T-Cell Changes in Peripheral Blood of Asthmatics During Bronchoconstriction Caused by Histamine Bronchoprovocation

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Abstract

To study the role of T lymphocyte subtypes in acute bronchoconstrictive responses caused by nonspecific stimuli, we performed bronchial histamine challenge tests in 24 stable asthmatic patients and in 10 nonatopic-nonasthmatic controls. None of the asthmatic patients had had an attack or suffered from an upper respiratory tract infection within the 8 weeks before the challenge test, nor had been receiving corticosteroids. Short acting β_2 agonists were stopped 6 hours and long acting β_2 agonists 12 hours before the challenge test. T lymphocyte subgroups, T lymphocyte activation markers and natural killer cells were determined in the peripheral blood before and after the histamine challenge test by two color flow cytometry. The results showed that while there were no changes in the percentages and absolute numbers of CD3+CD4+, CD16+CD56+, CD5+CD25+ and CD5+HLA-DR+ cells after the challenge test, CD3+CD8+ cells were higher both as percentages [23.9% (16.7-36.7) versus 24.5% (16.7-40.5), $p<0.05$] and as absolute numbers [574 (65-1204)/mm^3 versus 686 (163-1192)/mm^3, $p<0.05$] after the challenge test in the asthmatics. Control subjects showed no differences after nonspecific bronchoprovocation.
In view of these findings it can be stated that CD8+ T lymphocytes may have a role in the acute bronchoconstrictive response to nonspecific stimulus in asthmatic patients.

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Keywords: *asthma, bronchial challenge test, bronchial hyperressponsiveness, CD8, T lymphocytes*

Introduction

Asthma is characterized as a chronic inflammatory disease of the bronchi and it is well established that a variety of cells including mast cells, eosinophils and lymphocytes play a role in this process (1). Bronchial hyperresponsiveness (BHR) is a hallmark feature of asthma. A variety of factors may be responsible for the induction of airway hyperresponsiveness (2).
The role of T lymphocytes in asthma both in acute bronchoconstriction or in stable state have been suggested in several reports (3-6). In an experimental model, T cell mediated responses were stated to be responsible for induction of BHR (7). Since the lung has no afferent lymphatics, it is accepted that lymphocytes reach the lung via the blood stream (8). This is confirmed by previous studies showing the changes in lymphocyte numbers in blood and T cell recruitement from blood to airways after antigenic stimulation (9,10). The role of CD4+ T lymphocytes is well-defined in the de-

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Table 1. Median values (range) as percentages and absolute values of lymphocytes and T lymphocyte subtypes before and after the challenge test in control subjects

	Controls (n=10)		
	Before	After	p
Lymphocytes (mm^{-3}) (range)	2000 (1200-3500)	2060 (1250-3200)	NS
CD3+ (%) (range)	71.4 (62.4-77.9)	72.1 (62.8-80.3)	NS
(mm^{-3}) (range)	1451 (824-2439)	1467 (896-2272)	NS
CD3+CD4+ (%) (range)	40.9 (29.4-55.8)	42.0 (25.0-57.6)	NS
(mm^{-3}) (range)	746.7 (465.6-1559)	723.2 (400-1536	NS
CD3+CD8+ (%) (range)	25.9 (21-48.6)	23.8 (21.1-43.1)	NS
(mm^{-3}) (range)	542.4 (276-1263.6)	491 (285-1163.7)	NS
CD16+CD56+ (%) (range)	12.3 (6-26.5)	12.2 (5.1-24)	NS
(mm^{-3}) (range)	202.1 (78-927.5)	229.5 (87.5-720)	NS
CD5+ CD25+ (%) (range)	5.2 (1.8-6.7)	4.0 (2-8.1)	NS
(mm^{-3}) (range)	103.2 (23.4-198.1)	93.6 (25-188.8)	NS
CD5+HLA-DR+ (%) (range)	9.8 (3.6-36)	9.7 (4.2-37)	NS
(mm^{-3}) (range)	252.4 (67.2-936)	238 (62.7-999)	NS

NS= nonsignificant

velopment of the asthmatic response, but the role of CD8+ T cells is less clear. It has been shown that there is an influx of $CD8^+$ cells in single early asthmatic responders and an influx of $CD4^+$ cells in dual and late-phase responders (11).

