Relationship between IL-8, IL-10, MMP-9 level and morphological pattern of BAL fluid in interstitial lung diseases patients

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Abstract

There is an increasing evidence that imbalance between pro- and anti-inflammatory factors such as cytokines and proteases is able to direct course of inflammatory lung diseases. Possible relations between proinflammatory interleukin (IL) - 8 and proangiogenic metalloproteinase-9 (MMP-9) as well as antiinflammatory interleukin-10 in bronchoalveolar lavage fluid (BALf) of pulmonary sarcoidosis (BBS) (n=18), idiopathic pulmonary fibrosis (IPF) (n=10) and hypersensitivity pneumonitis (HP) (n=9) patients were examined. IL-8, IL-10 and MMP-9 concentrations in BAL supernatants were determined using ELISA test. A strong correlation ($p<0.01$) between concentration of IL-8 and MMP-9 in BALf of studied groups was found. Moreover, in IPF group the level of MMP-9 strongly correlated ($p<0.01$) with IL-10 as well as negatively with relative count of macrophages ($p<0.05$); while the relative count of neutrophils positively correlated with IL-10 ($p<0.01$) as well as MMP-9 ($p<0.01$). In BBS stage I group negative correlation between level of IL-10 and macrophages relative count ($p<0.05$) as well as positive correlation between IL-10 and lymphocytes relative count ($p<0.05$) was present. Activity of MMPs and their close relationship with proinflammatory and antiinflammatory factors such IL-8 and IL-10 suggest the contribution of angiogenesis in pathogenesis of interstitial lung disease. Further studies should reveal more data concerning the possible therapeutic approach involving both antiinflammatory and antiangiogenic activities.

Key words: *interstitial lung diseases, interleukin-8, interleukin-10, metalloproteinase-9, angiogenesis, bronchoalveolar lavage fluid*

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Introduction

Interstitial lung diseases are characterized as a chronic inflammatory disorders [1]. Alveolitis, a common finding in these diseases, leads to the lung injury and often fibrosis, that finally deranges alveolar capillary units causing their dysfunction and eventual loss. Tissue remodelling involved in this process is initiated by degrading of basement membranes - a thin matrices underlying most epithelium and endothelium in the lungs. On the other hand progress of pathological events in the lungs is usually associated with activation of alveolar macrophages and other cells, followed by accumulation of inflammatory cells as well as production of great number of proinflammatory factors such as cytokines and proteases.

Metalloproteinases (MMPs) are family of enzymes that play a central role in extracellular matrix (ECM) turnover and remodelling based on their ability to hydrolyze major protein components of ECM [2]. Two members of the family metalloproteinase (MMP)-2 and MMP-9 are considered to play an important role in angiogenesis. Both are known for their ability to degrade collagen present in a vascular basement membrane. Recent studies revealed their participation in development of some inflammatory disorders. Kumagi and

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colleagues demonstrated in the murine asthma model that MMP-2 and MMP-9 regulate the infiltration of inflammatory cells through the basement membrane and are able to induce airway hyperresponsivenes [3]. Their results implicate a key role of MMPs in the pathogenesis of asthma. According to Gibbs et al. macrophages-derived MMPs contribute to the neutrophil-dependent lung injury in mice proving a crucial role of MMP-2 and 9 in acute lung injury inflammation [4]. In recent studies increased expression of MMP-1, MMP-2, MMP-9 was detected in the lungs of patients with idiopathic pulmonary fibrosis (IPF) and histiocytosis X [5]. Elevated levels of MMP-9 were found in BALf of patients with adult respiratory distress syndrome [6] and asthma [7].

Apart from metalloproteinases, a considerable number of other proinflammatory markers such as IL-1, IL-2, IL-6, IL-8, tumor necrosis factor-α (TNF-α) was shown to be increased in BAL fluid originated from hypersensitivity pneumonitis (HP) [8], BBS and IPF patients [9-11].

IL-8 is a proinflammatory chemokine produced by different types of cells such as alveolar macrophages, activated lymphocytes, neutrophils as well as fibroblasts, epithelial and endothelial cells [8-10]. It acts as a specific chemoattractant mainly for neutrophils, but also for eosinophils, some lymphocytes T and NK cells. IL-8 activates neutrophils and lymphocytes stimulating their degranulation and migration to the sites of infammation that results in the acceleration of inflammatory response.

IL-10 is a pleiotropic Th-2 – type cytokine. It is known as an inhibitor of inflammatory cells - macrophages, monocytes, lymphocytes as well as resident cells such as airway epithelial and smooth muscle cells (11, 12). IL-10 down-regulates pro-inflammatory cytokines production (IL-6, IL-8, GM-CSF) and suppresses interferon gamma (IFN-γ) and IL-2 expression in Th-1 lymphocytes as well as IL-4 and IL-5 expression in Th-2 cells.

