

Chemokines Indicate Allergic Bronchopulmonary Aspergillosis in Patients with Cystic Fibrosis

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Rationale: Allergic bronchopulmonary aspergillosis (ABPA) is characterized by a Th2 immune response. Mouse models suggest a critical role for the Th2 chemokines thymus- and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC) in ABPA.

Objectives: To determine whether serum levels of TARC and MDC characterize ABPA in patients with cystic fibrosis (CF) and to examine longitudinally if levels of TARC and MDC indicate ABPA exacerbations in patients with CF.

Methods: Levels of TARC and MDC and levels of Th1 (IL-12 and IFN- γ) and Th2 (IL-4, IL-5, and IL-13) cytokines were analyzed in serum of 16 patients with CF with ABPA, six non-CF patients with asthma with ABPA, 13 patients with CF colonized with *Aspergillus fumigatus*, six patients with CF sensitized to *A. fumigatus*, 12 atopic patients with CF, and 13 non-CF atopic control subjects by ELISA. The longitudinal course of TARC, MDC, and IgE levels was assessed during ABPA episodes.

Results: Patients with ABPA had significantly higher serum levels of TARC compared with the other patient groups. Cytokine levels did not differ among the patient groups. Longitudinally, levels of TARC indicated ABPA exacerbations in patients with CF more clearly than IgE levels. In patients with CF and ABPA, levels of TARC correlated positively with specific IgE to *A. fumigatus* and rAsp f4.

Conclusions: Serum levels of TARC differentiate patients with CF or patients with asthma with ABPA from patients with CF colonized with or sensitized to *A. fumigatus*, atopic patients with CF, and atopic control subjects. Longitudinally, levels of TARC indicate ABPA exacerbations, suggesting TARC as a marker for identification and monitoring of ABPA in patients with CF.

Keywords: allergic bronchopulmonary aspergillosis; chemokines; cystic fibrosis; TARC

Allergic bronchopulmonary aspergillosis (ABPA) is a pulmonary hypersensitivity disease mediated by an allergic response to *Aspergillus fumigatus*. ABPA occurs in about 10% of patients with cystic fibrosis (CF). ABPA may lead to acute worsening of the respiratory status and requires corticosteroid treatment (1). Without early diagnosis and specific treatment, ABPA can progress to a chronic state and can lead to bronchiectasis and severe lung fibrosis (2, 3). The diagnosis of ABPA is based on the criteria according to Nelson (4), including wheezing, new pulmonary infiltrates, elevated total IgE serum levels, elevated specific IgE and IgG serum levels against *A. fumigatus*, positive skin prick test reactivity against *A. fumigatus*, and the detection of *A. fumigatus* in sputum. Despite these criteria, the diagnosis

of ABPA in patients with CF remains difficult because some of the major criteria (e.g., bronchial obstruction and new infiltrates on chest radiograph) are seen regularly in patients with CF without ABPA (5, 6). Especially in patients with CF who have recurrent ABPA episodes, the differentiation between an exacerbation due to ABPA or due to other pulmonary infections is difficult. Furthermore, ABPA shares characteristics with atopy, which is present in about 25% of patients with CF (7). Total serum IgE levels are commonly used for the diagnosis and the monitoring of ABPA episodes in patients with CF (8) but vary markedly with age and atopic background (9). Furthermore, a number of patients with ABPA have low total serum IgE levels (6, 8, 10). Thus, there is a need for serum markers for ABPA in patients with CF to initiate the correct clinical treatment as soon as possible and to detain disease progression. On the other hand, side effects of inappropriate corticosteroid treatment (e.g., diabetes mellitus, cataract, and growth retardation) should be avoided in patients with CF who do not have ABPA, especially because patients with CF are prone to the severe side effects of corticosteroids (11).

Animal and human studies have associated ABPA with a T-helper cell type 2 (Th2) immune response to *A. fumigatus* antigens (2, 12, 13). *A. fumigatus*-challenged mice exhibited a strong allergic response with accumulation of pulmonary Th2 cells (14). In patients with ABPA, the majority of T-cell clones was identified as CD4⁺ IL-4⁺ (Th2) clones (13). Peripheral blood mononuclear cells derived from patients with ABPA showed an increased sensitivity to IL-4 with an up-regulation of the low-affinity IgE receptor on B cells (15–17). Chemokines attract leukocytes through their corresponding receptors and are classified as Th1 and Th2 associated chemokines and chemokine receptors (18). Th2 cells preferentially express the chemokine receptor CCR4 and are attracted by the specific ligands thymus- and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC) (19, 20). In ABPA, the CCR4 knock out mouse model showed an attenuated airway hyperresponsiveness and a more rapid clearance of *A. fumigatus* conidia compared with wild-type mice, indicating a crucial role for CCR4 and TARC/MDC in the immune response to *A. fumigatus* (21).