To study the role of T lymphocytes subtypes in acute bronchoconstrictive response, we investigated T cell responses in the peripheral blood of asthmatic patients during bronchoconstriction caused by histamine challenge.

Materials and Methods

Twenty four asthmatic patients (5 males, 19 females) with a median age of 27 years (range 18-62 yrs) and 10 nonasthmatic subjects (2 males, 8 females) with a median age of 36.5 years (range 23-44 yrs) were subjected to a nonspecific bronchial challenge with histamine (NSBP). Asthma was diagnosed according to the criteria of the American Thoracic Society (12). None of the patients had an attack or upper respiratory tract infection within the 8 weeks before the challenge. All patients and controls were nonsmokers, were free of any other systemic disease and were not taking any other systemic drug. None had been receiving inhaled or systemic corticosteroids at least 4 months before the study. Short acting β_2 agonists were stopped 6 hours before and long acting β_2 agonists were stopped 12 hours before the histamine challenge test. NSBP was performed according to the method of Cockcroft (13), between 8^{30} – 10^{30} in the morning, with a Pari nebulizator.

After FEV_1 was determined (Vitalograph Model S; Buckingham, UK) histamine diphospate solutions were inhaled in doubling concentrations from 0.03 mg/ml to 16 mg/ml. Histamine concentration causing more than 20% fall in baseline FEV_1 was expressed as PC_{20} which was obtained from a log dose-response curve. Challenge was stopped if there was no response according to the above criterion to a dose of 16 mg/ml in nonasthmatics.

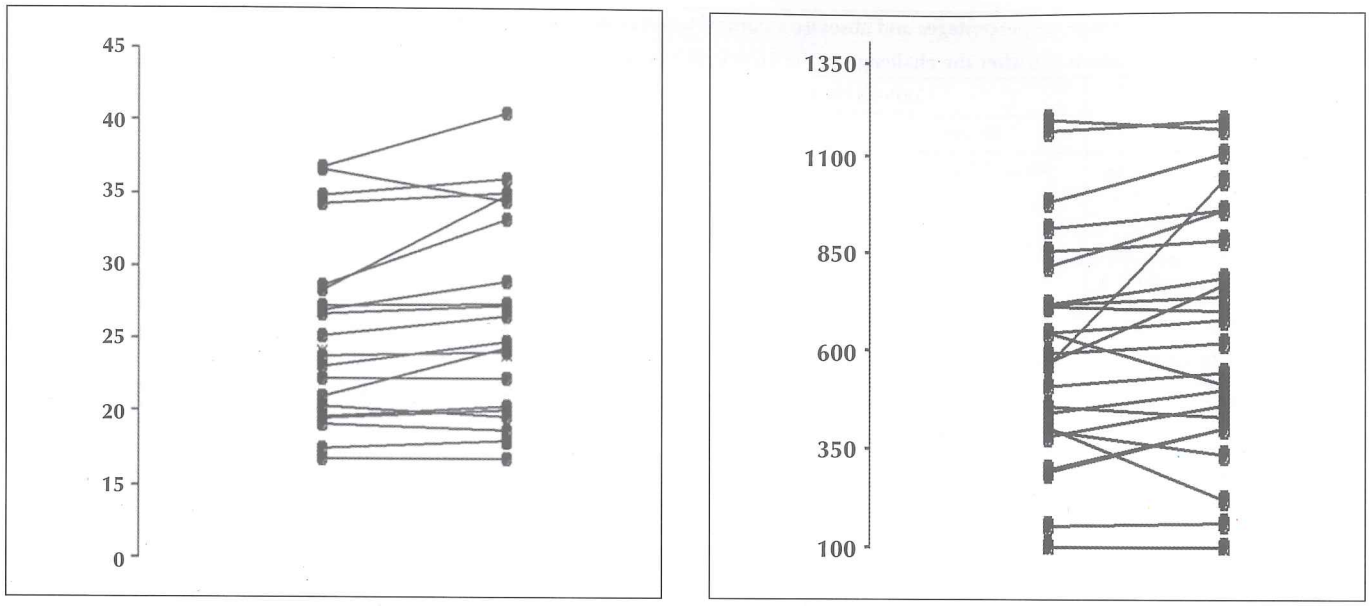
Peripheral blood was taken before and 20 minutes after the NSBP to determine T lymphocytes (CD3+) and T lymphocyte subtypes and activation markers (CD3+CD4+,CD3+CD8+, CD16+CD56+ =natural killer, CD5+CD25+ =IL-2R =interleukin 2-receptor and CD5+HLA-DR+), using two color flow cytometry (Faks Calibur, Becton Dickinson). T lymphocyte subtypes were determined as percentages and also in absolute values (mm^{-3}). Absolute values of the lymphocyte subtypes were calculated by multiplying the total numbers of lymphocytes by their percentages, detected by flow-cytometry analysis.

