

Pulmonary chemokines and their receptors differentiate children with asthma and chronic cough

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Background: Cough is a frequent symptom in children, but the differentiation of asthmatic cough from cough of other origins can be difficult. Chemokines recruit T lymphocytes to inflamed tissues, and the corresponding chemokine receptors are differentially expressed on T_H1 and T_H2 cells. **Objective:** We sought to determine whether levels of T_H1/T_H2-related chemokines and their receptors differ in bronchoalveolar lavage fluid (BALF) from 12 children with allergic asthma, 15 nonatopic children with chronic cough, and 10 children without airway disease.

Methods: The T_H1-related (IFN- γ -inducible protein of 10 kd [IP-10], IFN- γ -inducible T cell α chemoattractant [ITAC], monokine induced by IFN- γ [Mig], and IFN- γ) and T_H2-related (thymus- and activation-regulated chemokine [TARC], macrophage-derived chemokine [MDC], IL-5, and IL-4) chemokines and cytokines were quantified in BALF by ELISA and a particle-based multiplex array. Percentages of pulmonary lymphocytes expressing CXCR3⁺ and CCR5⁺ (T_H1) and CCR4⁺ and CCR3⁺ (T_H2) chemokine receptors were determined in BALF by flow cytometry.

Results: Pulmonary CCR4⁺CD4⁺ cells and levels of TARC and MDC were significantly increased in asthmatic children versus children with chronic cough or without airway disease. In asthmatic children CCR4⁺CD4⁺ cells correlated positively with levels of TARC, MDC, and serum IgE levels and negatively with FEV₁. In contrast, CXCR3⁺CD8⁺ cells and levels of ITAC were significantly increased in children with non-atopic chronic cough compared with the other groups. In children with chronic cough, CXCR3⁺CD8⁺ cells correlated with levels of ITAC and IFN- γ .

Conclusion: Pulmonary CCR4⁺CD4⁺ and CXCR3⁺CD8⁺ cells and their ligands TARC, MDC, and ITAC clearly differentiate asthmatic children from nonatopic children with chronic cough. The analysis of these markers could facilitate the diagnostic discrimination of asthma versus other reasons for chronic cough in children. (*J Allergy Clin Immunol* 2005;115:728-36.)

Key words: Chemokine, chemokine receptor, bronchoalveolar lavage, children, chronic cough, asthma, CXCR3, CCR4, TARC, MDC

Cough is one of the most common symptoms in childhood and a frequent symptom of asthma.^{1,2} The diagnostic differentiation between allergic asthma and non-allergic cough can be difficult, and the standard clinical and laboratory parameters are of limited value, particularly in young children. However, selection of patients who will benefit from a more extensive evaluation and antiasthmatic treatment is crucial. For these reasons, it is important to improve diagnostic tools for the evaluation of chronic cough of unknown origin. Asthma is believed to be a T_H2 cell-dominated disorder, and T cells producing IL-4, IL-5, and IL-13 have been identified in bronchoalveolar lavage fluid (BALF) from adult asthmatic patients.^{3,4} Selective trafficking of T cells to peripheral sites is controlled by adhesion molecules and through the interaction of chemokines with their counterpart receptors. Accordingly, a number of chemokine receptors are differentially expressed on lymphocytes in an organ- or disease-specific manner.⁵ Both *in vitro* and *in vivo* studies have shown that the expression of CXCR3^{6,7} and, to a lesser degree, CCR5⁸⁻¹⁰ is associated with T_H1 responses. CXCR3⁺ cells are attracted by the IFN- γ -inducible chemokines IFN- γ -inducible T cell α chemoattractant (ITAC), IFN- γ -inducible protein of 10 kd (IP-10), and monokine induced by IFN- γ (Mig).¹¹ CCR4 is a chemokine receptor with high selectivity for T_H2 cells that are attracted by the CCR4 ligands thymus- and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC).^{6,12} CCR3 is expressed on a subset of T_H2 cells.¹³ Amplified frequencies of CCR4⁺ and CCR8⁺ lymphocytes and increased expression of MDC and TARC were found in bronchial biopsy specimens from allergen-challenged adult asthmatic patients.¹⁴ Similarly, in BALF increased levels of MDC and TARC, as well as IP-10 and Mig, were observed in allergen-challenged adults with allergic asthma.^{15,16}

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Abbreviations used

BAL: Bronchoalveolar lavage
BALF: Bronchoalveolar lavage fluid
IP-10: IFN- γ -inducible protein of 10 kd
ITAC: IFN- γ -inducible T cell α chemoattractant
MDC: Macrophage-derived chemokine
Mig: Monokine induced by IFN- γ
TARC: Thymus- and activation-regulated chemokine

In nonchallenged asthmatic patients levels of MDC in BALF were increased compared with those seen in healthy subjects,¹⁷ and increased levels of TARC were found in induced sputum from adult asthmatic patients.¹⁸ Increased plasma levels of TARC were found in asthmatic children¹⁹ but not in adults.²⁰

