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Leukotriene B₄ receptor 1 is differentially expressed on peripheral T cells of steroid-sensitive and -resistant asthmatics

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ABSTRACT

Background: Numbers of CD8⁺ T cells expressing the leukotriene B₄ (LTB₄) receptor, BLT1, have been correlated with asthma severity.

Objective: To examine the activation and numbers of BLT1-expressing peripheral blood CD4⁺ and CD8⁺ T cells from patients with steroid-sensitive (SS) and steroid-resistant (SR) asthma.

Methods: CD4⁺ and CD8⁺ T cells isolated from peripheral blood of healthy human subjects and patients with SS and SR asthma were stimulated in culture with anti-CD3/anti-CD28 followed by analysis of BLT1 surface expression and cytokine production. Activation of CD8⁺ T cells after ligation of BLT1 by LTB₄ was monitored by changes in intracellular Ca²⁺ concentrations.

Results: The number of BLT1-expressing cells was larger in patients with asthma than in controls and larger on activated CD8⁺ than on CD4⁺ T cells. Addition of LTB₄ to activated CD8⁺ T cells resulted in increases in intracellular Ca²⁺ concentrations. Expansion of activated CD4⁺ T cells, unlike CD8⁺ T cells, was significantly decreased in the presence of corticosteroid. In patients with SS asthma, numbers of BLT1-expressing CD8⁺ T cells were lower in the presence of corticosteroid, unlike in those with SR asthma in whom cell expansion was maintained. Levels of interleukin-13 were highest in cultured CD8⁺ T cells, whereas interleukin-10 levels were higher in CD4⁺ T cells from controls and patients with SS asthma. Interferon- γ levels were lowest in patients with SR asthma.

Conclusion: Differences in BLT1 expression, steroid sensitivity, and cytokine production were demonstrated in T lymphocytes from patients with SS and SR asthma. The LTB₄–BLT1 pathway in CD8⁺ cells may play an important role in asthma and serve as an important target in the treatment of patients with SR asthma.

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Introduction

T lymphocytes are central to the induction and maintenance of the allergic inflammatory response, with an emphasis on the role of CD4⁺ T cells and their ability to produce T-helper type 2 (T_H2) cytokines, such as interleukin (IL)-4, IL-5, and IL-13.¹ CD8⁺ T cells

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also have been demonstrated in the bronchoalveolar lavage (BAL) fluid and sputum of patients with asthma^{2,3} and in lung tissue of subjects with fatal asthma,⁴ but their specific role in asthma pathogenesis has been somewhat controversial. Enhancing⁵ and protective⁶ roles for CD8⁺ T cells have been described.

Among the many mediators released during an asthmatic response, leukotriene levels, including leukotriene B₄ (LTB₄), have been reported to increase in the BAL fluid and breath condensates of patients with asthma⁷ and are potent mediators of asthma.^{8,9} LTB₄ is an important regulator of neutrophil chemotaxis to the lung.¹⁰ The authors and others have shown the importance of LTB₄ interacting with its high-affinity receptor, BLT1, in the accumulation of T cells in the lung.^{11–14} In a mouse model of experimental asthma, the authors found that BLT1-expressing effector memory CD8⁺ T cells were a potent source of IL-13 and required for the development of lung allergic responses.¹⁵ Moreover, these CD8⁺BLT1⁺ T cells were more resistant to corticosteroids than CD4⁺ T cells and

corticosteroids enhanced their activation and effector function by upregulating BLT1 expression through increased IL-2 receptor expression.¹⁶ In patients with asthma, the numbers of CD8⁺BLT1⁺ T cells were increased in the tissue and BAL fluid and numbers of CD8⁺BLT1⁺ T cells producing IL-13 correlated with decreased lung function.^{17,18} In patients with severe asthma, comparison of transcriptome analyses showed large changes in circulating CD8⁺, but not CD4⁺, T cells compared with patients with nonsevere asthma or controls.¹⁹

To further define potential differences in peripheral blood CD4⁺ and CD8⁺ T cells that might contribute to asthma pathogenesis, the authors compared responses of these T-cell subsets from patients with steroid-sensitive (SS) and steroid-resistant (SR) asthma. The results identified important differences in the responses of CD4⁺ and CD8⁺ T cells to cell activation and between patients with SS asthma and those with SR asthma in the induction of BLT1 expression, steroid sensitivity, and cytokine production in these subsets.

Methods

Subjects

Patients with a diagnosis of asthma according to American Thoracic Society criteria were selected. To qualify for study, baseline forced expiration volume in 1 second (FEV₁) had to be less than or equal to 80% predicted. All patients were nonsmokers (no smoking for >1 year with a total <10 packs/year) and had not experienced an upper respiratory tract infection for at least 6 weeks before enrollment. Patients with asthma had a methacholine provocation concentration that caused a decrease in FEV₁ of 20% no higher than 8 mg/mL if steroid naive or 16 mg/mL if on an inhaled corticosteroid; otherwise patients demonstrated an improvement of at least 12% in FEV₁ after albuterol. After baseline characterization, all patients received prednisolone (40 mg) orally for 7 days and were categorized as having SS asthma if the FEV₁ value improved by at least 10% or as having SR asthma if the FEV₁ improved by less than 10%. For controls, healthy adults with no history of atopic or respiratory disease were enrolled. None of the subjects received systemic corticosteroid therapy for at least 6 weeks before study. This study was approved by the institutional review board of National Jewish Health (Denver, Colorado).