The first and second measurements of T lymphocyte subtypes were compared using the Wilcoxon signed rank test. A value less than 0.05 was considered as significant. FEV_1 values and ages of the asthmatics and nonasthmatics were compared using the Mann-Whitney-U test. Results are reported as median and ranges. Correlations were determined using the Spearman correlation matrix. The personal computer program SPSS. WIN was used for all calculations.

Table 2. Median values (range) as percentages and absolute values of lymphocytes and T lymphocyte subtypes before and after the challenge test in the asthmatics

	Asthmatics (n=24)		
	Before	After	p
Lymphocytes (mm^{-3}) (range)	2380 (864-4100)	2650 (904-4020	NS
CD3+ (%) (range)	70.9 (58.8-78.6)	69.4 (57.7-78.4)	NS
(mm^{-3}) (range)	1830 (557-3091)	2010 (557-2682)	NS
CD3+CD4+ (%) (range)	41.7 (26.6-55.6)	40.1 (31.0-57.1)	NS
(mm^{-3}) (range)	1069 (388-1755)	1031 (378-1646)	NS
CD3+CD8+ (%) (range)	23.9(16.7-36.7)	24.5(16.7-40.5	p<0.05
(mm^{-3}) (range)	574 (65-1204)	686 (163-1192)	p<0.05
CD16+CD56+ (%) (range)	13.7 (3.7-22.8)	11.2 (2.7-1-23.5)	NS
(mm^{-3}) (range)	322.0 (115.0-685.0)	348.0 (115.0-868.0)	NS
CD5+CD25+ (%) (range)	5.35 (3.1-10.4)	5.3 (2.5-19.3)	NS
(mm^{-3}) (range)	96.0(31.0-601.0)	101.0 (32.0-264.0)	NS
CD5+HLA-DR+ (%) (range)	9.8 (3.6-36)	14.6 (4.2-32.3)	NS
(mm^{-3}) (range)	197.0(80.0-591.0)	259.0 (63.0-512.0)	NS

NS= nonsignificant



A: *CD3+CD8+ (%) (p<0.05)*

B: *CD3+CD8+ (mm^{-3}) (p<0.05)*

Figure 1. *Changes in individual values of CD3+CD8+ cells after non specific bronchial challenge as percentages (A) and absolute numbers (B) in the asthmatics.*

Results

The bronchial challenge test was well tolerated by all subjects. The median value of forced expiratory volume in one second (FEV_1) was 95.5 (range 75-126) as percent of predicted (FEV_1%) in the asthmatic patients and 92.5% (range 88%-103%) in controls. There was no statistically significant difference between asthmatics and controls with regard to age and FEV_1 values (p>0.05). The median value of PC_{20} was 1.57±0.63 mg/ml (range, 0.10 to 11 mg/ml) in the asthmatics, but higher than 16 mg/ml in all controls.

