Cytokine gene polymorphisms and BALF cytokine levels in interstitial lung diseases

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KEYWORDS
Lung fibrosis;
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Cytokines;
Chemokines;
Genetic polymorphism

Summary
Background: The aim of our study is to investigate correlations of TH1/TH2 cytokine gene polymorphisms and bronchoalveolar lavage fluid (BALF) cytokine values in interstitial lung diseases (ILD).

Methods: In 16 sarcoidosis, 7 idiopathic pulmonary fibrosis (IPF) and 8 hypersensitivity pneumonitis (HP) patients we evaluated IL-1α, -1R, -1RA, -2, -4, -4Rα, -6, -10, -12, IFN-γ, TGF-β1 and TNF-α gene polymorphisms in peripheral blood, and MCP-1, MIP-1α, MIP-1β, RANTES, ENA-78, FGF, G-CSF, GM-CSF, IFN-γ, IL-1α, IL-1RA, IL-1β, -2, -4, -5, -6, -10, -17, TNF-α, Tpo and VEGF values in BALF.

Results: We found higher TNF-α values in IL-1R pst 1970 TT homozygotes regardless of diagnosis (p = 0.0126). In the sarcoidosis group IL-4Rα(+1902)AA and IL-10(–1082)G allele correlated with higher BALF ENA-78 levels (p = 0.0258, p = 0.0230). In the HP group the IL-6 (–174)CG and IL-6(nt565)AG correlated with higher ENA-78 BALF levels (p = 0.0253). In the IPF group the IL-1β +3962 CC homozygotes had lower IL-1RA BALF values (p = 0.046). BALF chemokine values did not differ between ILD subgroups, except for IL-8, which was higher in stage III sarcoidosis patients compared to stage I.

Conclusion: Our results show a probable influence of gene polymorphisms, namely IL-4Rα and IL-10 on ENA-78 BALF levels in sarcoidosis, IL-6 on ENA-78 BALF levels in HP and IL-1-β on IL-1RA BALF values in the IPF group. The TNF-α BALF values correlated with IL-1R pst 1970 gene polymorphisms.

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Introduction

Idiopathic pulmonary fibrosis (IPF), hypersensitivity pneumonitis (HP) and sarcoidosis belong to a large group of interstitial lung diseases (ILDs) where an imbalance of regulatory, profibrotic and antifibrotic cytokines and also chemokines is supposed.

Among the most potent chemoattractants involved in pathogenesis of these diseases belong regulated on activation, T-cell expressed and secreted (RANTES)/CC ligand (CCL)5, macrophage inflammatory protein (MIP)-1α/CCL-3 and MIP-1β/CCL-4, interleukin (IL)-8/CXC ligand (CXCL)-8 and monocyte chemoattractant protein (MCP)-1/CCL2. The latter is also involved in angiogenesis via inducing the chemotaxis of endothelial cells and the formation of blood vessels. Chemokines that promote angiogenesis play a crucial role in all fibroproliferative disorders including ILDs. This group of molecules includes the CXC chemokine family members containing so-called ELR motif such as IL-8/CXCL-8, epithelial neutrophil-activating protein (ENA)-78/CXCL-5 and growth related genes (GROS)-α, β, γ/CXCL-1, -2, -3. The members of this family not containing this motif are potent angiogenesis inhibitors, i.e. monokine induced by interferon (IFN)-γ (MIG)/CXCL-9, IFN-γ inducible protein (IP)-10/CXCL-10 and IFN-γ inducible T-cell α chemoattractant (ITAC)/CXCL-11.

Idiopathic pulmonary fibrosis (IPF) is a serious lung disease with an unknown aetiology. The combination of genetic predisposition and environmental influence is supposed. Current data suggest that this disease is probably based on a pathologically accelerated growth rate and dysfunction of lung tissue cells, which would normally accomplish regular wound healing. The cellular immune response, regardless of the initial mechanism of damage, processes shifts to a so-called TH2 reaction, accompanied response, regardless of the initial mechanism of damage, accomplish regular wound healing. The cellular immune response, regardless of the initial mechanism of damage, processes shifts to a so-called TH2 reaction, accompanied by a relentless induction of mesenchymal growth during wound healing. Experimental data suggest that the immunomodulatory properties of biological factors of wound healing, such as some cytokines and their receptors, mentioned previously, may be responsible for this condition. An imbalance of angiogenic vs-angiostatic chemokines may contribute to the aberrant vasculogenesis seen in IPF.2–4