Human Peripheral Blood Mononuclear Cell Purification, Isolation of CD4⁺ and CD8⁺ T Cells, and Culture Procedures

Human peripheral blood mononuclear cells were isolated from heparinized venous blood by density gradient centrifugation and CD4⁺ and CD8⁺ T cells were isolated by negative selection using the magnetic bead human CD4⁺ T-cell isolation kit II and human CD8⁺ T-cell isolation kit II (Miltenyi Biotec, Auburn, California), respectively. Isolated CD4⁺ and CD8⁺ T cells (>95% purity) were stimulated in culture with antihuman CD3 monoclonal antibody (2 µg/mL; BD PharMingen, San Jose, California)/antihuman CD28 monoclonal antibody (2 µg/mL; BD PharMingen) and human recombinant IL-2 (100 U/mL; Peprotech, Rocky Hill, New Jersey) in the presence or absence of 100 nmol/L of dexamethasone (DEX; Sigma-Aldrich, St Louis, Missouri). Cells were cultured for 8 days at 37°C and 5% CO₂ in 12-well culture plates (Becton Dickinson, Franklin Lakes, New Jersey) at 1 × 10⁶ cells/mL per well. Medium containing IL-2, with or without DEX, was changed every other day and analysis of BLT1 surface expression was carried out at day 0 and day 8 of culture, the day of peak BLT1 expression.¹⁶ At all time points, cell viability remained greater than 90% as assessed by trypan blue dye exclusion.

Flow Cytometry

For staining of BLT1, cells were blocked with 10% human IgG for 15 minutes at 4°C. Fluorescein isothiocyanate–conjugated

anti-hLTB4 receptor (BLT1) monoclonal antibody (AbD Serotec, Raleigh, North Carolina) or isotype control was added and incubated for 30 minutes at 4°C and washed 3 times. BLT1 surface receptor staining was analyzed by flow cytometry using FlowJo software (Tree Star, Inc, Ashland, Oregon). Allophycocyanin-conjugated anti-CD3, peridinin chlorophyll protein–conjugated CD4, and phycoerythrin-conjugated and peridinin chlorophyll protein Cy5.5-conjugated CD8 were used for staining of CD4⁺ and CD8⁺ T cells.

Intracellular Ca²⁺ Monitoring

Activation of CD8⁺ T cells after ligation of BLT1 by LTB4 was monitored by changes in intracellular Ca²⁺ concentrations. Isolated CD8⁺ T cells (5 × 10⁶ cells/mL) were incubated for 45 minutes at 37°C with 5 µmol/L of indo-1 acetoxymethyl ester (Invitrogen, Carlsbad, California). Cells were resuspended and subjected to analysis by flow cytometry (LSR II; Becton Dickinson) with FlowJo software.¹⁶ After a baseline was established, cells were stimulated with LTB4 (100 and 1,000 nmol/L; Sigma-Aldrich) and intracellular Ca²⁺ concentrations were determined by measurement of the median fluorescence ratio of indo-1 acetoxymethyl ester at 390-nm and 490-nm emission.

Cytokine Levels in Cell Culture Supernatant Fluid

Equal numbers of isolated CD4⁺ and CD8⁺ T cells were stimulated in culture with anti-CD3/anti-CD28 in the presence of IL-2 for 48 hours. Culture supernates were harvested for analysis of cytokine levels (IL-4, IL-5, IL-10, IL-13, and interferon-γ [IFN-γ]) by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minnesota). The detection limits were 8.0 pg/mL for IFN-γ, 10 pg/mL for IL-4, 3.0 pg/mL for IL-5, 3.9 pg/mL for IL-10, and 32 pg/mL for IL-13.

Statistical Analysis

Patient clinical variables were analyzed using descriptive statistics, including proportions or means where appropriate. Data were expressed as mean ± SEM or SD. The paired *t* test was used to compare functional responses in the presence or absence of DEX from the same donors. Between-group (control, SS asthma, and SR asthma) comparisons were performed using 1-way analysis of variance. *P* values no greater than .05 were considered significant. Analysis for statistically significant differences for enzyme-linked immunosorbent assay data was performed using the unpaired 2-tailed *t* test and the nonparametric Mann-Whitney test, respectively. GraphPad Prism 4.01 (GraphPad, San Diego, California) was used for all statistical calculations.

Results

Demographics of Enrolled Subjects

Nineteen subjects (9 with asthma and 10 healthy controls) were enrolled (Table 1). Patients in the asthma group fulfilled the American Thoracic Society definition of asthma (>12% bronchodilator response) and exhibited atopy as demonstrated by multiple positive immediate skin prick responses to allergens. Serum IgE levels were highest and lung function the lowest in patients with SR asthma compared with controls (*P* < .05). Percentages of total lymphocytes in BAL fluid expressing CD8 and BLT1 and those expressing CD8, BLT1, and IL-13 were higher in patients with asthma than in controls, with the largest percentages in patients with SR asthma (Table 1).

BLT1 Expression on Isolated CD4⁺ and CD8⁺ T Cells

The BLT1 expression on isolated, live CD3⁺CD4⁺ T cells and CD3⁺CD8⁺ T cells was analyzed at baseline and in the presence or

Table 1

Patient demographics, pulmonary function, and BAL lymphocytes

Parameter	Group			P value
	CTL	SS	SR	
Subjects, n	10	5	4	
Age (y), mean $\pm$ SD	32.7 $\pm$ 11.0	30.6 $\pm$ 6.5	35.5 $\pm$ 9.9	
Men: women	5:5	3:2	2:2	
Race				
White	8	4	2	
Asian	2			
Hispanic		1	1	
African American			1	
Positive skin prick test results, average (range)	0	5.6+ (4+~9+)	5.0+ (1+~10+)	
Serum total IgE (IU/mL) (range)	76 (2~377)	177 (86~409)	333 (56~589) ^a	<.05 ^a
FEV ₁ % predicted, mean $\pm$ SD				
Before bronchodilator	95.1 $\pm$ 10.2	75.8 $\pm$ 8.2 ^a	65.8 $\pm$ 8.2 ^a	<.05 ^a
After bronchodilator	104.4 $\pm$ 10.6	89.8 $\pm$ 9.9	79.5 $\pm$ 13.0	<.05 ^a
FVC % predicted, mean $\pm$ SD				
Before bronchodilator	99.2 $\pm$ 3.9	90.8 $\pm$ 10.1	88.5 $\pm$ 19.8	NS
After bronchodilator	99.2 $\pm$ 5.9	97.4 $\pm$ 12.5	97.8 $\pm$ 26.1	NS
FEV ₁ /FVC				
Before bronchodilator	0.82 $\pm$ 0.03	0.70 $\pm$ 0.05	0.63 $\pm$ 0.06	<.05 ^a
After bronchodilator	0.86 $\pm$ 0.05	0.78 $\pm$ 0.08	0.69 $\pm$ 0.08	<.05 ^a
Lymphocyte phenotype in BAL				
BLT1 ⁺ CD8 ⁺	13.97 $\pm$ 1.6	25.32 $\pm$ 3.4	34.2 $\pm$ 5.1	<.05 ^a
BLT1 ⁺ CD8 ⁺ IL-13 ⁺	4.78 $\pm$ 1.2	13.4 $\pm$ 3.7	16.5 $\pm$ 2.3	<.05 ^a

