

Increased T-cell receptor V β 8⁺ T cells in bronchoalveolar lavage fluid of subjects with poorly controlled asthma: A potential role for microbial superantigens

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Background: T cells are thought to play an important role in the pathogenesis of chronic asthma. The immunologic triggers that contribute to poorly controlled asthma are unknown but may include infectious agents. Superantigens (SAGs), which stimulate T cells expressing selected T-cell receptor (TCR) β -chain variable (V β) regions, are known to be an important mechanism by which microbes can contribute to T-cell activation and disease pathogenesis.

Objective: We sought to determine the potential role of SAGs in T-cell activation of patients with poorly controlled asthma.

Methods: We studied the TCR-V β repertoire of bronchoalveolar lavage (BAL) cells and PBMCs from 9 subjects with poorly controlled asthma (FEV₁ <75%), 7 subjects with well-controlled asthma (FEV₁ >80%), and 8 normal control subjects with the use of anti-TCR-V β -specific mAbs and flow cytometry.

Results: Subjects with poorly controlled asthma had a significantly higher expression of V β 8⁺ T cells in BAL fluid than subjects with well-controlled asthma and normal control subjects ($P < .01$) and autologous PBMCs ($P < .05$). Increased V β 8⁺ BAL T cells were present in CD4⁺ ($P < .01$) and CD8⁺ ($P < .05$) subsets, suggesting activation by SAGs.

Conclusion: These results indicate that SAGs are a potential trigger of T-cell activation in poorly controlled asthma. (*J Allergy Clin Immunol* 1999;103:37-45.)

Key words: Asthma, lymphocytes, superantigens

Bronchial asthma is characterized by immune activation of the airways. Identification of the immunologic

Abbreviations used

BAL: Bronchoalveolar lavage
GC: Glucocorticoid
SAG: Superantigen
TCR: T-cell receptor
V β : Variable region of the TCR β chain

trigger or triggers that maintain chronic inflammation and give rise to disease progression in asthma is of critical importance in the development of novel therapies. A major pathogenic role has been assigned to activated T lymphocytes, which can be detected in bronchial mucosa, bronchoalveolar lavage (BAL) fluid, and peripheral blood of patients with symptomatic asthma.^{1,2}

Infections of the respiratory tract represent a major cause of asthma deterioration. An important mechanism by which microbes contribute to disease pathogenesis is through the secretion of toxins known as superantigens (SAGs).³ SAGs bind directly, without antigen processing, to MHC class II molecules on antigen-presenting cells and trigger a potent activation of T cells expressing selected T-cell receptor β -variable region (TCR-V β) elements in both CD4⁺ and CD8⁺ T cells. To date, involvement of SAGs has been studied in the pathogenesis of atopic dermatitis,⁴ psoriasis,⁵⁻⁷ Kawasaki disease,⁸ and rheumatoid arthritis.⁹ However, their role in asthma has not been previously studied. In this study we therefore examined the TCR-V β repertoire of BAL fluid and PBMCs from subjects with poorly controlled asthma to determine whether there was any evidence for superantigenic stimulation of their T cells.

METHODS

Patient selection

Patients with a diagnosis of asthma according to the criteria of the American Thoracic Society¹⁰ were selected for evaluation. None of the subjects was receiving systemic glucocorticoid (GC) therapy (Table I). Patients were classified as having poorly controlled asthma if they had a morning prebronchodilator FEV₁ value of less than 75% of predicted value on repeated occasions when their asthma was stable, and they were classified as having well-controlled asthma if they had an FEV₁ value of greater than 80% of predicted value.

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TABLE I. Patient characteristics

Parameter	Asthma		Normal control subjects
	Poorly controlled	Well controlled	
No. of subjects	9	7	8
Age, y (mean [range])	35 (24-50)	30 (19-42)	35 (26-47)
Sex (M/F)	5/4	2/5	2/6
Atopic subjects	6	5	0
Smokers	0	0	0
FEV ₁ before BD* (mean ± SEM)	59 ± 3 ^{†‡}	92 ± 4 [†]	102 ± 1
FEV ₁ after BD* (mean ± SEM)	75 ± 3 [‡]	104 ± 4	NA
Age at asthma onset, y (mean ± SEM)	11 ± 2	17 ± 4	NA
Duration of asthma, y (mean ± SEM)	24 ± 4	15 ± 4	0
Inhaled glucocorticoids [§] (yes/no)	4/5	1/6	0
Systemic glucocorticoids	0	0	0
Other medications	Albuterol as required, pirbuterol (1/9), theophylline (1/9)	Albuterol as required	0

BD, Bronchodilator; NA, not applicable.

*Percent predicted.

^{†‡}FEV₁ lower compared with normal control subjects[†] or subjects with well-controlled asthma[‡] ($P < .05$).[§]Subjects with poorly controlled asthma: fluticasone (1 mg/day) in 1 of 9, triamcinolone acetonide (800 µg/day) in 2 of 9, and beclomethasone (336 µg/day) in 1 of 9; subjects with well-controlled asthma: triamcinolone acetonide (800 µg/day) in 1 of 7.