Sarcoidosis is a multisystem inflammatory disease of unknown aetiology characterized by epithelial granuloma formation with accumulation of CD4+ T lymphocytes and macrophages. An imbalance of cytokine and chemokine production with a shift to so-called TH1 type immune reaction is supposed. The situation is not so clear in later phases of the disease, characterized by transition to fibrosis, where the TH2 type and pro-fibrotic cytokines and chemokines probably reach a dominant role.5,6

Hypersensitivity pneumonitis (HP) also belongs to the granulomatous diseases group and has different clinical stages in which different chemokines and cytokines prevail according to the stage, from granulomatous to fibrotic, as extreme poles of the disease phenotype.7–10

All the above-mentioned diseases probably share the same pathogenetic mechanism of fibroproduction, which could be the alternative activation of alveolar macrophages (AM) in the presence of prevalent TH2 type cytokines. It appears that macrophage colony-stimulating factor (M-CSF) can play a role in this alternative AM involvement and CCL2 production.11 These alternatively activated AMs induce an increase of collagen production by fibroblasts. This process seems to be mediated also by CCL18, which is overexpressed in the presence of interleukin IL-10, IL-4 and IL-13.12,13 The study of Capelli et al supported the later results, having revealed that CC chemokines, namely CCL2, CCL3 and CCL4, were involved in the mechanisms of pulmonary fibrosis.14 Zhu et al found that IL-13 is a potent stimulator of MCPs and other CC chemokines, and documented the importance of MCP-1-CCR2 signalling in the pathogenesis of the IL-13-induced pulmonary changes which include pulmonary inflammation stimulation, hyaluronic acid accumulation and tissue fibrosis.15 Antonioni et al proved the existence of distinct angiogenic profiles between IPF and sarcoidosis, indicating a potential different role of CXC chemokines in the local immunologic response in IPF and pulmonary sarcoidosis.16,17 The study of Keane et al supported the IL-8 and IP-10 influence on angiogenic activity regulation in IPF.18

There are more studies dealing with the expression of chemokines and cytokines in BALF in ILDs but only a few correlate the immunogenetic background with the BALF cytokine and chemokine expression in these patients. Navratilova et al studied MCP-1-2518 (A to G) single nucleotide polymorphism (SNP) in Czech patients with pulmonary sarcoidosis and found no difference in the SNP distribution compared to the healthy population, but a significantly higher proportion of G allele and GG genotype was observed in the patients with Löfgren syndrome (LS) compared to control subjects and patients without LS.19 Japanese authors did not find any substantial role for MCP-1 and MIP-1A genes in genetic predisposition but stated that these gene SNPs might be related to the recruitment of monocytes/macrophages to the alveolar spaces in sarcoidosis.20

We have described in our recent genetic studies the potential role of IL-1, IL-4, IL-12 and IFN-γ genes in the pathogenesis and clinical presentation of sporadic IPF. We have a pronounced suspicion of the pathogenic role of IL-4 promoter region (IL-4 –590, IL-4 –33) polymorphisms in IPF development.21 We have also found a correlation of CD4+ and CD8+ T-cell counts in bronchoalveolar lavage fluid (BALF) with IL-4 (~1098) polymorphisms and HLA DR+ T-cell counts with IL-1α (~889) polymorphisms, and a probable correlation of IL4Rα polymorphisms and the high resolution computed tomography (HRCT) alveolar score at the time of diagnosis. The interstitial score, i.e. the fibrotic changes, seemed to be correlated with IL-12 polymorphisms and the progression of lung fibrosis correlated with IL-1RA, IL4Rα and IL-4 –33 polymorphisms.22,23

The aim of our study is to investigate correlations of TH1/TH2 cytokine gene polymorphisms and BALF cytokines and chemokines levels in these patients. We hypothesize that the polymorphisms, especially of regulatory cytokines, could influence the expression of cytokines and chemokines in lung tissue and BALF and thus cause the alternative activation of AMs, which could lead to aberrant wound healing with excessive fibroproduction.
Material and methods

Study subjects

Sixteen sarcoidosis, 7 idiopathic pulmonary fibrosis (IPF) and 8 hypersensitivity pneumonitis (HP) patients were involved in our study. This study is a part of larger one dealing with the genetic and immunological background of ILDs. All patients with suspicion of ILD based on clinical investigation, functional parameters and HRCT studies undergo BALF investigation and genetic investigation of peripheral blood. The diagnosis of specific ILD is arrived at after stated procedures and in some patients also supported by transbronchial or videothoracoscopic biopsy. Then the patients are sorted into subgroups according to their definite diagnosis.