Although pathogenesis of BBS, IPF and HP is not yet fully understood, there is an increasing evidence that imbalance between the expression of pro- and anti-inflammatory factors such as cytokines and proteases is able to direct the course of these diseases. The aim of present study was to examine the possible relationship between proinflammatory IL-8 and proangiogenic MMP-9, as well as antiinflammatory IL-10 in the pathomechanism of BBS, IPF and HP.

Materials and methods

Patient characteristics

The study population consisted of 37 patients with interstitial lung diseases who underwent bronchoscopic bronchoalveolar lavage (BAL) for clinical reasons, either for the diagnostic purposes or to assess the disease activity. Patients were divided into three groups: group I consisted of 18 patients with sarcoidosis (9 male/9 female) who were diagnosed by clinical and roentgenological evaluation. Thirteen out of 18 had biopsy confirmation of pulmonary sarcoidosis. All patients but four had symptoms (cough, dyspnoea, fever, erythema nodosum, polyarthralgia) at the time of diagnosis. According to the chest X-ray findings, BBS was classified as stage I process in eight patients, stage II in eight and stage III in two. None of the patients was receiving oral or inhaled steroids at the time of BAL or in the previous 3 months.

Group II consisted of 9 patients with hypersensitivity pneumonitis (4 male/5 female). The diagnosis of HP was based on the history of antigen exposure, presence of serum IgG antibodies to these antigens, physical examination, chest X-ray and high resolution computed tomography (HRCT) findings. The diagnosis was confirmed by the open lung biopsy in 3 patients. One of the patients was receiving steroid therapy at the time of BAL.

Group III consisted of 10 patients: 9 with idiopathic pulmonary fibrosis and one with pulmonary fibrosis associated with sclerodermia (3 male/7 female). The diagnosis of pulmonary fibrosis was based on clinical (compatible history, digital clubbing, inspiratory crackles on auscultation, pulmonary function test results) and radiological signs (computed tomography scan with interstitial infiltrates and honeycombing present). A confirmatory open lung biopsy was performed in 4 patients. None of the patients was receiving oral steroids in the preceding 3 months.

Functional studies (table 3)

BAL and BAL analysis

BAL was performed in all patients for diagnostic purposees with informed consent. The control group consiting of healthy subjects was not formed for the ethical reasons, instead literature data of healthy control

Table 1. Patients characteristics

	Sarcoidosis	*Hypersensitivity Pneumonitis*	*Fibrosis*
Age, years	40.8±8.2	54.4±13.3	56.7±11.3
Smoking history	5S/13NS	1S/8NS	0S/10NS
Lung function parameters:			
FVC[a]	111.8±13.6	86.0±29.7	67.9±15.7
FEV1[b]	105.6±6.2	83.3±7.0	67.8±16.1
DLco[c]	96.1±22.9	76.3±5.7	48.1±4.4

a – forced vital capacity; b – forced expiratory volume in one second; c – diffusion capacity of carbon monoxide; S – smokers; NS – nonsmokers

group presented by M. John et al. [11] were used for comparison purposes. BAL was exercised by the standard procedure [13]. Fifty mililiters of sterile 0.9% saline solutions at the room temperature was instilled through a flexible fiberoptic bronchoscope four times to a total volume of a 200 ml with harvesting of the fluid under immediate gentle vacuum.

The recovered BAL was filtered through sterile gauze, and the cells were counted. Cytospine preparations were prepared for cells differential count. The fluid was centrifuged 400 x g for 10 min, the supernatant was collected and frozen at -80°C. Cells were >90% viable as assesed by trypan blue exclusion. Cytospined smears were stained with May-Grunwald-Giemsa differential staining and determined by counting of a minimum 600 cells. CD4/CD8 ratio was estimated by monoclonal antibody staining procedure using LSAB+ kit (DAKO).

Quantification of IL-8 , IL-10 and MMP-9 in BAL fluid

IL-8, IL-10 and MMP-9 concentration in BAL supernatants was determined by quantitative enzyme immunoassay technique (ELISA) using commercial kits (R&D). All standards and samples were measured in duplicate and the mean values of IL-8, IL-10 and MMP-9 were obtained. Their concentration was determined by interpolation of their absorbance from standard curve.

Statistical analysis

All data were presented as a mean ±SE. Student *t* test was used for mean values comparison and the Pearson test to analyze correlations between studied groups.

Results

BAL charateristics

BBS and HP patients presented BAL lymphocytosis when compared to the control group of healthy subjects presented by M. John et al. [11]. Both relative count of neutrophils and eosinophils were elevated in BAL fluid of patients with IPF. All patients show an increase of CD4/CD8 ratio.

The results are shown in Table 2.