No studies have been performed to investigate the role of CCR4 and its ligands TARC and MDC in patients with ABPA. We hypothesized that serum levels of TARC and MDC are increased in patients with CF who have ABPA. Therefore, levels of TARC and MDC together with levels of Th1 (IL-12 and IFN- γ) and Th2 (IL-4, IL-5 and IL-13) cytokines were analyzed in the serum of patients with CF who had ABPA as compared with non-CF patients with asthma who did not have ABPA, patients with CF colonized with *A. fumigatus* without ABPA, patients with CF sensitized to *A. fumigatus* without ABPA, atopic patients with CF, and atopic subjects without CF. Furthermore, we hypothesized that TARC and MDC serum levels indicate ABPA exacerbations in the clinical course of patients with CF. To test this hypothesis, TARC and MDC serum levels were assessed longitudinally from 16 wk before until 16 wk after an ABPA exacerbation peak in patients with CF.

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METHODS

Patient Groups

A total of 66 patients (35 men and 31 women; mean age of 21 ± 4.2 yr) were selected from the Allergy and Pneumology outpatient clinic at the Children's Hospital of the University of Munich, serving about 370 pediatric and adult patients with CF and about 500 allergic and patients with asthma per year. The diagnosis of CF was confirmed by clinical features consistent with CF and two independent positive sweat chloride tests (≥ 60 mEq/l).

Patients with CF and ABPA

ABPA in patients with CF was diagnosed based on the presence of at least six of the seven criteria of Nelson (4), which have been described in detail by Krane and Griese (22). These criteria included (1) wheezing, (2) new pulmonary infiltrates on X-ray, (3) detection of *A. fumigatus* in sputum culture, (4) positive skin prick test to *A. fumigatus*, (5) increased total IgE levels (> 150 kU/ml), (6) specific IgE to *A. fumigatus* (CAP class > 2), and (7) increased IgG antibodies specific to *A. fumigatus* (> 40 EU/ml). Following to these criteria, 16 patients with CF with ABPA (CF-ABPA) were selected for the study.

Control Groups

Patients without CF who had asthma with ABPA, patients with CF colonized with *A. fumigatus* without ABPA, patients with CF sensitized to *A. fumigatus* without ABPA, atopic patients with CF without ABPA, and atopic control subjects without CF were matched to the ABPA group by sex, age, disease severity, detected pathogens, and treatment characteristics.

Patients without CF with Asthma and ABPA

Six patients without CF with allergic asthma had a diagnosis of ABPA according to the presence of at least six of seven of the criteria of Nelson (4). Asthma was diagnosed based on recurrent episodes of wheezing and objective evidence of asthma as indicated by β_2 -agonist reversible airflow obstruction ($\geq 12\%$ improvement in FEV₁ predicted), bronchial hyperresponsiveness (exercise challenge), and $\geq 20\%$ intraday peak flow variability. These patients had *A. fumigatus* detected in their sputum and had an allergic reaction to *A. fumigatus* (i.e., positive skin prick test to *A. fumigatus*, increased total IgE levels, and increased specific IgE to *A. fumigatus*).

Patients with CF Colonized with *A. fumigatus*

Thirteen patients with CF were colonized with *A. fumigatus* but did not otherwise fulfill the criteria for ABPA. These patients had *A. fumigatus* detected in their sputum but had no allergic reaction to *A. fumigatus* (i.e., negative skin prick test to *A. fumigatus*, normal total IgE levels, and no specific IgE to *A. fumigatus* [below the detection limit of 0.35 kU/l]).

Patients with CF Sensitized to *A. fumigatus*

Six patients with CF were sensitized to *A. fumigatus* but did not otherwise fulfill the criteria for ABPA as listed previously. These patients had *A. fumigatus* detected in their sputum and had an allergic reaction to *A. fumigatus* (i.e., positive skin prick test to *A. fumigatus*, increased total IgE levels, and increased specific IgE to *A. fumigatus*).

Atopic Patients with CF

Twelve patients with CF were atopic but had no sensitization to *A. fumigatus* and no signs of ABPA. Atopic status was confirmed by positive skin prick testing (wheal diameter of ≥ 3 mm to at least one common allergen other than *A. fumigatus*) (23), elevated total serum IgE levels (> 150 kU/ml), and/or the presence of specific IgE to common allergens (CAP class > 2). None of the atopic patients with CF had *A. fumigatus* detected in the sputum or showed a sensitization to *A. fumigatus*, as confirmed by the absence of specific IgE to *A. fumigatus* (below the detection limit of 0.35 kU/L) and a negative skin prick test to *A. fumigatus*.

Atopic Subjects without CF

Thirteen atopic subjects without CF with positive skin prick testing (wheal diameter of ≥ 3 mm to at least one common allergen), elevated total serum IgE (> 150 kU/ml), and/or the presence of specific IgE to common allergens (CAP class > 2) but without sensitization to *A. fumigatus*, as confirmed by the absence of specific IgE to *A. fumigatus* (below the detection limit of 0.35 kU/L) and a negative skin prick test to *A. fumigatus*, were included in the study. These patients had a clinical diagnosis of allergic rhinitis ($n = 9$) or allergic dermatitis ($n = 4$).

The patient groups were evenly distributed and comparable in terms of sex, age, CRP values, and lung function (Table 1). Serum from all subjects was obtained after informed consent. The institutional review board approved the study protocol.

