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CHARACTERIZATION OF AIRWAY INFLAMMATION IN STABLE BRONCHIAL ASTHMA.

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ABSTRACT

Fifty stable mild bronchial asthma patients were studied to characterize the airway inflammation using the technique of bronchoalveolar lavage (BAL). 18 normal non-smoking subjects were studied as control subjects. Total inflammatory cells (29.6±18.4 x 10^6 /dl vs 16.0±6.6 x 10^6/dl, p(<0.001), absolute macrophages (24.5±15.9 x 10^6/dl vs 13.3 ± 5.5 x 10^6/dl. p<0.001), eosinophils (1.0±1.5 x 10^6 dl vs 0.1±0.1 x 10^6/dl, p<0.001) and lymphocytes (3.8±4.2 x 10^6/dl vs 2.4±1.5 x 10^6/dl, p<0.05) were significantly higher in BAL fluid from patients with bronchial asthma compared to normal control subjects. Abnormally elevated eosinophils (>4%) and lymphocytes (>28%) in BAL fluid were seen in 15 (30%) and five (10%) patients respectively. This study demonstrates that chronic lung inflammation characterised by abnormally elevated macrophages, eosinophils and lymphocytes persists even in patients with stable mild bronchial asthma, despite medication with oral beta stimulants or theophylline.

Introduction

Bronchial asthma is now considered an airway inflammatory disorder characterised by reversible airway obstruction and increased airway reactivity to both specific and non-specific stimuli(1). Bronchoalveolar lavage (BAL) is useful tool in the assessment of lower respiratorytract inflammation in various respiratory diseases including bronchial asthma(2,3-6). BAL studies in bronchial asthma had shown increased numbers of eosinophils and mast cells in the airways (7-9). These ceils were in an activated state as demonstrated by the presence of increased amounts of mast ceil products(e.g., histamine) and eosinophil products (e.g major basic protein) in the airways of asthmatics compared to controls (8,10). There were also sloughing of airway epithelial cells (8,11). The immediate response after exposure to allergen is mast cell degranulation and the late response is characterised by an increase in eosinophils, immature macrophages and T-cells (12,14). There were only a few Indian studies that had analysed brochoalveolar lavage cell profiles in bronchial asthma (15.16). A study was therefore, planned to evaluate the airway inflammatory process in stable bronchial asthma.

Materials and Methods

Fifty patients presenting with a history of intermittent wheeze, chest tightness, cough and sputum production were evaluated. Bronchial asthma had been previously diagnosed in all of them by an independent physician. Bronchial asthma was confirmed in these subjects by pulmonary function tests which had shown reversibility to inhaled bronchodilators. The following criteria were adopted for inclusion in the study as mild stable asthma:

1. No asthmatic symptoms, for at feast two weeks prior to the study.
2. No steriods have been used. Only oral salbutamol and oral theophylline had been used.
3. No acute asthma in the four weeks prior to study.
4. Chest radiograph normal.
5. FEV_1 was equal to or above 60% of predicted and ratio of FEV_1/FVC was above 60%

A full size posterio-anterior chest radiograph was evaluated in each subject. Other investigations included total and differential leucocyte counts in peripheral blood. Informed consent was obtained from each subject and the study was approved by the institutional ethical committee.

Pulmonary function

Pulmonary function tests that included forcedvital capacity (FVC) and forced expiatory volume in one second (FEV_1) were done on PK Morgan transfer test Model C (PK Morgan, Chatham, UK). FVC and FEV_1 were repeated after an inhalation of 200 µg of salbutamol through a spacehaler. Oral bronchodilators and theophylline were stopped 48 hours before pulmonary function tests. A response to the administration of the bronchodilator aerosol was considered significant when the improvement in FEV_1 and/or FVC is both larger than 12% and exceeds 200ml (17).