Abbreviations: BAL, bronchoalveolar lavage; BLT1, leukotriene B₄ receptor; CTL, control; FEV₁, force expiration in 1 second; FVC, forced vital capacity; IL-3, interleukin-3; NS, not significant; SR, steroid resistant; SS, steroid sensitive.

^aCompared with controls.

absence of DEX in controls and patients with SS and SR asthma. When CD4⁺ T cells from each group were compared, the percentages of BLT1⁺ cells at baseline was small and there was only a modest increase after activation of the CD4⁺ T cells with anti-CD3/anti-CD28 (Fig 1). Addition of DEX to the cultures had little effect on the percentages of BLT1 in CD4⁺ T cells ($P > .05$).

In contrast, when isolated CD8⁺ T cells were examined, activation with anti-CD3/anti-CD28 resulted in an increase in the percentages of CD8⁺ T cells expressing BLT1 in all groups. The increases were greater in isolated CD8⁺ T cells than in isolated CD4⁺ T cells after stimulation (Fig 2). The increases in BLT1-staining CD8⁺ T cells were greater in patients with asthma than in controls ($P < .05$) and these increases were greater in CD8⁺ T cells from patients with SR asthma than in CD8⁺ T cells from patients with SS asthma ($P < .05$). In patients with SS asthma (and controls), the percentages of BLT1-expressing CD8⁺ T cells on day 8 were smaller when cells were cultured in the presence of corticosteroid, unlike SR CD8⁺ T cells, where the numbers were maintained ($P < .05$). Individual responses in CD4⁺ and CD8⁺ T cells from controls and patients with SS and SR asthma are shown in eFigure 1.

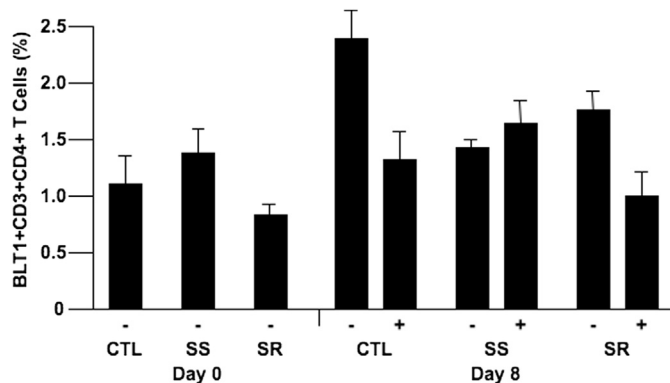


Figure 1. BLT1 expression on stimulated CD4⁺ T cells. Isolated CD4⁺ T cells were stimulated with anti-CD3/anti-CD28 for 8 days and BLT1 expression was assessed by flow cytometry. There were no significant differences among the groups. BLT1, leukotriene B₄ receptor; CTL, control; SR, steroid resistant; SS, steroid sensitive.

Ca²⁺ Influx in Isolated CD8⁺ T Cells

To determine the functional activity of the upregulated expression of BLT1 on activated CD8⁺ T cells, the authors determined the effect of adding LTB₄ to CD8⁺ T cells and monitored intracellular Ca²⁺ concentrations. When activated CD8⁺ T cells with upregulated BLT1 expression were exposed to 2 concentrations of LTB₄, intracellular Ca²⁺ concentrations increased in a dose-dependent manner (Fig 3). Activated CD8⁺ T cells expressing BLT1 from all groups showed similar increases in intracellular Ca²⁺ concentrations (data not shown).

Sensitivity to Corticosteroids

To further delineate the differences in CD4⁺ and CD8⁺ T cells in patients with SS and SR asthma, isolated subsets were stimulated and expanded with anti-CD3/anti-CD28 and IL-2 in the presence or absence of DEX. At the end of the culture period (day 8), cell counts were performed to determine the extent of expansion. When CD4⁺ T cells were stimulated in the presence of DEX, cell numbers on day 8 were significantly smaller than in cultures not containing DEX. In cultures of CD8⁺ T cells, the attenuation of anti-CD3/anti-CD28 and IL-2-induced expansion was lowest (Table 2) and the expansion of CD8⁺ T cells in the SR group was decreased to the lowest extent.

Cytokine Levels in Cell Culture Supernatant Fluid

The authors previously reported that in mice and in patients with asthma, CD8⁺ T cells were a source of the proallergic cytokine IL-13.^{15,18} The authors compared cytokine production in CD4⁺ and CD8⁺ T cells after culture for 48 hours in the presence of anti-CD3/anti-CD28 and IL-2. Levels of IL-13 were higher in supernates from cultured CD8⁺ T cells than from cultures of CD4⁺ T cells, whereas IL-10 levels were higher in CD4⁺ T cells from controls and patients with SS asthma than from cultures of CD4⁺ T cells from patients with SR asthma ($P < .05$; Fig 4). In cultures of CD4⁺ and CD8⁺ T cells, levels of IFN- γ were lowest from cells of patients with SR asthma ($P < .05$). IL-4 levels were at the level of detection and did not differ among groups, and levels of IL-5 did not differ among groups (data not shown).

