

Pulmonary T_H2 response in *Pseudomonas aeruginosa*-infected patients with cystic fibrosis

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Background: *Pseudomonas aeruginosa* infection determines the course of cystic fibrosis (CF) lung disease. Studies in human peripheral blood indicate that *P aeruginosa* infection is associated with a predominant T_H2 immune response, whereas T_H1 responses are accompanied by a better pulmonary outcome.

Objective: Analyses of peripheral blood may not correspond directly with the local pulmonary immune response. Therefore, we asked whether the T_H1/T_H2 response is altered in bronchoalveolar lavage fluid from *P aeruginosa*-infected patients with CF.

Methods: Bronchoalveolar lavage fluid was obtained from 12 patients with CF chronically infected with *P aeruginosa*, 11 noninfected patients with CF, and 8 healthy controls. Pulmonary CXCR3⁺ (T_H1) and CCR4⁺ (T_H2) expressing CD4⁺ and CD8⁺ lymphocytes were quantified by flow cytometry. Levels of T_H1-associated (IL-2, IFN- γ , IFN- γ inducible T cell- α chemoattractant, Monokine induced by IFN- γ , IFN- γ inducible protein of 10 kd) and T_H2-associated (IL-4, IL-5, IL-10, thymus and activation-regulated chemokine [TARC], macrophage-derived chemokine) cytokines and chemokines and a panel of proinflammatory molecules were quantified at the protein level. Chemokines mRNA levels were assessed by real time RT-PCR.

Results: *P aeruginosa*-infected patients with CF had significantly higher levels of pulmonary CCR4⁺CD4⁺ (T_H2) cells, IL-4, IL-13, and TARC and lower levels of IFN- γ compared with noninfected patients with CF and healthy controls. Bronchoalveolar lavage fluid levels of IL-4, IL-13, and TARC correlated inversely with FEV₁ in *P aeruginosa*-infected patients with CF.

Conclusion: These results reveal the prevalence of a pulmonary T_H2 immune response in *P aeruginosa*-infected patients with CF. The modulation of the pulmonary T_H2 response in *P aeruginosa* infection may be an option for the treatment of *P aeruginosa* lung disease in patients with CF. (J Allergy Clin Immunol 2006;117:204-11.)

Key words: Cystic fibrosis, *Pseudomonas aeruginosa*, bronchoalveolar lavage fluid, TARC, MDC, CCR4, T_H1/T_H2, cytokines

Progressive lung disease is the leading cause of morbidity and mortality in patients with cystic fibrosis (CF).¹ The majority of chronic pulmonary infections in patients with CF is caused by *P aeruginosa*,^{2,3} and approximately 80% of patients with CF become chronically infected.⁴ *P aeruginosa* infection in CF is associated with deterioration of pulmonary function and a higher mortality,^{5,6} especially when the bacterium converts to its mucoid form.⁷ Despite a cellular and humoral^{8,9} immune response against *P aeruginosa* with pulmonary accumulation of granulocytes,¹⁰ secretion of antimicrobial peptides,¹¹ lysozyme,¹² and lactoferrin,^{13,14} patients with CF are incapable of clearing chronic *P aeruginosa* lung infection even with intensive antibiotic treatment. In addition to host factors that limit the efficacy of immune reactions,¹⁵ *P aeruginosa* has developed several mechanisms to escape innate and adaptive immune responses. These include the formation of impenetrable biofilms,¹⁶ an intrinsic ability to develop resistance to antibiotics,¹⁷ and the release of a large number of virulence factors.¹⁸⁻²¹

Mouse models of chronic *P aeruginosa* infection showed that T_H1-reacting mice had a better disease outcome with an enhanced bacterial clearance, milder lung inflammation, and better survival compared with T_H2-reacting mice.^{22,23} Studies with PBMCs of patients with CF indicated that *P aeruginosa*-infected patients with CF responded to *P aeruginosa* outer membrane protein with a higher IL-4 secretion and lower IFN- γ secretion compared with noninfected patients. Because IFN- γ production correlated positively with lung function parameters, the hypothesis was put forward that shifting the immune response toward a T_H1 type may improve the pulmonary outcome in *P aeruginosa*-infected patients.²⁴ Similarly, a recent study showed that children chronically infected with *P aeruginosa* had greater IL-4 production in peripheral whole blood cultures than noninfected

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TABLE I. Patient characteristics

