

Quantitative and functional impairment of pulmonary CD4⁺CD25^{hi} regulatory T cells in pediatric asthma

Dominik Hartl, MD,^a Barbara Koller, MD,^a Alexander T. Mehlhorn, MD,^b Dietrich Reinhardt, MD,^a Thomas Nicolai, MD,^a Dolores J. Schendel, PhD,^{c,d} Matthias Griesel, MD,^a and Susanne Krauss-Etschmann, MD^{a,e} Munich and Freiburg, Germany

Background: Asthma is characterized by a T_H2 immune response. CD4⁺CD25^{hi} regulatory T cells (Tregs) have been proposed to prevent allergic diseases through suppression of T_H2 responses.

Objective: We sought to investigate the role of CD4⁺CD25^{hi} T cells in children with asthma.

Methods: CD4⁺CD25^{hi} Tregs and forkhead/winged-helix transcription factor FOXP3 mRNA levels were quantified in peripheral blood and bronchoalveolar lavage fluid (BALF) of 18 children with asthma, 10 children with chronic cough, and 13 control subjects without lung diseases. CD4⁺CD25^{hi} T cells were isolated from peripheral blood and BALF of asthmatic patients and control subjects, and their capacity to suppress proliferation and cytokine/chemokine production of autologous responder T cells was analyzed.

Results: CD4⁺CD25^{hi} T cells were decreased in BALF of asthmatic children compared with values in children with cough or control subjects. In children with asthma, inhaled corticosteroid treatment was associated with increased percentages of CD4⁺CD25^{hi} T cells in peripheral blood and BALF. Isolated BALF and peripheral blood CD4⁺CD25^{hi} T cells from nonasthmatic subjects suppressed proliferation and cytokine/chemokine production by CD4⁺CD25[−] responder T cells. BALF CD4⁺CD25^{hi} T cells from asthmatic subjects

failed to suppress proliferation and production of T_H2-associated cytokines and chemokines by CD4⁺CD25[−] responder T cells, which was restored after use of inhaled corticosteroids.

Conclusion: These findings provide the first evidence that pulmonary CD4⁺CD25^{hi} Tregs are impaired in pediatric asthma.

Clinical implications: Pulmonary Tregs might represent a therapeutic target in pediatric asthma. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Asthma, children, CD4⁺CD25^{hi} cells, regulatory T cells, FOXP3, bronchoalveolar lavage

Asthma is the most frequent chronic disease in childhood in Western countries and is characterized by a T_H2 immune response.¹ The parallel increase of both T_H2-associated allergic diseases and T_H1-associated autoimmune diseases²⁻⁵ has put forward the hypothesis that both allergic and autoimmune diseases might result from a functional or quantitative deficiency of regulatory T cells (Tregs) that control T_H1 and T_H2 immune responses.⁶

Tregs are a subgroup of T lymphocytes with immune suppressive properties on effector T cells.^{7,8} They are found within the CD4⁺CD25^{hi} population, whereas CD4⁺CD25^{intermediate/low} cells constitute a heterogeneous population of activated T cells.⁹ A further hallmark of CD4⁺CD25^{hi} Tregs is the selective expression of the forkhead/winged-helix transcription factor FOXP3.^{7,10} The first indication that Tregs might play a role in allergic disorders in human subjects came from the observation that FOXP3-deficient patients with immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome have atopic disease.¹¹ Subsequent studies analyzed Tregs in patients with allergic diseases.¹²⁻¹⁵ Peripheral blood-derived Tregs from nonatopic subjects and from the majority of patients with allergic rhinitis suppressed the production of both T_H1 and T_H2 cytokines.¹² Further studies showed an impaired suppressive capacity of Tregs in atopic patients, especially during the pollen season.^{13,15} Children who outgrew cow's milk allergy had increased numbers of circulating Tregs compared with children with persistent active allergic disease.¹⁴

Thus far, the role of Tregs in allergic disease in human subjects has been exclusively investigated in peripheral

From ^aChildren's Hospital of the Ludwig-Maximilians-University of Munich; ^bthe Medical Center, University of Freiburg; ^cthe Institute of Molecular Immunology and ^dClinical Cooperation Group "Immune Monitoring," GSF National Research Center for Environment and Health, Munich; and ^eGSF National Research Center for Environment and Health, Clinical Cooperation Group "Pediatric Immune Regulation," Munich.

Supported by grants from the Else-Kröner-Fresenius Stiftung and the Friedrich-Baur-Stiftung, a grant of the University and Science Program of the Ludwig-Maximilians-University (HWP), and by the Clinical Cooperation Groups "Pediatric Immune Regulation" and "Immune Monitoring." Dominik Hartl, MD, is supported by the Society for Pediatric Pneumology (GPP).

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication January 10, 2007; revised February 14, 2007; accepted for publication February 14, 2007.

Reprint requests: Susanne Krauss-Etschmann, and Dominik Hartl, MD, Children's Hospital of the Ludwig-Maximilians-University and GSF National Research Center for Environment and Health, Clinical Cooperation Group "Pediatric Immune Regulation," Lindwurmstr. 2a, D-80337 Munich, Germany. E-mail: dominik.hartl@med.uni-muenchen.de.

