CHARACTERISATION OF LOWER RESPIRATORY-TRACT INFLAMMATION IN PATIENTS WITH SMEAR-NEGATIVE PULMONARY TUBERCULOSIS

VIJAYAN, VK., SANKARAN, K., VENKATESAN, P. AND PRABHAKAR, R.

Cardio - Pulmonary Medicine Unit, Tuberculosis Research Centre, Indian Council of Medical Research, Madras 600 031.

(Received original January 1994; revised May 1994)

ABSTRACT

Bronchoalveolar lavage (BAL) studies were carried out in 27 sputum smear negative but radiographic probable pulmonary tuberculosis patients to characterize the inflammatory and Immune effector cells in the lower respiratory tract and in 17 normal subjects. The diagnosis of active pulmonary tuberculosis was confirmed by isolation of Mycobacterium tuberculosis in culture from sputum and/or lavage specimens. BAL was done first from radiologically normal lobe and then from radiologically abnormal lobe prior to treatment and the BAL fluids were analyzed separately. Two groups were identified based on lavage results. One group (macrophage predominant) had significantly elevated total cells and alveolar macrophages in both radiologically normal ($p<0.01$) and abnormal lobes ($p<0.001$) compared to normal controls. The other group (lymphocyte predominant) had expanded numbers of total cells ($p<0.01$), lymphocytes ($p<0.01$), neutrophlls ($p<0.05$) and eosinophils ($p<0.01$) in radiologically abnormal lobe. The cell profile in radiologically normal lobe in lymphocyte predominant group was within normal limits. These results suggest that two distinct cell profiles, in *one* group an increase in alveolar macrophages and in the other an increase in lymphocytes can occur In the lower respiratory tract of patients with sputum smear negative, but radiographic probable pulmonary tuberculosis.

Introduction

Although treatment for pulmonary tuberculosis p&vents the replication of mycobacteria and re duces the mycobacterial load thereby reducing the immune- mediated lung injury, the lung damage in tuberculosis results from host reaction rather than by direct bacterial effects (1). In conformity with this, it had been noticed that radiographic and pulmonary function abnormalities persist in a proportion of patients despite treatment with potent regimens (2-4). Thus in order to "treat" all pulmonary tuberculosis patients successfully, we should be able to prevent the lung damage and subsequent fibrosis. However, the pathogenesis and mechanism of lung injury and fibrosis in pulmonary tuberculosis are not well understood to formulate modalities of treatment that may prevent fibrosis. Bronchoalveolar lavage (BAL) Studies have been found to be useful in better understanding of pathogenesis and development of fibrosis in various interstitial lung diseases (5). There are also reports of cellular compositions of BAL fluid in pulmonary tuberculosis (6-10). Bronchoalveolar lavage studies of tuberculosis patients may, therefore, help to assess the role of inflammatory and immune effector cells in lower respiratory tract (LRT) in mediating injury and fibrosis. A preliminary study was, therefore, planned to characterise the inflammatory and immune effector cells in the lower respiratory tract of sputum smear negative, but radiographic probable pulmonary tuberculosis patients using BAL.

Materials and Methods

Study subjects

Sixty nine patients presenting with respiratory symptoms such as cough, fever, haemoptysis and loss of weight for less than four months, and at least four sputum smears negative for acid fast bacilli (AFB), but with radiographic appearances suggestive of pulmonary tuberculosis were evaluated by BAL during a four-year period. The duration of disease was calculated from the onset of symptoms as provided by the patient during initial interrogation by the chest physician (VKV). The diagnosis of tuberculosis was based on the demonstration of M. tuberculosis in culture in sputum

or BAL fluid. None had received any anti tuberculosis treatment in the past. Skin tests were done by the intradermal Mantoux technique with one tuberculin unit purified protein derivative (PPD RT 23 with Tween 80) and were examined at 48 or 72 hours. Full-size postero-anterior chest radiographs were assessed by the International Labour Organisation (ILO) criteria (11). None of them was suffering from diabetes mellitus as assessed by urine examination for sugar or receiving immunosuppressive drugs.