	Cystic fibrosis non- <i>Pseudomonas</i>	Cystic fibrosis <i>Pseudomonas</i>	Controls
N	11	12	8
Age, y	19.4 (17-37)	20 (16-37)	17.5 (12-19)
Sex, M:F	5/6	6/6	3/5
CFTR mutation	6 × Δ F508 4 × Δ F508/unknown 1 × R553/unknown	8 × Δ F508 3 × Δ F508/unknown 1 × R347P/Q493X	ND
Microorganisms in sputum	3 SA, 3 HI, 3 AF	4 SA, 2 HI, 4 AF, 3 O	0/8
Forced vital capacity (% predicted)	76 (69-121)	70 (52-106)	ND
FEV ₁ (% predicted)	67 (43-82)	62 (43-105)	ND
MEF ₂₅₋₇₅ (% predicted)	36 (27-64)	30 (14-80)	ND
TLC (% predicted)	85 (66-96)	99 (90-124)	ND
Inhaled glucocorticosteroids	5/11	6/12	0/8
Systemic glucocorticosteroids	0/11	0/12	0/8
Inhaled bronchodilators	8/11	10/12	0/8
Inhaled antibiotics	3/11*	9/12	0/8
BALF cells			
Viability (%)	75 (29-86)	79 (28-94)	82 (69-92)
Fluid recovery (%)	43 (19-62)	40 (14-66)	58 (45-72)
Total cells × 10 ³ /mL	2900 (60-3800)*	3430 (100-9740)	165 (94-233)
Neutrophils (%)	68 (53-79)	61 (10-96)	1 (0-3)
Eosinophils (%)	1 (0-3)	2 (0-4)	0 (0-1)
Macrophages (%)	29 (10-36)	31 (4-63)	87 (76-95)
Lymphocytes (%)	5 (4-10)	4 (1-22)	3 (1-8)

Results are expressed as medians with ranges shown in parentheses.

SA, *Staphylococcus aureus*; HI, *Hemophilus influenzae*; AF, *Aspergillus fumigatus*; O, others; CFTR, cystic fibrosis transmembrane conductance; MEF₂₅₋₇₅, midexpiratory flow between 25% and 75% of the forced vital capacity; TLC, total lung capacity.

**P* < .05: *Pseudomonas*-infected compared with noninfected patients with CF.

Abbreviations used

- BAL: Bronchoalveolar lavage
- BALF: Bronchoalveolar lavage fluid
- CF: Cystic fibrosis
- G-CSF: Granulocyte colony-stimulating factor
- IP-10: IFN- γ inducible protein of 10 kd
- ITAC: IFN- γ inducible T cell- α chemoattractant
- MCP: Macrophage chemotactic protein
- MDC: Macrophage-derived chemokine
- Mig: Monokine induced by IFN- γ
- MIP: Macrophage inflammatory protein
- OdDHL: N-(3-oxododecanoyl)-homoserine lactone
- TARC: Thymus and activation-regulated chemokine
- Tc: T cytotoxic cell type

patients with CF.²⁵ In another approach, the effect of single *P aeruginosa* signaling molecules on T_H1/T_H2 immune responses was studied. N-(3-oxododecanoyl)-L-homoserine lactone (OdDHL) is used by *P aeruginosa* to coordinate population behavior and the expression of numerous virulence factors in a process termed *quorum-sensing*.²⁶ However, these studies yielded conflicting results. Thus, OdDHL suppressed IL-12 production in LPS-treated murine macrophages and led to the formation of IgE antibodies in IL-4-stimulated PBMCs,²⁷ whereas

IFN- γ production was increased in OdDHL-treated murine splenic T cells.^{28,29}

In view of these conflicting data, we asked whether T_H1/T_H2-related immune responses toward pulmonary *P aeruginosa* infection could be assessed more precisely. Because analyses in peripheral blood may not correspond to the pulmonary immune response in CF, bronchoalveolar lavage fluid (BALF) was studied.

Chemokines attract T cells through their corresponding receptors.^{30,31} Thus, T_H2 cells express the chemokine receptor CCR4 and are attracted by the CCR4 ligands, thymus and activation-regulated chemokine (TARC), and macrophage-derived chemokine (MDC). In contrast, T_H1 cells express CXCR3 and are attracted by IFN- γ inducible T cell- α chemoattractant (ITAC), monokine induced by IFN- γ (Mig), and IFN- γ inducible protein of 10 kd (IP-10).^{32,33}

To assess the T_H1/T_H2 immune response in lungs of *P aeruginosa*-infected and noninfected patients with CF, the frequencies of CCR4⁺ (T_H2) and CXCR3⁺ (T_H1) T cells and levels of T_H1-related (IL-2, IFN- γ , IP-10, ITAC, Mig) and T_H2-related (IL-4, IL-5, IL-10, TARC, MDC) molecules were quantified in BALF. In addition, levels of proinflammatory cytokines and chemokines (TNF- α , granulocyte colony-stimulating factor [G-CSF], IL-1 β , IL-6, IL-8, macrophage inflammatory protein [MIP]-1 β , macrophage chemotactic protein [MCP]-1) were analyzed.