The median values of total lymphocytes, CD3+, CD4+CD3+, CD8+CD3+, CD5+CD25+, CD16+CD56+ and CD5+HLA-DR+ cells in the controls and in the asthmatics, before and after histamine challenge test are expressed as percentages and absolute values in Tables 1 and 2. As shown in the Table 1, no statistically significant differences were found in the percentages and absolute numbers of CD3+, CD4+CD3+, CD8+CD3+, CD5+CD25+, CD16+CD56+ and CD5+HLA-DR+ cells before and after histamine challenge test in the control group (p>0.05). In the asthmatics, the percentages and absolute numbers of CD3+CD8+ cells were increased (p<0.05) after the challenge while no changes were noted in the other cells (Table 2). Figure 1 shows the changes of individual values of CD3+CD8+ cells as percentages (A) and absolute numbers (B) after the challenge in asthmatic patients.

The difference between the first and the second values of CD8+CD3+ T lymphocytes was expressed as delta CD8 (ΔCD8). The median ΔCD8 for relative numbers was 0.6% (range: -2.2% to 6.5%) and the median ΔCD8 for absolute numbers was 42.0/mm^3 (range: –183 to 885). Neither the relative or absolute numbers of ΔCD8 showed a correlation with the values of PC_{20} (r=0.26 and r=0.20 respectively, p>0.05), baseline FEV_1 (r=-0.29 and r=0.18 respectively, p>0.05), nor with the fall in percent of FEV_1 (r=-0.19 and r=-0.14 respectively, p>0.05) after the challenge.

Discussion

The present study demonstrated that the relative and absolute numbers of CD8+ T lymphocytes showed an increase in the peripheral blood of asthmatic patients in response to bronchoconstriction in response to a nonspecific bronchial challenge.

T lymphocyte subpopulations are thought to be involved in the pathogenesis of bronchial asthma. There is an increase in activated T cells in bronchoalveolar lavage fluid and peripheral blood from subjects with asthma and is corrrelated with disease severity (4, 14). We also reported similar results showing an elevated T lymphocyte activation in chronic severe asthmatics and we found that BHR is related with the CD4/CD8 ratio (6).

Different T cell subsets have been implicated in various mechanisms of asthma pathogenesis. For instance, it has been suggested that T lymphocytes with a helper/inducer T cell phenotype (CD4) are depleted in the blood stream and are selectively retained in the lung after antigen challenge (10). CD8 (cytotoxic T) cells have cytotoxic activity and function to recognize and eliminate altered self cells. A number of studies mostly in animals showed that CD8+ T cells may have a role in asthmatic reactions and BHR. In experimental animal models, CD8+ T lymphocytes were implicated as important cells in the development of BHR (15, 16). After allergen exposure CD8+ T cells have been shown to be increased and presumed the role for regulation of events leading

to eosinophil inflammation and BHR (17). Huang et al. showed that CD8+ T cell depletion caused an increase in allergen induced BHR with an increase in eosinophil number in BAL supporting the idea that CD8+ cells may prevent ongoing BHR (18). Olivenstein et al reported that a depletion of CD8+ T cells before antigen challenge significantly increased the late asthmatic response in rats suggesting a protective role for CD8+ T cells in the asthmatic response (19). There is yet no available data for such effects in humans. In a previous study, selective mobilization of CD8+ T cells into the airways was thought to have a protective effect on the late asthmatic response in humans (20). Although there is a small increase in the levels of CD8+ T cells, our study supports the assumption that CD8+ T cells may play a part in the early asthmatic response in humans. It is possible that there are other events and contributing cells in this model. We were not able to study the serial changes in T cells, but our study, we believe, supports this protective idea during the response against nonspecific bronchoconstriction. Similar to our study, in atopic asthmatics CD8+ T cells were found to be increased in the bronchoalveolar lavage fluid and in the peripheral blood of atopic asthmatics after allergen challenge (21). These observations suggested the possibility that an increase in CD8+ T cells in bronchial hyperreactivity states may prevent the development of symptomatic asthma. We detected these changes only after bronchial obstruction occurred and suggested that CD8+ T cells may be contributing to the asthmatic response probably to prevent the organism from the late asthmatic response.