Occupational history revealed shop workers (eight) plumber and turner (two), executive, rickshaw puller, labourer, milkman, driver, tinkering and gardening work (one each). Three were unemployed and seven were housewives. None had been exposed regularly to dusts / chemical: at occupation. Four patients gave a family / contact history of tuberculosis. Informed consent was obtained from each subject and the study was approved by the Institutional Ethical Committee.

Bronchoalveolar lavage.

Bronchoalveolar lavages were performed with a flexible fibreoptic bronchoscope as previously described (12). The lavages were done from two sites, first from a radiologically abnormal lobe in patients with localised disease. The normal site selected for lavage was always from the contralateral normal lung and was usually middle lobe or lingula. Five 20 ml aliquots 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 from each lobe was analysed separately. Filter preparations were made for differential cell counts on lavage fluid (uncentrifuged cells) as reported by Saltini et al(13) and were stained with haematoxylin-eosin. A minimum of 400 cells was counted for differential cell counts on each filter preparation by two observers (VKV, KS) and recorded independently. Both observers agreed to within 5% of all lavage analysis and the mean value was used for analysis. The absolute numbers of different types of cells were derived from total cells times differential percentages.

Normal subjects.

Seventeen non-smoking individuals (13 males, four females) were evaluated as control subjects. The mean age of normal subjects was 25.8 ± 2.1 years (range 17-53 years). 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.

Classification of BAL results.

If lymphocytes in BAL fluid are more than 22% (more than one SD above normal mean) and macrophages less than 75% (less than one SD below normal mean) from radiologically abnormal lobe, it was classified as lymphocyte predominant. If lymphocytes are less than 22% and macrophages more than 75%, it was classified as macrophage predominant. We had not done subtyping of lymphocytes.

Statistical analysis

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

Results

Of the 69 patients assessed, 27 had bacteriological confirmation by sputum culture including 18 in whom BAL fluid cultures were also positive for M. tuberculosis. The mean age of these 27 subjects was 29.1 ± 1.63 Yrs (range 17 to 45 years) and the mean weight was 44.4 ± 1.03 Kg. (range 36.6 to 55.7 Kg.). There were 20 males and seven females Eleven subjects were smokers. Four had smoked more than 10 beedis/cigarettes daily for more than five years. The mean duration of symptoms was 1.7±0.24 months (range seven days to four months). and the mean reaction to PPD was 19.9±1.3 mm (range 10 to 40 mm.). All had radiographic abnormalities of category A (11) with exudative pattern confined to one of the upper zones and none had cavitary lesions. Based on the BAL results, two groups were identified. There were fourteen patients with predominance of macrophages (Group I) and 13 with predominance of lymphocytes (Group 2).

Group 1 (Macrophage Predominant)

There were nine males and five females in this group. The mean age was 29.1±2.2 Yrs (range 22 - 45 years) and six were smokers. There were no difference in lavage findings between smokers and non-smokers. The mean duration of symptoms was 1.5 ± 0.26 months (range seven days to three months) and the mean body weight was 43.9±1.4kg (range 36.6 to 52.0). The mean reaction to PPD in this group was 19.5±1.9 mm. (range 10 to 40 mm.). BAL results are shown in the Table.

TABLE

Inflammatory and Immune effector cells in the lower respiratory tract

(Mean ± SEM)