TABLE II. Primer sequences

mRNA target	Oligonucleotides (forward/reverse)
GAPDH	F: GAAGTGTAAGGTCTGGAGTC R: GAAGATGGTGATGGGATTTC
IL-8	F: TCTTGGCAGCCTTCCTGATT R: TCCAGACAGAGCTCTCTCCATC
TNF- α	F: AGGAACAGCACAGGCCTTAGTG R: AAGACCCCTCCCAGATAGATGG
IL-4	F: AGAAGACTCTGTGCACCGAGTTGA R: CTCTCATGATCGTCTTTAGCCTTT
IL-5	F: CTTGGCACTGCTTTCTACTCATC R: TGCAGGTAGTCTAGGAATTGGTT
IFN- γ	F: GCATCCAAAAGAGTGTGGAG R: ATGCTCTTCGACCTCGAAAC
IL-6	F: CTTTGGAGTTTGAGGTATACCTAG R: CGCAGAATGAGATGAGTTGTC
IL-1 β	F: CAGGGACAGGATATGGAGCAA R: ATGTACCAGTTGGGAACTG
IL-2	F: ACAGCTACAACCTGGAGCATT R: TGCTGTCTCATCAGCATATT
MCP-1	F: TGAAGCTCGCACTCTCGCCT R: GTGGAGTGAGTGTTCAAGTCs
MIP-1 β	F: CCTCACCTCTGAGAAAACC R: GCAACAGCAGAGAAACAGTGAC
G-CSF	F: TAGAGCAAGTGAGGAAGATCCAGG R: AGTTCCTCCATCTGCTGCCAGATG
IL-10	F: AGCTGAGAACCAAGACCCAGA R: GGGCTGGGTGAGTATCC
TARC	F: TTCTCTGAGCAGCATCC R: AAgACCTCTCAAGGCTTTG
MDC	F: AGGACAGAGCATGGCTCGCTACAGA R: TAATGGCAGGGAGGTAGGGCTCCTGA
ITAC	F: GCTATAGCCTTGCTGTGATATTGTG R: CTGCCACTTTCAGTCTTTTACC
IP-10	F: TCTAAGTGGCATTCAAGGAGTACC R: CAGTAAATTGATGGCCTTCGA
Mig	F: TGCAAGGAACCCAGTAGTGA R: GGTGGATAGTCCCTTGTTGG

METHODS

Patients and healthy controls

Twenty-three clinically stable patients with CF (mean FEV₁ 70% of predicted), including 17 patients with CF from a previous study,³⁴ were selected for this study. All patients were free from acute pulmonary exacerbation. The diagnosis of CF was confirmed by clinical features consistent with CF and 2 independent positive sweat chloride tests (≥ 60 mEq/L). Chronic *P aeruginosa* infection was diagnosed if the organism was isolated in at least 2 consecutive sputum samples with a minimum of a 6-month interval. All *P aeruginosa* sputum-positive patients also had *P aeruginosa* in BALF culture ($\geq 10^4$ colony-forming units/mL BALF^{21,35}).

Twelve patients with CF were infected with *P aeruginosa* (CF+P), whereas 11 patients had no detectable *P aeruginosa* infection (CF-P). All CF+P patients had mucoid forms of *P aeruginosa* and ≥ 2 elevated precipitating antibody titers against purified antigens from *P aeruginosa* (alkaline protease, exotoxin A, and elastase) as detected in serum by ELISA. None of the patients had received systemic antibiotic therapy within 2 weeks before the bronchoalveolar lavage (BAL). The patient characteristics and BALF cellular subsets are shown in Table I.

Eight healthy subjects were selected as control group. These patients were considered as healthy; that is, they had no systemic disease, no suspected or proven pulmonary disease, no atopic disease, were free of respiratory tract infections, and were overlapping in age with most of the patients with CF. These patients underwent elective tonsillectomy under general anesthesia. BAL was performed before the surgical procedure.