TABLE II. BAL cell differentials from subjects with poorly and well-controlled asthma

Cell type	Asthma	
	Poorly controlled	Well controlled
Total white blood cells, ×10 ⁴ /mL (median [range])	12.4 (5.1-23)	6.6 (3.5-12)
Macrophages/monocytes, % (mean ± SEM)	90.1 ± 1.9	86.6 ± 3.2
Polymorphonuclear cells, % (mean ± SEM)	0.8 ± 0.3	0.3 ± 0.1
Lymphocytes, % (mean ± SEM)	7.2 ± 1.0	11.6 ± 3.3
Eosinophils, % (mean ± SEM)	0.9 ± 0.4	0.5 ± 0.3
Epithelial cells, % (mean ± SEM)	1.5 ± 0.4	1.0 ± 0.3
Subjects undergoing cell differential	8/9	7/7

Subjects with poorly controlled asthma improved their FEV₁ by at least 15% after 2 inhalations of albuterol (90 µg per actuation). All asthmatic subjects showed no evidence for other types of lung disease. Normal control subjects had no history of either respiratory or allergic diseases. Informed consent was obtained from all patients and normal control subjects before enrollment in this study, which was approved by the National Jewish Medical and Research Center Institutional Review Board and the Scientific Advisory Committee of the University of Colorado Health Sciences Center General Clinical Research Center in Denver.

Specimen collection

PBMCs were isolated from heparinized blood by Ficoll-Hypaque (Pharmacia Biotech, Piscataway, NJ) gradient centrifugation. Fiber-optic bronchoscopies with BAL were performed according to the guidelines of the American Thoracic Society.¹¹ BAL cells were filtered through a 70-µm Nylon cell strainer (Becton Dickinson Labware, Franklin Lakes, NJ), spun at 200g for 10 minutes, washed 2 times, and resuspended in HBSS. Cell counts were performed by using a hemocytometer and trypan blue exclusion testing. BAL differentials were obtained on cytospin preparations by using a Diff-Quick (Scientific Products, McGraw Park, IL) stain, with 300 cells counted.

Flow cytometry

PBMCs and BAL cells were stained with mAbs against the following TCR-Vβ chains: Vβ2, 3, 5.1, 6.7, 8, 12, 13.1, 13.2, and 17.

Biotinylated mAbs against Vβ2, 3, 8, 12, and 17 were purchased from Immunotech (Westbrook, Me). Biotinylated mAbs against Vβ5.1 (derived from clone LC4), Vβ13.1 (from clone 131.6), and Vβ13.2 (from clone H132.8) were a kind gift from Dr Brian Kotzin (National Jewish Medical and Research Center, Denver, Colo). Streptavidin-phycoerythrin (PE) was purchased from Southern Biotechnology Associates (Birmingham, Ala). An FITC-conjugated mAb against Vβ6.7 was purchased from T cell Diagnostics (Woburn, Mass). mAbs against CD3, CD4, and CD8 (Becton Dickinson Immunocytometry Systems, San Jose, Calif) were used directly conjugated with FITC, PE, peridinin chlorophyll α-protein, or allophycocyanin. A mouse IgG2a-FITC/IgG1-PE from Antigenix America (Franklin Square, NY) was used as an isotype control. Stained cells were fixed with 1% methanol-free formaldehyde in PBS (Polysciences, Inc, Warrington, Pa) and analyzed with a Becton Dickinson FACScan or FACScalibur by using Lysis II or Cellquest software, respectively. By gating on CD3⁺ cells, only T cells were included in the analysis.

Statistical analysis

Comparisons of the TCR-Vβ repertoire for BAL cells and PBMCs between the study groups were made by using 2-way repeated-measures ANOVA. Comparisons of Vβ8⁺ BAL T cells among subjects with poorly controlled asthma who were receiving versus those not receiving inhaled steroid therapy were also performed by using ANOVA. Measurements of Vβ8⁺ T cells from BAL fluid were compared with those from autologous PBMCs within the