Laboratory Tests

Serum was obtained from venous blood by centrifugation at $1,000 \times g$ for 10 min. Aliquots of serum were stored at -20°C . Specific IgE antibodies to *A. fumigatus* were analyzed by radioallergosorbent test using a CAP system according to the manufacturer's instructions (Pharmacia, Uppsala, Sweden). Results are given as CAP Classes 0–6. The recombinant *A. fumigatus* allergens rAsp f1, f2, f3, f4, and f6 were analyzed by a CAP system (Pharmacia), and results are shown as CAP Classes 0 to 6 as described by the manufacturer (CAP Class 0: < 0.35 kU/l; Class 1: 0.35–0.7 kU/l; Class 2: 0.7 to < 3.5 kU/l; Class 3: 3.5 to < 17.5 kU/l; Class 4: 17.5 to < 50 kU/l; Class 5: 50 to 100 kU/l; Class 6: > 100 kU/l). Specific IgG antibodies to *A. fumigatus* were analyzed by ELISA using standardized DPC antigens (Diagnostic Products Corp., Los Angeles, CA). For the skin prick test, a commercial *A. fumigatus* extract was used (SmithKline Beecham, Neuss, Germany). Histamine dihydrochloride (10 mg/ml) was used as positive control, and glycerol/sodium chloride was used as negative control.

Study Design

All patients with CF were prospectively monitored for the presence of ABPA by clinical and serologic criteria according to Nelson (4). An ABPA episode was defined as increased wheezing and increasing levels of total serum IgE. The peak point of total serum IgE during an ABPA episode was considered as the peak of the ABPA episode.

In CF-ABPA patients, serum levels of TARC and MDC were longitudinally measured within an ABPA episode at nine time points beginning from 16 wk before until 16 wk after the peak IgE point. Serum levels of TARC and MDC in CF ABPA patients were calculated in two ways: (1) ABPA peak: serum levels of TARC and MDC analyzed at the peak IgE point during the ABPA episode and (2) ABPA average: serum levels of TARC and MDC analyzed at nine time points within an ABPA episode summarized and calculated as average levels for each patient.

Cytokine serum levels; specific IgE antibodies to *A. fumigatus*; and rAsp f1, f2, f3, f4, and f6 were analyzed closely around the IgE peak (± 1.2 wk) during an ABPA episode. The pulmonary function values (FEV₁) used for correlation analysis were from the last pulmonary function test done before the IgE peak during an ABPA episode (on average 2 ± 1.5 wk before the IgE peak).

Detection of Cytokines and Chemokines

Levels of IL-4, IL-5, IL-12, IL-13, IFN- γ , TARC, and MDC were analyzed in duplicates by sandwich ELISA according to the manufacturer's instructions (R&D Systems, Minneapolis, MN). In brief, samples were pipetted into antibody-precoated wells and detected by a horseradish peroxidase-linked polyclonal antibody specific for the respective chemokine. The optical density values were read in a microplate reader (MRX II; Life Sciences, Eglsbach, Germany) at 450/540 nm. The concentrations were calculated from standard curves. The detection limits were IL-4 (10–5,000 pg/ml), IL-5 (3–4,000 pg/ml), IL-12 (5–5,000 pg/ml), IL-13 (5–3,000 pg/ml), IFN- γ (8–3,000 pg/ml), MDC (15.6–2,000 pg/ml), and TARC (7–3,000 pg/ml).

Statistical Analysis

Data are given as medians with ranges in parentheses if not indicated otherwise. Differences between the patient groups were calculated using the nonparametric Kruskal-Wallis test. When a significant difference was found, the nonparametric Mann-Whitney *U* test was

TABLE 1. CHARACTERISTICS OF THE PATIENT GROUPS

	ABPA		CF			
	CF	Asthma	<i>A. fumigatus</i> Sensitization	<i>A. fumigatus</i> Colonization	Atopic	Non-CF Atopic
Number	16	6	6	13	12	13
Age, yr	18 (11–31) [†]	24 (21–30)	21 (15–28)	24 (11–42)	21 (14–37)	17 (7–19)
Sex (M/F)	10/6	3/3	3/3	6/7	7/5	6/7
CRP, mg/dl	0.6 (0–6.5)	0.3 (0–1.2)	0.4 (0–1.6)	0.6 (0–1.7)	0.8 (0–2)	0
IgE, IU/ml	3,254 (445–6,606) [‡]	1,899 (1,321–2,242) [§]	643 (395–988)	47 (1–120)	627 (338–2,395) [§]	838 (446–4,165) [§]
Specific IgE to <i>Aspergillus fumigatus</i> *	4 (0–6) [‡]	4 (0–5) [‡]	3 (0–4) [‡]	0 (0–0)	0 (0–0)	0 (0–0)
rAsp f4, kU/l	13.8 (0.2–53.4)	10.5 (1.2–34.5)	0.38 (0–4.9)	0 (0–0)	n.d.	n.d.
rAsp f6, kU/l	2.4 (0.1–8.3)	1.7 (0–7.1)	0.36 (0–5.2)	0 (0–0)	n.d.	n.d.
<i>A. fumigatus</i> in sputum culture	16/16 [‡]	6/6 [‡]	6/6 [‡]	13/13 [‡]	0/12	0/13
Blood eosinophils, %	10 (5–20) [§]	9 (3–18) [§]	5 (0–8)	2 (0–5)	7 (4–11) [§]	8 (2–10) [§]
FEV ₁ (% pred)	46 (32–81)	62 (41–79)	60 (24–93)	57 (15–104)	61 (37–92)	n.d.
FVC (% pred)	61 (34–89)	83 (65–95)	71 (45–84)	65 (36–91)	72 (41–93)	n.d.
MEF _{25–75} (% pred)	22 (12–39)	31 (17–35)	29 (19–37)	24 (15–41)	26 (17–40)	n.d.
Inhaled steroids	13/16	6/6	3/6	7/13	7/12	5/13
Systemic steroids	11/16 [‡]	4/6 [§]	0/6	1/13	1/12	0/13
Itraconazole	10/16 [‡]	4/6 [§]	0/6	3/13 [§]	0/12	0/13
<i>Pseudomonas aeruginosa</i>	10/16	0/6	2/6	9/13	6/12	0/13
<i>Stenotrophomonas maltophilia</i>	7/16	0/6	0/6	3/13	3/12	0/13
df508 homozygous/heterozygous/other	9/6/1	n.d.	4/2	3/6/4	5/5/2	n.d.