0091-6749/\$32.00

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doi:10.1016/j.jaci.2007.02.023

Abbreviations used

aTreg:	Adaptive regulatory T cell
BAL:	Bronchoalveolar lavage
BALF:	Bronchoalveolar lavage fluid
MDC:	Macrophage-derived chemokine
nTreg:	Naturally occurring regulatory T cell
PE:	Phycoerythrin
TARC:	Thymus- and activation-regulated chemokine
Treg:	Regulatory T cell

blood. No data are available on quantities or function of pulmonary Tregs in healthy or asthmatic human lungs. Because the characteristics of the pulmonary immune system differ from cellular subsets in peripheral blood,^{16,17} studies of lung-derived T cells might be of higher relevance than analyses of peripheral blood. We hypothesized that the T_H2 predominance in children with asthma results from a quantitative or functional lack of pulmonary $CD4^+CD25^{hi}$ Tregs. To test this hypothesis, we quantified $CD4^+CD25^{hi}$ Treg cells and FOXP3 mRNA levels in bronchoalveolar lavage fluid (BALF) and in peripheral blood of children with asthma, children with chronic cough, and control subjects without lung diseases. To examine the functional characteristics of pulmonary Tregs, $CD4^+CD25^{hi}$ T cells were isolated from peripheral blood and BALF of asthmatic patients and control subjects, and their capacity to suppress proliferation and cytokine/chemokine production of autologous responder T cells was analyzed. Our data indicate that children with asthma have a quantitative and functional impairment of pulmonary $CD4^+CD25^{hi}$ Tregs that is restored by corticosteroid treatment.

METHODS

Patients

Eighteen children with asthma, 10 children with chronic cough, and 13 control children without lung diseases were characterized^{18,19} and included in this study (see Table E1 in the Online Repository at www.jacionline.org). For a detailed patient characterization, see the Methods section in the Online Repository at www.jacionline.org.

Bronchoalveolar lavage

Bronchoscopy was performed by using a flexible bronchoscope with an outer diameter of 3.5 mm in children less than 10 years of age and a 4.9-mm diameter in older children. The bronchoscope was wedged in the lingula or one of its segments in all patients. In all children bronchoscopy was performed during sedation and topical or general anesthesia. Complications of bronchoscopy did not occur in any of the study patients. Bronchoalveolar lavage (BAL) was performed with 4×1 mL of 0.9% NaCl per kilogram warmed to body temperature. The obtained BALF was filtered through 2 layers of sterile gauze. The first aliquot was used for microbiologic assessment, and the remaining aliquots were pooled and centrifuged. The supernatant was stored at -80°C . Cells were used for differential cell count on May-Grünwald-Giemsa-stained cytospin preparations. Viability of cells was determined by using the erythrosin dye exclusion test.

Flow cytometry

Peripheral blood or BALF cells were analyzed by using 4-color flow cytometry (FACSCalibur; Becton-Dickinson, Heidelberg, Germany), as described previously.²⁰ The following mAbs were used: CD4-allophycocyanine mouse IgG1, CD8-phycoerythrin 5 mouse IgG1 (Immunotech, Marseille, France), CD25-fluorescein isothiocyanate mouse IgG1, CD45RO-phycoerythrin (PE) mouse IgG2a (BD PharMingen, San Diego, Calif), and CD69-PE mouse IgG2a (R&D Systems, Wiesbaden, Germany). Mouse IgG1-fluorescein isothiocyanate, mouse IgG2a-PE, mouse IgG1-phycoerythrin 5, and mouse IgG1-allophycocyanine (Immunotech, Marseille, France) were used as isotype controls. T cells in peripheral blood or BALF were identified as described previously.²⁰ $CD25^{hi}$ -expressing T cells were defined as $CD4^+$ T cells that had a fluorescence intensity for CD25 of greater than 2×10^2 and appeared as a tail to the right from the major population containing both $CD4^+CD25^{low}$ and $CD4^+CD25^-$ cells (Fig 1), according to Baecher-Allan et al.⁹ The gating and instrument settings were controlled in every experiment and were maintained throughout the whole study in all control and patient samples analyzed. $CD4^+CD25^{hi}$ T cells were referred to as Tregs, whereas $CD25^{low}$ -expressing T cells were assumed to be activated T cells. Calculations were performed with Cell Quest software 3.1 (Becton-Dickinson, Heidelberg, Germany).

For a detailed description of cell isolation, cell-culture reagents, and coculture suppression assays, see the Methods section in the Online Repository at www.jacionline.org.

Cytokine and chemokine production

Cell-free culture supernatants were collected after 4 days of culture and stored in aliquots at -70°C before analysis to quantify cytokine (IFN- γ , IL-4, and IL-13) production. For analysis of thymus- and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC) production, cells were cultured in the abovementioned conditions plus IL-4 (5 ng/mL). Human IL-4, IFN- γ , IL-13, TARC, and MDC levels were determined in duplicates by means of sandwich ELISA (R&D Systems, Minneapolis, Minn), according to the manufacturer's instructions. The minimum detection limits were 5, 7, 4, 15.6, and 7 pg/mL, respectively, and the maximum detection limits were 1000, 2000, 2000, 1000, and 2000 pg/mL, respectively.