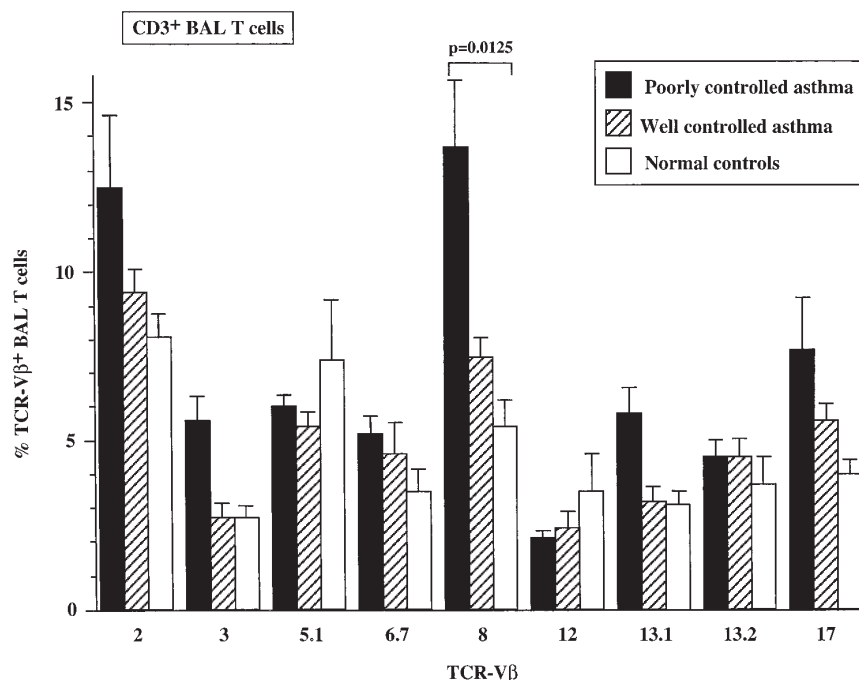


FIG 1. Percentages of Vβ⁺ T cells (mean ± 2 SEM) in the BAL fluid of 9 subjects with poorly controlled asthma, 7 subjects with well-controlled asthma, and 8 normal control subjects determined with the use of 9 different anti-Vβ mAbs. Subjects with poorly controlled asthma had a significantly higher ($P = .0125$) expression of Vβ8⁺ BAL T cells than normal control subjects. The expression of BAL T cells positive for Vβs other than Vβ8 was not significantly different among the study groups.

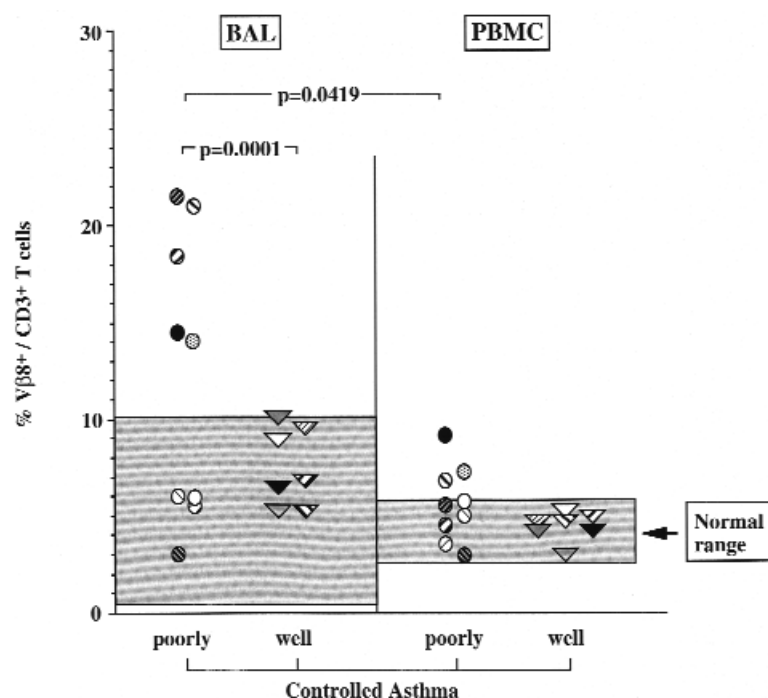


FIG 2. Percentages of Vβ8⁺ T cells in BAL fluid and blood of patients with poorly and well-controlled asthma determined with anti-Vβ8⁺ mAbs. Individual symbols are used for each patient. Shaded boxes represent normal ranges (mean ± 2SD) for the percentage of Vβ8⁺ T cells in BAL fluid and peripheral blood of the normal control subjects studied. Because subjects with poorly controlled asthma did not differ from normal control subjects in their expression of Vβ8⁺ T cells in BAL fluid and PBMCs, the combined results of both groups were compared with the expression of Vβ8⁺ T cells in subjects with poorly controlled asthma, demonstrating a significant increase of Vβ8⁺ BAL T cells in subjects with poorly controlled asthma compared with the combined group of subjects with well-controlled asthma and normal control subjects ($P = .0001$) and compared with their autologous PBMCs ($P = .0419$).

TABLE III. TCR-V β use (%) in peripheral blood (PBMCS) and BAL fluid of patients with poorly controlled asthma*†

V β	T cells	PC-1		PC-2		PC-3		PC-4	
		PBMCS	BAL fluid	PBMCS	BAL fluid	PBMCS	BAL fluid	PBMCS	BAL fluid
2	CD3 ⁺	8.8	24.1	9.3	14.1	9.4	7.6	9.2	28.1
	CD4 ⁺	10.6	38.7	9.7	15.7	11.0	9.8	8.6	28.4
	CD8 ⁺	7.3	7.9	8.5	9.8	6.8	4.6	10.5	30.0
3	CD3 ⁺	4.7	10.0	3.4	3.9	5.3	5.0	3.6	10.4
	CD4 ⁺	5.1	12.3	3.8	4.4	6.1	6.5	4.0	10.0
	CD8 ⁺	4.6	6.7	1.9	2.3	4.1	2.9	3.2	13.1
5.1	CD3 ⁺	4.2	5.8	4.6	6.9	5.1	7.2	4.9	7.1
	CD4 ⁺	5.3	8.1	5.3	7.6	6.5	9.3	5.4	8.1
	CD8 ⁺	3.2	3.7	2.3	5.4	2.4	4.3	4.3	3.8
6.7	CD3 ⁺	8.1	7.7	5.3	6.1	4.2	4.9	3.5	5.8
	CD4 ⁺	11.9	11.1	5.8	6.8	5.4	6.0	4.9	6.7
	CD8 ⁺	3.6	4.2	3.8	4.2	1.7	3.6	1.6	2.7
8	CD3 ⁺	5.8	5.9	4.5	18.4	9.2	14.5	5.6	21.6
	CD4 ⁺	6.2	6.3	5.1	19.8	7.6	17.4	5.5	30.0
	CD8 ⁺	5.9	6.2	2.4	15.7	5.0	11.2	6.1	18.0
12	CD3 ⁺	1.5	1.3	1.8	1.7	1.8	2.1	1.9	2.8
	CD4 ⁺	1.8	1.7	1.9	1.8	2.2	2.6	2.1	3.1
	CD8 ⁺	1.3	1.1	1.5	1.9	1.2	1.5	1.6	1.9
13.1	CD3 ⁺	4.8	4.9	2.0	10.0	1.8	2.1	3.5	5.1
	CD4 ⁺	5.4	6.8	2.2	11.0	2.2	2.6	2.2	5.0
	CD8 ⁺	4.5	3.1	1.3	7.9	1.2	1.5	5.5	5.8
13.2	CD3 ⁺	3.4	5.2	4.5	8.6	3.0	10.2	2.8	3.3
	CD4 ⁺	4.3	6.8	3.8	9.2	3.7	10.3	2.8	3.7
	CD8 ⁺	2.6	4.0	7.6	7.7	1.5	11.3	3.1	2.3
17	CD3 ⁺	5.3	7.4	5.3	12.7	4.7	2.9	6.1	15.2
	CD4 ⁺	5.7	5.0	6.0	14.4	4.5	3.0	5.3	16.5
	CD8 ⁺	5.2	12.0	2.6	7.8	6.0	3.1	7.7	11.4