Definition of abbreviations: ABPA = allergic bronchopulmonary aspergillosis; CF = cystic fibrosis; MEF_{25–75} = maximum expiratory flow rate at 25–75% of vital capacity; n.d. = not done.

* Specific IgE to *A. fumigatus* is shown as CAP Class 0–6.

[†] Data are given as medians with ranges shown in parentheses.

[‡] $p < 0.01$ as compared with the other patient groups, Kruskal-Wallis and Mann-Whitney *U* test.

[§] $p < 0.05$ as compared with the other patient groups, Kruskal-Wallis and Mann-Whitney *U* test.

applied for two-group comparisons. Correlations were verified with Spearman *rho* test. A probability of $p < 0.05$ was regarded as significant (SPSS statistical program, version 11.5; SPSS Inc., Chicago, IL) (24).

RESULTS

Cytokine Levels

Serum levels of the Th2 cytokines IL-4, IL-5, and IL-13 were significantly higher in CF-ABPA patients and in ABPA patients with asthma compared with patients with CF who were colonized with *A. fumigatus* but did not differ significantly from the other patient groups (Figure 1). Serum levels of IFN- γ and IL-12 did not differ among the patient groups. No association was found between serum levels of IFN- γ , IL-4, IL-5, IL-12, and IL-13 and levels of total IgE, specific IgE to *A. fumigatus*, recombinant *A. fumigatus* allergens rAsp f1–6, or FEV₁.

TARC Levels

Both patients with CF and ABPA and patients with asthma and ABPA had significantly higher serum levels of TARC as compared with all other patient groups, that is, patients with CF who were colonized with or sensitized to *A. fumigatus*, atopic patients with CF, and atopic control subjects without CF. In patients with CF and ABPA, this was significant for TARC levels at ABPA IgE peaks and for TARC average levels during ABPA episodes. In patients with CF, TARC serum levels at ABPA IgE peaks were significantly higher compared with TARC average levels during ABPA episodes (Figure 2A). Serum levels of TARC did not differ significantly among patients with CF sensitized to *A. fumigatus*, patients with CF who were colonized with *A. fumigatus*, atopic patients with CF, and atopic control subjects without CF. In patients with CF and ABPA, serum levels of TARC at IgE peaks correlated positively with levels of total IgE ($r = 0.4$; $p < 0.05$) and with levels of IL-4 ($r = 0.52$; $p < 0.01$) but showed a stronger correlation with

specific IgE ($r = 0.75$; $p < 0.01$) and with rAsp f4 ($r = 0.89$; $p < 0.01$). Thus, patients with CF and ABPA with a high CAP class to *A. fumigatus* had high TARC serum levels (Figure 3A). Similarly, patients with CF and ABPA with a high CAP class to rAsp f4 had high TARC serum levels (Figure 3B). No association was found between levels of TARC and rAsp f1, f2, f3, or f6. Furthermore, an inverse correlation between levels of TARC and FEV₁ was found in patients without ABPA with CF and those with asthma and ABPA ($r = -0.49$, $p < 0.05$; $r = -0.6$, $p < 0.01$) but was more prominent in patients with CF and ABPA ($r = -0.74$, $p < 0.01$). The four patients with CF and ABPA with the highest levels of TARC had the highest levels of IL-4 and the worst lung function (FEV₁ 32–36% of predicted) of all patients with ABPA. Patients with CF and ABPA and patients with asthma and ABPA with systemic corticosteroid therapy before the ABPA exacerbation had significantly lower TARC levels compared with patients with ABPA without systemic corticosteroid therapy (Figure 4). Inhaled corticosteroids did not show an association with TARC levels (data not shown).

During ABPA episodes, serum levels of TARC paralleled serum levels of total IgE (Figure 5A) but indicated the ABPA peak and the decline afterwards more clearly than levels of IgE did (Figure 5B). No correlation was found between serum levels of TARC and CRP (data not shown).