Bronchoalveolar lavage

Bronchoalveolar iavages were performed with a flexible fibreoptic bronchoscope as previously described(18). The lavages were done from right middle lobe and lingula. Five 20 ml aliquotes of normal saline solution at room temperature were instilled into each lobe and recovered immediately with the bronchoscope wedged in a subsegmental bronchus. The fluid was pooled and filter preparations were made for differential ceil count on lavage fluid and were stained with hematoxylin-eosin (19). A minimum of 400 cells were counted for differential cell counts on each filter preparation. The absolute number of different types of cells were derived from total cells times differential percentages.

Eighteen non-smoking individuals were evaluated as control subjects. The mean age of normal subjects, was 25.2±8.6 yrs (range 15-53 yrs). None of them had respiratory symptoms or abnormal physical findings and all had normal chest radiographs and normal pulmonary function tests. None of the subjects were on any medication

If either lymphocytes (>28%) or eosinophils (>4%) are more than two standard deviations above the normal mean, these are classified as abnormal elevations.

The results are presented as mean±SD. All the mean differences between groups were compared using Mann-Whitney U test.

Results

There was 26 males and 24 females in the study. The mean age of the 50 subjects was 23.9±7.2 years and the mean weight was 48.2±9.4 kg. Two males were smokers. The mean duration of symptoms was 7.3±5.7 years. All subjects gave history of allergy to multiple allergens that included dusts, certain food items. cold season, fumes etc. Family history of bronchial asthma was present in 15 subjects. One had a history of urticaria. All patients gave history of cough and wheezing, and all were on medication with oral bronchodilators ($ß_2$ stimulants or theophylline) for control of their asthma. None had received cromoglycate, Ketotifen or antihistaminics in the past. Physical signs especially rhonchi were heard in 21 subjects (42%). The mean total leucocyte count in the peripheral blood was 9600±3151 cells per cumm, and the differential count was as follows: neutrophils 51.9±11.6%, lymphocytes 37.2±9.5%, eosinophils 10.8±7.7% and monocytes 0.1±0.2%. The mean absolute eosinophil count in the peripheral blood was 1023±987 cells/cmm. The occupations of the study subjects were as follows: factory workers (cement, tannery, brass) (six), cooks(three), carpenters(two), drivers (two), tailors (two), shopsalesman (one), police man (one), peon in office(one). Eight subjects were students and 15 females were housewives. Nine patients were unemployed. The skiagrams chest were essentially normal except for increased bronchovascular markings in some subjects.

TABLE-1

Pulmonary function parameters in asthmatic subjects

	Observed	Predicted	%Predicted
FVC(L)	2.87 ±0.80	2.92 ±0.60	98.2 ±16.8
FEV_1(L)	2.31 ±0.70	2.56 ±0.60	90.5 ±19.4
FEV_1/FVC%	80.3 +8.1		

Pulmonary function results of the asthmatics are given in Table 1. The mean pre-bronchodilator FVC (%predicted) was 98±16.8% (range 64-139%) and FEV_1 (%predicted) was 90.5±19.4% (range 61-133%). The mean post bronchodilator FVC (%predicted) was 104.0±13.7% (range 76-143%) and FEV_1 (%predicted) was 98.7±17.1% (range 67-139%). The pre and post-bronchodilator FEV_1/FVC% were 80.3±8.1% and 82.8±8.3% respectively.

An analysis of the bronchoalveolar lavage fluid cells showed the following results. The total inflammatory cells (29.6±18.4 x 10^6/dl vs 16.0±6.6 x 10^6/dl, p<0.001), absolute macrophages (24.5±15.9 x 10^6/dl vs 13.3±5.5 x 10^6/dl, p<0.001), lymphocytes (3.8±4.2 x 10^6/dl vs 2.4±1.5 x 10^6/dl, p<0.05) and eosinophils (1.0±1.5 x 10^6/dl vs 0.1±0.1 x 10^6/dl, p <.001) were significantly higher in bronchial asthma patients compared to normal subjects (Table2). The eosinophil percentage (3.1±2.7% vs 1.0±1.1%, p<0.001) was also significantly higher in patients. Abnormal elevation of eosinophils (> 4%) and lymphocytes in BAL (> 28%) were present in 15 (30%) and five (10%) asthmatics respectively.

