Published in final edited form as:

J Allergy Clin Immunol. 2007 November ; 120(5): 1065–1072. doi:10.1016/j.jaci.2007.07.042.

Airway Remodeling and Lack of Bronchodilator Response in Steroid Resistant (SR) Asthma

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Abstract

Background—Steroid resistant (SR) asthma is characterized by airway inflammation that fails to resolve despite treatment with corticosteroids, raising concerns that resistance to steroid therapy in asthma could lead to airway remodeling.

Objective—To determine whether SR asthma is accompanied by decreased airflow reversibility and could lead to airway remodeling.

Methods—Spirometry was evaluated on 40 asthmatics defined as SR or steroid sensitive (SS) by 1 wk course of oral prednisone. 23 asthmatics underwent bronchoscopy with collection of broncho-alveolar lavage (BAL) to analyze markers of airway remodeling in BAL fluid (BALF) and cells.

Results—Prednisone significantly improved FEV₁% predicted in SS (62.0±10.9% [mean±SD] to 79.4±11.3%, p<0.001), but not SR asthmatics (66.9±10.0% to 65.9±12.1%). The bronchodilator response was significantly greater in SS than SR group (ΔFEV₁% predicted 33.5±22.5% vs. 15.2±7.9%, p=0.001) regardless of inhaled corticosteroids use. No difference in amounts of MMP-9, PMN elastase, VEGF were found in BALF from both groups. TIMP-1 levels were, however, significantly lower in BALF of SR compared to SS asthmatics (921.9±313.4 pg/ml vs. 2267.0±456.8 pg/ml, p<0.05), resulting in significantly higher MMP-9/TIMP-1 ratios in BALF of SR patients (0.24±0.04 vs. 0.11±0.03, p<0.01). Finally, DEX treatment induced TIMP-1 mRNA in SS BAL cells (p<0.01), but not in SR BAL cells.

Conclusion—Bronchodilator reversibility is impaired in SR asthma and is associated with a shift in MMP-9/TIMP-1 ratio due to inability of steroids to enhance TIMP-1 production, potentially promoting proteolytic activity in SR asthmatic airways and contributing to chronic airway remodeling.

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Clinical Implications—SR asthma may lead to irreversible airways disease.

Keywords

steroid resistant asthma; airway remodeling; bronchodilator response; BAL cells

INTRODUCTION

Glucocorticoids (GC) are the most effective agents for the treatment of inflammation in the management of chronic inflammatory diseases, such as asthma, inflammatory bowel disease, and autoimmune diseases.^{1–4} Current asthma therapy is aimed at controlling disease symptoms, with GCs being the gold standard for asthma treatment.⁵ However, recent studies suggest that there is substantial need for further improvement in asthma therapy, since a large group of asthma patients remains symptomatic despite optimal GC therapy. It is estimated that a subset of asthmatics (10% to 25%) do not respond to GC therapy.^{1,2,6,7} Steroid resistance complicates treatment of asthma since these patients have evidence of persistent airway inflammation and suffer from the unwanted side effects (e.g. osteoporosis, cataracts, diabetes, etc) of high dose prolonged GC therapy.^{1,8} Given the recent increase in asthma prevalence and severity worldwide, steroid resistance has become a challenging health problem that contributes to the high costs of asthma care.^{8,9}

Persistent asthma has been reported to contribute to airway remodeling which can result in permanent biomechanical and pathologic alteration of asthmatic airways.^{10–12} Structural changes seen in asthmatic patients can include thickening of the airway wall reticular basement membrane, formation of abnormal elastic fiber network, alterations in airway cartilage structure, angiogenesis, increase in airway smooth muscle mass.^{13–15} Tissue collagen deposition is known to be controlled by two classes of molecules – matrix metalloproteinases (MMPs) that degrade collagen, and tissue inhibitors of metalloproteinases (TIMPs).^{14,16} Studies of asthma patients demonstrate increased production of both MMP-9 and TIMP-1 by alveolar macrophages, elevated quantities of these mediators in the sputum, BAL fluid and biopsies.^{16–20} Of greatest importance is the balance of MMP-9 to TIMP-1, as this plays a role in modifying airway wall matrix deposition and remodeling.¹⁴

The current study explored the possibility that steroid resistance is associated with the development of airway remodeling by evaluating bronchodilator response in steroid resistant (SR), as compared to steroid sensitive (SS) asthmatics, and measurement of airway remodeling markers in BAL fluid from these patients. As well, steroid regulation of metalloproteinase production by BAL macrophages was studied in these two patient populations.

