PROTEIN LEVELS OF CC CHEMOKINE LIGAND (CCL)15, CCL16 AND MACROPHAGE STIMULATING PROTEIN IN BAL FROM PATIENTS WITH SARCOIDOSIS

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Abstract

The objective of this study was to assess protein levels for candidate cytokines, chemokines, growth factors, matrix metalloproteinases and their inhibitors in bronchoalveolar lavage fluid (BALF) in patients with polar forms of pulmonary sarcoidosis (Löfgren's syndrome (LS) and more advanced, chest X-ray (CXR) stage III).
Twenty-four inflammatory molecules were analyzed in BALF samples from 10 patients with sarcoidosis with CXR stage III and 10 patients with LS by semiquantitative protein array. The differentially expressed mediators were then quantified by ELISA in a second cohort of 68 sarcoidosis patients and 17 control subjects.
Protein levels of CCL15, CXCL8, CXCL10, IL16, MSP and MMP1 were increased in CXR stage III patients when compared to patients with LS ($p<0.05$). CCL15 and MSP upregulation in CXR stage III patients was confirmed by ELISA. Further quantitative analyses showed an increase of MSP, which was associated with a requirement for treatment ($p=0.001$) and also an elevation of CCL15, which accompanied disease progression at two-year follow-up ($p=0.016$). CCL16 levels were increased in the combined patient group compared to controls ($p<0.05$), but no difference was observed in patient subgroups.
In conclusion, our data for the first time implicates chemokines CCL15, CCL16 and MSP in the pathogenesis of sarcoidosis and suggests that CCL15 and MSP may affect disease course.
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Background

Sarcoidosis is an inflammatory disorder characterized by the accumulation of CD4+ helper T-cell type 1 lymphocytes and macrophages with subsequent granuloma formation at the site of disease, notably in the lung.
It is graded from Chest X-ray (CXR) stage I to stage IV with stages III and IV being the most advanced disease manifestation forms, characterized by parenchymal involvement and fibrosis with a low rate of spontaneous resolution (less than 20% for stage III, zero for stage IV).
Löfgren's syndrome (LS), characterized by an acute onset, fever, erythema nodosum, arthralgias and bilateral hilar lymphadenopathy, is a distinct sarcoidosis clinical phenotype, which has a better long-time prognosis than other disease forms
There is strong evidence that a wide spectrum of inflammatory molecules is involved in sarcoidosis development and progression. A number of immune mediators, namely chemotactic cytokines, have been implicated in T cell and macrophage infiltrations at sites of inflammation in sarcoidosis, thus leading to granuloma formation.

HYPOTHESIS

We hereby hypothesize that the character of inflammatory profile may reflect the pathogenic events in different clinical phenotypes of sarcoidosis

AIM

To assess protein levels for candidate cytokines, chemokines, growth factors, matrix metalloproteinases and their inhibitors in bronchoalveolar lavage fluid (BALF) in patients with polar forms of pulmonary sarcoidosis, LS and more advanced, CXR stage III.