Quantitative RT-PCR

BALF and whole peripheral blood cells were lysed in Trizol LS Reagent (Invitrogen, Life Technologies, Karlsruhe, Germany) and stored at -20°C until RNA extraction. Total RNA was isolated according to the manufacturer's instructions and reverse transcribed into cDNA. Contamination with genomic DNA was excluded by RNA controls without reverse transcriptase in the cDNA synthesis reaction. The following mRNA-specific oligonucleotide primers were used: FOXP3 (5'-CAGCACATTCCCAGAGTTCTCTC-3'; 5'-GCGTGTGAACCAAGTGGTAGATC-3') and 18-S rRNA (5'-AGTCCTGCCCCTTGTACACA-3'; 5'-GATCCGAGGGCCTCACTAAC-3'), as previously published by Yagi et al.²¹ Expression levels were determined in duplicates by using real-time RT-PCR with SYBR green and the iCycler iQ detection system (Bio-Rad, Hercules, Calif), according to the manufacturer's instructions. Threshold cycle values for genes of interest were normalized to 18-S rRNA and used to calculate the relative mRNA expression.

Statistical analysis

The nonparametric Kruskal-Wallis test was applied to compare all 3 patient groups. When a statistically significant difference was found, the nonparametric Mann-Whitney *U* test was used for comparison between 2 groups. Correlations were verified with the Spearman

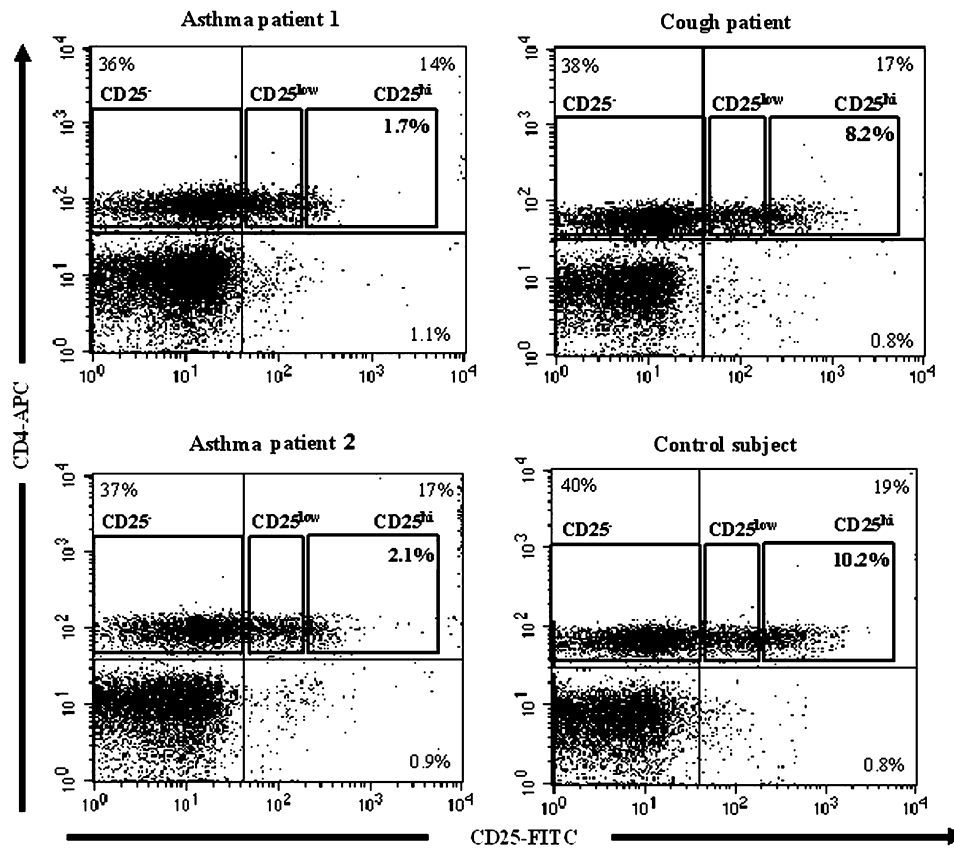


FIG 1. Characterization of CD4⁺CD25^{hi} T cells in BALF. BALF lymphocytes were identified as described previously.²⁰ Electronic gates were set on CD4⁺CD25⁻, CD4⁺CD25^{low}, and CD4⁺CD25^{hi} T cells on the basis of the CD25 fluorescence intensity, as shown in the dot plots. Only CD4⁺CD25^{hi} T cells were considered Tregs. Representative examples of BALF T cells of 2 individual children with asthma (upper and lower left), one child with chronic cough (upper right), and one control child (lower right) are shown. APC, Allophycocyanine; FITC, fluorescein isothiocyanate.