METHODS

Subjects

Patients with a diagnosis of asthma according to American Thoracic Society criteria²¹ were selected for evaluation. Forty adult subjects (>18 years) with asthma were enrolled in this study. To qualify, subjects were required to have a diagnosis of asthma with a baseline FEV₁% predicted ≤80% and to demonstrate either significant airway hyperresponsiveness (PC₂₀ methacholine <8mg/mL) or significant bronchodilator responsiveness (>12% improvement in FEV₁% predicted after 180µg albuterol by metered-dose inhaler) as described.²² The patients enrolled were required to have FEV₁% predicted lower than 80% to allow evaluation of lung function improvement after prednisone burst as published by us earlier.^{22–25} The patients were recruited for this study by advertisement. The majority of these patients were not followed at National Jewish Medical and Research Center and many were previously untreated asthmatics. After baseline characterization, all subjects received prednisone, 20mg by mouth

twice daily for seven days and were categorized as being SS if the FEV₁% predicted improved by $\geq 15\%$ and as SR if the FEV₁% predicted improved by $< 12\%$ (Tables I, II). Adherence to therapy was monitored by a significant drop ($\geq 50\%$) in morning plasma cortisol level. Four healthy non atopic control donors with no history of the lung diseases were enrolled as well. Informed consent was obtained from all patients before enrollment in this study. The Institutional Review Board at National Jewish Medical and Research Center, Denver, CO approved the study.

Specimen collection

None of the subjects had received systemic GC therapy for at least one month prior to bronchoscopy. During the study, the patients using inhaled steroids were asked to withhold them 24 hr prior to bronchoscopy to avoid interference with the *in vitro* steroid response tests performed. The patients continued to use short acting beta-agonists as needed. Fiberoptic bronchoscopies with BAL were performed in a subset of patients recruited for the study according to the guidelines of the American Thoracic Society.²⁶ There was no specific selection of the patients for this procedure aside from the patient's agreement to undergo this procedure. In general bronchoscopy was performed not sooner than one month after oral prednisone course.

BAL cells were filtered through a 70- μ m Nylon cell strainer (Becton Dickinson Labware, Franklin Lakes, NJ), spun at 200g for 10 min, washed two times, and resuspended in HBSS. BAL differentials were obtained on cytopspin preparations by using a Diff-Quick (Scientific Products, McGraw Park, IL) stain, counting a minimum of 500 cells. BAL samples consisted mostly of macrophages (over 90%) (Table III), with $5.5 \pm 4.2\%$ vs. $6.6 \pm 4.3\%$ lymphocytes for the SR and SS asthma groups, respectively. Significantly higher levels of eosinophils were found in BAL samples from SS asthma patients ($p=0.02$) (Table III). In contrast, no significant difference was noted in the number of neutrophils in BAL samples from both study groups (Table III).

For the study, BAL cells were resuspended in RPMI 1640 (BioWhittaker) containing 10% heat activated charcoal filtered GC-free FCS (Gemini Bio Products, Calabasas, CA), 40 μ mol/L L-glutamine, 100 U/ml penicillin, 100 U/ml streptomycin, and 20 mmol/L HEPES buffer solution (GIBCO BRL Life Technologies).

ELISA

Collected BAL fluid was preserved in sterile tubes at -80°C before analysis. To determine whether the difference in bronchodilator response between the two study groups was related to the production of airway remodeling enzymes, MMP-9, TIMP-1, PMN elastase and VEGF – known markers of airway remodeling^{13–15} – were measured in BAL fluid from SR, SS asthmatics and healthy control subjects by ELISA according to the manufacturer's recommendations (R&D systems, Minneapolis, MN) and TIMP-1, MMP-9, VEGF and PMN elastase levels in BAL fluid and normalized to the total protein amounts in each sample detected by Bradford protein assay (Bio-Rad, Hercules, CA).

Real time PCR

BAL cells from five SR and five SS asthmatics were cultured with or without 10^{-6}M DEX for 4 hr at 37°C and preserved in RLT buffer. RNA was extracted according to the manufacturer's guidelines (Qiagen, Valencia, CA), transcribed into cDNA, and analyzed by real-time PCR using the dual-labeled fluorogenic probe method on an ABI Prism 7000 sequence detector (Applied Biosystems, Foster City, CA) as described.²⁷ Primers and probes for human TIMP-1 and GAPDH mRNA were purchased from Applied Biosystems. Standard curves for TIMP-1 and GAPDH were generated using the fluorescent data from two-fold serial dilutions of total

RNA of the highest expression sample. Quantities of each target gene in test samples were normalized to the corresponding levels of the housekeeping gene (GAPDH) in each sample.