These findings led us to ask whether a differential expression of T_H1/T_H2-associated chemoattractants and cells bearing their respective chemokine receptors could be used for the differentiation of asthmatic children versus children with nonatopic chronic cough. We hypothesized that T_H2-related chemokines are increased in the airways of asthmatic children, whereas T_H1-associated chemokines are not. As a consequence, this should lead to an immigration of pulmonary T cells bearing T_H2-associated chemokine receptors without a concomitant increase of T cells positive for T_H1-associated chemokine receptors. To address this question, BALF levels of TARC, MDC, IP-10, ITAC, and Mig, together with IL-5, IL-4, and IFN- γ , were quantified in samples obtained from a well-characterized pediatric study population that included children with allergic asthma, nonallergic children with chronic cough, and control children without airway disease. The chemokine levels were correlated with the frequencies of the corresponding chemokine receptor-positive CD4⁺ and CD8⁺ pulmonary T cells.

METHODS

Patients

Children and adolescents requiring fiberoptic bronchoscopy with bronchoalveolar lavage (BAL) at the Department of Pulmonology and Allergology of the University Children's Hospital of Munich from 1999 through 2004 were considered for inclusion in this study. The study population was set up retrospectively by an independent investigator (M.G.) who was blinded to the experimental results. Patients were selected if allergic asthma or chronic cough could be diagnosed according to the criteria described below. The parents provided their informed consent before bronchoscopy. The institutional review board approved the protocol.

Characterization of the patients

A child was classified as having allergic asthma when he or she had positive atopic status (see below), recurrent episodes of wheezing, objective evidence of asthma as indicated by β_2 -agonist reversible airflow obstruction ($\geq 12\%$ improvement in FEV₁ % predicted), bronchial hyperresponsiveness (exercise challenge), and

20% or greater intraday peak flow variability. Atopic status was confirmed on the basis of positive skin prick test results (wheal diameter of ≥ 3 mm to at least one common allergen), increased total serum IgE levels (>150 kU/mL; IgE-Elecsys, Fa Roche, Basel, Switzerland), and/or the presence of specific IgE (RAST class >2). RASTs were performed for 40 inhalation and food allergens (Sanofi Diagnostics Pasteur, Inc, Chaska, Minn). Spirometry and flow volume curves were performed according to the American Thoracic Society guidelines.²¹ Patients with 3 or more months per year of persistent or recurrent nonproductive cough for at least 2 years who had no signs of atopy and none of the aforementioned criteria for asthma were classified as having chronic cough. An underlying immune deficiency, cystic fibrosis, immotile cilia syndrome, malformations, foreign bodies, microaspirations from gastroesophageal reflux disease, chemical exposures, tracheomalacia, or nicotine abuse were excluded in all children. Children without suspected or proved pulmonary disease and without atopic disease were selected as the control group.

The children underwent elective tonsillectomy after achievement of general anesthesia. BAL was performed before the surgical procedure. Flexible bronchoscopy with BAL was performed during a period free of exacerbation and pulmonary infiltration, as evaluated by means of C-reactive protein measurement. Pulmonary infiltrates and other abnormalities were excluded on the basis of chest radiographs. None of the children had received antibiotics, oral glucocorticosteroids, or antihistamines within the last week before bronchoscopy, and inhaled glucocorticosteroids were withheld for 12 hours before bronchoscopy.

BAL

Bronchoscopy was performed as described previously.²² The BALF was filtered through 2 layers of sterile gauze. The first aliquot was used for microbiologic evaluation, and the remaining aliquots were pooled and centrifuged. The supernatant was stored at -80°C . Cells were used for differential cell counts on May-Grünwald-Giemsa-stained cytospin preparations. Viability of cells was determined by means of erythrosin dye exclusion testing. Residual material from the BALF was used for flow cytometry and quantitation of chemokines.

Antibodies

The following antibodies were used: CD4-allophycocyanine mouse IgG1, CD19-phycoerythrin (PE) mouse IgG2a, CCR5-PE mouse IgG2a, CCR4-PE mouse IgG2a (BD Pharmingen, Heidelberg, Germany), CD8-phycoerythrin 5 mouse IgG1 (Immunotech, Marseille, France), CD69-PE mouse IgG2a, CXCR3-FITC mouse IgG1, and CCR3-FITC rat IgG2a (R&D Systems, Wiesbaden, Germany). Mouse IgG1-FITC, mouse IgG2a-PE (Immunotech, Marseille, France), and rat IgG2a-FITC (kindly provided by Dr E. Kremmer, GSF-Institute of Molecular Immunology, Munich, Germany) were used as isotype controls.