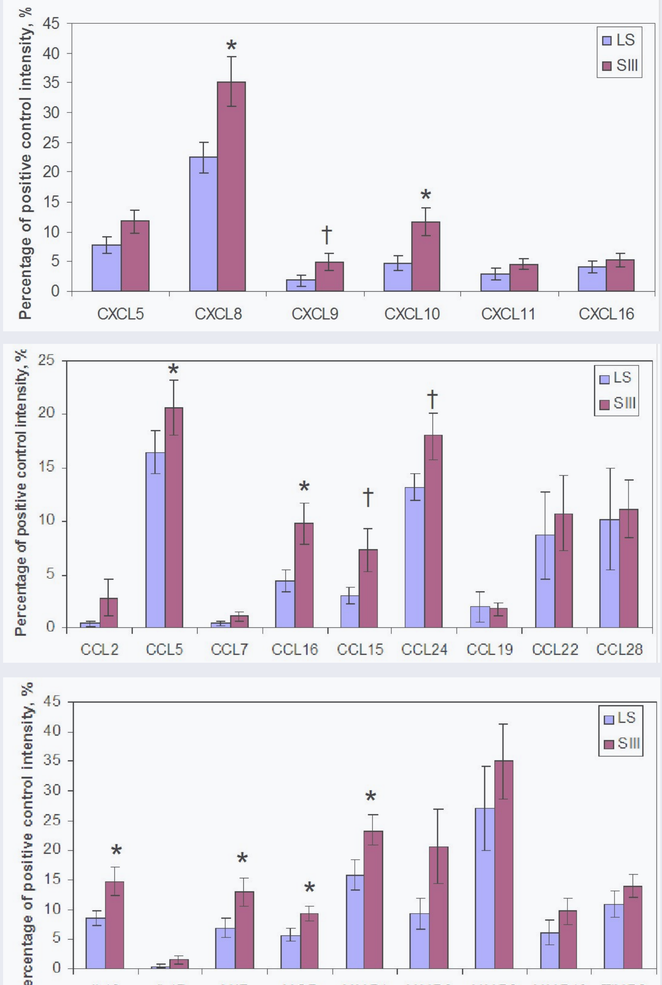
Subjects

- 20 patients (10 patients with CXR stage III (SIII) and 10 patients with Löfgren's syndrome (LS) were included in the screening phase studies.
  The second cohort consisted of sarcoidosis patients (n = 68, LS = 17, CXR stage I (SI) = 13, CXR stage II (SII) = 22, CXR stage III (SIII) = 16) and controls (n = 17).
- Diagnosis of sarcoidosis (Statement on Sarcoidosis, 1999): clinical features + granuloma on biopsy + CD4+ lymphocytic alveolitis.
- Control group: no clinical signs of lung inflammation; no lung disease in medical history; normal BALF cytology, immunology and microbiology.

Methods

- BAL samples (15 ml) were centrifuged and the BALF was separated from cells. Fluid was then aliquoted and stored at -80°C until analysis.
- The protein levels of 24 inflammatory molecules were screened using RayBio Custom cytokine antibody array (RayBiotech Inc, Georgia, USA).
- Concentrations of CCL15, CCL16, MSP and MIF proteins in the BALF were measured by commercially available ELISA kits. The detection limits of the CCL15, CCL16, MSP and MIF ELISA were 10, 8, 8, and 17pg/ml, respectively.
- Group differences in the levels of studied molecules were evaluated with Student's t-test for independent samples and ANOVA. Data are presented as mean ± S.E.M; $p < 0.05$ was considered as significant, and $p < 0.1$ was considered as trend.

Fig 1. Semiquantitative levels of CC , CXC chemokines and other inflammatory molecules in LS vs SIII



Results

Screening phase: Of 24 tested molecules, seven (CCL16, CXCL8, CXCL10, IL16, MIF, MMP and MSP) were increased in patients with SIII compared to LS + three further proteins (CCL15, CCL24 and CXCL9) tended to have increased levels in BALF samples of SIII patients compared to LS (Figure 1).

Verification and extension: CCL15 and MSP levels were increased in BALF from patients with SIII compared with LS patients ($p < 0.001$), CCL16 showed no difference between the groups (Fig. 2A). Furthermore, BALF CCL15, CCL16, and MSP levels were higher in the combined group of sarcoidosis patients compared to control subjects (Fig 2B).

CCL15, MSP and disease progression: MSP levels were increased in the patients requiring corticosteroid treatment (Fig 3B) and higher CCL15 levels were associated with disease progression at 2 years follow-up (Fig 3A).