Statistical analysis

Patient's clinical variables were analyzed using descriptive statistics, including proportions or means when appropriate. To check if the data fits Gaussian distribution Kolmogorov-Smirnov test was performed. Spirometry data between the asthma groups were analyzed with nonparametric Kruskal-Wallis test using 95% confidence interval with Dunn's post test, spirometry data within groups were analyzed with repeated measures nonparametric Friedman test with Dunn's post test. Analysis for statistical significant differences for the data gathered by real-time PCR and ELISAs was performed with unpaired two-tailed *t*-test and nonparametric Mann-Whitney test, respectively. Graph Pad Prism, version 4.01 (San Diego, CA) was used for all statistical calculations. *p* values ≤ 0.05 were considered significant.

RESULTS

Reduced bronchodilator response in SR asthmatics

The characteristics of patients that enrolled in this study are summarized in Table I. In terms of asthma severity, both groups were equivalent (Table I) with similar levels of baseline FEV₁% predicted (62.0 $\pm$ 10.9% and 66.9 $\pm$ 10.0% for SR and SS asthma groups, respectively). No active smokers were recruited for this study. Patients were divided into SR and SS groups based on FEV₁% predicted responses after the one week burst with oral prednisone. Prednisone significantly improved FEV₁% predicted in SS asthmatics (62.0 $\pm$ 10.9% (mean $\pm$ SD) to 79.4 $\pm$ 11.3%, *p*<0.001), with no change in SR asthmatics (66.9 $\pm$ 10.0% to 65.9 $\pm$ 12.1%) (Table I, Figure 1A).

Significantly greater bronchodilator response was detected in SS compared to SR group (delta FEV₁% predicted 33.5 $\pm$ 22.5% vs. 15.2 $\pm$ 7.9%, *p*=0.001) (Figure 1B). Furthermore, after a prednisone burst, the bronchodilator response demonstrated up to 43.7 $\pm$ 24.9% improvement in FEV₁% predicted in the SS group compared to the baseline FEV₁% predicted measured one week earlier, while SR group showed only 12.2 $\pm$ 12.5% improvement (*p*<0.001) (Figure 1B).

Controller medication consisted only of ICS in a subset of both groups: 9/21 in the SR group and 7/19 in SS group. The change in (or delta) FEV₁% predicted for the SR and SS asthma patients on ICS vs. patients not on ICS is summarized in Table II. In terms of asthma severity, based on the gathered data about frequency of the nocturnal asthma symptoms and rescue medication use, no significant differences between SR and SS asthma groups were noted (Table II). A greater bronchodilator response was observed in both groups of SS asthmatics (patients on ICS, and not on ICS), as compared to SR asthmatic subgroups (Table II). This observation led us to explore markers of airway remodeling in the BAL fluid of SR and SS patients.

Markers of airway remodeling in BAL fluid of SR and SS asthmatics

No difference in the amounts of MMP-9, PMN elastase and VEGF was found between the SS vs. SR asthmatics studied (Figure 2). However, TIMP-1 levels were found to be significantly lower in BAL fluid of SR compared to SS asthmatics (Mean $\pm$ SE: 921.9 $\pm$ 313.4 pg/ml vs. 2267 $\pm$ 456.8 pg/ml, *p*<0.05), resulting in significantly higher MMP-9/TIMP-1 ratios in BAL fluid of SR patients (0.26 $\pm$ 0.04 vs. 0.11 $\pm$ 0.03, *p*<0.01) (Figure 3). MMP-9, TIMP-1, VEGF and PMN elastase were also measured in BAL fluid from four healthy control subjects. The mean $\pm$ SE levels of these proteins in BAL fluid were as follows: MMP-9-137.9 $\pm$ 68pg/ml; TIMP-1-792.2 $\pm$ 217.2pg/ml; VEGF - 257.6 $\pm$ 37.7pg/ml; PMN elastase - 468.8 $\pm$ 126.6 pg/ml.