The IL-8 concentration in BAL fluid of studied group

There were no significant differences in IL-8 levels between HP, BBS or IPF patients. However, IL-8 concentration in BALf of examined patient groups was increased in comparison to the control group ($p<0.001$), Table 3.

Table 2. BAL characteristics of the studied groups

	Sarcoidosis **n=18**	***Hypersensitivity Pneumonitis*** **n=9**	***Fibrosis*** **n=10**	**Controls[a]** **n=10**
Total cell count x 10^4/ml	13.7±1.6	20.6 ±2.4	17.2±2.4	14.4±4.2
Macrophages (%)	71.1±3.6*	60.2±6.7	59.1±5.3**	84.9±3.3
Lymphocytes(%)	27.2±3.7*	34.9±7.3*	17.4±3.2	11.3±2.8
Neutrophils (%)	1.2±0.2	2.6±1.0	16.9±5.2**	2.1±0.6
Eosinophils (%)	0.5±0.1	2.1±1.1	6.4±1.8*	1.0±0.7
CD4/CD8	5.0±0.7**	2.1±0.2**	2.3±0.6**	0.7±0.2

*a – data of healthy subjects according to M. John et al. [11]; * – p<0.05 versus control ; ** – p<0.01 versus control*

Table 3. BALF concentration of IL-8, IL-10 and MMP-9 in studied groups

	Sarcoidosis	***Hypersensitivity Pneumonitis***	***Fibrosis***	***Controls***
IL-8 (pg/ml)	42.61±2.3 (3.4-184) n=18	26.37±7.8 (9.8-78.9) n=9	50.0±17.3 (11.3-182.1) n=10	5.0±2.2[a] n=10
IL-10 (pg/ml)	0.76±0.05 (0.14-19.7) n=16	0.44±0.06 (1.0-9.3) n=9	1.2±0.3 (0.70-4.75) n=10	130.0±61[b] n=8
MMP-9 (ng/ml)	2.86±1.56 (0.44-1.16) n=12	4.02±0.97 (0.16-0.76) n=9	6.8±3.05 (0.65-32.3) n=10	3.2±0.9[c] n=15

a – according to LE Girgis et al. [13], b – according to L Borish et al. [14], c – according to MT Henry et al. [15]

The IL-10 concentration in BAL fluid of studied group

The HP group was characterized by the lowest concentration of IL-10 (HP vs. BBS, $p<0.01$), while the IPF group BALf contained the highest amount of that cytokine (IPF vs. BBS, $p<0.01$). The mean concentration of IL-10 measured in each patients group was dramatically reduced in comparison to the control group ($p<0.001$), Table 3.

The MMP-9 concentration in BAL fluid of studied group

There were no significant differences in MMP-9 amount in BALf between HP, BBS or IPF patients. MMP-9 concentration calculated in patient's groups was comparable to that of control group (no significant differences). Table 3.

Correlations in hypersensitivity pneumonitis group

A positive correlation ($r=0.67$; $p<0.05$) between the level of IL-8 and MMP-9 was present in HP group of patients.

Correlations in idiopathic pulmonary fibrosis group

A strong correlation between the concentration of IL-8 and MMP-9 ($r=0.73$; $p<0.05$) as well as between concentration of IL-10 and MMP-9 ($r=0.93$; $p<0.01$) was found in IPF group. The relative count of Neu strongly correlated with IL-10 ($r=0.90$; $p<0.01$) and with MMP-9 ($r=0.806$; $p<0.01$). The negative correlation between MMP-9 and macrophage relative count ($r=-0.702$; $p<0.05$) was also found in this group of patients.

Correlations in pulmonary sarcoidosis group

BBS patients were divided into two subgroups:

A. BBS Stage I (8 patients),
B. BBS Stage II (8 patients) and BBS Stage III (3 patients).

In group A the level of IL-8 strongly correlated with MMP-9 level (0.95; $p<0.05$), while IL-10 negatively correlated with macrophages relative count ($r=-0.87$; $p<0.05$) as well as positively with lymphocytes relative count ($r=0.87$ $p<0.05$).

In group B concentration of IL-8 in BAL fluid strongly correlated with MMP-9 concentration ($r=0.967$; $p<0.01$).

Discussion

At the first stages of interstitial lung diseases airway resident cells are destroyed and basement membrane and ECM components are damaged. Epithelial and endothelial cells increase the expression of adhesion molecules: ICAM-1 as well as VCAM-1 and start to produce a great number of inflammatory mediators causing progression of the disease [1].

Interestingly, the starting point of the angiogenesis process seems to be quite similar. Angiogenesis, a new blood vessels formation, begins from the production of MMPs and serine proteases capable of basement membranes breakdown through the digestion of ECM components (16). It is known that in chronic inflammatory diseases inflammation is usually accompanied by angiogenesis. Therefore, MMPs activity might be perceived as a bridge between processes of inflammation and angiogenesis, since MMPs are known to play an important role in the course of inflammatory disorders and are vital during angiogenesis [2, 16].