All seven patients with IPF (mean age 63.7 years, 5 men and 2 women) were diagnosed according to the American Thoracic Society (ATS)/European Respiratory Society (ERS) consensus classification.24 We used the following criteria: insidious onset of dyspnoea, bilateral basal crackles and digital clubbing, restrictive ventilatory pattern and lowered diffusion capacity for carbon monoxide, typical radiological changes on high resolution computed tomography of the lungs (HRCT) with prevailing fibrotic changes and granulocytic bronchoalveolar lavage (BAL). The videothoracoscopic lung biopsy was performed in four patients who did not meet all the above-mentioned criteria, and the histopathological investigation revealed the characteristic changes of usual interstitial pneumonia (UIP).

The 16 sarcoidosis patients (mean age 51 years, 6 men and 10 women) were diagnosed according to the American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and Granulomatous Disorders statement on sarcoidosis, based on history, clinical symptoms, standard chest radiography, HRCT and laboratory tests (serum angiotensin converting enzyme, calcaemia and calcuiatra). Four patients had radiological stage I, 7 patients stage II, and 5 patients stage III. All patients underwent transbronchial biopsy or transbronchial lymph node puncture or videothoracoscopic lung biopsy with histopathological evidence supporting the diagnosis of sarcoidosis.

The diagnosis in 8 patients with HP (mean age 64 years, 4 men and 4 women) was based on history of exposition to suspect antigen, typical clinical course, radiological (HRCT) changes compatible with HP, BALF cell count and the levels of specific IgG to the suspect antigen. The diagnosis was supported with videothoracoscopic lung biopsy in 3 patients. According to clinical stage of the disease, with one exception all patients had chronic HP.

The patients all signed the Informed Consent Form before submitting a blood sample for genotyping. The study design and Informed Consent Form was approved by the Central Ethical Committee of the Thomayer University Hospital and the Institute for Clinical and Experimental Medicine. Basic demographic data such as age and sex were collected. The basic demographic data and BALF cell counts are stated in Table 1. Interestingly the percentages of lymphocytes were lower in patients with sarcoidosis and HP than in those with IPF. This was probably caused by the fact that the majority of HP patients had the chronic form of the disease and also in the sarcoidosis group the majority of the patients were stage I or III where the BALF lymphocytosis need not be prominent (Table 1).

Methods

Polymorphisms in the promoter regions of the IL-1α, IL-1β, interleukin-1 receptor (IL-1R), IL-1RA, IL-2, IL-4, IL-6, IL-10, IL-12, TNF-α, IFN-γ as well as polymorphisms in the translated regions of the transforming growth factor (TGF)-β, IL-1β, IL-2, IL-4 and IL-4Rx genes were characterized.

Deoxyribonucleic acid (DNA) extraction

Ten millilitres of peripheral blood was collected in ethylene-diamine-tetraacetic acid (EDTA) tubes and red cell lysis buffer was added; after 20 min the tube was spun for 10 min at 1300 × g and the supernatant was removed. White cell lysis buffer was then added to sediment with proteinase and soybean dodecyl sulphate (SDS). The mixture was incubated on a rotator for 18 h at 37°C. After incubation 6 M NaCl and chloroform were added and mixed for 15 s before being centrifuged for 25 min at 1300 × g. The supernatant was added to 4 ml of absolute ethanol in a clean tube. The precipitated DNA was removed, re-suspended in sterile water and stored at 4°C.

Cytokine genotyping

We evaluated polymorphisms of thirteen different cytokine genes utilizing CYTOKINE GENOTYPING KIT (Dynal, Biotech, Norway). The test is designed as a polymerase chain reaction (PCR) with sequence-specific primers. More closely, each well of a 48 well tray contains specific primer pair for amplifying desired unique sequence. Whole procedure was performed according to the manufacture’s manual. Obtained pattern of positive and negative PCR is documented and interpreted according to the manufacture’s worksheet.