The parents and/or patients gave their informed consent before BAL, and the institutional review board approved the study protocol.

BAL

Bronchoscopy and BAL (4×1 mL/kg body weight 0.9% NaCl) were performed as described previously.³⁶ The obtained BALF was filtered through 2 layers of sterile gauze. The first aliquot of BALF was used for microbiology, whereas the remaining 3 fractions were pooled. The sample preparation and processing were performed immediately on ice. After centrifugation at 200g for 10 minutes, the supernatant was stored at -80°C for biochemical analysis. The cell pellet was resuspended in 5 mL PBS and used for preparation of cytospin slides, flow cytometry, and mRNA extraction.

Flow cytometry

Bronchoalveolar lavage fluid cells were analyzed by 4-color flow cytometry (FACSCalibur, Becton-Dickinson, Heidelberg, Germany) as described previously.³⁷ The following antibodies were used: CD4-allophycocyanine mouse IgG1, CD8-phycoerythrin 5 mouse IgG1 (Immunotech, Marseille, France), CCR4-phycoerythrin mouse IgG2a (BD Pharmingen, Heidelberg, Germany), and CXCR3-fluorescein isothiocyanate mouse IgG1 (R&D Systems, Wiesbaden, Germany). Mouse IgG1-fluorescein isothiocyanate and mouse IgG2a-phycoerythrin (Immunotech, Marseille, France) were used as isotype controls.

Detection of cytokines and chemokines

Levels of TNF- α , G-CSF, IFN- γ , IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, MIP-1 β , and MCP-1 were quantified in BALF by a multiplex assay (Bio-Rad Laboratories, Minneapolis, Minn) as described by the manufacturer. The detection limits for all cytokines were 1.5 to 2.5 pg/mL (minimum) and 1000 pg/mL (maximum). Human TARC (7-2000 pg/mL), MDC (15.6-1000 pg/mL), IP-10 (1.7-1000 pg/mL), ITAC (13.9-3000 pg/mL), Mig (3.8-1000 pg/mL), and IL-8 (2-2000 pg/mL) were determined in duplicates by a sandwich ELISA (R&D Systems, Minneapolis, Minn) according to the manufacturer's instructions.

Quantitative RT-PCR

Bronchoalveolar lavage fluid cells were lysed in Trizol LS Reagent (Invitrogen, Life Technologies, Karlsruhe, Germany). Total RNA (200-600 ng) was isolated according to the manufacturer's instructions and reverse-transcribed into cDNA. Expression levels of cytokines and chemokines (Table II) were determined in duplicates by real-time RT-PCR using SYBR green and the iCycler iQ detection system (Biorad, Hercules, Calif). Cycle threshold (Ct) values for genes of interest were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and used to calculate the relative quantity of mRNA expression.

Statistical analyses

Nonparametric tests (Kruskal-Wallis, Mann-Whitney *U* test) were applied to determine differences between the patient groups. Correlations with clinical parameters were verified by using Spearman ρ test. A probability of $P < .05$ was regarded as significant (SPSS statistical program, version 11.5; SPSS Inc, Chicago, Ill).³⁸

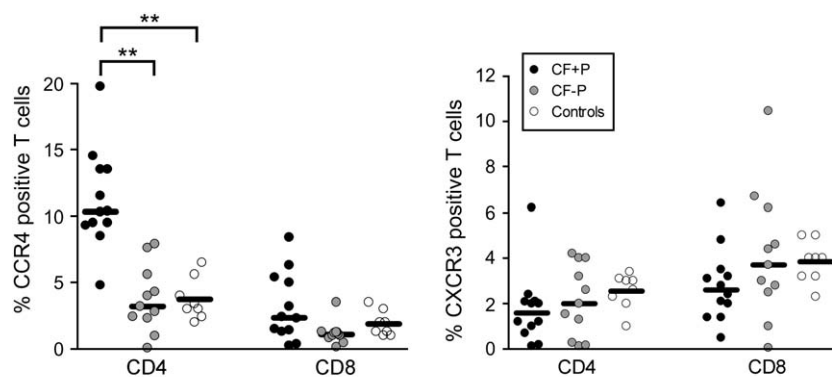


FIG 1. CCR4⁺ and CXCR3⁺ T cells in BALF. CCR4⁺ (T_H2) and CXCR3⁺ (T_H1) CD4⁺ and CD8⁺ T cells were determined in BALF by 4-color flow cytometry. Percentages of double-positive cells within total BALF lymphocytes are shown. ***P* < .01; horizontal bars represent median values.