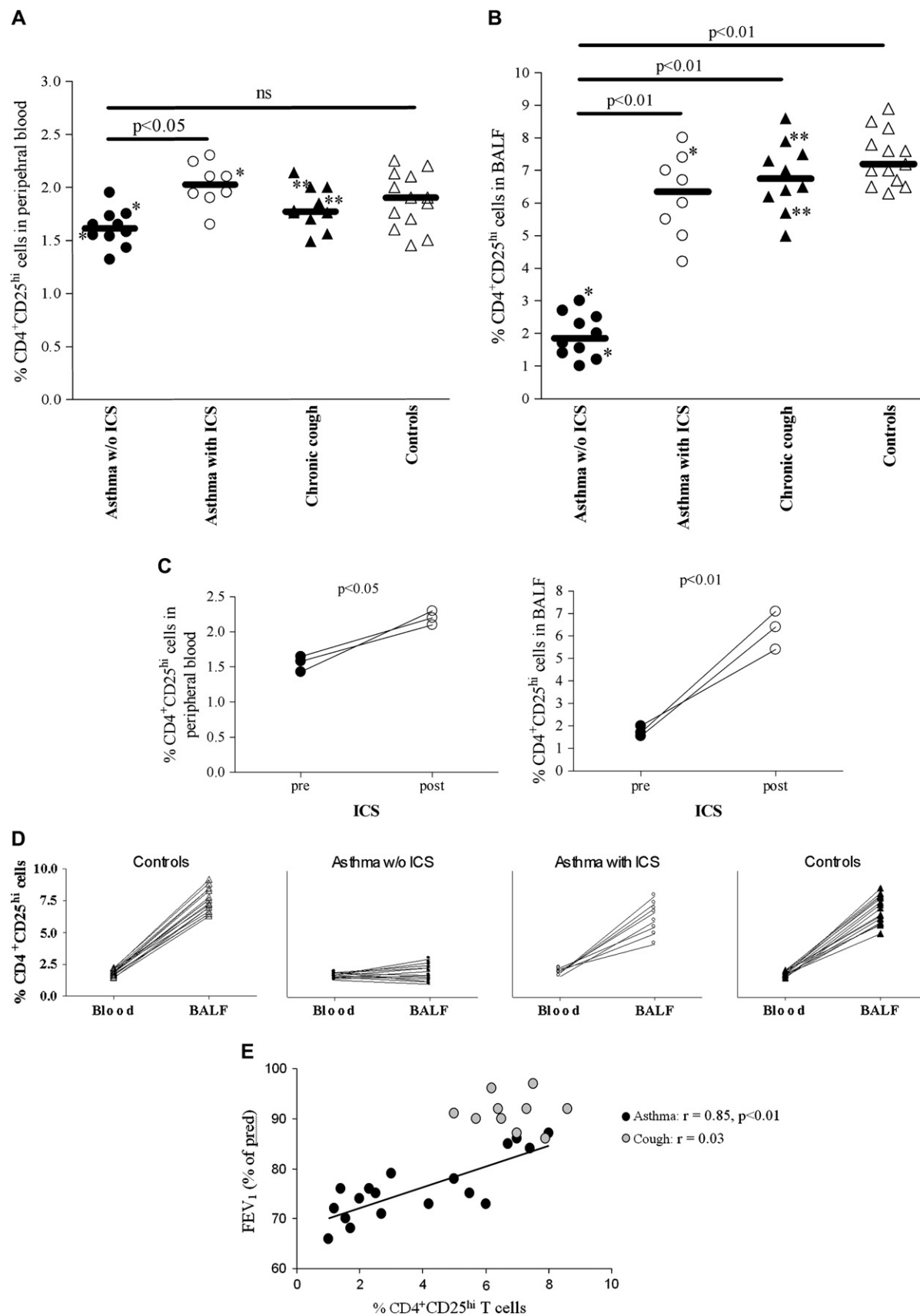
rank correlation test ($r > 0.3$). A P value of less than .05 was regarded as significant (SPSS Statistical Program, version 11.5; SPSS Inc, Chicago, Ill).²²

RESULTS

Decreased CD4⁺CD25^{hi} T cells in BALF of children with asthma

Taking all groups together, peripheral blood T cells included a median of 5% total CD4⁺CD25^{hi+low} T cells (range, 4% to 8%) and a median of 1.9% CD4⁺CD25^{hi} T cells (range, 1.4% to 2.4%), whereas BALF T cells contained a median of 12% CD4⁺CD25^{hi+low} T cells (range, 2% to 19%) and a median of 6.5% CD4⁺CD25^{hi} T cells (range, 1% to 10.2%). CD4⁺CD25^{hi} T cells in peripheral blood and BALF were CD45RO⁺ (95% and 90%, respectively) and did not express the early activation marker CD69. In peripheral blood the percentages of CD4⁺CD25^{hi} cells were slightly lower in children with asthma not treated with corticosteroids compared with those in children with asthma receiving inhaled corticosteroids, whose levels were similar to those of the 2 control

groups (Fig 2, A). In BALF percentages of CD4⁺CD25^{hi} T cells were markedly lower in untreated children with asthma compared with those in children with asthma receiving inhaled corticosteroids, whose levels again were similar to those of the 2 control groups (Fig 2, B). After 4 weeks of treatment with inhaled corticosteroids in asthmatic subjects who had not used corticosteroids previously, CD4⁺CD25^{hi} T cells were increased in peripheral blood and even more so in BALF (Fig 2, C). In control subjects, children with chronic cough, and asthmatic subjects treated with inhaled corticosteroids, percentages of CD4⁺CD25^{hi} T cells were higher in BALF than in peripheral blood (Fig 2, D). In contrast, in nontreated asthmatic subjects the percentages of bronchoalveolar CD4⁺CD25^{hi} T cells were similar to those in peripheral blood. Percentages of CD4⁺CD25^{hi} T cells in BALF were positively associated with FEV₁ in children with asthma but not in children with chronic cough (Fig 2, E). Percentages of CD4⁺CD25^{hi} T cells in BALF correlated inversely with levels of the T_H2-associated chemokines TARC and MDC in BALF ($r = -0.76$, $P < .01$ and $r = -0.79$, $P < .01$, respectively; data not shown).