***Bold** values are greater than the normal upper range (mean + 2SD of normal control subjects).

†Percentages of positive cells were determined by immunofluorescence analysis by using the appropriate anti-V β mAb.

study groups. For testing the larger set of pairwise means comparisons for the TCR-V β repertoire between the study groups, the more conservative Tukey-Kramer multiple comparison was used. To further minimize the multiple comparison problem, overall hypotheses were tested by contrasting the percentage of TCR-V β 8⁺ cells from the remainder of the TCR-V β profile and contrasting the poorly controlled asthma group from the other 2 groups. This provides an explicit test of whether the extent to which subjects with poorly controlled asthma have an increased percentage of V β ⁺ cells is magnified for V β 8. Age, disease onset and duration, and FEV₁ values were compared by ANOVA (Tukey-Kramer). BAL cell counts and differentials of subjects with poorly and well-controlled asthma were compared by using the nonparametric Wilcoxon test. All results were considered significant at the .05 level.

RESULTS

Patient characteristics

Patient characteristics are summarized in Table I. Compared with 7 patients with well-controlled asthma, 9 subjects with poorly controlled asthma had a significantly lower baseline FEV₁ ($P < .05$) and a significantly lower FEV₁ after bronchodilator therapy ($P < .05$). At both time points, the mean FEV₁ of subjects with poorly controlled asthma remained below the predicted normal range, although they had a greater than 15% improvement after bronchodilator therapy. The mean FEV₁ of

subjects with well-controlled asthma was below the normal range ($P < .05$) at baseline and within the normal range after bronchodilator therapy. Both study groups contained patients taking inhaled GCs. None of the patients were treated with oral GCs. Patients had stopped oral GC therapy for greater than 1 month before study entry. All asthmatic patients were receiving inhaled albuterol on an as-required basis.

The mean ages of subjects with poorly controlled asthma (mean, 35 years; range, 24 to 50 years) and subjects with well-controlled asthma (mean, 30 years; range, 19 to 42 years) were not significantly different. The mean age of asthma onset in subjects with poorly controlled asthma (mean, 11 years; range, 3 to 26 years) was lower than that found in subjects with well-controlled asthma (mean, 17 years; range, 3 to 38 years); however, this was not significantly different. This was reflected in a longer mean duration of asthma in subjects with poorly controlled asthma (mean, 24 years; range, 10 to 40 years) than in subjects with well-controlled disease (mean, 15 years; range, 4 to 29 years), but this was also not significant. Sixty-seven percent of subjects with poorly controlled asthma and 71% of subjects with well-controlled asthma were atopic, which was confirmed by a positive history, a positive skin prick test response to inhalant allergens, or