Bronchoalveolar lavage

BAL was performed during the fiberoptic bronchoscopy under local anaesthesia. Five fractions of 50 ml of lukewarm

| Table 1 Demographic data and BAL cell counts in ILD patients according to the groups. |
|---|---|---|
| Sarcoïdosis (16) | IPF (7) | HP (8) |
| Mean age (years) | 51.5 (SD 13.1) | 63.7 (SD 9.4) | 63.9 (SD 18.7) |
| Men/women | 6/10 | 5/2 | 4/4 |
| BAL lymphocyte count in % | 12 (0–71) | 18 (4–72) | 15 (0–78) |
| BAL neutrophil count in % | 3 (0–62) | 6 (2–70) | 7 (1–75) |
| BAL eosinophil count in % | 0 (0–4) | 0 (0–27) | 0 (0–6) |
| Smokers/Ex-smokers/Non-smokers | 5/2/9 | 1/4/2 | 1/1/6 |

BAL cell counts are stated in median values and a range.
saline were instilled into the segmental part of the middle lobe where the bronchoscope was wedged. After instillation the fluid was recovered by syringe suction and put into sterile containers. The first three parts were processed by ultra-centrifugation and Giemsa staining for a cytological investigation and the fourth part was sent for flow cytometric evaluation. The sample was determined to be valid if the recovery was above 20 ml per fraction and the significant admixture of polymorphic bronchial epithelial cells was not found; otherwise the patient was not enrolled in our study. The recovered BALF volume did not significantly differ between enrolled patients. The supernatant was discarded and stored frozen for later cytokine and chemokine identification.

The BALF cytokine levels were investigated with Fluorokine Multianalyte Profiling Kits with Luminex 100 platform, Human cytokine panel A: MCP-1, MIP-1α, MIP-1β, RANTES, ENA-78, FGF, G-CSF, GM-CSF, IFN-γ, IL-1α, IL-1RA, IL-1β, IL-2, -4, -5, -6, -8, -10, -17, TNF-α, thrombopoietin (Tpo) and vascular endothelial growth factor (VEGF) (R&D Systems, Inc. Minneapolis, USA), according to the manufacturer’s protocol. The results were pronounced in relative fluorescence units (RFU).

Statistical analysis

The genotype frequencies and allele carriage frequencies were determined by direct counting. The basic statistical characteristics, i.e. mean values, standard deviation and median values, were counted from quantitative variables (age, cell counts, cytokine values). The Kruskal–Wallis test with multiple comparisons was applied for comparison of cytokine BALF values between the different ILD groups and for correlation of BALF cytokine values with genotype in different ILD groups. TNF-α values were measurable only in some patients, thus we counted the values only as not present (TNF-α not measurable) and present (measurable) and stated as a percentage of the whole group. Pearson’s chi-square and Fisher’s exact test were used for testing of association of TNF-α presence in BALF with genotype. Spearman’s correlation coefficient was applied for testing of BALF chemokine values association with the sarcoidosis stage. Statistical analysis was performed using MedCalc statistical software. A p value of less than 0.05 was considered significant.

Results

Not all of the investigated chemokines were detected in BALF of the studied ILD patients. For instance, IFN-γ was detected only in one patient from the whole study group, and IL-2, IL-4 and IL-10 in none of them. We have considered for the statistical evaluation only the chemokines whose values were detectable in BALF in a significant number of patients. VEGF, IL-8, and IL-1RA were detected in BALF in all of the patients. The VEGF BALF values ranged from 0.29 to 182.84, IL-8 values from 3.98 to 335.43 and IL-1RA values from 61.53 to 5353.26. ENA-78 values were measurable in BALF of almost all patients except three individuals and its values ranged from 0 to 154.54.

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<th>Genotype (No. of patients)</th>
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<td>AA (11)</td>
<td>35.744 (42.167)</td>
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<td>AG (4)</td>
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TNF-α values ranged from 0 (undetectable) to 2.28 in one of the patients with IPF. Five of 16 sarcoidosis patients had measurable BALF TNF-α (range 0.15–1.43). One of them was stage I, 3 stage II and only 1 stage III of the disease, compared with 11 sarcoidosis patients with undetected BALF TNF-α, where 6 of 11 had stage III of the disease. In the IPF group only 2 of 7 patients had measurable TNF-α levels and in the HP group, where the majority of patients had the chronic form of the disease, only 2 of 8 patients had detectable TNF-α BALF values. The cytokine BALF values did not differ significantly between the different ILD groups. TNF-α was identified only in some patients and its presence in BALF did not differ significantly between the ILD groups.