		Normal Subjects (n:17)	Macrophage Predominant		Lymphocyte Predominant	
			RNS (n:14)	RAS (n:14)	RNS (n:13)	RAS (n:13)
Total cells, (x 10^6/dl)			16.1±1.6	25.9±3.8**	32.6±3.3***	29.0±7.3
33.6±7.0**						
Macrophages	%	83.5±1.5	89.1±2.2*	90.2±1.4**	77.1±4.9	56.1±5.2*** ££
	x 10^6/dl	13.4±1.4	23.8±3.8**	29.5±3.1***	23.8±7.1	17.1±3.7
Lymphocytes,	%	14.9±1.6	8.9±1.9*	7.3±1.1**	20.6±4.9	40.0±5.3*** ££
"	x 10^6/dl	2.5±0.4	1.7±0.3	2.2±0.3	4.8±1.5	15.1±4.7** £
Neutrophils.	%	0.7±0.2	1.0±0.3	1.1±0.5	0.9±0.2	1.7±0.4**
"	x 10^6/dl	0.1±0.03	0.2±0.05	0.4±0.2	0.3±0.1	0.8±0.3*
Eosinophils,	%	0.9±0.3	1.0±0.3	1.4±0.4	1.4±0.4	2.2±0.6**
"	x 10^6/dl	0.1±0.03	0.2±0.05	0.5±0.2	0.3±0.1	0.7±0.2**
% Recovery		59.6±1.6	62.6±4.1	63.4±4.2	64.6±0.8	59.3±1.3

RNS : Radiologically normal site

RAS : Radiologically abnormal site

p values * < 0.05, ** <0.01, *** <0.001 as compared to normal subjects

£ <0.05, ££ <0.01, as compared radiologically normal site.

Total inflammatory ceils and absolute (total) numbers of alveolar macrophages were significantly higher in both radiologically abnormal (p < 0.001) and normal (p < 0.01) lobes compared to normal subjects. Other types of cells were similar in both abnormal and normal lobes on comparison with normals. This was true whether all,, individuals or only non-smokers were considered. Because of a significant rise in macrophage percentages at both sites. there was a reciprocal significant reduction in lymphocyte percentages. However, total lymphocytes were normal at both sites. The fluid recovery was similar in normal subjects and patients in both lobes.

Group 2 (Lymphocyte Predominant)

Of the 13 patients in this. group, two were females and five were smokers. BAL results between smokers and non-smokers were similar. The mean age was 29.0 ± 2.5 Yrs (range 17- 44 years). The mean duration of symptoms was 2.1 ± 0.5 months (range 15 days to four months) and the mean body weight was 45.1±1.5 kg (38.5 to 55.7). The mean reaction to PPD in these patients was 20.6±1.6 mm. (range 14 to 28 mm.). BAL results in Group 2 patients are given in Table. The total inflammatory ceils (p<0.01), lymphocytes (p<0.01), neutrophils (p<0.05) and eosinophils (p<0.01) were significantly higher in radiologically abnormal site compared to normal subjects. This was true whether all individuals or only non-smokers were considered. There was also a significant increase in the proportion of lymphocytes, neutrophils and eosnophils and a significant reduction in proportion of alveolar macrophages at radiologically abnormal site. Total lymphocytes (p<0.05) and eosinophils (p<0.05) were also significantly higher at radiologically abnormal site in comparison with radiologically normal site. All types of cells were similar except for a suggestion of an increase in total inflammatory cells (p = 0.06) in radiologically normal site in comparison with normal subjects. Fluid recovery was similar in normal subjects and patients in this group.

Discussion

We have demonstrated in this study that two distinct cell profiles, one group with an increase in

LUNG INDIA (1994) XII, NO (P. 63 - 68)

alveolar macrophages and the other with an increase in lymphocytes can occur in the tower respiratory tract of patients with sputum smear-negative but radiographic probable, pulmonary tuberculosis . We had earlier reported that a third type of cell profile with an increase in granulocytes (neutrophils and eosinophils) can also occur in smear negative pulmonary tuberculosis (14). Since the age, smoking habit, fluid recovery during BAL, the reaction to PPD, the nutritional status as assessed by body weight and the radiological abnormalities were similar in both groups. the reason for the observed difference in cell profile between the two groups could not be explained, except for a suggestion that the duration of illness (1.5±0.26 vs 2.1±0.5 months; is longer in lymphocyte predominant group. Neutrophils are the first cells to appear in tuberculosis exudate (15) and the BAL findings of granulocyte accumulation in tuberculosis patients (14) may thus represent the earliest response after inhalation of Mycobacterium tuberculosis into an alveolus. The demonstration of abnormally elevated macrophages in the radiologically normal and abnormal lobes of pulmonary tuberculosis patients in this study may represent recruitment of monocytes to the lungs(16) from the circulating pool of peripheral blood monocytes (17) by the chemotactins released by neutrophils(18). Expanded numbers of alveolar macrophages alone in lower respiratory tract had been described in early stages of pneumoconioses (19,20) and subacute phase of toxic gas exposure (12). Macrophage activation is characterised by spontaneous release of reactive oxygen species and a number of a microbicidal enzymes by these cells (21-23). We had reported previously that alveolar macrophages from tuberculous patients were activated as evidenced by spontaneous release of hydrogen peroxide (H_2O_2) by these cells (24).