FOXP3 mRNA expression

Overall, CD4⁺CD25^{hi} T cells in peripheral blood or BALF expressed high levels of FOXP3 mRNA, whereas CD4⁺CD25^{low} expressed no FOXP3 mRNA or at low levels only (data not shown). In peripheral blood FOXP3 mRNA expression levels were lower in untreated asthmatic subjects compared with those in asthmatic subjects treated with inhaled corticosteroids, whose levels were similar to those of the 2 control groups (see Fig E1, A, in the Online Repository at www.jacionline.org). In BALF asthmatic children without inhaled corticosteroid treatment had significantly lower levels of FOXP3 mRNA in BALF cells compared with levels in corticosteroid-treated children with asthma, whose levels were again similar to those of the 2 control groups (see Fig E1, B, in the Online Repository at www.jacionline.org). Asthmatic children receiving inhaled corticosteroid therapy had higher FOXP3 mRNA expression levels in BALF compared with those in children not using inhaled corticosteroids. FOXP3 expression levels increased significantly in peripheral blood and BALF after 4 weeks of corticosteroid treatment (see Fig E1, C, in the Online Repository at www.jacionline.org). Similar to CD4⁺CD25^{hi} T cells, FOXP3 mRNA levels were higher in BALF compared with those in peripheral blood in control subjects and patients with chronic cough, whereas in noncorticosteroid-treated asthmatic subjects the BALF FOXP3 mRNA expression levels were similar to those found in peripheral blood (see Fig E1, D, in the Online Repository at www.jacionline.org). BALF FOXP3 mRNA expression levels correlated positively with frequencies of CD4⁺CD25^{hi} T cells in BALF ($r = 0.79$, $P < .01$; data not shown). Furthermore, FOXP3 mRNA expression levels in BALF cells correlated positively with FEV₁ ($r = 0.68$, $P < .01$) in asthmatic children but not in children with chronic cough.

Impaired pulmonary Treg function in children with asthma

To verify whether percentages of CD4⁺CD25^{hi} T cells in BALF indeed corresponded to Tregs, CD4⁺CD25^{hi} T cells were isolated from BALF and peripheral blood, and their suppressive capacity on proliferation and cytokine production of peripheral and lung responder T cells was analyzed in an experimental cross-design. Overall, BALF-isolated CD4⁺CD25^{hi} T cells suppressed the proliferation (Fig 3, A) and cytokine (Fig 3, B-D) and chemokine (Fig 4) production by autologous responder T cells to a similar extent as blood-derived Tregs. Peripheral blood CD4⁺CD25^{hi} T cells from asthmatic subjects showed similar suppressive characteristics compared with those

