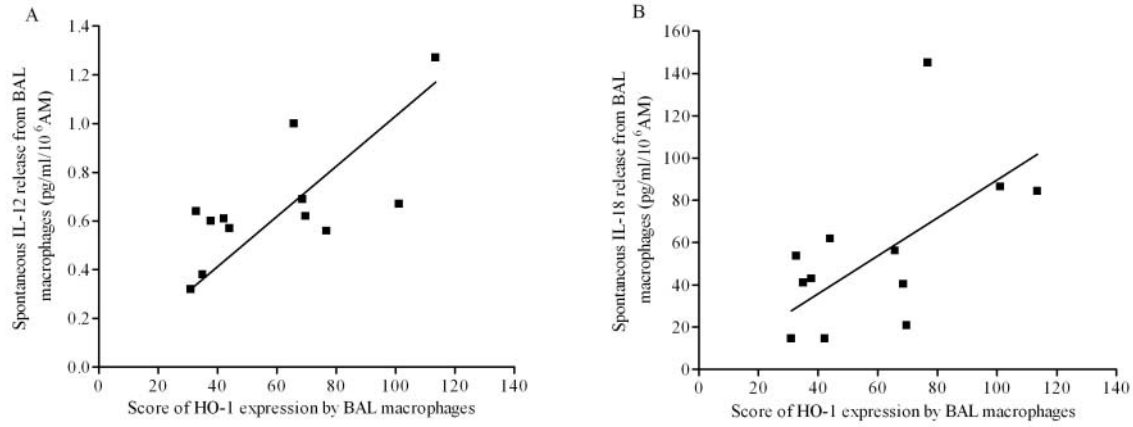

Figure 5 The concentration of total bilirubin (panel A) and ferritin (panel B) in the macrophage culture supernatants of patients with idiopathic pulmonary fibrosis (IPF) (n=12) and the granulomatous lung disorders (n=12).