CD8+ T cells exert their effects via some cytokines. Among these IFN-γ (interferon gamma) is a characteristic of asthma and is found in increased levels in asthmatics (22). IFN-γ produced by CD8+ T cells has been shown to reduce asthmatic inflammation suppressing the infiltration of CD4+ T cells (23). In addition, IFN-γ inhibited IL-5 which is a central proinflammatory cytokine in asthma (24). More recently Magnan et al. reported that IFN-γ producing CD8+ T cells were highly linked to asthma and the degree of bronchial hyperreactivity supporting the regulatory role for CD8+ T cells in asthma (25). Eosinophils and other leukocytes are directly drawn into the lung by local production of chemokines that act primarily as chemoattractans. Eotaxin, one of the C-C chemokine family which is the most active on eosinophils, monocytes, lymphocytes, natural killer cells and basophils, plays an important role in the selective recruitement of eosinophils into the airways. In a recent study, CD8+ T cells from sensitized rats has been shown to prevent the late asthmatic response and eosinophilia (26). The authors also showed that the effect of CD8+ T cells are related with decreased production of eotaxin and increased production of IFN-γ mRNA.

To date, the mechanism by which T lymphocytes get into the lungs has not been demonstrated. It is likely that these cells reach the lung via the blood stream (8). Recently, Schuster et al reported migration of the lymphocytes from blood to the lung in an animal model, supporting the speculation that changes in the peripheral blood may reflect changes in the lungs (27). Thus, although we measured the changes in peripheral blood in our subjects, our findings may well reflect the lymphocyte trafficking into the airway mucosa.

We observed an increase in CD8+ T lymphocytes after a bronchial challenge test in the subjects with BHR but not in normal subjects. So, also considering the experiences cited above, one could say that CD8+ T cells play a part in the events which cause the asthmatic response, probably by contributing to the regulation of the bronchoconstrictive response. Further studies with serial measurements, showing changes in cytokines, may help to confirm the exact role of T cells in asthma.

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CORRECTION

The sentence beginning with "**Significant**... on line 8th of the abstract of the article published in Turkish Respiratory Journal Vol 5. No. 1, page 22-26, should have been "**No significant**". We apologize for the mishap. The corrected abstract is as follows.

Effects of Antioxidant Therapy on Lung Clearance, Pulmonary Function Tests and Oxidant Stress in Patients With Chronic Obstructive Pulmonary Disease

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To investigate the effects of antioxidant therapy on pulmonary function tests (PFTs) and oxidant-antioxidant balance on COPD patients, pulmonary function tests (PFTs), arterial blood gas (ABG) analyses, alveolo-capillary permeability measurements and plasma levels of superoxide dismutase (SOD), malondialdehyde (MDA), vitamin E, and vitamin C at the initiation of the study and after six weeks of antioxidant therapy were assessed in 13 patients with stable COPD. **No significant** changes were observed in the PFTs and ABG after therapy, while a slight but not significant decrease was noted in lung clearance with antioxidant therapy (p>0.05). MDA levels decreased from 7.71 0.8 to 6.11 0.29 nmol/mL (p:0.000) whereas SOD levels increased from 20.41 2.4 to 23.71 2.7 U/mL with therapy (p<0.001). Significant increases were observed in mean vitamin E (0.76.2 vs 0.821 7.2 mg%) (p:0.000) and vitamin C (4.31 0.2 vs 5.110.4 mg/dL) levels (p:0.000). Our findings indicate that antioxidant therapy as an adjunct to diet is effective on oxidant-antioxidant balance but has no demonstrable effects on PFTs and alveolocapillary permeability.