TIMP-1 mRNA regulation by short term steroid exposure in BAL cells

Airway macrophages are the major producers of airway TIMP-1.²⁸ To analyze whether lack of steroid response contributed to the difference in regulation of TIMP-1 production in SR asthma group vs. SS asthma group, BAL macrophages from five SR and five SS asthmatics were stimulated *in vitro* with 10^{-6} M DEX for 4 hr or media alone. It was found that steroids significantly increased MMP-9 inhibitor TIMP-1 mRNA production by BAL cells from SS asthmatics, while TIMP-1 mRNA production was unchanged in SR asthma BAL cells ($p<0.01$) (Figure 4).

DISCUSSION

Current asthma treatment guidelines support the use of steroids to control airway inflammation and decrease airway remodeling. Several studies suggest that steroids can reduce airway remodeling.^{5,13,17,29} However, the effects of steroid treatment on airway remodeling are controversial. There had been reports about beneficial effects of steroids on the reduction of the subepithelial membrane thickness³⁰ and airway vascularity.³¹ Importantly, there are also studies reporting considerable degree of airway remodeling in the peripheral airways and inability of ICS to modulate collagen deposition³² or demonstrating that only high doses steroids are able to slow basement membrane thickening.³³ SR asthmatics have persistent airway inflammation despite treatment with steroids, and therefore could be predisposed to increased airway remodeling and irreversible lung disease. This concern is supported by data from the current study.

Indeed, the current study suggests that the degree of bronchodilator reversibility is impaired in SR, as compared to, SS asthmatics and this is supported by the observation that there is a shift in MMP-9/TIMP-1 ratio in SR asthma due to the inability of steroids to enhance TIMP-1 production in this group of asthmatics. Importantly, this observation applied to SR patients whether they were taking ICS or not, i.e. they demonstrated lower bronchodilator response than SS patients on ICS or off ICS. Our study suggests that resistance to steroid therapy is an additional factor that contributes to the development of the airway remodeling features. This is presumably because chronic airway inflammation, not responsive to corticosteroid therapy, could also lead to airway remodeling and therefore a vicious cycle that results in irreversible airways disease. This suggests that SR patients need to be identified early and put on alternative non-steroidal, anti-inflammatory medications to reduce the risk of developing irreversible airway obstruction due to uncontrolled airway remodeling.

The current study was performed on a group of SR and SS asthmatics defined by one week course of oral prednisone treatment as previously described by our group.^{22–25} Spirometry studies revealed significantly greater bronchodilator response in SS group than in SR group. These differences in post bronchodilator response suggest that SR, as compared to SS, asthma is associated with decreased reversibility of airway obstruction. BAL samples from SS asthmatics revealed significantly higher amounts of eosinophils as compared to BAL samples from SR patients. No difference in the numbers of neutrophils between the two groups studied was noted.

Airway inflammation contributes to structural changes in asthma lungs. Many clinical studies that have used airway biopsy specimens have shown a decrease in airway inflammatory cell numbers after inhaled corticosteroid therapy.^{17,23} However, there is very little information on the effects of asthma medication on the structural components of the airways. Both the synthesis and degradation of many extracellular matrix components can be affected by the asthmatic process, resulting in remodeling and altered gene expression in the airways. The balance between MMP-9 and TIMP-1 is critical in the control of extracellular matrix remodeling with an imbalance in these proteins resulting in pathological changes of the airway

wall.^{12,14} In our current study we measured MMP-9, TIMP-1, VEGF and PMN elastase expression in BAL fluid from SR and SS asthmatics, and 4 healthy non atopic control donors. The levels of MMP-9,^{20,34} TIMP-1,²⁰ VEGF³⁵ and neutrophil elastase³⁶ reported by us are comparable to what has been previously reported in other asthma studies: with higher levels of MMP-9 in BAL fluid as compared to normal control subjects. TIMP-1 levels were found to be significantly lower in BAL fluid of SR compared to SS asthmatics.

Our current study demonstrates that lack of responses to steroids in SR asthma group supports airway remodeling with significantly lower TIMP-1 expression and significantly greater MMP-9/TIMP-1 ratio in BAL fluid of SR patients as compared to SS patients. It also appears that both SR patients on ICS and not on ICS have this change in the MMP-9/TIMP-1 ratio. Uncontrolled proteolysis and continuous degradation of the extracellular matrix structure as well can be associated with structural changes in the airways due to an injury-repair cycle since changes in the extracellular matrix support alterations in smooth muscle mass, microvascular alterations, regulate epithelial shedding and migration of the inflammatory cells, potentially contributing to enhanced bronchoconstriction.^{20,32}