PC-5		PC-6		PC-7		PC-8		PC-9	
PBMCs	BAL fluid	PBMCs	BAL fluid	PBMCs	BAL fluid	PBMCs	BAL fluid	PBMCs	BAL fluid
8.9	7.6	7.7	6.7	10.1	11.4	9.9	9.5	7.2	9.3
11.0	7.4	8.9	7.9	11.0	12.7	11.0	8.4	9.3	13.0
6.0	7.9	6.3	5.6	8.5	9.1	7.9	12.0	5.5	5.2
3.9	3.6	3.9	5.6	1.0	5.5	2.2	2.9	2.6	3.9
1.6	3.1	4.5	7.0	1.1	6.0	2.0	2.3	3.8	4.2
3.0	6.1	3.1	3.9	0.9	4.7	3.0	4.0	1.4	4.2
5.4	5.1	6.6	5.2	4.9	4.6	7.5	6.2	3.2	2.8
7.4	5.2	7.9	7.6	6.0	4.6	8.6	7.0	5.0	3.7
2.1	4.5	5.0	2.1	2.7	4.9	5.0	5.6	1.4	1.8
6.4	6.4	0.8	1.9	3.7	5.6	2.1	2.7	21.6	6.3
7.1	7.1	1.0	2.5	4.8	6.4	2.5	2.8	6.5	7.2
5.9	1.6	0.5	1.0	1.7	4.2	1.0	2.8	40.4	6.4
2.9	2.9	5.0	6.0	6.8	21.1	7.3	14.1	3.5	5.5
3.4	2.9	5.6	7.8	6.9	22.5	5.1	13.0	5.0	6.8
2.4	2.8	4.6	3.8	6.7	19.1	15.3	17.1	2.0	4.4
1.6	3.1	1.9	2.2	1.6	2.0	1.4	3.6	1.5	1.0
2.0	3.1	1.8	3.2	3.3	2.1	1.5	4.3	2.0	0.8
0.9	2.3	2.5	0.9	1.1	1.8	1.3	3.0	1.0	1.4
0.8	2.3	4.4	2.4	2.2	4.7	3.7	5.4	1.8	7.4
1.0	2.2	5.7	3.7	2.9	5.5	4.0	4.5	3.1	10.0
0.4	2.8	2.3	0.7	0.9	3.1	3.4	7.1	0.5	4.7
2.4	2.1	1.1	2.4	3.0	4.8	1.1	2.9	3.1	6.6
2.7	1.7	1.1	3.2	3.4	6.3	1.0	2.0	4.1	9.4
2.1	4.1	1.3	1.3	2.3	1.6	1.4	4.4	2.2	3.5
2.6	4.2	5.3	5.3	5.7	17.8	5.6	4.5	4.3	3.6
3.2	4.2	5.9	5.9	6.6	18.2	6.3	5.1	6.0	3.9
1.7	3.9	4.6	4.9	4.2	17.9	4.1	4.3	2.9	3.8

both. With respect to age, gender distribution, and FEV₁ after bronchodilator use, patients with well-controlled asthma did not differ from the group of 8 healthy volunteers. The normal control subjects (mean, 35 years; range, 26 to 47 years) had no history of respiratory or atopic disease. All study subjects were nonsmokers.

BAL cell differentials

In 8 of 9 subjects with poorly controlled asthma and all subjects with well-controlled asthma, a BAL cell differential was performed. As shown in Table II, the median of white blood cell counts per milliliter of BAL fluid from subjects with poorly controlled asthma (mean, $12.4 \times 10^4/\text{mL}$; range, 5.1 to $23 \times 10^4/\text{mL}$) was higher than that found in subjects with well-controlled asthma (mean, $6.6 \times 10^4/\text{mL}$; range, 3.5 to $12 \times 10^4/\text{mL}$); however, this difference was not statistically significant. There also were no significant differences in the mean percentages of the different BAL cell types between subjects with poorly and well-controlled asthma. However, 4 subjects with poorly controlled asthma treated with inhaled steroids had a significantly lower mean percentage of eosinophils in their BAL fluid than 4 subjects with poorly controlled asthma without inhaled steroid treatment ($0.2\% \pm 0.2\%$ vs $1.8\% \pm 0.7\%$; $P < .05$).

TCR-V β repertoire analysis

To determine whether there was any evidence for superantigenic stimulation of T cells from subjects with poorly controlled asthma, we examined the TCR-V β repertoire in their PBMCs and BAL cells compared with that found in subjects with well-controlled asthma and normal control subjects. The analysis was restricted to TCR-V β 2, 3, 5.1, 6.7, 8, 12, 13.1, 13.2, and 17 because of limitations in cell numbers and previous reports of their elevations in human cells stimulated by SAg.^{3,12} The use of these TCR-V β s in PBMCs of patients with poorly controlled asthma revealed no significant differences compared with subjects with well-controlled asthma and normal control subjects. However, some individuals had percentages of T cells expressing a specific TCR-V β in their peripheral blood, which were increased above the upper range of normal control subjects (mean $\pm$ 2SD) (Tables III and IV). This was most prominent in 2 patients with poorly controlled asthma (Table III). Patient PC-9 had an elevated percentage of V β 6.7⁺/CD3⁺ T cells (21.6%) present in the CD8⁺ subset (40.4%), and patient PC-8 showed an increased percentage of V β 8⁺/CD8⁺ T cells (15.3%).

In contrast, the TCR-V β repertoire analysis of BAL T

TABLE IV. TCR-V β (%) use in peripheral blood (PBMCs) and BAL fluid of patients with well-controlled asthma**

