CCL7, CCL8 and CCL13 are augmented during the pathogenesis of pulmonary sarcoidosis

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Pulmonary sarcoidosis

➢ A Th1 cell-mediated inflammatory disease characteristic by CD4+ lymphocyte alveolitis mediated by **chemokines** with subsequent granuloma formation at the site of disease

➢ Cleavage of **chemokines CCL2, CCL7, CCL8 and CCL13** by MMP12 results in shorter chemokine forms antagonising inflammatory response

➢There has been little information about expression of these **CC chemokines and MMP12 in sarcoidosis and clinical disease subtypes**

Aims

➢ To investigate mRNA/protein expression of 4 candidate chemokines CCL2 (MCP-1), CCL7 (MCP-3), CCL8 (MCP-2), CCL13 (MCP-4) and MMP12 in unseparated bronchoalveolar lavage (BAL) cells from sarcoidosis patients and control subjects

➢To analyse chemokine expression profiles in patient subgroups based on specific clinical phenotypes

Methods

➢ **Quantitative RT-PCR** (RotorGene 3000 system, Corbett Research) was used to investigate mRNA expression of studied molecules in unseparated BAL cells, PSMB2 was used as a reference gene *(Kriegova et al. BMC Mol Biol. 2008)*

➢ **Used primers/probes:** Assays-on-*Demand*™ Gene Expression (Applied Biosystems), LNA primers/probes (Roche, Universal Probe Library)

➢ Relative expression was calculated using **second derivative method** (RotorGene Software 6.1.71, Corbett Research)

➢ **Immunohistochemistry** with anti-chemokine antibodies was used to localize corresponding proteins on lung biopsies using Benchmark XT automatic tissue staining apparatus (Ventana Medical Systems Inc., USA) **(Fig. 4)**

Patient characteristics

➢ **Sarcoidosis patients** (S, n=82) (diagnosis according to the Statement on Sarcoidosis, 1999), clinical features + granuloma on biopsy + CD4+ lymphocytic alveolitis)

➢ **Control subjects** (C, n=25) (patients without inflammation, normal BAL profile)

Subgroups based on disease phenotypes:

➢ as assessed by chest X-ray (CXR) stage: CXR stage I (S-I, n= 20), CXR stage II (S-II, n=48), CXR stage III (S-III, n=14)

Results

➢ Of studied chemokines, sarcoid BAL cells expressed higher mRNA levels of CCL7 (p=0.02), CCL8 (p=0.0003) and CCL13 (p=0.003) when compared to control subjects. CCL2 mRNA expression did not differ between sarcoidosis patients and controls (p>0.05). **(Fig. 1)**

➢ MMP12 mRNA was up-regulated in sarcoidosis *vs.* controls (p=0.000002); no difference in its expression was observed between studied phenotypes. **(Fig. 2)**

➢ Subanalysis of expression profiles in clinical phenotypes as assessed by chest X-ray (CXR) showed higher number of CCL2, CCL7, CCL8 and CCL13 transcripts in patients with lung parenchymal involvement in comparison to those with only hilar lymphadenopathy (CXR-stage I *vs.* stages II-III: p<0.05). **(Fig. 3)**

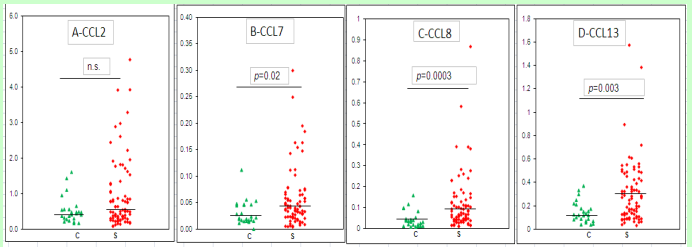

Fig. 1: mRNA expression of CCL2, CCL7, CCL8 and CCL13 in BAL cells from sarcoidosis patients (S) and control subjects (C).

Y axis represents the relative mRNA expression of target gene/PSMB2; group medians are indicated by *horizontal bars*; n.s. not significant.

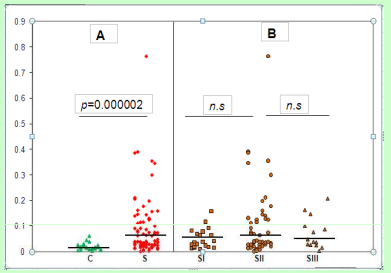

Fig. 2: mRNA expression of MMP12 in BAL cells

A) from sarcoidosis (S) and control subjects (C)

B) in patient groups according CXR stages.

Y axis represents the relative mRNA expression of MMP12/PSMB2; group medians are indicated by *horizontal bars*; n.s. not significant.

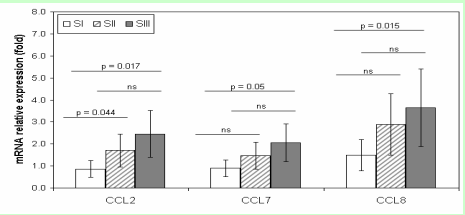

Fig. 3: Chemokine expression profiles in patient subgroups according to the CXR stages. The data are presented as a mean fold change of relative expression compared to CXR stage I (normalized to 1); the whiskers on each box represent the SD values.

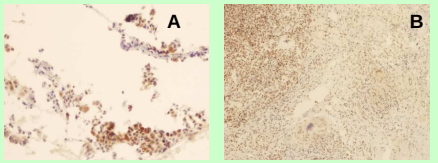

Fig. 4: Immunohistochemistry for CCL8 protein expression in lung sections of sarcoidosis patients - representative example.

CCL8 was expressed by epithelioid macrophages (A), lymphocytes and multinucleated giant cells (B). Original magnification x200

Conclusions

➢ Pulmonary sarcoidosis is associated with an up-regulation of CCL7 (MCP-3), CCL8 (MCP-2), CCL13 (MCP-4) and MMP12 expression, mainly in patients with lung parenchymal involvement.

➢ The hypothesis that upregulated MMP12 cleaves the chemokines present in the sarcoid lung into molecules with antiinflammatory action needs to be further investigated.

Support: IGA MZ CR NS/10267, MSM 6198959205, IGA PU project SV LF_2010_008