Lymphocytes which are building blocks of granuloma are recruited to the site of lesion and activated by interleukin-I and gamma-interferon released by activated macrophages (25). Thus lymphocytosis at radiologically abnormal but *not* in radiologically normal lobe in this study may represent the localisation of granuloma at the site of lesion. Other cell types such as neutrophils and eosinophils also contribute to granuloma formation (16) and in conformity with this, there is a significant elevation of neutrophils and eosinophils in radiologically abnormal lobe in this study as well.

Previous workers had demonstrated BAL lymphocytosis in sputum smear positive pulmonary tuberculous patient (6-8). It had also been observed that lymphocytosis occurred in active tuberculous lesion, but not in non-affected parts of the lungs (8), as seen in this study. In a study of sputum smear negative pulmonary tuberculosis, lymphocytosis was, observed in 55% of patients at local site (9) and this was similar to the observation of lymphocytosis in 48% (13 of 27) of patients in our series. Generalised lymphocytosis in lung has been described in other granulomatous lung diseases such as sarcoidosis, hypersensitivity pneumonitis and berylilosis (5). The main type of lymphocytes in tuberculous granuloma is T cells (6,26) and an increase in CD4+ T cells is also demonstrated in tuberculous pleural effusion (27). Activated T cells are capable of spontaneously releasing lymphokines such as interleukin-2, gamma interferon and tumor necrosis factor and play an important role in immune response in tuberculo sis (16,25). The demonstration of expanded numbers of alveolar macrophages in both radiologically normal and abnormal lobes of patients with shorter duration of symptoms may suggest the possibility that monocyte/macrophage recruitment to the lung precedes lymphocyte recruitment and granuloma formation at the site of lesion following tuberculous infection. This may further be suggested by the fact that abnormal accumulation of lymphocytes and granulocytes were seen only in radiographically abnormal lung segments of patients with longer duration of symptoms. However, there was no significant difference in duration of symptoms between the two groups for a definite conclusion.

A dissociation of cell mediated immunity (CMI) and delayed type hypersensitivity (DTH) was described in tuberculosis and it was suggested that cellular types of immune responses were involved in both (28). CMI was defined as a beneficial host response and DTH as the immunological reaction that caused caseous necrosis (23). Although tuberculin-positive hosts with good CMI and tuberculin-positive hosts with poor CMI could arrest bacillary growth, the host with good CMI might recover whereas the host with poor CMI might suffer excessive tissue destruction (23). All patients in Our study had strong tuberculin reaction (> 10 mm) and the observation of two types of cell profile may. therefore, suggest the possibility that one group may have tuberculin- reactive good CMI and the other tuberculin-reactive poor CMI. It had also been

suggested that two mechanisms of cell mediated responses referredto as the Listeria-type and the Koch- type could occur in infections with mycobacteria and the Listeria-type is thought to provide protective immunity (29,30). It had also been described previously that healing could occur in a proportion of tuberculous patients without treatment (31, 32) which might be due to the development of good CMI in some of these patients. The finding of generalised recruitment of macrophages to the lung in one group may, therefore, suggest the inability of the host to localise the immune response as r e served in lymphocyte predominant group. We have recently reported that lymphocytic alveolitis in tuberculosis patients is associated with increased bactericidal activity against Staphylococcus aureus, whereas no lymphocytic alveolitis is associated with reduced activity (33). Ethical considerations will not permit the follow up without treatment to know the fate of these patients. However; Immunological studies of the cytokines (34,35) released from lung immune and inflammatory cells to assess the immunologic competence of the host may aid in understanding the pathogenesis of tissue destruction and healing in pulmonary tuberculosis.