V β	T cells	WC-1		WC-2		WC-3	
		PBMCs	BAL fluid	PBMCs	BAL fluid	PBMCs	BAL fluid
2	CD3 ⁺	9.8	10.9	10.1	9.3	8.7	9.4
	CD4 ⁺	8.8	10.9	12.2	9.9	13.1	11.7
	CD8 ⁺	12.3	11.4	5.8	5.6	7.9	4.7
3	CD3 ⁺	3.1	3.3	2.7	1.7	2.0	1.3
	CD4 ⁺	3.4	3.3	3.1	1.6	2.7	1.3
	CD8 ⁺	3.1	3.3	1.9	3.0	2.2	1.5
5.1	CD3 ⁺	4.5	6.8	4.4	6.0	3.6	5.5
	CD4 ⁺	6.0	8.2	5.5	6.3	5.5	6.7
	CD8 ⁺	2.5	2.8	1.9	4.0	3.1	3.3
6.7	CD3 ⁺	3.0	4.2	5.0	10.0	3.0	5.5
	CD4 ⁺	4.0	5.1	6.4	11.1	3.7	7.3
	CD8 ⁺	1.6	1.5	2.1	2.6	4.1	1.3
8	CD3 ⁺	4.3	6.5	4.2	10.1	3.1	5.3
	CD4 ⁺	4.5	6.7	5.8	10.5	3.3	5.6
	CD8 ⁺	4.5	6.2	3.4	7.8	4.8	5.8
12	CD3 ⁺	1.5	2.8	2.1	3.4	1.4	1.0
	CD4 ⁺	1.9	3.0	2.1	3.6	1.8	1.0
	CD8 ⁺	0.9	2.1	2.3	2.6	1.6	1.3
13.1	CD3 ⁺	2.1	3.3	1.9	2.4	1.7	1.6
	CD4 ⁺	3.8	3.9	2.2	2.5	2.7	1.7
	CD8 ⁺	2.9	4.0	1.5	2.0	1.4	1.9
13.2	CD3 ⁺	2.6	7.1	3.4	5.9	2.3	2.6
	CD4 ⁺	2.4	5.7	3.5	6.3	2.7	2.8
	CD8 ⁺	3.0	11.6	3.3	3.3	3.1	3.0
17	CD3 ⁺	5.5	7.4	4.9	—	3.7	3.4
	CD4 ⁺	6.5	7.4	5.8	—	4.3	4.2
	CD8 ⁺	4.3	7.6	3.0	—	1.8	2.0

***Bold** values are greater than the normal upper range (mean + 2SD of normal control subjects).

†Percentages of positive cells were determined by immunofluorescence analysis by using the appropriate anti-V β mAb.

cells revealed a significantly increased level of V β 8⁺ BAL T cells in subjects with poorly controlled asthma compared with normal control subjects ($P = .0125$) (Fig 1). BAL T cells positive for other TCR-V β s were not significantly increased in subjects with poorly controlled asthma. Of note, the extent to which V β 8⁺ BAL T cells were increased in subjects with poorly controlled asthma was also highly significant ($P = .0037$) compared with other TCR-V β s. Normal control subjects and subjects with well-controlled asthma did not differ significantly from each other in their BAL TCR-V β repertoire. Therefore we compared the expression of V β 8⁺ BAL T cells in subjects with poorly controlled asthma with the combined average expression of V β 8⁺ BAL T cells in subjects with well-controlled asthma and normal control subjects. As shown in Fig 2, this was also highly significant ($P = .0001$).

To evaluate whether V β 8⁺ T cells were selectively expanded in the lungs compared with the peripheral blood, we compared the expression of V β 8⁺ T cells between BAL cells and PBMCs within the different study groups. The increase of V β 8⁺/CD3⁺ BAL T cells in subjects with poorly controlled asthma was also present when compared with their autologous peripheral blood ($P = .0419$), as shown in Fig 2. Of note, in 3 of 5 subjects

with poorly controlled asthma with elevated V β 8⁺/CD3⁺ BAL T cells, an increase of V β 8⁺/CD3⁺ T cells above the normal range could also be detected in their peripheral blood, but not to as great an extent as found in their respective BAL fluid. However, there was no significant correlation between the percentage of V β 8⁺ T cells in BAL fluid and PBMCs from subjects with poorly controlled asthma, either in their total CD3⁺ or in their CD4⁺ or CD8⁺ subpopulations. In contrast to subjects with poorly controlled asthma, subjects with well-controlled asthma and normal control subjects showed no significant differences between the TCR-V β repertoire from BAL fluid and PBMCs. When compared with the average increase of V β 8⁺ BAL T cells versus PBMCs in combined subjects with well-controlled asthma and normal control subjects, the extent to which V β 8⁺/CD3⁺ BAL T cells from subjects with poorly controlled asthma were increased in comparison to their peripheral blood was also significant ($P = .0178$).

To exclude the possibility that anti-inflammatory therapy with inhaled glucocorticoids had affected the expression of TCR-V β 8⁺ T cells in BAL fluid and PBMCs of subjects with poorly controlled asthma, we compared the expression of V β 8⁺ T cells in BAL fluid and PBMCs from subjects with poorly controlled asthma receiving

WC-4		WC-5		WC-6		WC-7	
PBMCs	BAL fluid	PBMCs	BAL fluid	PBMCs	BAL fluid	PBMCs	BAL fluid
6.7	13.0	6.6	5.1	7.6	8.9	6.8	9.0
10.0	13.5	9.7	9.5	9.1	9.8	7.8	12.2
3.6	10.6	3.9	2.1	4.9	5.1	5.0	7.0
3.4	3.3	0.7	1.3	4.1	2.9	2.5	4.8
4.0	2.5	0.0	1.4	4.5	2.7	2.9	5.7
2.9	4.9	0.5	1.4	3.5	4.8	1.9	4.4
4.0	6.8	3.9	3.0	6.6	5.6	4.4	3.9
6.3	6.5	6.1	5.2	6.5	5.9	5.6	5.1
1.6	4.7	1.9	1.6	7.4	4.8	2.3	3.2
1.9	0.2	3.9	3.2	3.8	5.3	3.1	3.6
2.2	0.1	6.1	7.0	4.6	5.9	3.9	2.7
1.8	0.2	1.9	0.4	2.2	1.9	1.5	4.6
6.0	8.9	4.7	5.3	4.8	9.6	5.7	7.0
4.3	6.6	7.0	10.1	5.4	10.5	6.0	7.4
8.5	13.5	2.5	1.9	3.9	5.8	5.4	7.3
1.3	1.4	1.5	0.9	2.0	1.8	2.1	5.4
1.5	1.3	2.4	1.4	2.1	1.9	2.3	5.4
1.0	1.6	0.6	0.7	1.9	1.3	1.7	6.0
1.7	2.2	3.0	2.9	2.5	5.4	3.6	3.6
1.7	2.2	3.7	4.1	2.7	5.7	3.8	4.6
1.8	2.9	2.5	2.4	2.2	4.8	3.4	3.1
1.8	3.5	3.8	6.0	2.5	4.1	2.4	2.5
2.3	3.7	3.6	10.1	2.5	4.1	2.2	2.8
1.2	3.4	4.5	3.6	2.7	4.7	3.0	2.4
3.2	5.9	4.7	6.5	5.1	4.1	5.6	6.3
4.5	5.5	6.0	4.7	5.6	4.1	6.1	6.1
1.9	6.8	3.9	9.6	4.7	5.1	4.9	7.0