When we compared the presence of TNF-α in BALF with cytokine genotype regardless of diagnosis, we found TNF-α was significantly more frequently measurable in IL-1R pst 1970 TT homozygotes (p = 0.0126) (Table 2). In the sarcoidosis group, IL-4Rα(þ1902)AA and IL-10(–1082)G allele correlated with higher ENA-78 levels (p = 0.0258, p = 0.0230) in BALF (Tables 3 and 4). In the HP group the IL-6(–174)CC and IL-6(nts565)AG both correlated with higher ENA-78 BALF levels (p = 0.0253 for both) (Tables 5 and 6). In the IPF group the IL-1β+ 1970 TT homozygotes had lower IL-1RA values in BALF (p = 0.046) (Table 7).

According to the sarcoidosis stage, we did not observe statistically significant differences in BALF chemokine values, except for IL-8, which was higher in patients with stage III compared with stage I (r = 0.574, p < 0.05) (Fig. 1).

Discussion

TNF-α was identified only in some patients in our study and its presence in BALF did not differ significantly between the ILD groups. When we compared the presence of TNF-α in BALF with cytokine genotype regardless of diagnosis, we found TNF-α significantly more frequently measurable in IL-1R pst 1970 TT homozygotes (p = 0.0126) (Table 2). The functional role of this polymorphism is not known to date, but one can suppose the potential influence of the polymorphism on IL-1R signalling and inflammatory cascade which could influence also TNF-α expression.

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In our study, 5 of 16 sarcoidosis patients had measurable BALF TNF-α levels and in the HP group, where the majority of patients had the chronic form of the disease, only 2 of 8 patients had detectable TNF-α BALF values.

In the study of Tahanovich et al the group of sarcoidosis patients with a poor clinical outcome was characterized by a lower percentage of lymphocytes in BALF and rather small amounts of TNF-α compared to those with a favourable clinical outcome. In our study, 5 of 16 sarcoidosis patients had measurable BALF TNF-α. One of them was stage I, 3 stage II, and only 1 stage III of the disease, compared with those 11 sarcoidosis patients with undetected BALF TNF-α, where 6 of 11 had stage III of the disease. Kuroki et al showed on the model of TNF-deficient mice with bleomycin-induced lung fibrosis that TNF also had an anti-inflammatory function. He demonstrated persistent and intense inflammation in TNF-deficient mice due to reduced apoptosis of inflammatory cells and revealed that TNF is essential for repressing pulmonary inflammation in bleomycin-induced pneumopathy. These results support our finding of measurable TNF-α levels mostly in patients with prognostically favourable stages of sarcoidosis, though the differences between the stages were not statistically significant. According to the sarcoidosis stage, we observed statistically significant differences in BALF chemokine values only for IL-8, which was higher in patients with stage III compared with stage I (Fig. 1). These results could support the findings of Antoniou et al who described higher IL-8 BALF levels in patients, where the advanced lung fibrosis is supposed, compared with sarcoidosis patients. IL-10 and TGF-β 1 are anti-inflammatory cytokines that play important roles in the immunoregulatory processes of numerous granulomatous diseases. In sarcoidosis polymorphisms within these cytokine genes were suspected of modifying the course of the disorder. Previously the IL-10 −1082 polymorphisms were proved to have an influence on IL-10 production. Allele A was correlated with low IL-10 production and allele G with high IL-10 production. ENA-78 and IL-8 BALF levels in IPF patients were found to be significantly higher compared with sarcoidosis patients in the study of Antoniou et al. In the study of Sugiyama et al the ENA-78 BALF levels in patients with sarcoidosis were significantly higher than those in control subjects and were more increased in stage III sarcoidosis, showing that ENA-78 may be associated with lung parenchymal disease in pulmonary sarcoidosis.