Flow cytometry

Resuspended BALF cells were analyzed by means of 4-color flow cytometry (FACSCalibur; Becton-Dickinson, Heidelberg, Germany). Because lymphocyte gating in BALF according to forward-scatter/side-scatter (FSC/SSC) characteristics is hampered by cellular overlay (Fig 1, A), BALF lymphocytes were identified by the surface expression of CD8 (Fig 1, B) and then gated back into an FSC/SSC plot (Fig 1, C). The cells falling into this FSC/SSC gate were used for further phenotypic analyses. The identity of the lymphocyte subsets was proved by gating CD4 versus CD8 (Fig 1, D). The percentage of chemokine receptor-positive cells was determined on CD4⁺ and CD8⁺ cells (Fig 1, E) after defining

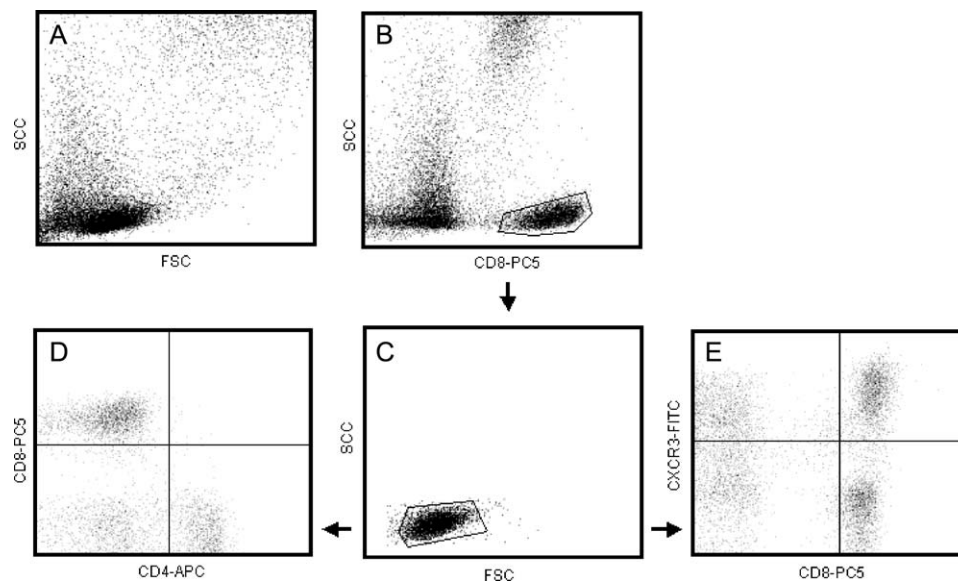


FIG 1. Lymphocyte gating in BALF according to FSC/SSC characteristics is hampered by cellular overlay (**A**). Therefore lymphocytes were identified by the surface expression of CD8 (**B**) and then gated back into an FSC/SSC plot (**C**). Because CD4⁺ and CD8⁺ lymphocytes have identical FSC/SSC characteristics, the cells falling into this FSC/SSC gate (Fig 1, **C**) were used for further phenotypic analyses (**D** and **E**).

TABLE I. Patient characteristics

	Asthmatic patients	Patients with chronic cough	Control patients
N	12	15	10
Age (y)	10 (5.3-16.3)*	7 (5.2-17.3)	6.3 (3.7-15.6)
Sex (F/M)	6/6	8/7	6/4
Total IgE (IU/mL)	250 (159-922)‡	25 (4-67)	20 (14-76)
Specific IgE (RAST class ≥2)	11‡	0	0
Blood eosinophils (%)	4 (0-8) †§	1.5 (0-4)	1 (0-3)
Family history of atopy	10†§	3	2
Skin prick positive	12‡	0	0
Wheezing	12‡	3†	0
FEV ₁ (% predicted)	72 (66-87)§	90 (76-110)	—
FEV ₁ /FVC	71 (62-83)	88 (75-120)	—
MEF ₂₅₋₇₅ (% predicted)	54 (23-74)	76 (65-117)	—
Differential cell counts in BALF			
Total cells (× 10 ³ /mL)	240 (95-870)†	270 (26-1080)†	89 (83-97)
Macrophages (%)	85 (31-95)	83 (15-89)	94 (81-92)
Lymphocytes (%)	9 (2-42)†	10 (2-35)†	4 (2-13)
Neutrophils (%)	5 (0-39)†	6 (1-29)†	2 (0-3)
Eosinophils (%)	1 (0-2)	0 (0-2)	0 (0-1)
Mast cells (%)	0 (0-6)	1 (0-2)	0 (0-0)

*Results are expressed as medians, with ranges shown in parentheses.

†*P* < .05 compared with control patients.

‡*P* < .01 compared with control patients.

§*P* < .05, asthmatic patients versus patients with chronic cough.

||*P* < .01, asthmatic patients versus patients with chronic cough, Mann-Whitney *U* test.

a cutoff value according to the isotype control. Calculations were performed with Cell Quest software 3.1 (Becton-Dickinson).