MDC Levels

Patients with CF and ABPA had significantly higher MDC serum levels analyzed at IgE peaks as compared with patients with CF sensitized to *A. fumigatus*, patients with CF colonized with *A. fumigatus*, atopic patients with CF, and atopic control subjects without CF. CF-ABPA average levels were significantly higher only when compared with patients with CF colonized with *A. fumigatus*, but not compared with the other patient groups. Patients with asthma and ABPA did not differ from the other

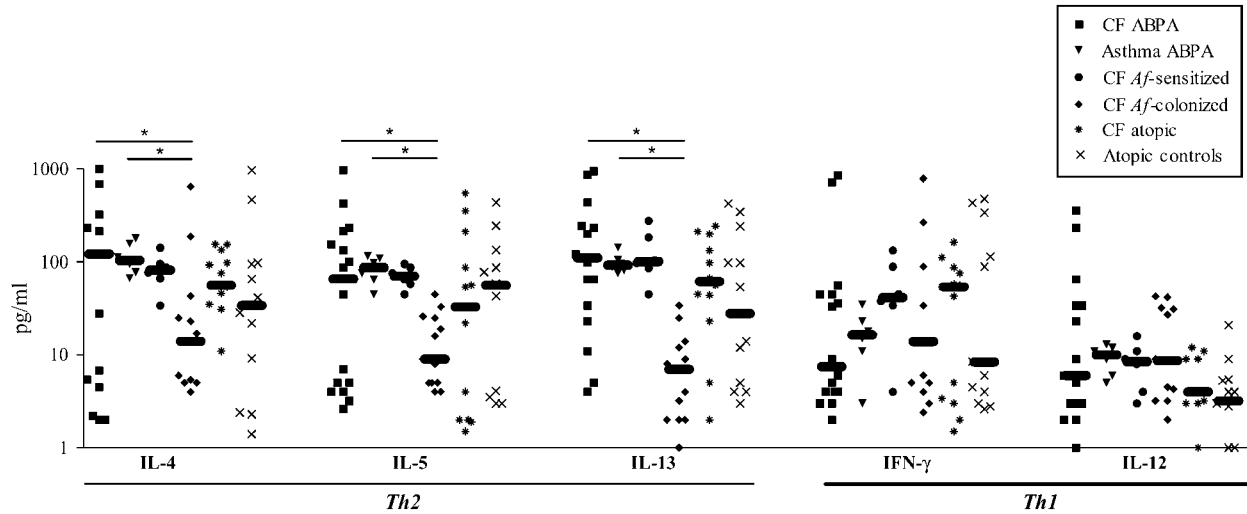


Figure 1. Cytokine levels. Levels of IL-4, IL-5, IL-13, IFN- γ , and IL-12 in the serum of patients with cystic fibrosis (CF) and allergic bronchopulmonary aspergillosis (CF-ABPA), patients without CF with asthma and ABPA (Asthma-ABPA), patients with CF sensitized to *A. fumigatus* without ABPA (CF *Af*-sensitized), patients with CF colonized with *Af* without ABPA (CF *Af*-colonized), atopic patients with CF without ABPA (CF atopic), and atopic control subjects without CF (Atopic controls). Median values are shown by horizontal bars. Differences between all patient groups were tested with the Kruskal-Wallis test. Differences between two patient groups were calculated with the Mann-Whitney *U* test. **p* < 0.05.

patient groups (Figure 2B). Similar to TARC, serum levels of MDC did not differ among patients with CF sensitized to *A. fumigatus*, patients with CF colonized with *A. fumigatus*, atopic patients with CF, and atopic control subjects without CF.

MDC serum levels were not associated with systemic or inhaled corticosteroid therapy (data not shown). A slight inverse correlation between levels of MDC and IFN- γ was found ($r = -0.4$, $p < 0.5$). In contrast to TARC, MDC serum levels did not