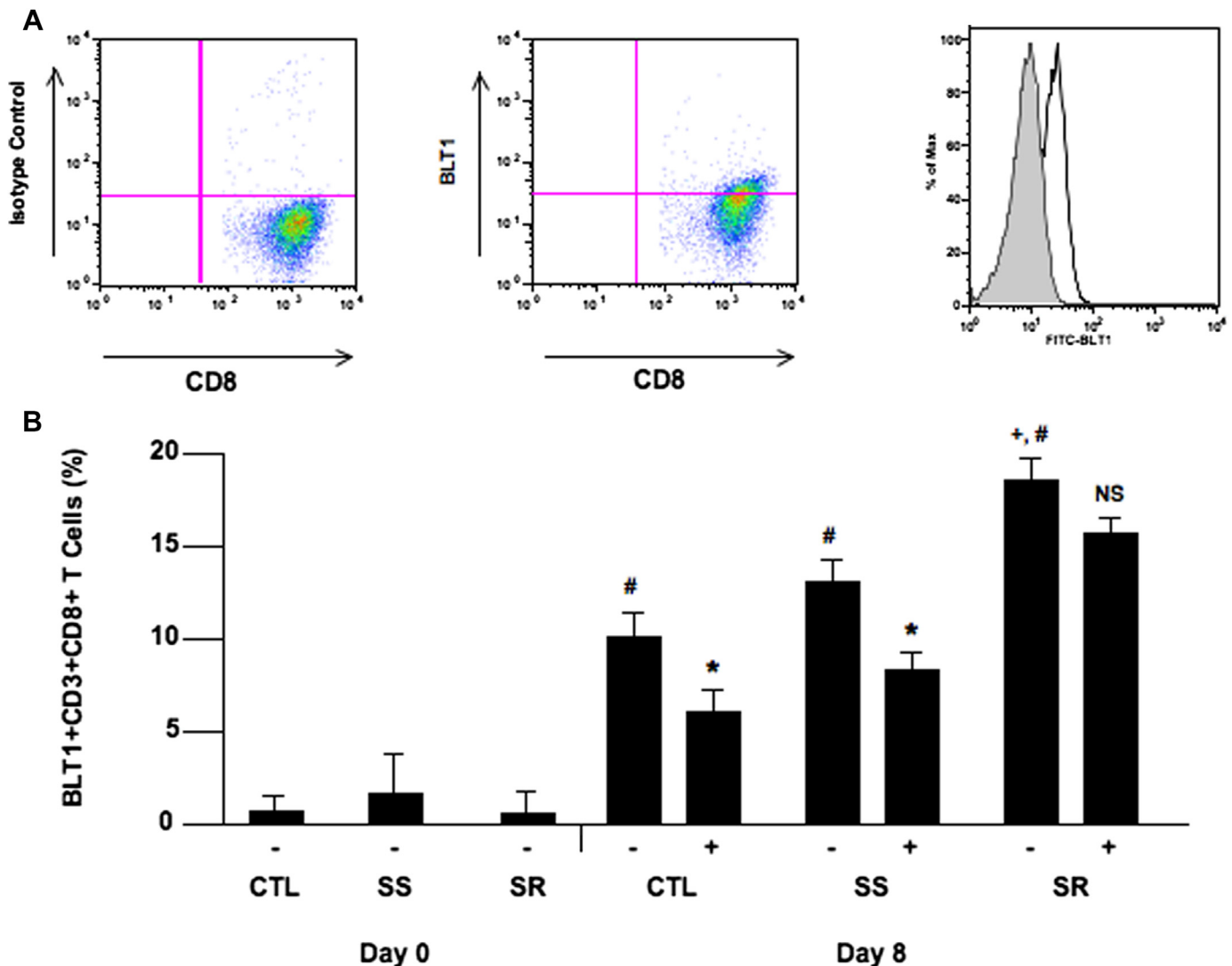


Figure 2. BLT1 expression on stimulated CD8⁺ T cells. Isolated CD8⁺ T cells were stimulated with anti-CD3/anti-CD28 for 8 days and BLT1 expression was assessed by flow cytometry. (A) Representative plots are illustrated. Representative data show isotype control staining (left) and BLT1 expression (center). (Right) Flow analysis of BLT1 staining and discrimination between the FITC isotype control and FITC BLT1 monoclonal antibody staining. (B) BLT1 expression on unstimulated and stimulated CD8⁺ T cells in the presence (+) or absence (–) of dexamethasone. #*P* < .05, unstimulated vs stimulated group; **P* < .05, steroid-treated vs untreated group; +*P* < .05, SR vs CTL and SS groups. BLT1, leukotriene B₄ receptor; CTL, control; FITC, fluorescein isothiocyanate; NS, not significant; SR, steroid resistant; SS, steroid sensitive.

Discussion

The contributions of CD4⁺ T-cells in the development of allergic airway inflammation are well established.¹ A growing body of evidence suggests that CD8⁺ T cells also may have the capacity to

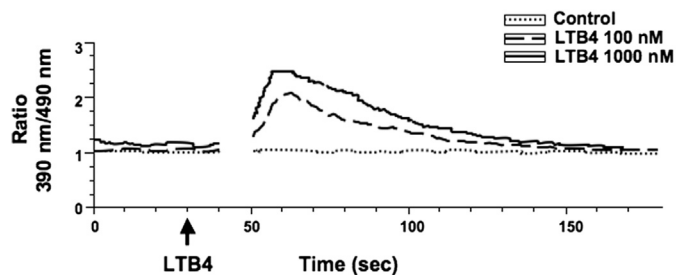


Figure 3. Addition of leukotriene B₄ (LTB₄) to stimulated CD8⁺ T cells results in increased intracellular Ca²⁺ concentrations. Isolated CD8⁺ T cells stimulated with anti-CD3/anti-CD28 for 8 days were incubated with LTB₄ (100 or 1,000 nmol/L) and intracellular Ca²⁺ concentrations were monitored comparing the median fluorescence ratio of indo-1 to indo-2 as described in the “Methods” section.

