CHARACTERIZATION OF AIRWAY INFLAMMATION IN STABLE BRONCHIAL ASTHMA.

Vijayan VK., Subramaniam P, Venkatesan P., Sankaran K. and Gnanaguruparan KS.
Cardio-Pulmonary Medicine Unit, Tuberculosis Research Centre,
Indian Council of Medical Research, Madras - 600 031 and Department of Medicine.
Government Stanley Medical College, Madras - 600 001.

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 ($29.6 \pm 18.4 \times 10^6$ /dl vs $16.0 \pm 6.6 \times 10^6$/dl, p(<0.001), absolute macrophages ($24.5 \pm 15.9 \times 10^6$/dl vs $13.3 \pm 5.5 \times 10^6$/dl. p<0.001), eosinophils ($1.0 \pm 1.5 \times 10^6$ dl vs $0.1 \pm 0.1 \times 10^6$/dl, p<0.001) and lymphocytes ($3.8 \pm 4.2 \times 10^6$/dl vs $2.4 \pm 1.5 \times 10^6$/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 in haled 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=18)	Bronchial asthma (n=50)
Total cells ' x 10^6.dl	16.0±6.6	29.6±18.4 *
Macrophages % " x 10^6.dl	83.7±6.3 13.3±5.5	82.8±13.0 24.5±15.9
Lymphocytes % " x 10^6/dl	14.6±6.4 2.4±1.5	13.4±122 3.8±4.2 **
Eosinphils % " x 10^6/pl	1.0±1.1 0.1±0.1	3.1±2.7 * 1.0±1.5 *
Neutrophils % " x 10^6.dl	0.7±0.8 0.1±0.1	0.6±2.1 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 hyper-responsivenessinmildasthrnapromptedseveraiinvestigators 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 eosi-nhils. It had been suggested that activation of TH, 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 pathogen-

esis 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.

REFERENCES

1. International Consensus Report on Diagnosis and treatment of asthma. Eur Respir J 1992; 5: 601-41.

2. Hunnighake GW, Gadek JE, Kawanami O, Ferrans VJ, and Crystal RG: inflammatory and immune process in the human lung in health and disease: evaluation of bronchoalveolar lavage; Am J Pathol 1979; 97: 147-206.

3. Godard P. Chaintreuil J. Damon M, Coupe M. Flandre O, Crastesde Paulet A and Michel FB. Functional assessment of alveolar macrophages; Comparison of cells from asthmatics and normal subjects. J Allergy Clin Immunol 1982; 70, 88-93.

4. Rankin J, Snyder PE, Schachter EN and Matthay RA. Bronchoalvolarlavage. Its safety in subjects with mild asthma. Chest 1984; 85, 723-8.

5. Fick RB, Richarson HB, Zavala DC and Hunnighake GW. Bronchoalveolar lavage in allergic asthmatics. Am Rev Respir Dis 1987; 135: 1204-8.

6. Crump JW, Pueringer RJ and and Hunninghake GW. Bronchoalveolar lavage and lymphocytes in asthma. Eur Respir J 1991; 4 (Suppl); 39S-46S.

7. Metzger WJ, Nugent K, Richerson HB, Moseley P, Lakin R. Zavala DC and Hunninghake GW. Methods for bronchoalveolar lavage in asthmatic patients following bronchoprovocation and local antigen challenge, Chest 1985; 87 S: 16 S-19 S.

8. Wardlaw AJ, Dunnette S, Gleich GJ, Collins JV and Kay AB. Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma. Am Rev Respir Dis 1988; 37; 62-9.

9. Kirby JG, Hargreave FE, Gleich GJ and O'Byrne PM. Bronchoalveolar cell profiles of asthmatic and non-asthmatic subjects. Am Rev Respir Dis 1987; 136; 379-83.

10. Zehr BB, Casale TB. Wood D, Floerchinger C, Richerson HB and Hunninghake GW. Use of segmental airway lavage to obtain relevant mediators from the lungs of asthmatic and control subjects. Chest 1989; 95; 1059-63.

11. Beasley R. Roche WE, Roberts JA. and Hdgate ST. Cellular events in the bronchi in mild asthma and after bronchial provocation. Am Rev Respir Dis 1989; 739: 806-17.

12. Metzger WJ, Richerson HB, Worden K, Monick M and Hunninghake GW. Bronchoalveolar lavage of allergic asthmatic patients following allergic bronchoprovocation. Chest 1986; 89; 477-83.

13. Lam S, Le Riche, J. Phillips D and Chan-Yeung M. Cellular and protein changes in bronchial lavage fluid after late asthmatic reaction in with red cedar asthma. J. Allergy Clin Immunol 1987; 80 ; 44-50.

14. Diaz P, Gonzalez MC. Galleguilos FR, Ancic P, Cromwell O, Shepherd D, Durham SR, Gleich GF and Kay AB. Leucocytes and mediators in bronchoalveolar lavage during allergen- in-

duced late-phase asthmatic reactions. Am Rev Respir Dis 1989; *139;* 1383-9.