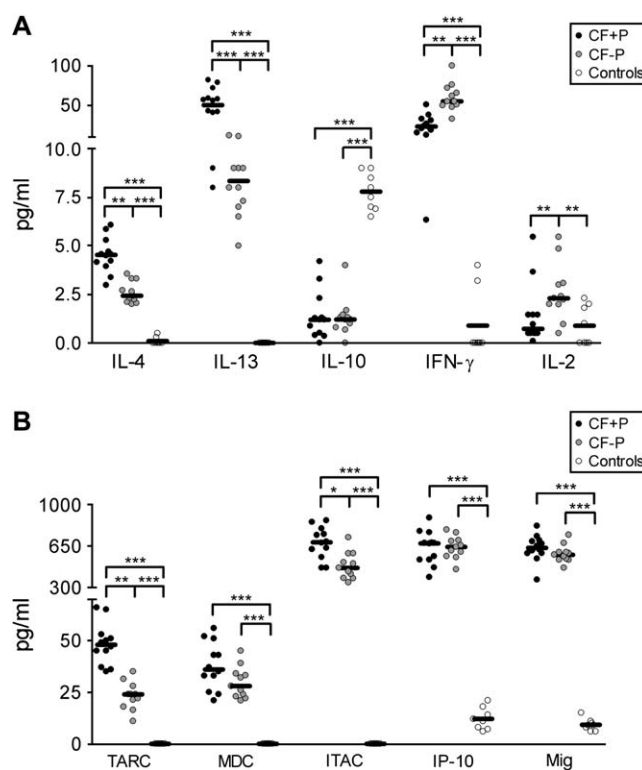


FIG 2. Protein levels of T_H1/T_H2 cytokines and chemokines in BALF. Protein levels of cytokines (A) and chemokines (B) in BALF. BALF cytokines were determined by a multiplex assay. TARC, MDC (CCR4 ligands) and ITAC, IP-10, and Mig (CXCR3 ligands) were quantified by sandwich ELISA. All cytokine and chemokine levels are shown as pg/mL BALF. **P* < .05, ***P* < .01, ****P* < .001; horizontal bars represent median values.

RESULTS

Pulmonary CCR4⁺ (T_H2) and CXCR3⁺ (T_H1) cells

Percentages of CCR4⁺CD4⁺ T (T_H2) cells were significantly higher in BALF of *P. aeruginosa*-infected patients with CF compared with noninfected patients and healthy controls (Fig 1). In contrast, CCR4⁺CD8⁺

(Tc2), CXCR3⁺CD4⁺ (T_H1) and CXCR3⁺CD8⁺ T cells (T cytotoxic cell type [Tc] 1) were present at low levels and did not differ among the groups. Patients with CF infected with *P. aeruginosa* had slightly more total cells compared with noninfected patients with CF. Percentages of neutrophils, macrophages, total lymphocytes, mast cells, and plasma cells did not differ significantly between CF+P and CF-P patients (Table I).

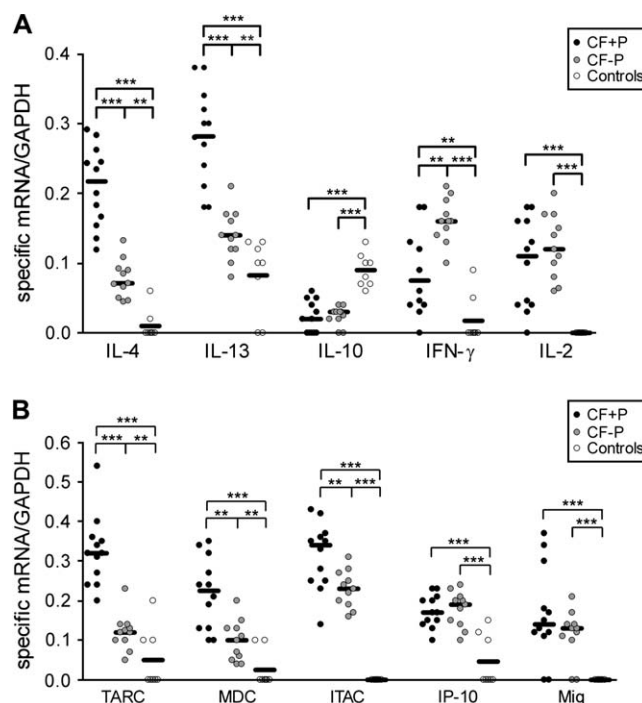


FIG 3. mRNA levels of T_H1/T_H2 cytokines and chemokines in BALF. mRNA levels of cytokines (**A**) and chemokines (**B**) in BALF cells. Total RNA was extracted from unstimulated whole BALF cells (Trizol). mRNA levels of T_H1/T_H2 cytokines (**A**) and T_H1/T_H2 associated chemokines (**B**) were quantified by mRNA specific primers and real-time RT-PCR. Threshold cycle values for genes of interest were normalized to GAPDH and used to calculate the relative quantity of mRNA expression. ** $P < .01$, *** $P < .001$; horizontal bars represent median values.