trigger allergic inflammatory processes in the lungs that contribute to clinically relevant immunopathologic processes.^{1,5,18,20,21} A cross-sectional relation between CD8⁺ T cells and asthma outcomes has been observed in fatal asthma.²¹ Studies examining a potential role for CD8⁺ T cells in asthma have noted a positive correlation among CD8⁺ T-cell numbers, disease severity, and decrease in lung function.^{5,18} The authors compared the phenotype and activation outcomes of CD4⁺ and CD8⁺ T cells in 2 subsets of patients with asthma defined as SS or SR. The percentages of cells that expressed BLT1, the high-affinity receptor for the lipid mediator LTB₄, were larger in patients with asthma than in those without asthma, and in patients with SR asthma, the largest percentages of BLT1-expressing cells were detected in activated CD8⁺ T cells. This increase in BLT1-expressing cells in SR asthmatic CD8⁺ T cells was maintained in the presence of corticosteroid and was functional as determined by the intracellular Ca²⁺ response to ligation of the receptor by LTB₄. CD8⁺ T cells produced the highest levels of the cytokine IL-13, important in asthma and mucus production.²² SR asthmatic CD4⁺ T cells produced the lowest levels of the anti-inflammatory cytokine IL-10 and IFN-γ levels were the lowest in cultures of SR asthmatic CD4⁺ and CD8⁺ T cells. Importantly, CD8⁺

Table 2Expansion of CD4⁺ and CD8⁺ T cells and effect of DEX treatment

Group	CD4 ⁺ T cells		CD8 ⁺ T cells	
	Total cell count ($\times 10^6$), mean $\pm$ SD	Decrease in cell expansion, %	Total cell count ($\times 10^6$), mean $\pm$ SD	Decrease in cell expansion, %
CTL DEX ⁻	24.4 $\pm$ 11.7		31.7 $\pm$ 14.8	
CTL DEX ⁺	8.5 $\pm$ 8.6 ^a	65	16.0 $\pm$ 8.2 ^a	50
SS DEX ⁻	38.4 $\pm$ 22.1		44.1 $\pm$ 26.0	
SS DEX ⁺	7.8 $\pm$ 4.4 ^a	80	25.5 $\pm$ 15.5 ^a	60
SR DEX ⁻	16.3 $\pm$ 9.0		21.4 $\pm$ 5.5	
SR DEX ⁺	3.3 $\pm$ 1.9 ^a	80	17.6 $\pm$ 5.3 ^{NS}	19 ^b

Abbreviations: CTL, control; DEX, dexamethasone; NS, not significant; SR, steroid resistant; SS, steroid sensitive.

^a*P* < .05 vs non-DEX-treated group.^b*P* < .05 vs CTL and SS groups.

T cell expansion, especially in SR asthma, was relatively resistant to corticosteroid compared with CD4⁺ T cells.

Although LTB4 was initially described as a chemoattractant for myeloid leukocytes,²³ it was shown that LTB4 could bind to a small proportion of human peripheral blood T cells.²⁴ Consistent with these findings, LTB4 was shown to induce chemotaxis of human peripheral blood lymphocytes.²⁵ Elevated levels of LTB4 have been reported in various allergic diseases and these levels have been related to disease activity and response to treatment. LTB4 levels were increased in the sputum, plasma, and BAL fluid of patients with asthma but not of healthy subjects.^{7,26} Many studies have shown that LTB4, although perhaps not a primary mediator of allergic disease, may be important in specific conditions such as severe persistent asthma.²⁶ BAL LTB4 levels⁸ and bronchial wall CD8⁺ T cell numbers have been correlated with disease severity,⁵ supporting a potential link between these previously assumed independent findings.

Corticosteroids effectively suppress inflammatory responses through repression of many immune genes and therefore are widely used for the treatment of various allergic diseases. T_H2 cytokine-producing CD4⁺ T cells and eosinophils play important roles in the pathogenesis of allergic diseases, and numbers of these cells, but not of CD8⁺ T cells, were dramatically decreased in peripheral blood and inflamed bronchial tissues of patients with asthma after initiation of corticosteroid treatment.²⁷ Although considered immunosuppressive and anti-inflammatory, several lines of investigation have demonstrated that corticosteroids may increase immune and inflammatory responses and even enhance adaptive immune responses.²⁸ As confirmed in the present study, human CD8⁺ T cells have been shown to be relatively steroid resistant compared with CD4⁺ T cells,²⁹ similar to findings in mice.¹⁶ It has been well established that a proportion of patients with asthma develop a decrease in lung function despite high doses of inhaled or oral corticosteroid treatment and have been labeled as steroid insensitive or steroid refractory.³⁰ Because the pathway leading to the generation of lipid mediators is activated in many inflammatory diseases but is resistant to corticosteroid treatment,⁸

the LTB4–BLT1 axis in steroid-insensitive CD8⁺ T cells may be a major factor in SR asthma.

The present study confirmed this notion in patients with SS and SR asthma and demonstrated, despite small numbers of patients, significant differences between CD4⁺ and CD8⁺ T cells. Activation with anti-CD3/anti-CD28 resulted in the expansion of BLT1-expressing CD8⁺ T cells in all groups in contrast to CD4⁺ T cells. The numbers of BLT1⁺CD8⁺ T cells were larger in patients with asthma than in the controls and the increases were greater in CD8⁺ T cells from patients with SR asthma than from patients with SS asthma. Expansion of activated CD4⁺ T cells with anti-CD3/anti-CD28 and IL-2 was significantly decreased on day 8 in the presence of DEX, whereas the effects of DEX on expansion of activated CD8⁺ T cells was lower and the smallest effects were seen in the CD8⁺ T cells from patients with SR vs SS asthma.