inhaled GC therapy with that of similar subjects not receiving therapy. The expression of V β 8⁺ T cells in BAL fluid and PBMCs from 4 subjects with poorly controlled asthma receiving inhaled GC therapy did not differ significantly from 5 patients who were not treated with inhaled steroids.

The significant increase of V β 8⁺/CD3⁺ BAL T cells in subjects with poorly controlled asthma was accounted for by 5 patients who had values higher than the normal range (Fig 2). In 4 of these 5 subjects with poorly controlled asthma, the elevation of V β 8⁺ BAL T cells was present in both their CD4⁺ and CD8⁺ subsets, which is suggestive of a superantigenic trigger (Fig 3). The fifth patient with poorly controlled asthma with increased V β 8⁺ BAL T cells showed the elevation only in the CD4⁺ subset. The increased expression of V β 8⁺ BAL T cells in CD4⁺ and CD8⁺ subsets from subjects with poorly controlled asthma was significant for V β 8⁺/CD4⁺ ($P = .0001$) and V β 8⁺/CD8⁺ ($P = .0191$) BAL T cells when compared with the average expression of V β 8⁺ BAL T cells in CD4⁺ and CD8⁺ subsets from combined subjects with well-controlled asthma and normal control subjects.

Although other TCR-V β s did not show a significantly increased overall expression in BAL cells from subjects with poorly controlled asthma, individual patients demon-

strated expansions of TCR-V β s other than V β 8 above the normal range (Table III). This was most prominent in patient PC-1 who had increased V β 2⁺/CD3⁺ (24.1%) BAL T cells present only in the CD4⁺ subset (38.7%), suggesting a conventional antigen response. Patient PC-4 additionally had elevated levels of V β 2⁺, V β 3⁺, and V β 17⁺ BAL T cells, and patient PC-7 had increased V β 17⁺ BAL T cells (Table III). Interestingly, these elevations were present in both their CD4⁺ and CD8⁺ subsets, which is suggestive of superantigenic activation. Increased expression of T cells positive for different TCR-V β s was also present in BAL T cells in individuals with well-controlled asthma (Table IV), but these elevations were present in either the CD4⁺ or the CD8⁺ subset, suggesting exclusively a conventional antigen response in these patients.

DISCUSSION

This study demonstrates for the first time a potential role of microbial SAg in the pathogenesis of poorly controlled asthma. Although the TCR-V β repertoire from the peripheral blood did not differ between the study groups, there was a significant increase in V β 8⁺ BAL T cells of subjects with poorly controlled asthma. The overexpression of V β 8⁺ BAL T cells was present in 5 of 9 subjects