Pulmonary T_H1/T_H2 -related cytokines and chemokines

We next asked whether increased percentages of $CCR4^+CD4^+$ T_H2 cells were associated with increased levels of the T_H2 cytokines IL-4 and IL-13. CF+P patients had significantly higher pulmonary levels of IL-4 and IL-13 as compared with CF-P patients, who in turn had higher levels than healthy controls (Fig 2, A). Although IFN- γ was significantly higher in all patients with CF compared with healthy controls, this increase was higher in CF-P patients, leading to a significant difference between CF+P and CF-P patients. IL-10 was significantly decreased in all patients with CF compared with healthy controls without a difference between CF+P and CF-P patients. Levels of IL-2 were significantly higher in CF-P patients compared with CF+P patients and healthy controls. $CCR4^+CD4^+$ T cells are attracted by TARC and MDC. *P aeruginosa*-infected patients with CF had significantly higher levels of TARC compared with noninfected individuals and healthy controls (Fig 2, B). The second $CCR4$ ligand, MDC, tended to be higher in infected patients, but this was not statistically significant. These findings were confirmed at the mRNA level, where MDC was significantly higher in *P aeruginosa*-infected patients (Fig 3, A and B).

$CCR4^+CD4^+$ lymphocytes correlated positively with levels of TARC ($r = 0.73$; $P < .01$), IL-13 ($r = 0.85$; $P < .01$), and IL-4 ($r = 0.65$; $P < .01$), but not with MDC. Levels of IL-4 and IL-13 were highly correlated with levels of TARC ($r = 0.67$, $P < .01$; $r = 0.86$, $P < .01$) and MDC ($r = 0.42$, $P < .05$; $r = 0.68$, $P < .01$). IL-5 was undetectable in the majority of patients or present at very low levels at both protein and mRNA levels (data not shown).

We then asked how increased cytokine and chemokine levels from *P aeruginosa*-infected patients related to pulmonary disease severity. Pulmonary protein levels of IL-4 (Fig 4, A), IL-13 (Fig 4, B), and TARC (Fig 4, C) correlated inversely with lung function (FEV_1) in CF+P but not in CF-P patients.

Among T_H1 -related chemokines, only ITAC was higher in infected patients with CF compared with noninfected patients and healthy controls, whereas Mig and IP-10 levels did not differ between CF+P and CF-P patients (Fig 2, B). $CXCR3^+CD8^+$ T cells correlated positively with levels of the $CXCR3$ ligands ITAC ($r = 0.62$; $P < .05$), Mig ($r = 0.71$; $P < .01$), and IP-10 ($r = 0.74$; $P < .05$) in all patients. *P aeruginosa*-infected patients with CF had significantly higher BALF levels of the proinflammatory cytokines IL-1 β , IL-6, and TNF- α compared with noninfected patients with CF and healthy controls (Fig 5).

In all patients with CF (CF+P and CF-P), protein levels of IL-1 β correlated inversely with forced vital capacity ($r = -0.68$; $P < .01$).

DISCUSSION

This study shows that BALF of *P aeruginosa*-infected patients with CF is characterized by increased percentages of T_H2 (CCR4⁺CD4⁺) cells. This is accompanied by decreased levels of IFN- γ and increased levels of IL-4, IL-13, and TARC, which correlate positively with CCR4⁺CD4⁺ cells. Furthermore, the levels of IL-4, IL-13, and TARC are correlated with disease severity (FEV₁) in *P aeruginosa*-infected patients with CF.