Although much of the previous work in asthma has focused on the role of CD4⁺ T_H2 cells and their capacity for proallergic cytokine production, including IL-13, the authors previously showed that mouse¹⁵ and asthmatic¹⁸ CD8⁺ T cells are a potent source of IL-13. When cytokine levels in cultures of CD4⁺ and CD8⁺ T cells activated with anti-CD3/anti-CD28 were assessed, levels of IL-13 were higher in CD8⁺ T cells than in CD4⁺ T cells. In contrast, levels of the anti-inflammatory cytokine IL-10 were higher in CD4⁺ T cells from controls and patients with SS asthma compared with CD4⁺ T cells from patients with SR asthma. This inverse relation between IL-13 and IL-10 (increased IL-13 and decreased IL-10 levels in BAL fluid) has correlated with disease severity.^{31,32} Corticosteroids may exert some of their beneficial effects through the induction of IL-10 production.^{31,33} Levels of IFN- γ , which may downregulate T_H2 cytokine production and asthmatic responses, also have been shown to be lowest in CD4⁺ and CD8⁺ T cells from patients with SR asthma.

These studies extend previous findings implicating an important role for CD8⁺ T cells in a subset of patients with asthma. The data presented identify impressive differences between CD4⁺ and CD8⁺ T cells and in patients with SS and SR asthma. In patients with asthma, numbers of BLT1⁺CD8⁺ T cells were larger and

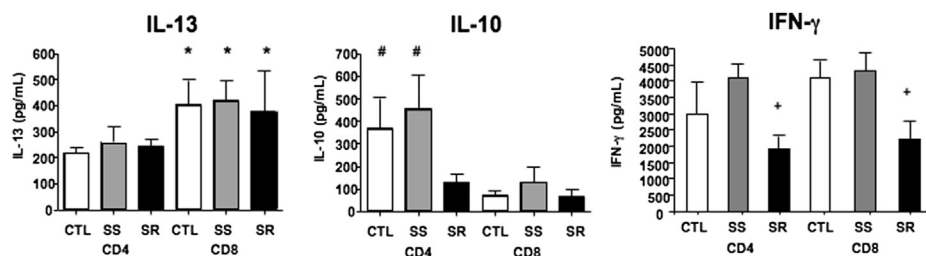


Figure 4. Cytokine production from cultures of CD4⁺ and CD8⁺ T cells. Isolated CD4⁺ and CD8⁺ T cells from CTL, SS, and SR subjects were stimulated with anti-CD3/anti-CD28 for 48 hours and culture supernates were harvested and assayed by enzyme-linked immunosorbent assay. **P* < .05 vs CD4⁺ T cells; #*P* < .05 vs SR CD4⁺ T cells and CD8⁺ T cells; +*P* < .05, SR cells vs CTL or SS cells. CTL, control; IFN- γ , interferon- γ ; IL, interleukin; SR, steroid resistant; SS, steroid sensitive.

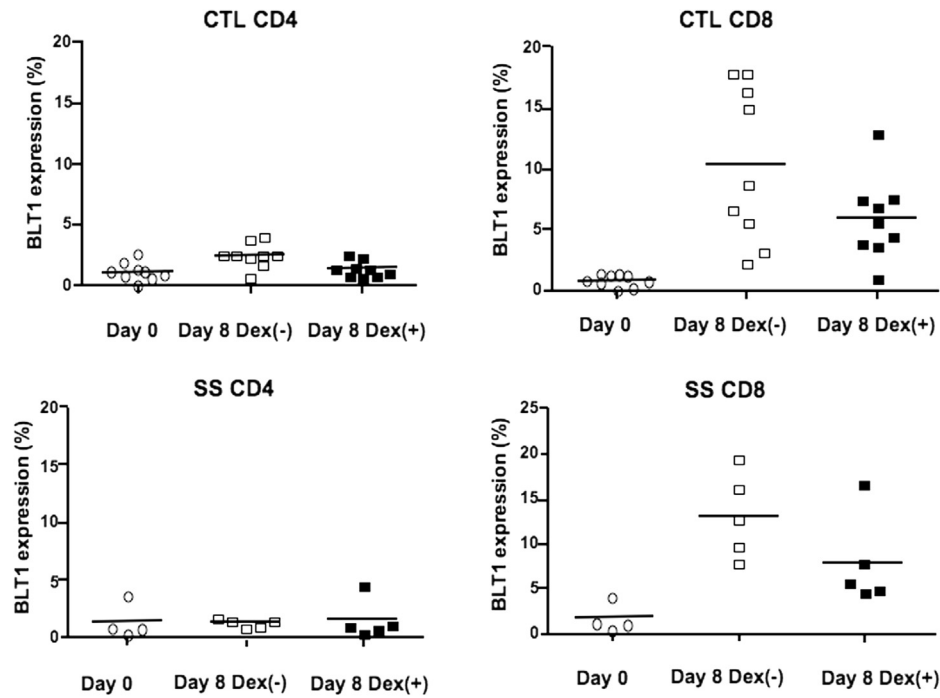
demonstrated relative corticosteroid insensitivity and capacity for IL-13 production. These differences appeared enhanced in CD8⁺ T cells from patients with SR asthma. Severe asthma has been defined as abnormal lung function despite treatment with high doses of corticosteroids.³⁴ Because of differences in baseline lung function in patients with SR asthma and differences in steroid sensitivity, it is difficult to distinguish whether the differences in CD8⁺ T cell responses are related in part to disease severity or steroid responsiveness. Nonetheless, the findings identify the need for the development of newer therapeutic approaches that target these pathogenic CD8⁺ T cells if the course of severe asthma is to be altered, especially when corticosteroids do not achieve asthma control.