Detection of cytokines and chemokines

Human IP-10, ITAC, Mig, MDC, and TARC levels were determined in duplicate by sandwich ELISA (R&D Systems, Minneapolis, Minn). In brief, samples were pipetted into antibody-

precoated wells and detected with 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 a standard curve. The minimum detection limits were 1.7, 13.9, 3.8, 15.6, and 7 pg/mL, and the maximum detection limits were 1000, 3000, 1000, 1000, and 2000 pg/mL, respectively. IFN- γ , IL-5, and IL-4 were measured in

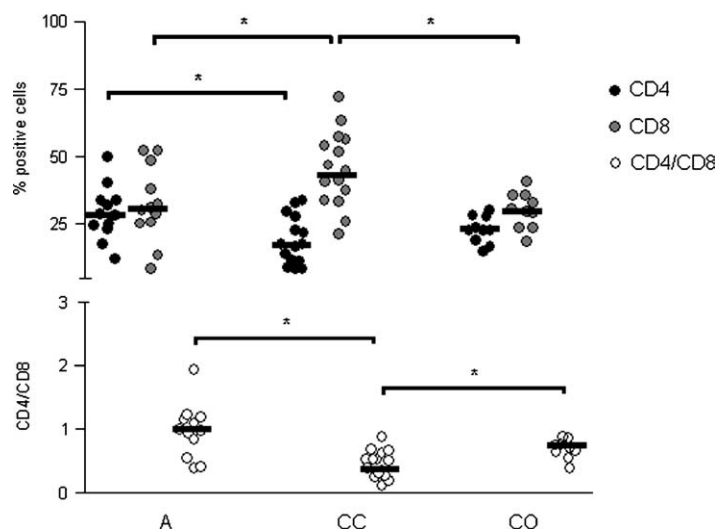


FIG 2. Percentages of CD4⁺ and CD8⁺ T cells and CD4/CD8 ratios in BALF of asthmatic patients (A), patients with chronic cough (CC), and control patients (CO). Median values are shown by horizontal bars. Differences between the patient groups were tested with the Mann-Whitney U test (* $P < .05$).

duplicate by using a multiplex, particle-based, flow cytometric assay (BioPlex; Bio-Rad Laboratories, Hercules, Calif). The minimum detection limits were 1.5, 1.2, and 2.3 pg/mL, and the maximum detection limits for all were 1000 pg/mL. Monoclonal antibodies coupled to dyed 5.5- μ m polystyrene beads and directed against IFN- γ , IL-5, and IL-4, respectively, were incubated with the sample or with a cytokine standard. After a series of washes to remove unbound protein, a biotinylated antibody with specificity for another epitope on the analyte was added to the reaction. The bound biotinylated antibody was further detected by the addition of streptavidin-phycoerythrin. The reaction mixture was analyzed with a flow cytometric microtiter plate reader system. The concentrations of cytokines were calculated by using the Bio-Plex software program with a standard curve derived from the cytokine standard.

Statistical analysis

The nonparametric Mann-Whitney U test was applied. Correlations were verified with the Spearman rank correlation test ($r > 0.5$). A P value of .05 or less was regarded as significant (SPSS statistical program, version 11.5; SPSS Inc, Chicago, Ill).

RESULTS

Patients

Thirty-seven children (21 male, 16 female patients; median age, 8.3 years; age range, 5.2-17.3 years) were selected for this study (Table I). Twelve children fulfilled the criteria for allergic asthma. These children were atopic, as shown by positive skin prick test results, increased total serum IgE levels, and specific serum IgE levels. According to the National Institutes of Health classification,²³ all asthmatic children had moderate-to-severe persistent asthma with symptoms for at least 3 years, despite use of long-acting inhaled β_2 -bronchodilators and maintenance therapy with medium-to-high-dose inhaled glucocorticosteroids. Bronchoscopy and BAL were ordered by the attending physician for further diagnostic evalua-

tion. Fifteen children had severe cough for at least 3 months per year without any detectable underlying cause. There was no evidence for infection with *Chlamydia*, *Mycoplasma*, or *Bordetella pertussis* species. These children had no respiratory tract infections, as shown by means of chest imaging and C-reactive protein measurement; no signs of atopy; and an unsatisfactory response after therapy with β_2 -bronchodilators. Ten patients who were free of respiratory tract infections and had no asthma or atopic diseases were enrolled in a previous study with entirely different research questions from the study presented here²² and were included as control subjects. None of these children had any signs of airway infection and never had asthma or other allergic diseases. Patients with asthma were slightly older and had significantly higher levels of total and specific serum IgE levels and eosinophil counts in the peripheral blood compared with those in the chronic cough and control groups.

Leukocyte subsets in BALF

The recovery of BALF and the viability of cells did not differ significantly among the 3 patient groups. Asthmatic patients and patients with chronic cough had significantly more total cells, lymphocytes, and neutrophils in the differential cell counts of BALF compared with children without airway disease. Eosinophils were significantly increased in asthmatic children. CD8⁺ cells were the predominant T-cell population in 32 of 37 patients. Patients with asthma had slightly higher percentages of CD4⁺ T cells ($P < .05$) in BALF compared with those in patients with chronic cough or control subjects (Fig 2). In contrast, patients with chronic cough showed higher percentages of CD8⁺ T cells ($P < .05$) in BALF compared with those in patients with asthma or control patients. This resulted in significantly higher CD4/CD8 ratios in asthmatic patients versus patients with chronic cough or