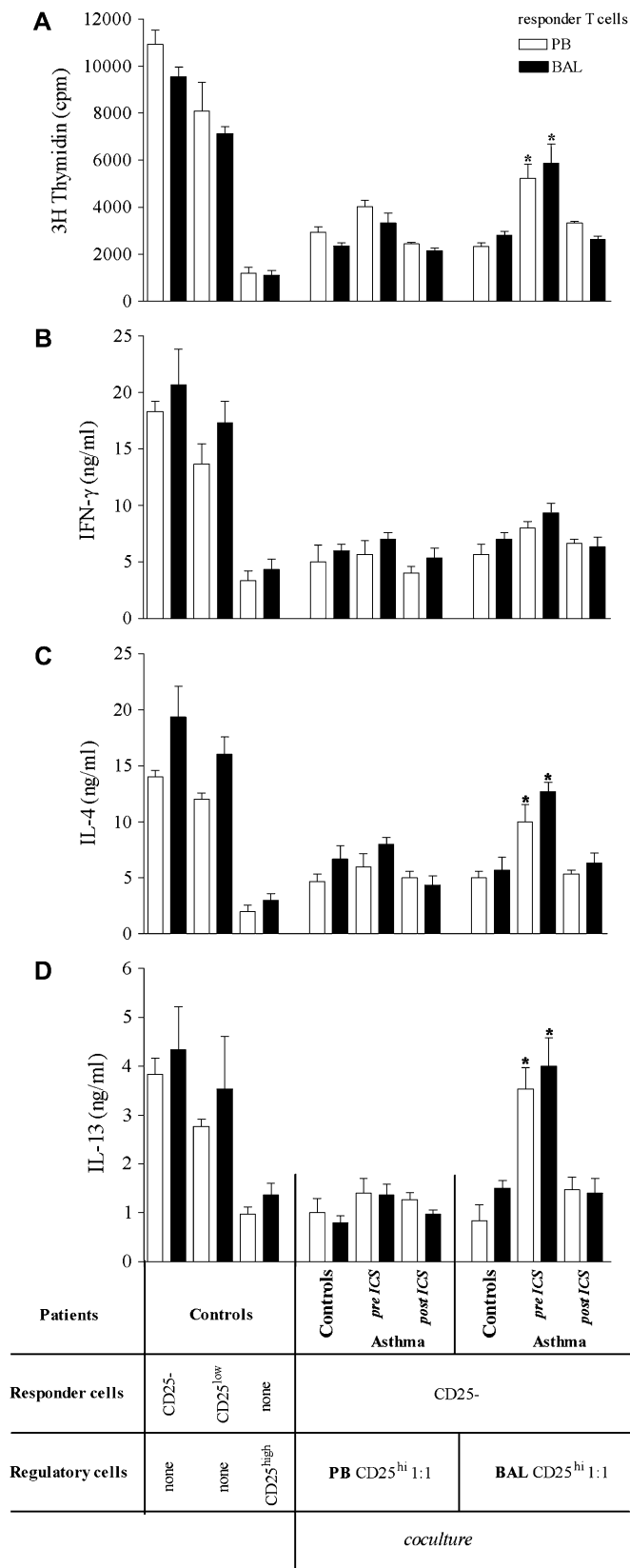
seen in control subjects both before and after corticosteroid treatment. However, CD4⁺CD25^{hi} Tregs isolated from BALF of untreated asthmatic subjects suppressed the T-cell proliferation and IL-4, IL-13, TARC, and MDC production of autologous responder T cells significantly less than Tregs from control subjects, whereas IFN- γ production remained unaffected. Corticosteroid treatment restored the suppressive capacity of BALF-isolated CD4⁺CD25^{hi} Tregs on responder T cells in asthmatic subjects. The suppressive capacity of CD4⁺CD25^{hi} Tregs on peripheral blood or BALF-derived responder T cells was similar. Similar to blood-isolated Tregs, the suppressive capacity of BALF-derived Tregs decreased with titration from 1:1 to 1:10 (see Fig E2 in the Online Repository at www.jacionline.org). Remarkably, CD4⁺CD25^{hi} Tregs from BALF were less effective in suppressing T-cell proliferation at a ratio of 1:5 compared with that seen in control subjects. Again, this effect was reversed after corticosteroid treatment. Finally, we asked whether blocking of IL-10, TGF- β , or cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) affects the suppressive capacity of blood- or BALF-derived CD4⁺CD25^{hi} Tregs. Although blocking of TGF- β reduced the suppressive effect of BALF-derived CD4⁺CD25^{hi} Tregs on proliferation of autologous blood or BALF responder T cells, no effects were observed for IL-10 or CTLA-4 (see Fig E3 in the Online Repository at www.jacionline.org).

DISCUSSION

The present work demonstrates, for the first time in human subjects, that CD4⁺CD25^{hi} T cells are enriched in the bronchoalveolar space compared with in peripheral blood and are fully capable of suppressing T-cell responses. However, in asthmatic children pulmonary CD4⁺CD25^{hi} T cells are decreased in number and fail to suppress T_H2 responses. Inhaled corticosteroid treatment in asthmatic subjects increases pulmonary CD4⁺CD25^{hi} T cells and restores their suppressive function.

With few exceptions,^{23,24} most studies point to a protective role of Tregs in asthma.²⁵⁻²⁸ Previous studies on human asthma have been exclusively performed in peripheral blood. However, the frequency and functional characteristics of Tregs might differ between peripheral blood and the site of inflammation, as suggested by studies of Tregs from synovial fluid of patients with rheumatoid arthritis.²⁹⁻³¹ Therefore we performed quantitative and functional analyses of CD4⁺CD25^{hi} T cells that were directly obtained from the bronchoalveolar space without any additional cellular stimulation. Percentages of CD4⁺CD25^{hi} T cells were increased in BALF compared

FIG 2. CD4⁺CD25^{hi} T cells in children with asthma, children with chronic cough, and control subjects. Percentages of CD4⁺CD25^{hi} T cells in peripheral blood (A) and BALF (B) of children with asthma without inhaled corticosteroids (*Asthma w/o ICS*), children with asthma receiving inhaled corticosteroids (*Asthma with ICS*), children with chronic cough, or control children are shown. *Children with asthma without atopy; **children with chronic cough and atopy. Median values are shown by horizontal bars. ns, Nonsignificant. C shows CD4⁺CD25^{hi} T cells in peripheral blood (left) and BALF (right) in 3 asthmatic children before (*pre*) and after (*post*) 4 weeks of inhaled corticosteroid therapy. D shows the percentages of CD4⁺CD25^{hi} T cells in peripheral blood compared with BALF. E shows the correlation of CD4⁺CD25^{hi} T cells with FEV₁.