Previous studies investigated the T_H1/T_H2 immune response in *P aeruginosa* infection exclusively in mouse models or in peripheral blood of patients with CF. Thus, Moser et al analyzed the role of T_H1/T_H2 responses in lungs of *P aeruginosa*-infected mouse models^{22,23} and in peripheral blood of patients with CF with *P aeruginosa* lung infection.²⁴ *P aeruginosa* infection in patients with CF was characterized by a T_H2 response (IL-4 production) in peripheral blood,^{24,25} whereas a higher IFN- γ production was associated with a better pulmonary outcome, suggesting a protective role for T_H1-associated immune responses in *P aeruginosa* lung infection. However, analyses in peripheral blood may not mirror the pulmonary immune response. To reflect the pulmonary situation of patients with CF *in vivo* as closely as possible, lymphocytes and soluble mediators were analyzed in BALF without any additional artificial stimulation. Because protein levels of some mediators are very low in BALF (eg, IL-4), cytokines and chemokines were studied both at the protein and mRNA levels to detect changes even at low transcriptional levels.

Thymus and activation-regulated chemokine and MDC specifically attract CCR4⁺CD4⁺ cells to the pulmonary tissue, where they might act as T_H2 lymphocytes. The association of increased levels of IL-4 and IL-13 with increased percentages of CCR4⁺CD4⁺ T cells in BALF of *P aeruginosa*-infected patients with CF is in agreement with the hypothesis that CCR4⁺CD4⁺ cells correspond to T_H2 cells in the pulmonary immune response. However, functional analyses are necessary to investigate whether CCR4⁺CD4⁺ T cells in BALF of *P aeruginosa*-infected patients secrete these cytokines and therefore indeed act as T_H2 cells.

Levels of IL-4, IL-13, and TARC correlated inversely with lung function in *P aeruginosa*-infected patients, further supporting the contribution of these molecules to the pulmonary pathology in *P aeruginosa*-infected patients with CF. Although these findings strengthen the concept that *P aeruginosa* infection is characterized by a pulmonary type 2 response, a strong T_H2 immune response seems unlikely because CCR4⁺CD4⁺ T cells and levels of TARC and MDC in BALF were clearly lower than those found in BALF of children with asthma analyzed by the identical methods.³⁷

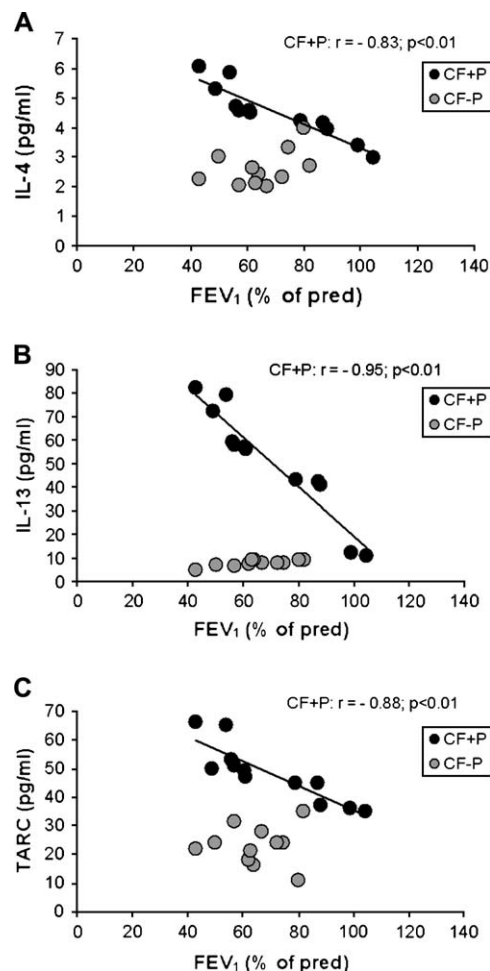


FIG 4. T_H1/T_H2 cytokines and lung function. Protein levels of IL-4 (A), IL-13 (B), and TARC (C) in BALF and FEV₁. The regression line and the correlation coefficient are shown for CF+P patients. No correlation was found for CF-P patients.

ITAC, Mig, and IP-10 attract CXCR3⁺ T cells and have been associated with T_H1/Tc1 immune responses.^{30,39} On the other hand, increased levels of ITAC, Mig, and IP-10 were found in several inflammatory conditions and may therefore act as proinflammatory chemokines.^{40,41} Patients with CF chronically infected with *P aeruginosa* had significantly increased BALF levels of ITAC compared with noninfected patients with CF. However, this was not associated with increased frequencies of CXCR3⁺ T cells in BALF of these patients, suggesting that higher levels of ITAC, IP-10, and Mig may be required to attract CXCR3⁺ T cells into the bronchoalveolar compartment. Alternatively, CXCR3⁺ T cells may be trapped in the pulmonary tissue and therefore may not be recovered in BALF. BALF levels of ITAC, Mig, and IP-10 in our patients with CF were clearly higher than those found in BALF of healthy control subjects and patients with mild chronic bronchitis,³⁷ suggesting that the levels of these chemokines may relate to the degree of pulmonary