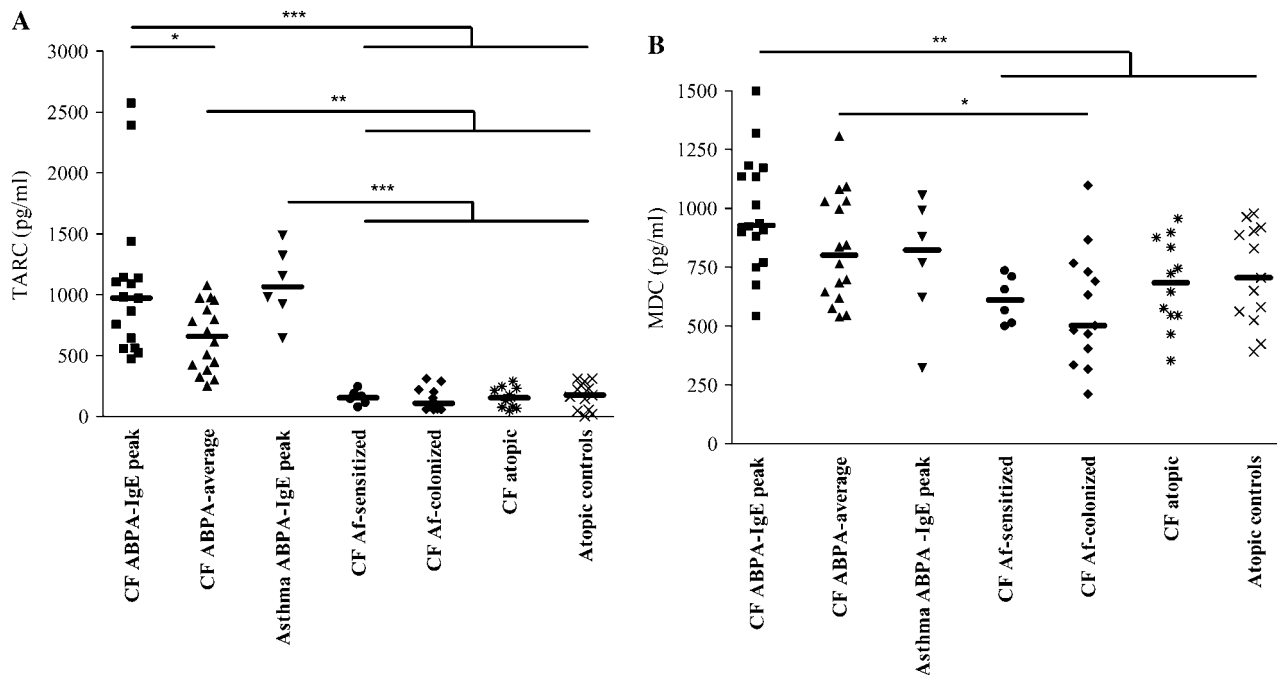


Figure 2. Levels of thymus- and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC), TARC (A), and MDC (B) serum levels are shown at peak IgE points during allergic bronchopulmonary aspergillosis (ABPA) episodes in patients with cystic fibrosis (CF) (CF ABPA-IgE peak), as average levels during ABPA episodes in patients with CF (CF ABPA-average), at peak IgE points during ABPA episodes in patients without CF with asthma (Asthma ABPA-IgE peak), in patients with CF sensitized to *A. fumigatus* (CF *Af*-sensitized), in patients with CF colonized with *A. fumigatus* (CF *Af*-colonized), in atopic patients with CF without ABPA (CF atopic), and in atopic control subjects without CF (Atopic controls). Median values are shown by horizontal bars. Differences between all patient groups were tested with the Kruskal-Wallis test. Differences between two patient groups were calculated with the Mann-Whitney *U* test. **p* < 0.05; ***p* < 0.01; ****p* < 0.001.

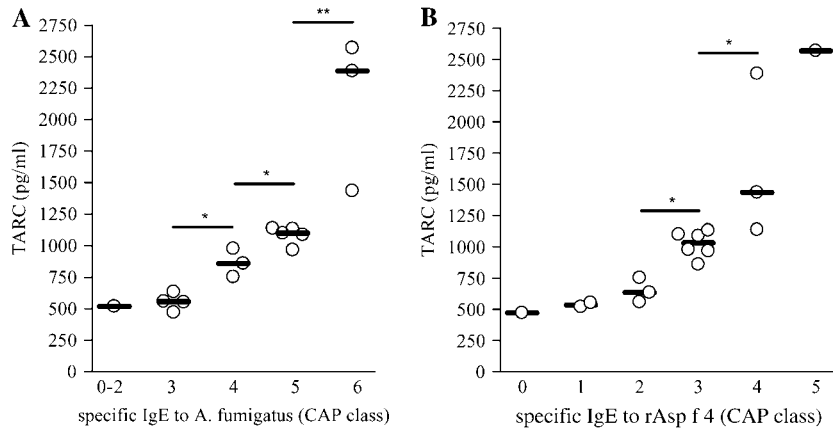


Figure 3. Thymus- and activation-regulated chemokine (TARC) levels and specific IgE to *A. fumigatus* and rAsp f4 TARC serum levels in relation to CAP class groups of specific IgE to *A. fumigatus* (A) and specific IgE to the recombinant *A. fumigatus* allergen rAsp f4 (B) in patients with allergic bronchopulmonary aspergillosis (ABPA) at peak IgE points during ABPA episodes. CAP class 0: < 0.35 kU/l; Class 1: 0.35–0.7 kU/l; Class 2: 0.7 to < 3.5 kU/l; Class 3: 3.5 to < 17.5 kU/l; Class 4: 17.5 to < 50 kU/l; Class 5: 50–100 kU/l; Class 6: > 100 kU/l. Median values are shown by horizontal bars. Differences between all CAP class groups were tested with the Kruskal-Wallis test. Differences between two CAP class groups were calculated with the Mann-Whitney *U* test. **p* < 0.05; ***p* < 0.01.

correlate with levels of IL-4, IgE, rAsp f1–6, or FEV₁. Levels of MDC measured longitudinally within an ABPA episode in patients with CF did not show an association with serum levels of IgE (Figure 5C).