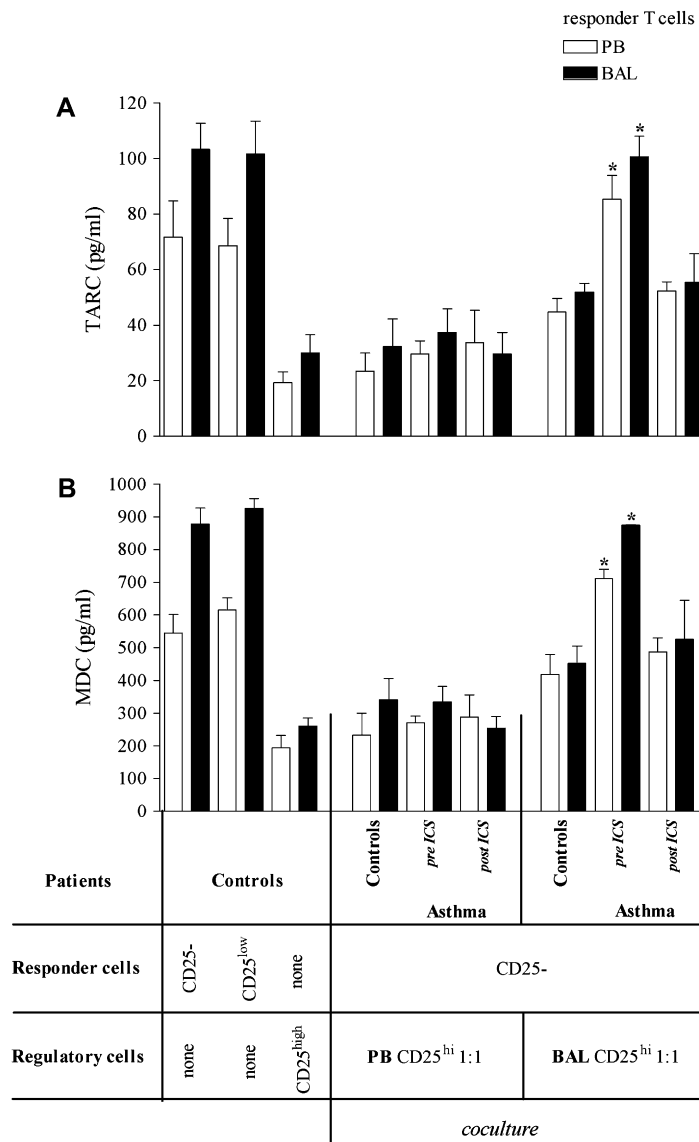


FIG 4. Suppression of chemokine production by bronchoalveolar CD4⁺CD25^{hi} T cells. FACS-sorted CD4⁺CD25⁻, CD4⁺CD25^{low}, and CD4⁺CD25^{hi} T-cell populations were obtained from peripheral blood (PB; open bars) and BALF (solid bars) of control subjects (n = 3) and asthmatic subjects (n = 3) before (pre ICS) and after (post ICS) 4 weeks of treatment with inhaled corticosteroids. The cells were cocultured with CD4⁺CD25^{hi} T cells isolated from peripheral blood or BALF at a ratio of 1:1 in the presence of 10 μ g/mL plate-bound anti-CD3, 10 μ g/mL soluble anti-CD28 mAbs, and IL-4 (5 ng/mL). After 4 days of culture, cell-free culture supernatants were collected and stored in aliquots at -70°C before analysis of TARC (A) and MDC (B). * $P < .05$ versus control subjects.

FIG 3. Suppression of T-cell proliferation and cytokine production by bronchoalveolar CD4⁺CD25^{hi} T cells. FACS-sorted CD4⁺CD25⁻, CD4⁺CD25^{low}, and CD4⁺CD25^{hi} T-cell populations were obtained from peripheral blood (PB; open bars) and BALF (solid bars) of control subjects (n = 3) and asthmatic subjects (n = 3) before (pre ICS) and after (post ICS) 4 weeks of treatment with inhaled corticosteroids. The CD4⁺CD25^{hi} cells were cocultured with CD4⁺CD25^{hi} T cells isolated from peripheral blood or BALF at a ratio of 1:1 in the presence of 10 μ g/mL plate-bound anti-CD3 plus 10 μ g/mL soluble anti-CD28 mAbs. After 4 days of culture, tritiated thymidine (37 KBq per well) was added for an additional 16 hours, and proliferation was measured (A). For detection of cytokine production (B-D), cell-free culture supernatants were collected and stored in aliquots at -70°C before analysis of IFN- γ (Fig 3, B), IL-4 (Fig 3, C), or IL-13 (Fig 3, D). * $P < .05$ versus control subjects.

with in peripheral blood in control children and children with chronic cough. In contrast, in untreated asthmatic children percentages of CD4⁺CD25^{hi} T cells were similar in BALF and peripheral blood. Thus percentages of BALF CD4⁺CD25^{hi} T cells were significantly reduced in children with asthma compared with those in children with chronic cough and control children. It has been demonstrated that Tregs can be induced on activation of CD4⁺CD25⁺ cells.³² The higher percentages of CD4⁺CD25^{hi} T cells in BALF compared with peripheral blood might be the result of constant antigen exposure in the lung. However, the underlying cause for the lack of pulmonary CD4⁺CD25^{hi} T cells in asthmatic children remains unclear. Verhagen et al³³ found an absence of CD4⁺CD25⁺ T cells in the skin of patients with atopic dermatitis. This study, together with our results, suggests that Tregs might be lacking at local sites of allergic inflammation. This raises the question of to what extent the lack of pulmonary Tregs in children with asthma might be caused by an underlying atopic disease. However, we did not find associations between total or specific IgE levels and percentages of CD4⁺CD25^{hi} T cells in BALF or peripheral blood. Furthermore, CD4⁺CD25^{hi} T cells and FOXP3 mRNA levels did not differ between atopic and nonatopic asthmatic children or between atopic and nonatopic children with chronic cough. These findings suggest that underlying atopic disease is not responsible for reduced pulmonary CD4⁺CD25^{hi} T cells in children with asthma.