Supplementary Data

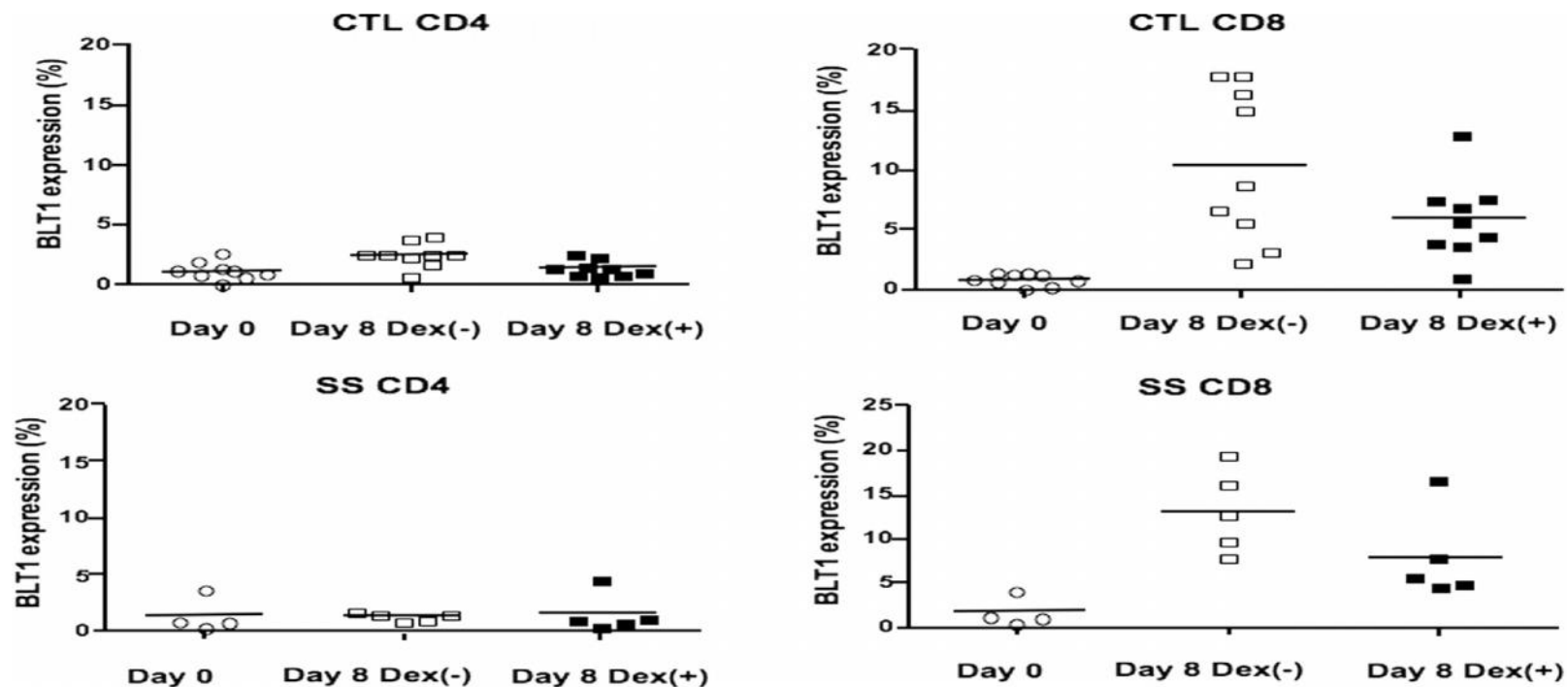
Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.anaai.2013.12.006>.