DISCUSSION

The mouse model of allergic aspergillosis reveals a crucial role for the CCR4-TARC axis in the immune response to *A. fumigatus* (21). This study shows that TARC is useful to identify and characterize ABPA in patients with CF. Serum levels of TARC are increased in patients with CF and ABPA compared with patients with CF sensitized to *A. fumigatus*, patients with CF colonized with *A. fumigatus*, atopic patients with CF, and atopic control subjects without CF. Within the course of an ABPA episode, serum levels of TARC longitudinally indicate ABPA peaks in patients with CF. In patients with CF and ABPA, levels of TARC are positively associated with specific IgE to *A. fumigatus* and to the recombinant *A. fumigatus* allergen rAsp f4. ABPA patients with systemic corticosteroid therapy have decreased levels of TARC compared with ABPA patients without systemic steroid therapy.

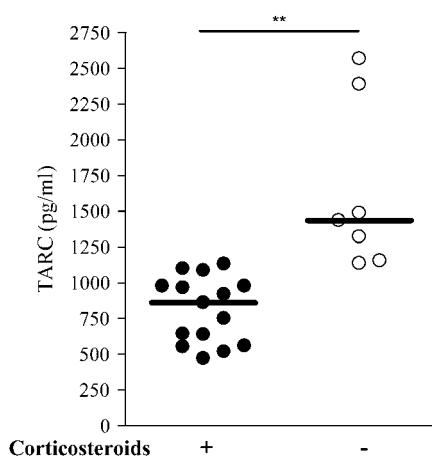


Figure 4. Systemic corticosteroid therapy and thymus- and activation-regulated chemokine (TARC) levels. Levels of TARC in patients with cystic fibrosis and allergic bronchopulmonary aspergillosis (CF-ABPA) (*n* = 16) and asthma-ABPA (*n* = 6) patients with (+, *n* = 15) or without (–, *n* = 7) systemic corticosteroid therapy. Median values are shown by horizontal bars. Differences between two groups were calculated with the Mann-Whitney *U* test. ***p* < 0.01.

Because *A. fumigatus* colonization occurs in up to 60% of patients with CF (4) but only a minority of patients develops ABPA, we compared patients with CF and ABPA with patients with CF chronically colonized with *A. fumigatus* or sensitized to *A. fumigatus* but without further criteria for ABPA. Patients with CF with colonization or sensitization to *A. fumigatus* but without clinically active ABPA did not show elevated TARC levels, suggesting that increased levels of TARC in patients with ABPA may not be due to the underlying *A. fumigatus* colonization or sensitization, but might represent a specific phenomenon in clinically active ABPA. Thus, TARC serum levels might be clinically useful to identify ABPA episodes especially in patients with CF already colonized with or sensitized to *A. fumigatus*. Patients with CF and ABPA had significantly higher TARC serum levels compared with the other patient groups at ABPA IgE peaks and as average levels during ABPA episodes. This suggests TARC as a marker for ABPA in patients with CF not only at ABPA peaks but also during the clinical course in between. The recombinant *A. fumigatus* allergens rAsp f4 and rAsp f6 were suggested as specific markers for clinically active ABPA (25–28). We found a positive association between serum levels of TARC and rAsp f4 in patients with CF. Furthermore, TARC serum levels were related positively to specific IgE to *A. fumigatus*. To determine whether serum levels of TARC are increased in ABPA in general or are a CF-related phenomenon, TARC levels were measured in the serum of patients without CF with allergic asthma and ABPA. Because TARC serum levels were likewise increased in the serum of patients with ABPA with or without CF, we rule out that CF-specific processes may account for the elevation of TARC serum levels in patients with CF and ABPA. The lack of correlation between levels of TARC and CRP suggests that serum levels of TARC may be not due to underlying infections in patients with CF.

TARC levels correlated inversely with lung function (i.e., FEV₁). This correlation was most prominent in patients with ABPA but was not found for MDC, supporting a pathophysiologic role for TARC in the Th2 immune response in patients with ABPA. Corticosteroids are known to suppress the immunologic responses to *A. fumigatus* (29). Patients with CF and those with asthma and ABPA with systemic corticosteroid therapy had significantly lower TARC levels compared with patients with ABPA without systemic steroids, suggesting that immunosuppressive treatment may be able to reduce the high serum levels of TARC in patients with ABPA. Thus, TARC serum levels might be useful as a marker for the effect of corticosteroid treatment in patients with CF and in patients with asthma and ABPA and should be evaluated in future trials.