Discussion

- CXCL8, CXCL10, IL16 and MMP1 protein levels were upregulated in BALF of SIII patients compared to LS. These results are in line with the hypothesis that the intensity of the inflammatory process may result in a different clinical course in sarcoidosis.
- Increased levels of MSP in SIII disease may provide evidence for a mechanism for the propagation of the inflammatory process in patients with a more progressive form of sarcoidosis via stimulation of the NF-kappaB pathway.
- The possible action for CCL15 in the lung is the attraction of neutrophils along with CXCL8. It is known that the number of neutrophils in BAL is elevated in advanced stages of disease, where highest concentration of CCL15 protein were detected.
- CCL16 protein levels were increased in sarcoidosis patients as a whole,thus, may non-specifically amplify inflammation by inducing the expression of proinflammatory cytokines and chemokines (CCL2, IL-1beta, TNF-alpha, and IL-12).

Figure 2. Quantitative levels of CCL15, CCL16 and MSP in BALF of sarcoidosis patients and controls

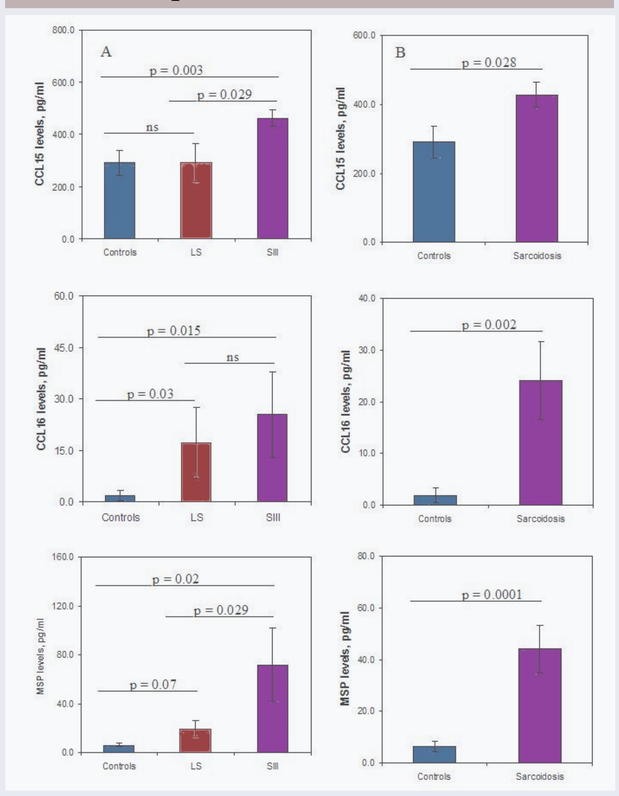
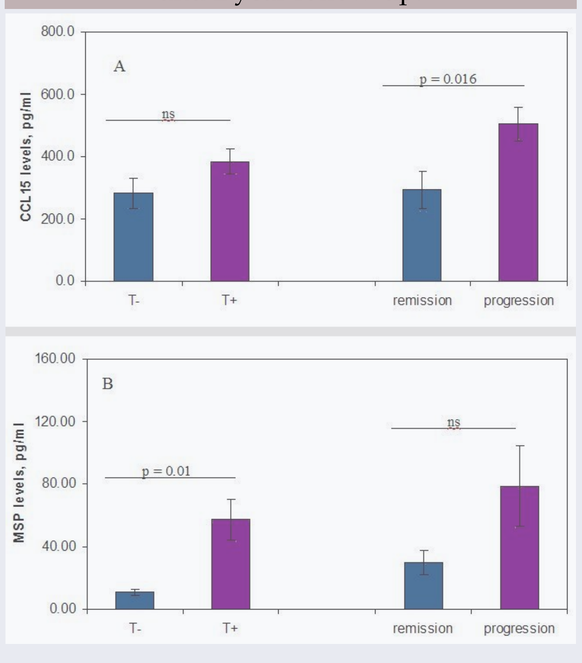


Fig 3. Levels of CCL15 (A) and MSP (B) in patients with different treatment requirement and 2 year follow-up.



The results of the present study for the first time implicate chemokines CCL15, CCL16 and Macrophage stimulating protein in the pathogenesis of sarcoidosis and suggest that CCL15 and MSP may affect disease course. Hence, the exact role of these novel candidate molecules in pathomechanisms of sarcoidosis should be elucidated in future studies.

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