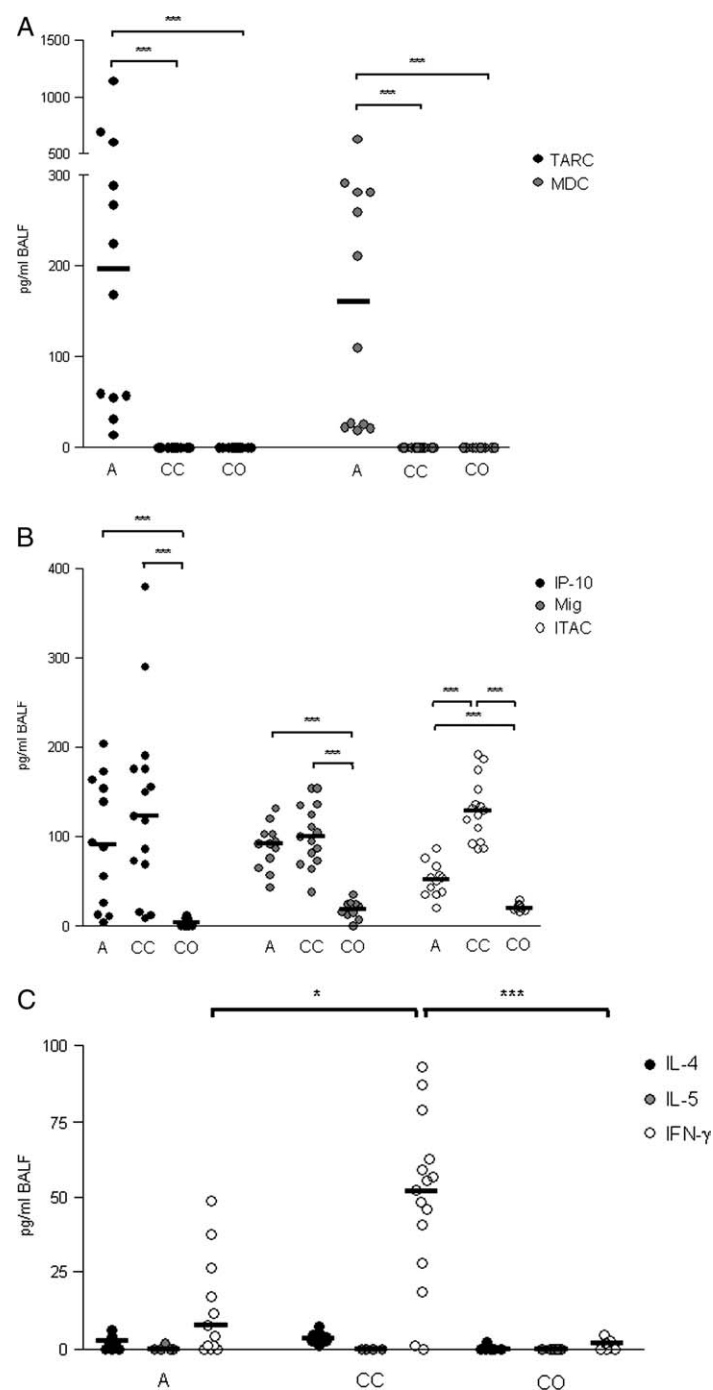


FIG 3. Levels of the CCR4 (T_H2) chemokines TARC and MDC (**A**); the CXCR3 (T_H1) chemokines IP-10, Mig, and ITAC (**B**); and IL-4, IL-5, and IFN-γ (**C**) in BALF of asthmatic patients (A), patients with chronic cough (CC), and control patients (CO). Median values are shown by horizontal bars. Differences between the patient groups were tested with the Mann-Whitney U test (* $P < .05$, *** $P < .001$).

control patients. B lymphocytes, defined as CD19⁺ cells, were present in 9 samples, albeit at very low percentages (data not shown).

Chemokine and cytokine levels in BALF

TARC and MDC were only found in patients with asthma, whereas none of the patients in the chronic cough

or control groups had detectable amounts (Fig 3, A). TARC and MDC showed a strong significant positive correlation with each other ($r = 0.96$, $P < .001$). In addition, the quantities of TARC ($r = 0.66$, $P < .01$) and MDC ($r = 0.86$, $P < .01$) correlated significantly with serum IgE levels in asthmatic patients but not in patients in the chronic cough or control groups. The 2 patients with