TIMP-1 is produced by multiple cell types, including peripheral blood monocytes and lymphocytes,³⁷ airway macrophages and epithelial cells.²⁵ In this study we explored TIMP-1 mRNA production by BAL cells from asthmatics. As shown, the great majority (over 90%) of these cells are macrophages.²² *Ex vivo* studies in the current report revealed that short term treatment with steroids (4 hr of DEX) induced MMP-9 inhibitor TIMP-1 mRNA in BAL cells from SS asthmatics, but did not change TIMP-1 mRNA expression in SR BAL cells suggesting inability of steroids to enhance TIMP-1 production by BAL macrophages of SR patients. The majority of BAL samples that were analyzed in this study, were also used in a recent report in which we found that BAL samples from SR asthmatics had lack of glucocorticoid receptor alpha translocation in response to steroids and had greater amounts of glucocorticoid receptor beta mRNA than SS BAL samples.²² Thus, lower TIMP-1 induction by DEX in BAL cells from SR patients could be due to the lack of glucocorticoid receptor translocation in BAL macrophages in response to steroids and/or increased expression of the glucocorticoid receptor beta dominant negative isoform in BAL cells that alters normal function of the glucocorticoid receptor.²²

A limitation of the current study is that no biopsies were obtained to demonstrate whether any of the biomarkers of remodeling measured in this study correlated with levels of airway remodeling. Although a reduced bronchodilator response has been used as a surrogate for airway remodeling, to date there are also no biopsy studies in the literature demonstrating that lack of a bronchodilator response is an evidence of remodeling.

Cumulatively these data suggest that steroid resistance in asthma needs to be recognized early and their therapy carefully monitored, especially if it only involves ICS, to prevent potential onset of irreversible structural changes in the airways leading to airway obstruction. Future therapeutic studies to assess the effects of the combination of ICS with LABA or anti-IgE on airway remodeling in SR asthmatics would be of great interest.

Acknowledgments

The authors would like to thank Dr. Stanley J. Szefer for his critical reading of the manuscript. The authors also thank Maureen Sandoval for her help in preparing this manuscript.

Work supported by NIH Grants HL36577, AR41256, AI070140, and HL37260. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute Of Allergy And Infectious Diseases, National Heart, Lung, and Blood Institute or the National Institutes of Health

ABBREVIATIONS

BAL	bronchoalveolar lavage
BD	bronchodilator
DEX	dexamethasone
GC	glucocorticoid
ICS	inhaled corticosteroids
LABA	long acting beta agonists
MMP	matrix metalloproteinase
PMN	neutrophil
TIMP	tissue inhibitor of metalloproteinases
VEGF	vascular endothelial growth factor

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Figure 1.

FEV₁ % predicted (A) and ΔFEV₁ % predicted (B) in response to bronchodilator and prednisone burst in SS (filled circles) and SR (open circles) asthma groups. Each dot represents spirometry measurements for each individual analyzed. S/P BD – status post bronchodilator; S/P prednisone – status post one wk of oral prednisone; S/P prednisone+BD – status post one wk of oral prednisone in response to BD; *BD response after a one wk of oral prednisone as

compared to baseline FEV₁% predicted measured one wk earlier; **BD response after a one wk of oral prednisone as compared to FEV₁% predicted in response to prednisone.

Figure 2.

Markers of airway remodeling - MMP-9 (A), TIMP-1 (B), VEGF (C), PMN elastase (D) - in the BAL fluid from SR and SS asthmatics as detected by ELISA (open circles - patients on ICS, filled circles - patients not on ICS).

Figure 3. Significantly increased MMP-9/TIMP-1 ratio in BAL fluid samples from SR asthmatics (open circles - patients on ICS, filled circles – patients not on ICS).

Figure 4.

Inability of steroids to enhance TIMP-1 production by BAL cells from SR asthmatics. BAL cells were cultured with media only or with 10^{-6} M DEX for 4 hr. TIMP-1 mRNA induction by DEX in the cell isolates as compared to media treated cells was analyzed by real-time PCR (open circles - patients on ICS, filled circles – patients not on ICS).