References

- [1] Larche M, Robinson DS, Kay AB. The role of T lymphocytes in the pathogenesis of asthma. *J Allergy Clin Immunol*. 2003;111:450–463.
- [2] Walker C, Bauer W, Braun RK, et al. Activated T cells and cytokines in bronchoalveolar lavages from patients with various lung diseases associated with eosinophilia. *Am J Respir Crit Care Med*. 1994;150:1038–1048.
- [3] Lee SY, Kim SJ, Kwon SS, et al. Distribution and cytokine production of CD4 and CD8 T-lymphocyte subsets in patients with acute asthma attacks. *Ann Allergy Asthma Immunol*. 2001;86:659–664.
- [4] Faul JL, Tormey VJ, Leonard C, et al. Lung immunopathology in cases of sudden asthma death. *Eur Respir J*. 1997;10:301–307.
- [5] van Rensen EL, Sont JK, Evertse CE, et al. Bronchial CD8 cell infiltrate and lung function decline in asthma. *Am J Respir Crit Care Med*. 2005;172:837–841.
- [6] Takeda K, Dow SW, Miyahara N, et al. Vaccine-induced CD8⁺ T cell-dependent suppression of airway hyperresponsiveness and inflammation. *J Immunol*. 2009;183:181–190.
- [7] Wardlaw AJ, Hay H, Cromwell O, Collins JV, Kay AB. Leukotrienes, LTC4 and LTB4, in bronchoalveolar lavage in bronchial asthma and other respiratory diseases. *J Allergy Clin Immunol*. 1989;84:19–26.
- [8] Peters-Golden M, Henderson WR Jr. Leukotrienes. *N Engl J Med*. 2007;357:1841–1854.
- [9] Ohnishi H, Miyahara N, Gelfand EW. The role of leukotriene B(4) in allergic diseases. *Allergol Int*. 2008;57:291–298.
- [10] Martin TR, Pistoresse BP, Chi EY, Goodman RB, Matthay MA. Effects of leukotriene B4 in the human lung. Recruitment of neutrophils into the alveolar spaces without a change in protein permeability. *J Clin Invest*. 1989;84:1609–1619.
- [11] Miyahara N, Takeda K, Miyahara S, et al. Leukotriene B4 (BLT1) is essential for allergen-mediated recruitment of CD8⁺ T cells and airway hyperresponsiveness. *J Immunol*. 2005;174:4979–4984.
- [12] Miyahara N, Takeda K, Miyahara S, et al. Requirement for the leukotriene B4 receptor-1 in allergen-induced airway hyperresponsiveness. *Am J Respir Crit Care Med*. 2005;172:161–167.
- [13] Taube C, Miyahara N, Ott V, et al. The leukotriene B4 receptor (BLT1) is required for effector CD8⁺ T-cell-mediated, mast cell-dependent airway hyperresponsiveness. *J Immunol*. 2006;176:3157–3164.
- [14] Goodarzi K, Goodarzi M, Tager AM, Luster AD, von Andrian UH. Leukotriene B4 and BLT1 control cytotoxic effector T cell recruitment to inflamed tissues. *Nat Immunol*. 2003;4:965–973.
- [15] Miyahara N, Takeda K, Kodama T, et al. Contribution of antigen-primed CD8⁺ T cells to the development of airway hyperresponsiveness and inflammation is associated with IL-13. *J Immunol*. 2004;172:2549–2558.
- [16] Ohnishi H, Miyahara N, Dakhama A, et al. Corticosteroids enhance CD8⁺ T cell-mediated airway hyperresponsiveness and allergic inflammation by upregulating leukotriene B4 receptor 1. *J Allergy Clin Immunol*. 2008;121:864–871.
- [17] Gelfand EW, Dakhama A. CD8⁺ T lymphocytes and leukotriene B4: novel interactions in the persistence and progression of asthma. *J Allergy Clin Immunol*. 2006;117:577–582.
- [18] Dakhama A, Collins ML, Ohnishi H, et al. IL-13–producing BLT1-positive CD8 cells are increased in asthma and are associated with airway obstruction. *Allergy*. 2013;68:666–673.
- [19] Tsitsiou E, Williams AE, Moschos SA, et al. Transcriptome analysis shows activation of circulating CD8⁺ T cells in patients with severe asthma. *J Allergy Clin Immunol*. 2012;129:95–103.
- [20] Cho SH, Stanciu LA, Holgate ST, Johnston SL. Increased interleukin-4, interleukin-5, and interferon-gamma in airway CD4⁺ and CD8⁺ T cells in atopic asthma. *Am J Respir Crit Care Med*. 2005;171:224–230.
- [21] O'Sullivan S, Cormican L, Faul JL, et al. Activated, cytotoxic CD8(+) T lymphocytes contribute to the pathology of asthma death. *Am J Respir Crit Care Med*. 2001;164:560–564.
- [22] Zhu Z, Homer RJ, Wang Z, et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J Clin Invest*. 1999;103:779–788.
- [23] Ford-Hutchinson AW, Bray MA, Doig MV, Shipley ME, Smith MJ. Leukotriene B, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature*. 1980;286:264–265.
- [24] Payan DG, Missirlian-Bastian A, Goetzl EJ. Human T-lymphocyte subset specificity of the regulatory effects of leukotriene B4. *Proc Natl Acad Sci U S A*. 1984;81:3501–3505.
- [25] Bacon KB, Camp RD, Cunningham FM, Woollard PM. Contrasting in vitro lymphocyte chemotactic activity of the hydroxyl enantiomers of 12-hydroxy-5,8,10,14-eicosatetraenoic acid. *Br J Pharmacol*. 1988;95:966–974.
- [26] Sampson AP, Castling DP, Green CP, Price JF. Persistent increase in plasma and urinary leukotrienes after acute asthma. *Arch Dis Child*. 1995;73:221–225.
- [27] Hoshino M, Nakamura Y, Sim JJ, Tomioka H. A comparative study of the effects of ketotifen, disodium cromoglycate, and beclomethasone dipropionate on bronchial mucosa and asthma symptoms in patients with atopic asthma. *Respir Med*. 1998;92:942–950.
- [28] Mittelstadt PR, Monteiro JP, Ashwell JD. Thymocyte responsiveness to endogenous glucocorticoids is required for immunological fitness. *J Clin Invest*. 2012;122:2384–2394.
- [29] Li L-B, Leung DYM, Strand MJ, Goleva E. ATF2 impairs glucocorticoid receptor-mediated transactivation in human CD8⁺ T cells. *Blood*. 2007;110:1570–1577.
- [30] Martin RJ, Szeffler SJ, King TS, et al. The Predicting Response to Inhaled Corticosteroid Efficacy (PRICE) trial. *J Allergy Clin Immunol*. 2007;119:73–80.
- [31] Borish L, Aarons A, Rumblyrt J, Cvetusa P, Negri J, Wenzel S. Interleukin-10 regulation in normal subjects and patients with asthma. *J Allergy Clin Immunol*. 1996;97:1288–1296.
- [32] Matsumoto K, Inoue H, Fukuyama S, et al. Decrease of interleukin-10–producing T cells in the peripheral blood of severe unstable atopic asthmatics. *Int J Arch Allergy Immunol*. 2004;134:295–302.
- [33] John M, Lim S, Seybold J, et al. Inhaled corticosteroids increase interleukin-10 but reduce macrophage inflammatory protein-1-alpha, granulocyte-macrophage colony-stimulating factor, and interferon-gamma release from alveolar macrophages in asthma. *Am J Respir Crit Care Med*. 1998;157:256–262.
- [34] American Thoracic Society. Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions. *Am J Respir Crit Care Med*. 2000;162:2341–2351.



eFigure 1. BLT1 expression on stimulated CD4⁺ and CD8⁺ T cells from individual samples. Isolated CD4⁺ and CD8⁺ T cells were stimulated with anti-CD3/anti-CD28 for 8 days and BLT1 expression was assessed by flow cytometry. BLT1 expression on unstimulated (day 0) and stimulated CD4⁺ and CD8⁺ T cells in the presence (+) or absence (–) of Dex. BLT1, leukotriene B₄ receptor; CTL, control; Dex, dexamethasone; SR, steroid resistant; SS, steroid sensitive.



eFigure 1 BLT1 expression on stimulated CD4 + and CD8 + T cells from individual samples. Isolated CD4 + and CD8 + T cells were stimulated with anti-CD3/anti-CD28 for 8 days and BLT1 expression was assessed by flow cytometry. BLT1 expression on unstimul...

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Leukotriene B4 receptor 1 is differentially expressed on peripheral T cells of steroid-sensitive and -resistant asthmatics

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