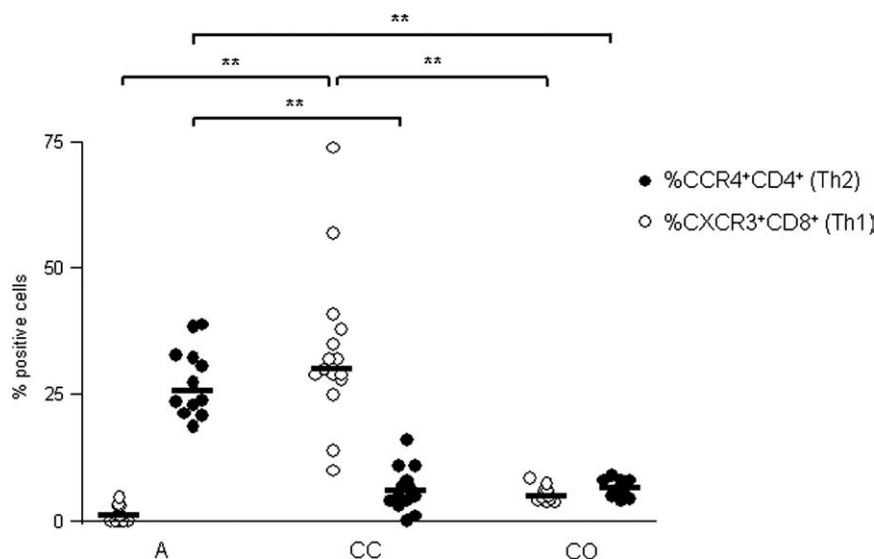


FIG 4. Percentages of CXCR3⁺CD8⁺ (Th1) and CCR4⁺CD4⁺ (Th2) double-positive cells in BALF of asthmatic patients (A), patients with chronic cough (CC), and control patients (CO). Median values are shown by horizontal bars. Differences between the patient groups were tested with the Mann-Whitney *U* test (***P* < .01).

the highest levels of TARC had also the highest IgE levels of all patients, and the patient with the highest TARC level had the lowest FEV₁ (66% of predicted value) of all patients and used high-dose inhaled glucocorticosteroids. Levels of ITAC were significantly higher in patients with chronic cough compared with those in the asthmatic and control groups, whereas IP-10 and Mig levels did not differ between children with asthma and those with chronic cough (Fig 3, B). IL-5 and IL-4 were not verifiable or only present in very low amounts in all 3 patient groups. IFN- γ was detected at slightly higher levels in the chronic cough group (Fig 3, C). In addition, ITAC and IFN- γ correlated with each other ($r = 0.72$, $P < .01$).

Chemokine receptor-expressing BALF lymphocytes

We next asked whether increased levels of MDC and TARC are associated with an increased frequency of CCR4⁺ T cells in asthmatic patients and, as a second step, whether ITAC is related to the frequency of CXCR3⁺ T cells. In addition, the contribution of CCR5⁺ and CCR3⁺ T cells to the local cellular infiltrate was evaluated.

CCR4 was expressed on a higher percentage of CD4⁺ cells compared with CD8⁺ cells. The majority of CCR4⁺CD4⁺ T cells were activated, as shown by CD69 expression (median CD69⁺ cells, 80%). The percentages of CCR4⁺CD4⁺ cells were significantly higher in asthmatic patients compared with those seen in children with chronic cough or control children (Fig 4). TARC and MDC were significantly correlated with CCR4⁺CD4⁺ T cells in asthmatic patients but not in patients of the other groups (Fig 5, A and B). Similarly, percentages of CCR4⁺CD4⁺ T cells correlated positively with serum IgE levels ($r = 0.6$, $P < .01$) and negatively with FEV₁ (Fig 5, C) only in

asthmatic patients. CCR3⁺ lymphocytes were not detectable in BALF (data not shown).

In contrast to CCR4, CXCR3⁺ was expressed in all patients on a higher percentage of CD8⁺ T cells compared with CD4⁺ T cells. The majority of CXCR3⁺CD8⁺ T cells were activated (median CD69⁺ cells, 71%). A significantly higher percentage of CXCR3⁺CD8⁺ lymphocytes was found in patients with chronic cough versus that in patients with asthma and control patients (Fig 4). Seven asthmatic patients had no CXCR3⁺CD8⁺ lymphocytes in BALF. In an analysis that was restricted to patients with chronic cough, strong correlations between percentages of CXCR3⁺CD8⁺ T cells and ITAC (Fig 6) and IFN- γ ($r = 0.7$, $P < .01$; data not shown) levels were found. No such correlations existed in asthmatic or control patients.

CCR5⁺ lymphocytes were present in all patients, without differences between the groups (data not shown).

Patients without diagnosis

We further asked whether levels of TARC, MDC, and ITAC and percentages of CCR4⁺CD4⁺ and CXCR3⁺CD8⁺ T cells differentiate patients with cough who can not be clearly assigned to the atopic asthma or the chronic cough group. Therefore we added 2 patients with cough in whom a clear clinical diagnosis could not be made. Neither patient showed clinical symptoms of asthma, and both had normal pulmonary function. One patient was atopic (RAST and skin prick testing). Percentages of BALF CD4⁺ T cells were 25% (nonatopic patient) and 22% (atopic patient), and percentages of CD8⁺ T cells were 34% (nonatopic) and 30% (atopic). IL-4 and IL-5 were not detectable in either patient. IFN- γ levels were 56 pg/mL BALF in the nonatopic individual and 39 pg/mL BALF in the atopic patient. Both patients