TABLE- 2

Bronchoalveolar lavage results in controls and asthmatics

(n=18)	Control (n=50)	Bronchial asthma
Total cells ' x 10^6.dl	16.0±6.6	29.6±18.4 *
Macrophages %	83.7±6.3	82.8±13.0
" x 10^6.dl	13.3±5.5	24.5±15.9
Lymphocytes %	14.6±6.4	13.4±122
" x 10^6/dl	2.4±1.5	3.8±4.2 **
Eosinphils %	1.0±1.1	3.1±2.7 *
" x 10^6/pl	0.1±0.1	1.0±1.5 *
Neutrophils %	0.7±0.8	0.6±2.1
" x 10^6.dl	0.1±0.1	0.3±1.4
% Recovery	60.2±6.9	68.2±8.8

p value * < 0.001 ** < 0.05

Discussion

This study demonstrates that there is an abnormal accumulation of inflammatory cells as evidenced by significantly high total cells in the lower respiratory tract of stable mild bronchial asthma patients. This inflammation is characterised by abnormally elevated macrophages, lymphocytes and eosinophils. All patients were asymptomatic

at the time of study and had only mild ventilatory defect and were taking oral beta stimulants or theophylline. The persistence of chronic inflammatory process in the lower respiratory tract in these patients suggests that oral bronchodilator therapy may not be suffiiient to suppress the inflammation.

Autopsy studies from patients dying in status asthmaticus had demonstrated mucus plugging of airways, goblet cell hyperplasia, subepithelial deposition of collagen, disruptionof airway epithelium, smooth muscle hypertrophy and infiltration of airway walls by inflammatory cells (20-22). Thus severe bronchial asthma is associated with marked airway inflammation. The demonstration of airway hyperresponsivenessinmildasthrnapromptedseveraiinvestigators to put forth the hypothesis that airway inflammation is present even when the disease is quiescent, and broncholaveolarand endobronchial biopsy studies in individuals with mild asthma had shown evidence of airway inflammation (11, 23-27). Based on these studies, the expert panel of the National Heart Lung and Blood Institute concluded that airway inflammation is present in virtually all patients with asthma (28). Thus, the finding of significantly elevated total cells in our study is in conformity with the earlierobservationof airway inflammation in mild bronchial asthma.

Although eosinophilic inflammatory process is the characteristic feature of bronchial asthma, large numbers of lymphocytes and macrophages had been demonstrated in autopsy and biopsy of airways in bronchial asthma patients (25,29,30). T-cell infiltrates inairways had been found to be helper T cells which appear to be activated (25). Several studies have suggesteda role for T-cell derived cytokines in the initiation of eosinophilic inflammation (20,25). interleukin-5 (IL-5), granulocyte macrophage-colony stimulatingfactor (GM-CSF) and IL-3 causetissue localization, prologation of survival, maturation and activation of eosinophils. It had been suggested that activation of TH_2 like subset of CD4+T-lymphocytes might contribute to eosinophil infiltration (31). Antigen-specific IgE is required for release of mast cell mediators in bronchialasthmaand IgE production is dependent on lymphocytes (32,33,34). Alveolarmacrophages present allergen in the context of appropriate class II HLA antigens to helper T cells. Activated helper T cells cause antigen-specific B cells to differentiate into plasma cells which produce IgE. In addition, it had been demonstrated that lymphocytes produce histamine releasing factor (HRF) which facilitates the release of histamine from mast cells (35). T cells also produce chemotactic factors that attract eosinophils to the site of disease (36). Inconformity with these studies, the demonstration of abnormally elevated macrophages, lymphocytes and eosinophils in this study validates the proposed mechanism that several cell types are involved in the pathogenesis of bronchial asthma. The absence of neutrophilic response in arthmatics requires further evaluation. Its significance is unknown.

In conclusion, this study demonstrates that chronic lung inflammation characterised by abnormally elevated macrophages, eosinophils and lymphocytes persists even in patients with stable mild bronchial asthma, despite medication with oral beta stimulants or theophylline.

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