REFERENCES

1. Beck JS. Skin changes in the tuberculin test. Tubercle 1991; *72:* 81 - 7.
2. Fox W. Whither Short-Course Chemotherapy ? Brit J Dis Chest 1981; *75:* 331-57.

   Wilcox PA and Ferguson AC. Chronic obstructive airways disease following treated pulmonary tuberculosis. Respiratory Med 1989; *83:* 195 - 8.

4. Tuberculosis Research Centre: Study of Chemotherapy regimens of 5 and 7 months duration and the role of corticosteroids in the treatment of sputum - positive patients with pulmonary tuberculosis in South India. Tubercle 1983; *64:* 73 - 91.
5. Crystal RG, Bitterman PB, Rennard SI, Hance AJ and Keogh BA. Interstitial lung diseases of unknown cause. Disorders characterised by chronic inflammation of the lower respiratory tract. N Engl J Med 1984; *310:* 154 - 65, 235 - 44.
6. Dhand R, De A, Ganguly NK, Gupta N, Jaiswal S, Malik SK and Kohli KK. Factors influencing the cellular response in bronchoalveolar lavage and peripheral blood-of patients with pulmonary tuberculosis. Tubercle 1988; *69:* 161 - 73.
7. Baughman RP, Cohn MN, Loudon RG, and Trame PT. Bronchoscopy with bronchoalveolar lavage in tuberculosis and fungal infections. Chest 1991; *99:* 92 - 7.
8. Ozaki T, Nakahira S, Tani K, Ogushi F, Yasuoka S and Ogura T. Differential cell analysis in broncho-alveolar lavage fluid from pulmonary lesions of patients with tuberculosis. Chest 1992; *102:* 54 - 9.
9. Ainslie GM, Solomon JA and Bateman. Lymphocyte and lymphocyte subset numbers in blood and in bronchoalveolar lavage and pleural fluid in various forms of human pulmonary tuberculosis at presentation and during recovery, Thorax 1992; *47:* 513-8.
10. Vijayan VK, Jawahar MS, Reetha AM, and Prabhakar R. Persisting alveolitis in miliary tuberculosis despite treatment with short-course chemotherapy. Indian J Chest Dis All Sci 1990; *32:* 49 - 53.
11. International Labour Office. Guidelines for the use of ILO. International classification of radiographs of pneumoconiosis. ILO Geneva: 1980. Occupational Safety. Health Services No. 22 (Rev 80).
12. Vijayan VK, Pandey VP, Sankaran K, Mehrotra Y, Darbari BS and Mira NP. Bronchoalveolar lavage study in victims of toxic gas leak at Bhopal. Indian J Med Res 1989; *90:* 407 - 14.
13. Saltini C, Hance AJ, Ferrans VJ, Basset F, Bitterman PB and Crystal RG. Accurate quantification of cells recovered by bronchoalveolar lavage. Am Rev Respir Dis 1984; *130:* 650-8.
14. Vijayan VK, Reetha AM, Jawahar MS, Sankaran K and Prabhakar R. Pulmonary eosinophilia in pulmonary tuberculosis. Chest 1992; *10:* 1708 - 9.
15. Montgomery LG and Lemon WS. The cellular reaction of the pleura to infection with Mycobacterium tuberculosis. J Thoracic Surg 1933; *2:* 429 - 39.
16. Edwards C and Kirpatrick CH. The Immunology of Mycobacterial Diseases. Am Rev Respir Dis 1986; *134:* 1062 - 71.
17. Blusse Van Ovd Alblas, Van Der Linden - Schrever B and Van Furth R. Origin and Kinetics of pulmonary macrophages during an inflammatory reaction induced by intra alveolar administration of aerosolised heat-killed BCG. Am Rev Resp Dis 1983; *128:* 276 -81.
18. Antony VB, Sahn SA, Antony AO and Repin JE. Bacillus - Calmette - Guerin - stimulated neutrophils release chemotaxins for monocytes in rabbit pleural spaces and in vitro. J Clin Invest 1985; *76:* 1514 - 21.
19. Rom WN, Bitterman PB, Rennard SI, Canter AC and Crystal RG. Characterisation of the lower respiratory tract inflammation of non-smoking individuals with interstitial lung disease associated with chronic inhalation of inorganic dusts. Am Rev Respir Dis 1987; *136:* 1429 - 34.
20. Takemura T, Rom WN, Ferrans VJ and Crystal RG: Morphological Characterisation of alveolar-macrophages from subjects with occupational exposure to inorganic particles. Am Rev Respir Dis 1989; *140:* 1674 - 85.
21. Dannenberg AM Jr. Pathogenesis of pulmonary tuberculosis. Am Rev Respir Dis 1982; *125:* 25 - 9.
22. Cannenberg AM Jr. Pathogenesis of tuberculosis. In : Fishman AP, ed. Pulmonary diseases and disorder% New York: McGraw - Hill, 1980: 1264-81.
23. Danneberg AM Jr. Delayed - type hypersensitivity and cell mediated immunity in the pathogenesis of tuberculosis. Immunology Today 1991; *12:* 228-33.
24. Selvaraj P, Raji Swamy, Vijayan VK, Prabhakar R and Narayanan PR. Hydrogen peroxide producing potential of alveolar macrophages and blood monocytes in pulmonary