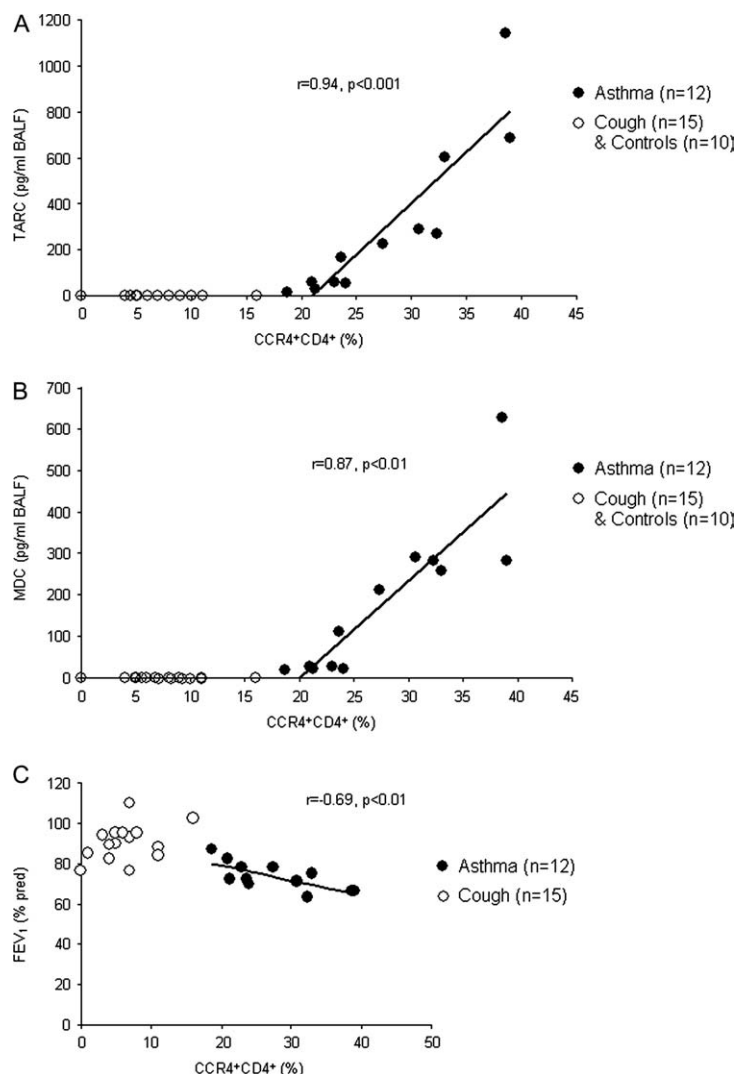


FIG 5. Correlations between percentages of CCR4⁺CD4⁺ T cells, levels of TARC (**A**), levels of MDC (**B**), and FEV₁ (**C**). The regression line and the correlation coefficient are only shown for children with asthma.

had high pulmonary levels of TARC (nonatopic, 192 pg/mL BALF; atopic, 324 pg/mL BALF) and MDC (nonatopic, 124 pg/mL BALF; atopic, 211 pg/mL BALF). BALF levels of ITAC, IP-10, and Mig were low. Furthermore, these children showed high percentages of CCR4⁺CD4⁺ cells (nonatopic, 23%; atopic, 31%), whereas CXCR3⁺CD8⁺ cells were low or absent (nonatopic, 5%; atopic, 0%). Therefore on the basis of the BALF analysis, these patients with cough of unknown origin would be assigned to the asthma group.

DISCUSSION

The present work demonstrates that TARC and MDC levels in BALF were increased in asthmatic children, whereas ITAC and, to a lesser degree, IFN- γ levels were increased in nonatopic, nonasthmatic children with chronic cough. These findings were accompanied by

increased frequencies of BALF CCR4⁺CD4⁺ T cells in children with asthma, whereas CXCR3⁺CD8⁺ lymphocytes were significantly more frequent in children with chronic cough compared with children in the asthmatic or control groups. Significant positive correlations between frequencies of CCR4⁺CD4⁺ T cells and levels of TARC, MDC, and IgE were present in asthmatic children but not in children with chronic cough or control children. A significant negative correlation was found between CCR4⁺CD4⁺ T cells and FEV₁ in asthmatic children. Vice versa, levels of ITAC correlated with CXCR3⁺CD8⁺ BALF T cells in children with chronic cough. CCR3⁺ is another putative marker of T_H2 lymphocytes but was undetectable in BALF T cells, which is in accordance with findings in the adult human lung.^{14,24,25} Asthmatic children were somewhat older than children of the other 2 groups. There is a possibility that children with chronic cough or control children will still have allergic asthma, which would signify an overlap

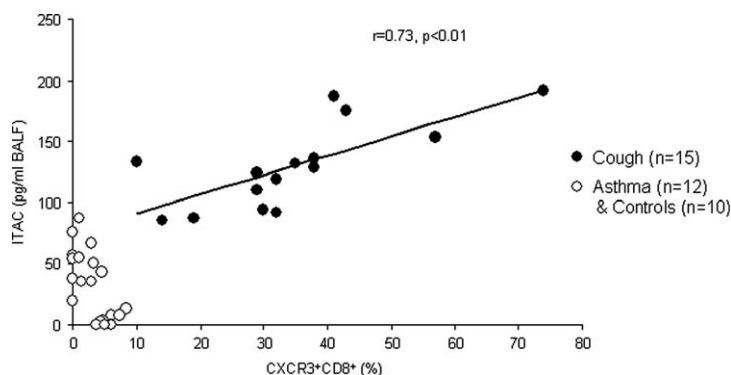


FIG 6. Correlation between levels of ITAC and percentages of CXCR3⁺CD8⁺ T cells. The regression line and the correlation coefficient are only shown for children with chronic cough.

between the groups that would only become apparent later. However, this seems unlikely because no signs of atopic sensitization could be demonstrated in these children, and a positive family history of allergic disease, which is a predictor for the development of allergies, was clearly less frequent in this group. In addition, no correlation of any immune marker with age was demonstrated for all groups. Because the study population was set up retrospectively, an investigator who was blinded for all experimental results performed the selection of patients. To minimize the effect of causative overlap, we defined clear criteria for the diagnoses of asthma and chronic cough.