In our study the cytokine BALF values did not differ significantly between the different ILD groups. We suppose that this finding could be ascribed to the greater number of patients with advanced, i.e. fibrotic, disease in HP and sarcoidosis groups. When correlating the gene polymorphisms with BALF cytokine levels IL-4Rα(−1902)AA and IL-10(−1082)G allele correlated with higher BALF ENA-78 levels (p = 0.0258, p = 0.0230) in the sarcoidosis group (Tables 3 and 4). This could prove the influence of genetically based IL-10 production up-regulation on increased ENA-78 levels and thus on poor clinical outcome. Unfortunately, the results of ENA-78 BALF values in the different sarcoidosis stages did not support this idea in our study. Nevertheless we might suppose that the clinical course of the disease in those patients with higher ENA-78 might be less favourable, but to date we have not carried out a longitudinal study.

Lu et al have stated that the presence of high-expression polymorphisms at position −174 of the IL-6 gene (allele G) significantly increases the risk for bronchiolitis obliterans syndrome development after lung transplantation. Gruters et al hypothesized that the IL-6 −174 C allele might play a role in sarcoidosis severity or a progression towards pulmonary fibrosis in a particular subgroup. In our study the IL-6 polymorphisms (IL-6 −174CG and IL-6 nt565 AG) correlated with higher ENA-78 BALF levels only in the HP group (p = 0.0253 for both), which showed the possible role also of IL-6 polymorphisms on ENA-78 production and thus on the prognosis in HP (Tables 5 and 6). Nevertheless we should be aware of the low number of HP patients in our study.

Shimoji et al found that IL-1RA levels were decreased in healthy smokers as well as IPF and sarcoidosis patients, compared to healthy non-smokers, and suggested that decreased expression of IL-1RA gene may contribute to the development of chronic low-grade inflammation of the lung. In our study, in the IPF group the IL-1α +3962 CC homozygotes had lower IL-1RA values in BALF (p = 0.046),

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<th>Table 5</th>
<th>Correlation of IL-6(−174) polymorphisms with BALF ENA-78 values in HP (p = 0.0253).</th>
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<td>Genotype (No. of patients)</td>
<td>Mean value (SD)</td>
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<td>CG (5)</td>
<td>85.04 (27.418)</td>
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<td>GG (3)</td>
<td>16.583 (23.400)</td>
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<th>Table 6</th>
<th>Correlation of IL-6 nt 565 polymorphisms with BALF ENA-78 values in HP (p = 0.0253).</th>
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<tr>
<td>Genotype (No. of patients)</td>
<td>Mean value (SD)</td>
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<tr>
<td>AG (5)</td>
<td>85.04 (27.418)</td>
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<th>Table 7</th>
<th>Correlation of IL1β(+3962) polymorphisms with BALF IL-1RA values in IPF (p = 0.046).</th>
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<tr>
<td>Genotype (No. of patients)</td>
<td>Mean value (SD)</td>
</tr>
<tr>
<td>CC (3)</td>
<td>1079.3 (673.6)</td>
</tr>
<tr>
<td>CT (3)</td>
<td>4585.1 (1238.3)</td>
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which could support the hypothesis of the IL-1 genes group role in the pathogenesis of this disease (Table 7).

Conclusion

Our results show a probable influence of cytokine gene polymorphisms on cytokine/chemokine levels in BALF, especially for IL-4Ra and IL-10 on ENA-78 BALF levels in sarcoidosis, IL-6 on ENA-78 BALF levels in HP, and IL-1β on IL-1RA BALF values in IPF. The cytokine BALF values did not differ significantly between the ILD subgroups, only within the sarcoidosis subgroup did patients with stage III have higher IL-8 BALF values as compared to stage I. TNF-α was detectable only in some patients, mostly in those with unadvanced sarcoidosis and BALF TNF-α detectability correlated with IL-1R pst 1970 gene polymorphisms.

We suppose that cytokine gene polymorphisms, especially of so-called regulatory cytokines, probably influence cytokine mRNA expression and thus also the level of secreted protein and its functional status (activity). The changes in cytokine levels could thus influence chemokine levels in target tissues and organs, i.e. lung, and form a milieu enhancing inflammation and granuloma formation, or on the other hand excessive fibroproduction in answer to an unknown, probably mostly common, stimuli.

Conflict of interest statement

None of the authors have a conflict of interest to declare in relation to this work.

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References