Our study has shown the strong association between concentration of proinflammatory IL-8 and proangiogenic MMP-9 in BALf of patients with BBS, HP, and IPF. That relationship might be more common and concern also many other interstitial lung diseases. If so, than the close association between inflammation, pathologic angiogenesis and tissue remodeling in pathogenesis of interstitial lung diseases should exist.

Some of our previous studies confirmed the important role of angiogenesis in the inflammatory lung diseases development [17]. The sera obtained from BBS patients were shown to stimulate the angiogenic activity of healthy donors mononuclear leukocytes [18]. Weber et al. proved that BAL fluid from BBS patients induced migration and chemotaxis of endothelial cells and fibroblasts[19].

IL-10 concentrations present in BAL fluid of all studied groups of BBS, HP and IPF patients were significantly decreased when compared to controls (as presented by Borish et al. [14]). Similar results were shown by other authors. Borish et al revealed that asthmatic patient's BALf was characterized by diminished concentration of IL-10 (9±18 pg/ml) when measured up to that of normal controls (130±61 pg/ml). Martinez et al described the reduced amount of IL-10 in BALf of IPF patients while, paradoxically, the expression of IL-10 mRNA in BAL macrophages was increased as compared to healthy controls [20]. Interestingly, the down-regulated production of IL-10 in mice was proved to be associated with amplified lipolysaccharide (LPS)-induced MMP-2 and 9 release from inflammatory BAL cells [21]. Therefore, the significant reduction of anti-inflammatory IL-10 concentration in BALf shown in our study might be the indication of the on-going abnormal inflammatory process characterizing interstitial lung diseases.

High levels of IL-8 in BALf of all patient's groups shown in our study are in consistence with other studies [8-10]. These results strongly indicate the substantial contribution of IL-8 in the pathogenesis of interstitial lung diseases.

On the other hand the concentration of MMP-9 was comparable in the control and the studied groups. Although, the differences between studied groups were not statistically signifcant, the MMP-9 value for IPF patients was at the highest level. The up-regulated expression of MMPs by alveolar macrophages isolated from BALf of severe IPF patients has been already shown by others [22], while John et al. proved that alveolar macrophages from BBS and IPF patients as well as healthy individuals produce comparable amouts of MMP-9 [11].

It was clearly demonstrated that in the course of active IPF alveolar macrophages were responsible for accumulation of neutrophils (and other inflammatory cells) in the lungs [9, 22] that are presently regarded as cellular one of the key elements in the pathogenesis of IPF [10]. Both activated macrophages and neutrophils spontaneously produce and release considerable amounts of pro-inflammatory mediators (including MMPs and IL-8) responsible for the alveolar epithelium and alveolar basement membranes damage. Positive correlation between neutrophils relative count and MMP-9 as well as IL-10, and negative correlation between MMP-9 and macrophages relative count shown in our study support that opinion. The augmented MMPs concentration, closely associated with neutrophils

number as well as inflammatory mediators production obviously reflects the on-going, active inflammatory process crucial in the pathogenesis of IPF.

In BBS group the strong negative correlation between macrophages relative count and IL-10 as well as positive correlation between lymphocytosis and IL-10 were observed, representing their involvement in the regulation of inflammatory process. These results are in accordance with conclusions of Thomas at al., who suggested that granulomatosus lessions in pulmonary sarcoidosis might be a result of macrophage and T lymphocyte dysfunction [23].

Moreover, correlations shown for IL-10 and cellular markers of inflammatory process activity in both BBS and IPF groups strongly imply the regulatory role of IL-10 in these diseases. Silvestre et al. revealed the ability of IL-10 to regulate the production of MMP-2 and 9 [24]. Similarly, it was demonstrated that this anti-inflammatory Th2 cytokine inhibited both angiogenesis as well as MMP-2 and 9 production by alveolar macrophages from BALf of BBS patients [14, 16]. Stern et al have also shown that overexpression of MMP-2 and MMP-9 in prostate tumor cells was acompanied by IL-10 deficiency [25]. Finally, the strong correlation between IL-10 and MMP expression was established in inflammatory bowel disease [26]. All these data support our results and confirm anti-inflammatory function of IL-10 in the regulation of inflammatory response.

Observed close inter-relation of MMPs and pro- as well as anti-inflammatory factors such as IL-8 and IL-10 appears as a strong encouragement to study further the contribution of angiogenesis in the pathogenesis of interstitial lung diseases and to reconsider new possibilities of therapeutic approach involving both antiinflammatory and antiangiogenic activities.

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