In the majority of patients, CD8⁺ cells predominated in BALF, which is in line with previously published data in children.²⁶⁻²⁸ We observed slight but significantly higher frequencies of CD8⁺ cells in children with chronic cough compared with the other 2 groups. However, these distinctions were not absolute and were not useful in the clear differentiation of asthma versus nonasthmatic chronic cough, as expected from the results of other studies.^{29,30} IFN- γ and IL-4 in BALF were not helpful in this respect either because they were almost undetectable (IL-4) or showed overlap between the patient groups (IFN- γ). IL-5 was almost undetectable, which contradicts observations in infants with acute asthma made by Kim et al.²⁹ Beneath slight differences in the patient characterization, we have no clear explanation for this discrepancy. In contrast, levels of TARC, MDC, and, to a lesser degree, ITAC had a better discriminatory capacity. The same applies to the frequency of CXCR3⁺CD8⁺ and CCR4⁺CD4⁺ BALF cells. One study in adults also reported increased levels of MDC in nonchallenged asthmatic patients¹⁷ that were in the range of the MDC levels observed in this study. Although levels of MDC and TARC were clearly lower than those reported for allergen-challenged individuals, these asthma baseline levels seem to have a biologic relevance because they were accompanied by increased frequencies of CCR4⁺CD4⁺ T cells. One study encompassing a small number ($n = 4$) of adult asthmatic subjects did not observe differences in the frequency of chemokine receptor-positive BALF lymphocytes from asthmatic

patients compared with those from healthy control subjects.²⁴ In addition to the difference of age, our patients had more severe asthma compared with the patients of Campbell et al.²⁴ TARC and MDC are produced by airway epithelial cells^{31,32} and attract T_H2 lymphocytes. Our finding that TARC and MDC showed a significant positive association with serum IgE levels further supports their contribution to the pathogenesis of asthma. Consistent with current concepts of a T_H2 lymphocyte-based pathology of type I allergies, a significant negative correlation of CCR4⁺CD4⁺ T cells with FEV₁ was found in the asthmatic patients. To test whether TARC, MDC, and ITAC and CCR4⁺CD4⁺ and CXCR3⁺CD8⁺ T cells clearly differentiate patients with cough who cannot be assigned to the atopic asthma or the chronic cough group, we added 2 patients with cough in whom a clear clinical diagnosis could not be made. On the basis of the analysis of levels of TARC, MDC, and ITAC and percentages of CCR4⁺CD4⁺ and CXCR3⁺CD8⁺ T cells in BALF, these patients would be assigned to the asthma group.

In the majority of our children with chronic cough, an infection with a viral agent might be suspected because cystic fibrosis, foreign bodies, immune deficiencies, bacterial infections, microaspirations from gastroesophageal reflux disease, tracheobronchomalacia, dyskinetic cilia syndrome, chemical exposures, or nicotine abuse could be excluded as triggers for chronic cough. Cytotoxic type 1 T cells are the main effector cells for viral infections, and pulmonary CXCR3⁺CD8⁺ cells produce IFN- γ .³³ This suggests that in chronic cough of non-allergic origin, the bronchial epithelial cells release the IFN- γ -inducible chemokines IP-10, Mig, and ITAC. These in turn predominantly attract CXCR3⁺ effector T cells. Our finding that IFN- γ and ITAC correlated strongly with CXCR3⁺CD8⁺ cells in the patients with chronic cough further supports this hypothesis. If CXCR3⁺CD8⁺ cells predominate, they might further enhance the release of IFN- γ -inducible chemokines by the synthesis of IFN- γ and thereby initiate, amplify, and/or maintain a cytotoxic type 1 T-cell response. The finding that children with allergic asthma had markedly lower frequencies of such cells supports this hypothesis.

In summary, an inverse relationship between CXCR3⁺CD8⁺ and CCR4⁺CD4⁺ T cells and some of their respective ligands exists in asthmatic children versus children with nonasthmatic chronic cough. Therefore we speculate that these markers facilitate the discrimination of asthma versus chronic cough. To substantiate these initial observations, prospective studies need to be conducted in children with chronic cough of unknown origin. Because BAL is a comparatively invasive procedure, especially in children, induced sputum should be tested as a potential alternative to obtain material for the quantitation of the markers described here. Furthermore, direct functional analyses of the cells present in BALF will help to clarify their precise role in asthma or chronic cough in children.

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