Brief report

Decreased interleukin-18 expression in BAL cells and peripheral blood mononuclear cells in adult cystic fibrosis patients

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Abstract

Lymphocytes from cystic fibrosis (CF) patients secrete less interferon-γ (IFN-γ) upon stimulation compared to controls. Expression of interleukin (IL)-18 as an IFN-γ inducing factor and of IL-10 as an IL-18 inhibiting factor were determined in bronchoalveolar lavage (BAL) cells from CF patients (n = 5) and from normal control subjects (n = 9) as well as in peripheral blood mononuclear cells (PBMC) from patients (n = 12) and from control subjects (n = 9) with RT-PCR. IL-18 and IL-10 serum protein levels were measured using ELISA.

BAL cells and PBMC of CF patients expressed significantly less IL-18 compared to controls (p < 0.05). There was no significant difference for IL-10 in BAL cells. However, PBMC from patients expressed significantly more IL-10 mRNA (p < 0.05). IL-18 serum protein levels were decreased in the patient group, whereas IL-10 serum concentrations were elevated. Stimulation with rhIL-10 reduced IL-18 expression in PBMC from CF patients.

Decreased IL-18 expression in CF patients may contribute to decreased IFN-γ production. IL-10 may contribute to inhibit IL-18 expression in PBMC in CF.

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1. Introduction

A primary dysregulation of pulmonary inflammation with a relative defect in Th1 immune response has been proposed as a mechanism leading to airways disease in cystic fibrosis (CF) [1]. Peripheral blood lymphocytes from CF patients produce significant lower levels of the Th1 cytokine interferon-γ (IFN-γ) upon stimulation compared to control lymphocytes [2]. Since interleukin-18 (IL-18) induces IFN-γ production [3] we sought to investigate the expression of IL-18 and IL-10 (as IL-18 inhibiting factor) in the lungs and peripheral blood of adult CF patients.

2. Material and methods

2.1. Patients and control subjects

Twelve adult CF patients were investigated (5 male, 7 female, mean age 28.3 years ranging from 23 to 39). *Pseudomonas aeruginosa* was found in sputum cultures of all CF patients. Nine healthy non-smoking volunteers served as control group (4 male, 2 female, mean age 27.4 years ranging from 23 to 31).

2.2. Bronchoalveolar lavage and blood samples

Bronchoalveolar lavage (BAL) was performed in five patients and in all control subjects. Peripheral blood samples of all patients and control subjects were obtained for serum and peripheral blood mononuclear cells (PBMC).

2.3. RT-PCR and semiquantitative PCR analysis

RNA preparation, reverse transcription and PCR were performed as previously described [4]. The oligonucleotide

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primers for human \(\gamma\)-actin, IL-18 and IL-10 have been published before [5,6]. PCR products were analyzed, densitometric analysis and semiquantitative RNA quantification was performed as previously described [4].

2.4. Interleukin-18 and interleukin-10 enzyme-linked immuno-sorbent assay

An IL-18 enzyme-linked immuno-sorbent assay (ELISA) (R&D Systems, Minneapolis, USA) was used to determine the concentration of IL-18 in the sera of both groups and PBM cell culture supernatants of patients. An IL-10 ELISA (R&D Systems) was used to determine the concentration of IL-10 in sera of both groups. Minimum detectable doses were less than 15 pg/ml (IL-18) and less than 3.9 pg/ml (IL-10) as indicated by the manufacturer.

2.5. Stimulation of PBMC with recombinant human IL-10

PBMC from four patients were cultured in RPMI medium without and in the presence of 0.4 \(\mu\)g/ml recombinant human (rh) IL-10 as indicated by the manufacturer (Strathmann Biotech, Hannover, Germany). IL-18 mRNA expression and IL-18 protein levels in cell culture supernatants were determined after 18 h.

2.6. Statistics

IL-18 and IL-10 expression as well as IL-18 and IL-10 serum protein levels between patients and control subjects were compared using the Mann–Whitney \(U\)-test. IL-18 expression without and in the presence of rhIL-10 was compared with the paired \(t\)-test. A \(P\) value of less than 0.05 was considered significant. All values are given as mean \(\pm\) S.E.M. if not otherwise stated.

3. Results

3.1. Expression of IL-18 and IL-10 mRNA in BAL cells and PBMC

BAL cells of investigated patients \((n = 5)\) expressed significantly less IL-18 mRNA compared to controls \((1.44 \pm 0.80 \text{ vs. } 3.68 \pm 0.53; \, p<0.05)\). IL-18 mRNA expression was significantly lower in PBMC of patients compared to PBMC of control subjects \((0.41 \pm 0.09 \text{ vs. } 1.59 \pm 0.55; \, p<0.05)\) (Fig. 1A).

IL-10 mRNA expression in BAL cells was very low in both groups \((0.02 \pm 0.02 \text{ vs. } 0.01 \pm 0.01, \, p>0.05)\). PBMC of patients expressed significantly more IL-10 mRNA in comparison with control subjects \((0.73 \pm 0.24 \text{ vs. } < 0.01; \, p<0.05)\) (Fig. 1B).

3.2. IL-18 and IL-10 serum protein levels

Eight patients and eight control subjects had detectable IL-18 serum protein levels. The median level of patients was lower than that of control individuals \((42.91 \text{ pg/ml vs. } 74.78 \text{ pg/ml}; \, p>0.05)\). While IL-10 serum protein levels were below minimum detectable dose in all control
subjects, seven patients showed detectable IL-10 serum protein concentrations with a median value of 8.38 pg/ml ($p < 0.05$).

3.3. Effect of stimulation with rhIL-10 on IL-18

Stimulation with rhIL-10 significantly reduced IL-18 mRNA expression in PBMC of patients compared to without stimulation (0.46 ± 0.11 vs. 0.89 ± 0.19; $p < 0.05$) (Fig. 2). IL-18 protein levels were detectable in only one patient but stimulation with rhIL-10 decreased protein concentrations (12.87 pg/ml) in comparison to without (16.47 pg/ml).

4. Discussion

Reduced IL-18 protein levels have been shown in BAL fluid of CF children [7] but expression in BAL cells and PBMC of adult patients have not been investigated yet. In the present study, we found decreased IL-18 expression in both BAL cells and PBMC of CF patients compared to control subjects. In that previous study an IL-18 degrading factor has been postulated to be present in CF BAL fluid [7]. Since P. aeruginosa was found in sputum cultures of the patients it is tempting to speculate that P. aeruginosa exotoxin A may decrease IL-18 expression in BAL as shown in lungs of mice [8].

IL-10 is known to inhibit IL-18 mRNA expression [9]. In BAL cells of patients and controls IL-10 was very low. This agrees with previous data [10]. However, IL-10 expression in PBMC (as shown before, ref. 2) and IL-10 serum concentrations in patients were increased. Moreover, stimulation of patient PBMC with rhIL-10 significantly decreased IL-18 expression. Therefore IL-10 may inhibit IL-10 in CF PBMC thus decreasing IFN-γ production.

5. Conclusions

Increased IL-10 expression in PBMC may inhibit IL-18 expression in PBMC of CF patients. Other factors (e.g., P. aeruginosa exotoxin A) than IL-10 may decrease IL-18 in CF BAL cells. Decreased IL-18 expression in adult CF patients may reduce IFN-γ production which may lead to a relative defect in Th1 immune response.

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References