Corticosteroids are widely used in the treatment of asthma, but it remains unclear whether they act beneficially in asthma by restoring the number, function, or both of Tregs. *In vitro* glucocorticoids induced IL-10³⁴ and FOXP3³⁵ expression in CD4⁺ T cells. Dexamethasone increased the frequency of murine Tregs.³⁶ Karagiannidis et al³⁵ reported that adult asthmatic patients receiving glucocorticoids had higher levels of CD4⁺CD25⁺ Tregs and FOXP3 mRNA expression in peripheral blood compared with levels seen in untreated asthmatic patients.³⁵ In line with these results, our asthmatic children treated with inhaled corticosteroids had higher frequencies of CD4⁺CD25^{hi} T cells and higher FOXP3 mRNA expression levels in peripheral blood and BALF compared with values seen in untreated asthmatic patients. Four weeks of treatment with inhaled corticosteroids increased percentages of CD4⁺CD25⁺ Tregs and FOXP3 mRNA expression levels in peripheral blood and BALF. In addition, corticosteroid treatment in asthmatic subjects restored the capacity of bronchoalveolar CD4⁺CD25^{hi} T cells to suppress autologous CD4⁺CD25⁺ cells. Overall, these data might imply that inhaled corticosteroids in children with bronchial asthma could act, at least partly, by increasing the percentages and function of pulmonary CD4⁺CD25^{hi} T cells.

CD4⁺CD25^{hi} lymphocytes comprise naturally occurring (nTregs) and adaptive (aTregs) Tregs.^{37,38} nTregs develop in the thymus, whereas aTregs are suggested to be generated in the periphery from naive T cells after antigen encounter^{9,39} or by rapid turnover of memory CD4⁺

T-cell populations.⁴⁰ nTregs are suggested to control immune responses in homeostatic conditions, whereas aTregs are believed to suppress T_H1/T_H2 effector cells at sites of inflammation through IL-10, TGF- β , or both.^{37,38} We found that the suppressive capacity of BALF-derived CD4⁺CD25^{hi} T cells was dependent on TGF- β , whereas the function of CD4⁺CD25^{hi} T cells isolated from peripheral blood was independent of TGF- β , as also shown by Bellinghausen et al.¹² On the basis of these observations, we speculate that the bronchoalveolar CD4⁺CD25^{hi} T cells of our patients might predominantly represent aTregs.

How reduced, impaired, or both CD4⁺CD25^{hi} Tregs could contribute to the pathophysiology of asthma is unclear at present. Four mechanisms, alone or in combination, are proposed.

First, CD4⁺CD25^{hi} T cells in BALF are primarily deficient in children with asthma. This seems unlikely because adults also have asthma, contradicting a primary Treg deficiency.

Second, the accumulation of bronchoalveolar T_H2 cells in asthmatic children leads to a numeric imbalance between T_H2 cells and CD4⁺CD25^{hi} Tregs, which might, in turn, result in a lack of suppression of bronchoalveolar T_H2 immune responses by Tregs. The inverse correlation between CD4⁺CD25^{hi} T cells and the T_H2 chemokines TARC and MDC in BALF of our patients supports this concept. However, the initial events leading to this imbalance remain unexplained.

Third, Baecher-Allan et al^{9,39} show that strong T-cell receptor-mediated signals render effector T cells refractory to suppression through Tregs. In patients with rheumatoid arthritis, activated responder T cells isolated from synovial fluid were less susceptible to CD4⁺CD25⁺ T cell-mediated suppression compared with responder T cells isolated from peripheral blood of the respective patients.³⁰ Because 80% of the bronchoalveolar T_H2 cells of our asthmatic children expressed CD69⁺ (data not shown), we speculate that activated bronchoalveolar T_H2 cells might escape the regulation through bronchoalveolar CD4⁺CD25^{hi} T cells.

Fourth, the local T_H2 cell accumulation might suppress the immigration, expansion, and/or function of bronchoalveolar CD4⁺CD25^{hi} T cells. No data are available to test these hypotheses in human subjects thus far.

In summary, the present study demonstrates that in children with asthma, pulmonary CD4⁺CD25^{hi} T cells are decreased and fail to suppress pulmonary T_H2 responses. Corticosteroids increase the frequencies and restore the impaired function of pulmonary Tregs in asthmatic subjects. The local imbalance between CD4⁺CD25^{hi} T cells and T_H2 cells might contribute to the disturbed pulmonary immune response in patients with asthma. These findings suggest that pulmonary Tregs could be a future therapeutic target in children with asthma.

We thank Nicolas Rieber, MD, Children's Hospital of the Ludwig-Maximilians-University of Munich, and David A. Hafler, Center for Neurologic Diseases, Department of Neurology, Brigham and

Women's Hospital, Harvard Medical School, Boston, Massachusetts, for critical revision of the manuscript.

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