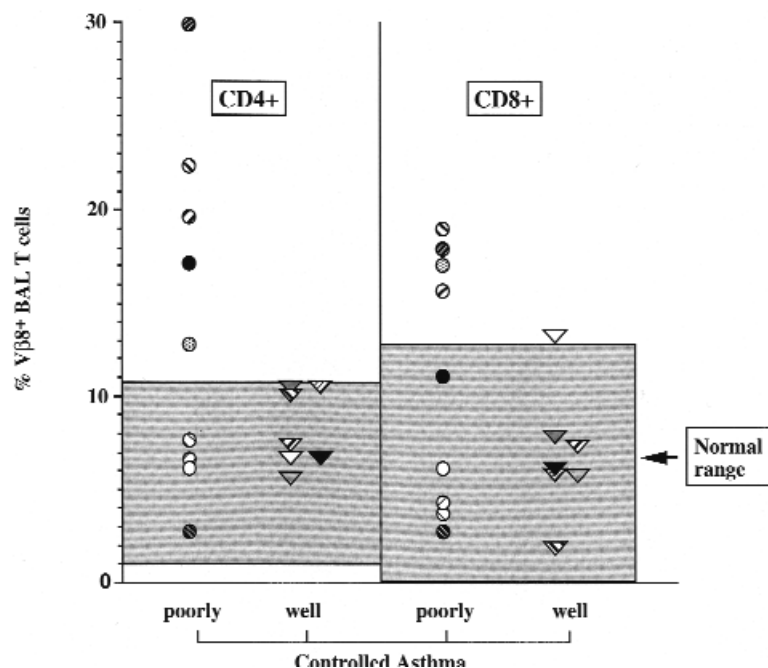


FIG 3. Percentages of V β 8⁺ T cells in CD4⁺ and CD8⁺ BAL T cells of individual patients with poorly and well-controlled asthma. Symbols for individual patients correspond to symbols used in Fig 2. Shaded boxes represent normal ranges (mean $\pm$ 2SD) for the percentage of V β 8⁺/CD4⁺ and V β 8⁺/CD8⁺ BAL T cells of the normal control subjects studied. Subjects with well-controlled asthma did not differ from normal control subjects in their expression of V β 8⁺ T cells in CD4⁺ and CD8⁺ BAL T cells. V β 8⁺ T cells from subjects with poorly controlled asthma were significantly elevated in their CD4⁺ ($P = .0001$) and CD8⁺ ($P = .0191$) BAL T cells compared with the combined group of subjects with well-controlled asthma and normal control subjects.

with poorly controlled asthma and was present in both the CD4⁺ and CD8⁺ T cell subsets in 4 of these subjects. This result strongly suggests a superantigenic trigger in these patients because SAgS are known to activate T cells through interaction with specific TCR-V β S independent of their CD4 and CD8 phenotype.³ In 2 subjects with poorly controlled asthma with evidence for superantigenic stimulation, an increased expression of V β 8⁺ BAL T cells could also be detected in their PBMCs but to a lesser extent than that observed in their BAL cells. This observation might reflect T cells recirculating from bronchial lymph nodes. The overall expression of V β 8⁺ T cells in subjects with poorly controlled asthma was significantly higher in the BAL fluid than in the peripheral blood, indicating a local T-cell activation in the airways.

Evidence for local T-cell activation in poorly controlled asthma by a variety of SAgS is supported by the increased expression of BAL T cells positive for TCR-V β S other than V β 8. This was observed for V β 2, V β 3, and V β 17 in the CD4⁺ and CD8⁺ T cell subsets of 2 patients. These V β expansions were observed in their BAL fluid but not their PBMCs.

We also found evidence for antigen-driven T-cell activation in some subjects with poorly controlled asthma who had increased numbers of T cells positive for specific TCR-V β S in the CD4⁺ or CD8⁺ subsets from BAL cells or PBMCs. This was most prominent in 1 subject with poorly controlled asthma who demonstrated an

increased level of TCR-V β 8⁺/CD4⁺ T cells in the BAL fluid, and to a lesser extent in PBMCs, when compared with our normal control subjects. These observations are consistent with a previous report of allergen-induced expansions of BAL T cells in atopic asthma.¹³ Expansions of T cells in CD4⁺¹⁴⁻¹⁶ or CD8⁺ subpopulations^{17,18} have also been described in other inflammatory diseases.

The mean duration of asthma in subjects with poorly controlled asthma was 9 years longer than that found in subjects with well-controlled asthma (Table I). Furthermore, the mean age of asthma onset was different between these patient groups, with the disease developing 6 years earlier in the poorly controlled asthma group. However, mean duration of asthma and mean age of disease onset did not differ significantly between subjects with poorly and well-controlled asthma, which might be explained by the wide ranges of these 2 parameters. Because there is clearly a tendency to longer disease duration and earlier onset of asthma in subjects with poorly controlled asthma, more data are needed to identify the effect of asthma duration on disease control and the concurrent BAL T-cell repertoire.

Although the subjects with poorly controlled asthma showed a trend toward a higher number of white blood cells in their BAL fluid compared with subjects with well-controlled asthma, the BAL cell differentials did not differ significantly between these two groups. However, subjects with poorly controlled asthma receiving inhaled

GCs had a significantly lower ($P < .05$) percentage of BAL eosinophils than subjects with poorly controlled asthma not receiving inhaled GC therapy. This result emphasizes the necessity for effective anti-inflammatory treatment and asthma control.

Our data suggest that poorly controlled asthma is associated with ongoing airway inflammation, which results in skewing of the BAL T-cell repertoire by several different stimuli, including microbial SAGs and antigens. In contrast, there was no evidence for SAG-induced T-cell activation in patients with well-controlled asthma. SAG-producing microbes, as compared with allergens, may cause a more potent T-cell activation in the airways, which maintains chronic inflammation if not appropriately treated. The role of microbial SAGs has already been demonstrated in the pathogenesis of atopic dermatitis, in which they trigger and enhance skin inflammation^{4,19} and stimulate IgE synthesis.²⁰ To date, we have not been able to identify the SAG-producing microbe in our patients with poorly controlled asthma and V β -specific T-cell activation. However, a pathogenic mechanism similar to the SAG-induced process in atopic dermatitis can be suggested for these subjects.

Increasing evidence for microbes in the pathogenesis of asthma support this concept. Infections with *Chlamydia pneumoniae*²¹ and *Mycoplasma pneumoniae*²² can trigger acute asthma exacerbations, but more importantly, infections with *C pneumoniae* have been shown to precede severe chronic asthma.^{23,24} Furthermore, Kraft et al²⁵ detected *M pneumoniae* in the lower airways of adults with chronic asthma. A *Mycoplasma*-derived SAG²⁶⁻²⁸ and its pathogenic role in rheumatoid arthritis²⁹ have already been described. Further studies, however, will be needed to identify the relevant microbial agent or agents that result in the immune activation associated with poorly controlled asthma.

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