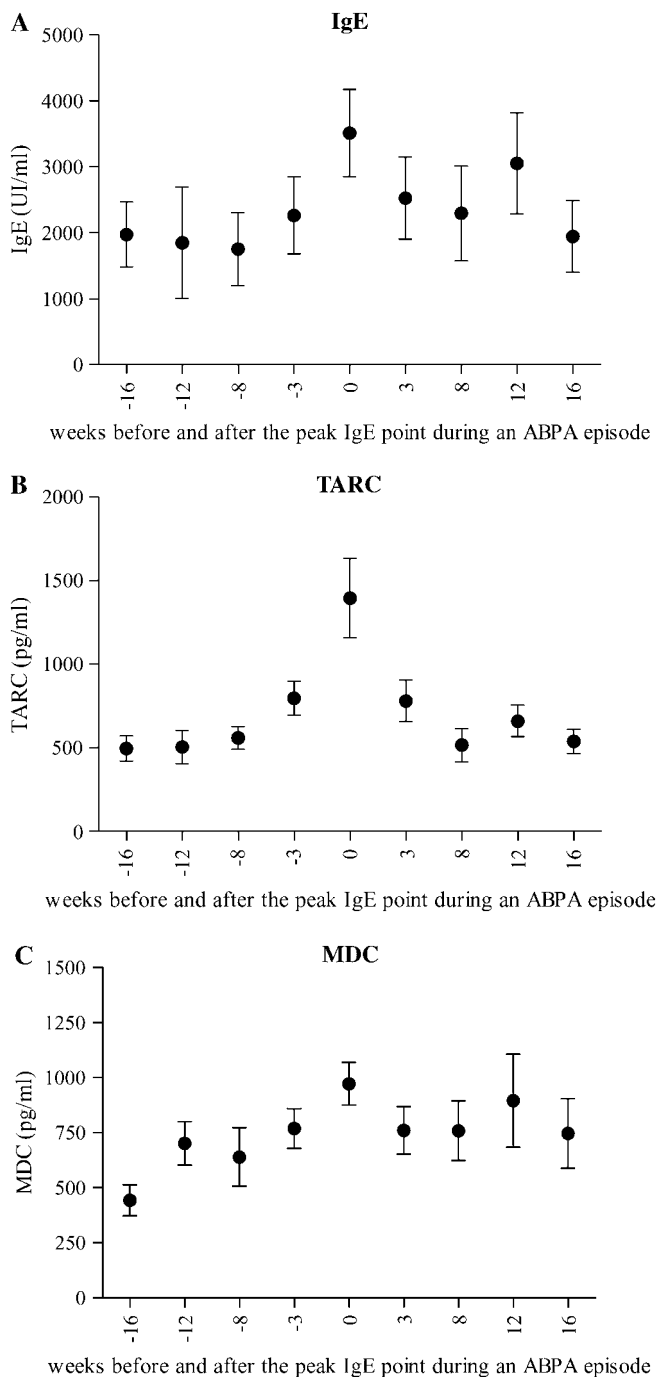


Figure 5. Longitudinal course of IgE, thymus- and activation-regulated chemokine (TARC), and macrophage-derived chemokine (MDC) levels during allergic bronchopulmonary aspergillosis (ABPA) episodes. Serum levels of IgE (A), TARC (B), and MDC (C) in the course of an ABPA episode. Data are shown as mean $\pm$ SEM for 16 wk before until 16 wk after the peak IgE point during an ABPA episode. The number in parentheses indicates the number of patients at the respective time points: -16 (7), -12 (4), -8 (7), -3 (5), 0 (16), 3 (11), 8 (7), 12 (5), 16 (6).

During ABPA episodes in patients with CF, serum levels of TARC, but not MDC, paralleled serum levels of total IgE but indicated the peak of an ABPA episode and the amelioration afterward more clearly than IgE levels did. TARC serum levels at the peak of an ABPA episode were about two times higher

than the TARC levels before and after the peak. This increase of TARC levels during ABPA episodes was more obvious than the increase of IgE levels, suggesting TARC as a sensitive marker for the follow-up of patients with CF and ABPA, especially in patients with recurrent ABPA episodes. Large studies are needed to prove the clinical value of TARC serum levels for the longitudinal follow-up of ABPA in patients with CF.

TARC is suggested to recruit CCR4⁺ and CCR8⁺ Th2 cells, whereas MDC recruits exclusively CCR4⁺ Th2 cells (30). This might be one explanation for the discrepancy between TARC and MDC levels in identifying ABPA episodes in patients with CF. It remains unclear which cells secrete TARC and MDC in patients with ABPA and how this process is regulated. T cells, B cells, monocytes, and dendritic cells (DCs) were identified as the main producers of TARC and MDC (30, 31). DCs in the respiratory tract are suggested as initiators of immune responses to pulmonary pathogens. After pathogen recognition, pulmonary DCs mature, migrate to lymph nodes, and instruct T cells how to react to the invading pathogen (32). This is of interest in the immune response to *A. fumigatus* because DCs were found to internalize conidia and hyphae of *A. fumigatus* and to initiate a Th2 immune response (33). In patients with ABPA, *A. fumigatus*-primed DCs might secrete TARC and MDC and/or may trigger Th2 cells to secrete TARC and MDC, but experimental evidence is lacking.

Kawasaki and colleagues demonstrated that treatment of ovalbumin-sensitized mice with an anti-TARC antibody before challenging with aerosolized ovalbumin prevented airway eosinophilia, led to decreased percentages of infiltrating CD4⁺ lymphocytes and decreased levels of Th2 cytokines compared with untreated mice (34). Although no studies in ABPA have been performed, TARC inhibitors may provide a promising tool to inhibit the strong Th2 immune response in patients with ABPA.

In summary, TARC serum levels are useful to identify and follow-up ABPA in patients with CF. Further studies on the role of TARC in ABPA are necessary because TARC might be a possible therapeutic target in patients with ABPA.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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