15. Rishi JP, Govil A, Agarwal A, Arya A, Goyal M, Mehta S and Juneja R. Bronchoalveolar lavage cells in bronchial asthma. Lung India 1991; *9:* 49-51.

16. Pinkston P, Vijayan VK, Nutman TB, Rom WN, O'Donnell KM, Cornelius MJ, Kumaraswami V, Ferrans VJ, Takamura T, Yenokida G, Thiruvengadam KV, Tripathy SP, Ottesen EA and Crystal RG. Acute tropical pulmonary eosinophilia. Characterisation of the lower respiratory tract inflammation and its response to therapy. J Clinical Invest 1987; *80* :216-25.

17. American Thoracic Society. Lung function testing; Selection of reference values and interpretative strategies. Am Rev Respir Dis 1991; *144:* 1202-18.

18. Vijayan VK, Pandey VP, Sankaran, K, Mehrotra Y, Darbari BS and Misra NP. Bronchoalveolar lavage study in victims of toxic gas leak at Bhopal. Ind J Med Res 1989, *90:* 407-14.

19. Saltini C, Hance AJ, Ferrans VJ, Besset F, Bitterman PB and Crystal RG. Accurate quantification of cells recovered by bronchoalveolar lavage. Am Rev Respir Dis 1984; *130;* 650-8.

20. Azzawi M, Johnston PW, Majumdar S, Kay AB and Jeffery PK. T lymphocytes and activated eosinophils in airway musoca in fatal asthma and cystic fibrosis. Am Rev Respir Dis 1992; *145:* 1477-82.

21. Dunhill MS, Massarella GR and Anderson JA. A comparison of the quantitative anatomy of the bronchi in normal subjects, in status asthmatics, in chronic bronchitis and in emphysema. Thoraz 1969; *24* :176-9.

22. Richards Wand Patrick JA; Death from asthma in children. Am J Dis Child 1965; *110:* 4-23.

23. Laitinen LA, Heino M, Laitinen A, Kava T and Haathela T. Damage of the airway epithelium and bronchial reactivity in patients with asthma. Am Rev Respir Dis 1985; *131:* 599-606.

24. Wardlaw AJ, Dunnette S. Gleich GJ, Collins JV and Kay AB. Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma; relationship to bronchial hyperreaactivity Am Rev Respir Dis 1988; *137;* 62-9.

25. Azzawi M, Bradley B, Jeffery PK, Frew AJ, Wardlas AJ, Knowles G, Assoufl, B, Collins JV, Durham S and Kay AB. Identification of activated lymphocytes and eosinophils in bronchial biopsises in stable atopic asthma; Am Rev Respir Dis 1990; *142;* 1407-13.

26. Djukanovic R. Roche WR, Wilson JW, Beasley CRW, Twentyman OP, Howarth PH and Holgate ST. Mucosal inflammation in asthma. Am Rev Respir Dis 1990; *142* : 434-57.

27. Jeffery PK, Wardlaw AJ, Nelson FC, Collins JV and Kay AB. Bronchial biopsies in asthma - an ultrastructural quantification study and correlation with hypersensitivity; Am Rev Respir Dis 1989; *140:* 1745-53.

28. National Heart, Lung and Blood Institute National Asthma Education program. Expert panel Report; guidelines for the diagnosis and management of asthma. J Allergy Clin Immunol 1988 ; 425-534.

29. Kay AB; Leucocytes in asthma. Immunol Invest 1988; *17:* 679-705.

30. Thurbeck WM. Pathology of status asthmaticus In: Status asthmaticus. ED. Weirs EB. University Park Press. Baltimore 1982; PP 19-31.

31. Robinson DS, Hamid O. Sun Ying, Tsicopoulos A, Barkans J. Bentley AM, Corrigan CJ, Durham SR and Kay AB, Evidence for a predominant 'Th 2 -type' bronchoalveolar lavage T-lympho-cyte population in atopic asthma. New Eng J Med 1992; *326:* 298-304.

32. Ishizaki T. Conrad DH, Schulman ES, Stork AR. Ko CG. and Ishizaki K. IgE-mediated triggering signals for mediator release from mast cells and basophils. Fed Proc 1984; *43:* 2840-5.

33. Sustiel A and Rocklin R. T cell responses in allergic rhinitis, asthma and atopic dermtatitis. Clin Exp Allergy 1989; *19;* 1-18.

34. Dinarello CA and Mier JW. Lymphokines. New Engl J Medl 1987; *317:* 940-5.

35. Sedgwick JD, Holt PG, and Turner KJ. Production of a histamine-releasing lymphokine by antigen-or mitogen stimulated human peripheral T cells. Clin Exp Immunol 1981; *45:* 409-18.

36. Parish WE, Lymphocyte substances controlling eosiniphilia. Clin Allergy 1982; *12:* 47-51.