tuberculosis. Indian J Med Res 1988; *88:* 124 - 9.

25. Garrett KC, Richerson HB and Hunninghake GW. Mechanisms of granuloma formation. Am Rev Respir Dis 1984; *130:* 477-83.
26. Rossi GA, Balbi B, and Manca F. Tuberculous pleural effusions. Evidence for selective presence of PPD = specific T-lymphocytes at site of inflammation in the early phase of the infection. Am Rev Respir Dis 1937; *136:* 575 - 9.
27. Lucivero C, Pierucci G, and Bonoma L. Lymphocyte subsets in peripheral blood and pleural fluid. Eur Resp J. 1988; *1:* 337 - 40.
28. Youmans GP. Relation between delayed hypersensitivity and cell mediated immunity Am Rev Respir Dis 1975; *111:* 373-7
29. Stanford JL, Shield MJ and Rook GAW. How environmental mycobacteria may predetermine the protective efficacy of BGG. Tubercle 1981: *62:* 55-62.
30. Rook GAW, Bahr GM and Stanford JL. The effect of two distinct forms of cell-mediated response to mycobacteria on the protective efficacy of BCG. Tubercle 1981; *62:* 63-8.
31. National Tuberculosis Institute, Bangalore. Tuberculosis in a rural population of South India. Bull Wld Hlth Org. 1974; *51:* 473-88.
32. Grzybowski S. Cost in tuberculosis control. Tubercle 1987; *68:* 33-37.
33. Selvaraj P, Venkataprasad N, Vijayan VK and Narayanan PR. Altered bactericidal activity against Staphylococcus aureus in tuberculous broncho alveolar lavage fluids. Eur Resp J 1994; *7:* 121-6.
34. Rook GAW and Attiyah R AI. Cytokines and the Koch phenomenon. Tubercle 1991; *72:* 13-20.
35. Kovacs EJ: Fibrogenic cytokines the role of immune mediators in the development of scar tissue. Immunology Today 1991; *12:* 17 - 23.

Correspondence / request for reprints : Dr. V.K. Vijayan Deputy Director and Head, Cardio-Pulmonary Medicine and TB Research Centre, Indian Council of Medical Research Madras 600 031.