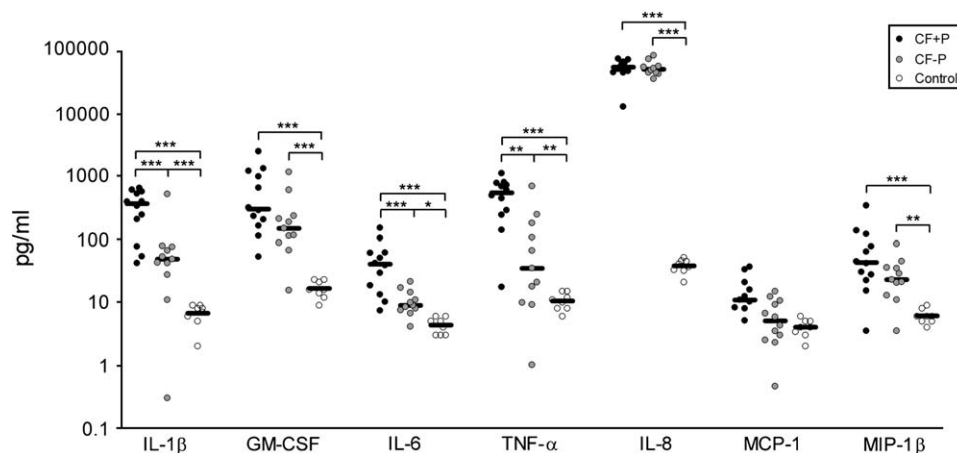


FIG 5. Proinflammatory cytokines and chemokines in BALF. Levels of TNF- α , G-CSF, IL-1 β , IL-6, MIP-1 β , and MCP-1 were quantified in BALF by a multiplex assay. Levels of IP-10, ITAC, Mig, and IL-8 were determined in BALF by a sandwich ELISA. All cytokine and chemokine levels are shown as pg/mL BALF. * $P < .05$, ** $P < .01$, *** $P < .001$; horizontal bars represent median values.

inflammation. Therefore, we speculate that levels of ITAC, Mig, and IP-10 in BALF of patients with CF may represent inflammation rather than a pulmonary T_H1 response. The finding that the proinflammatory cytokines IL-1 β , IL-6, and TNF- α were significantly increased in BALF of *P aeruginosa*-infected patients with CF compared with noninfected patients with CF supports this contention. Similar to our observations, Bonfield et al⁴² found significantly increased BALF levels of TNF- α , IL-1 β , and IL-8 and decreased levels of IL-10 in patients with CF versus healthy controls. The levels of proinflammatory cytokines tended to be higher in *P aeruginosa*-infected versus noninfected patients with CF, although this did not reach statistical significance.

The pulmonary T_H2 response might represent an appropriate effort of the immune system to defend against *P aeruginosa*. However, the overwhelming inflammatory response in CF lungs may provide an unfavorable background for the proper functioning of T_H2 (effector) cells. A dysregulated T_H1/2 response might, in turn, contribute to physical and biochemical factors that avert the clearance of *P aeruginosa*.

The main limitation of this study is the small number of patients included. Therefore, these initial observations need to be verified in larger patient groups. Because some of the *P aeruginosa*-infected patients with CF were also infected with other CF-typical bacteria (*Staphylococcus aureus*, *Hemophilus influenzae*), an alteration of the analyzed immunological parameters by these organisms cannot be excluded with certainty. However, these microbes were almost equally distributed between the 2 patient groups. Furthermore, longitudinal studies of chronically *P aeruginosa*-infected patients with CF are necessary to analyze the contribution of T_H1/T_H2 cytokines/chemokines to the pulmonary outcome of *P aeruginosa*-infected patients with CF.

In summary, these results reveal the prevalence of a pulmonary T_H2 immune response in chronically *P aeruginosa*-infected patients with CF. The positive correlations between pulmonary levels of IL-4, IL-13, and TARC with FEV₁ suggest a pathophysiological relevance of these molecules in lungs of *P aeruginosa*-infected patients with CF. The further characterization of the T_H2 response in *P aeruginosa* infection may help to develop new concepts for the treatment of *P aeruginosa* lung infection in patients with CF.

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