Table I

Patient characteristics

Parameter	Asthmatics	
	SR	SS
Number of subjects	21	19
Age, y (mean±SD)	36.9±11.6	32.6±8.1
Sex (M/F)	11/10	10/9
Atopic subjects *	21	19
Active smokers	0	0
Pre-bronchodilator FEV ₁ % predicted before prednisone burst (mean±SD)	66.9±10.0	62.0±10.9
Pre-bronchodilator FEV ₁ % predicted after prednisone burst (mean±SD)	65.9±12.1 [†]	79.4±11.3
Post-bronchodilator (albuterol) FEV ₁ % predicted before prednisone burst (mean±SD)	76.9±11.1	81.3±12.3
Post-bronchodilator (albuterol) FEV ₁ % predicted after prednisone burst (mean±SD)	74.8±13.0 [‡]	89.5±10.0
Medications:		
ICS only [§]	9/21	7/19
Systemic CS	None	None
Other maintenance medications	None	None

* Patients were defined as atopic based on degree of skin prick responses to the set of common allergens.

[†] p<0.001;

[‡] p<0.001 – significantly lower FEV₁% predicted in SR asthma group as compared to the corresponding parameter in SS asthma group.

[§] The patients selected for this study were not on ICS+LABA. (ICS – inhaled corticosteroids, CS – corticosteroids, LABA – long acting beta-agonists).

Table II Spirometry data for patients on ICS vs. patients not on ICS ($\Delta FEV_1\%$ predicted)

Parameter	Asthmatics			
	SR patients on ICS (group I)	SR patients not on ICS (group II)	SS patients on ICS (group III)	SS patients not on ICS (group IV)
Number of subjects	9	12	7	12
Baseline $FEV_1\%$ predicted (mean $\pm$ SD)	71.3 $\pm$ 7.1	63.6 $\pm$ 10.8	62.9 $\pm$ 13.1	61.4 $\pm$ 10.0
$\Delta FEV_1\%$ predicted response to prednisone burst (mean $\pm$ SEM)	-3.2 $\pm$ 3.2 (p<0.001) *	-0.9 $\pm$ 2.6 (p<0.001) †	27.7 $\pm$ 10.3	23.3 $\pm$ 7.7
$\Delta FEV_1\%$ predicted bronchodilator response (mean $\pm$ SEM)	16.5 $\pm$ 2.8 (p=0.11) *	14.2 $\pm$ 2.2 (p=0.01) †	29.7 $\pm$ 8.3	35.6 $\pm$ 7.1
$\Delta FEV_1\%$ predicted prednisone burst+bronchodilator response compared to baseline response (mean $\pm$ SEM)	13.1 $\pm$ 4.8 (p<0.05) *	10.9 $\pm$ 2.5 (p<0.001) †	44.8 $\pm$ 13.4	50.1 $\pm$ 7.9
$\Delta FEV_1\%$ predicted bronchodilator response as compared to prednisone burst response $FEV_1\%$ (mean $\pm$ SEM)	17.0 $\pm$ 3.8	12.5 $\pm$ 3.3	12.3 $\pm$ 6.5	14.6 $\pm$ 3.5
Albuterol use, puffs/day ‡	5.1 $\pm$ 4.5	2.3 $\pm$ 3.3	6.8 $\pm$ 6.3	4.7 $\pm$ 5.4
Frequency of asthma symptoms at night/month §	10.3 $\pm$ 11.6	0.7 $\pm$ 1.6	6.8 $\pm$ 13.1	2.3 $\pm$ 4.5

* compared to SS asthmatics on ICS.

† compared to SS asthmatics not on ICS.

‡ Albuterol use data was collected for 6 out of 9, 8 out of 12, 4 out of 7 and 9 out of 12 patients from groups I, II, III and IV, respectively.

§ Frequency of asthma symptoms at night data was gathered for 6 out of 9, 6 out of 12, 5 out of 7 and 7 out of 12 patients from groups I, II, III and IV, respectively.

Table III

BAL cell differentials for the samples from SR and SS asthmatics

Cell type	Asthmatics	
	SR group (n=10)	SS group (n=13)
Total white blood cells, $\times 10^6$ (mean $\pm$ SD)	12.6 $\pm$ 4.4 $\times 10^6$	15.6 $\pm$ 6.4 $\times 10^6$
Macrophages/monocytes, % (mean $\pm$ SD)	92.9 $\pm$ 5.2	90.6 $\pm$ 5.8
Polymorphonuclear cells, % (mean $\pm$ SD)	1.0 $\pm$ 0.9	0.7 $\pm$ 0.4
Lymphocytes, % (mean $\pm$ SD)	5.5 $\pm$ 4.2	6.6 $\pm$ 4.3
Eosinophils, % (mean $\pm$ SD)	0.7 $\pm$ 0.7	2.4 $\pm$ 2.3 [*]

*
p=0.02 as compared to SR asthmatics.