ALVEOLAR MACROPHAGES IN PATIENTS WITH PULMONARY TUBERCULOSIS

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ABSTRACT

Total and HLA-DR positive alveolar macrophages were enumerated in the broncho-alveolar lavage fluids of active pulmonary tuberculosis, inactive pulmonary tuberculosis (treated cases), non-tuberculous lung disease patients and normal healthy individuals. A significantly increased number of macrophages was found in the patient groups than the normal individuals: however, there was no difference in HLA-DR positive cells. Among the different patient groups, smokers had more macrophages as well as more HLA-DR positive macrophages than non-smokers and normal subjects. Total number of alveolar macrophages of the active tuberculosis non-smoker group was significantly higher than the inactive tuberculosis non-smoker group and normal individuals. On the contrary, a slightly decreased number of HLA-DR positive cells was seen in the non-smoker group of active-TB patients. The present study suggests that infection and smoking induce the accumulation of macrophages in the lung. The implication of these findings in relation to tuberculosis is discussed.

Introduction

Immunologically active cells lining the alveolar region of the lung are predominantly alveolar macrophages. These cells are mainly involved in eliminating the inhaled irritants and defence against pathogens. Macrophages appear to be important for bacterial phagocytosis and killing(1). Further, these cells are involved in host resistance to M. tuberculosis (2,3). These mononuclear phagocytes produce reactive oxygen intermediates such as super oxide anion and hydrogen peroxide. These reactive oxygen intermediates are well known for their microbicidal function. This represents cellular capacity for microbicidal function and the host defense function against intracellular pathogens. (4,5,6,7).

The human leukocyte antigen system (HLA) is divided into Class-I (HLA-A, -B & -C) and Class-II (HLA-DR, -DP, & -DQ) antigens. The Class-II antigens are known as Ia antigens (immune region associated antigen) (8). It is well known that activation of macrophages by pathogens or any other antigens or lymphokines will increase the expression of immune region associated Ia antigen and alters other functions of the macrophages (9,10). It is well established that these Ia antigens are responsible for the immunological functions such as antigen presentation to T-lymphocytes that leads to T-cell proliferation and these cells are responsible for specific cell-mediated immunity(11). The present study was carried out to enumerate the number of total alveolar macrophages and Ia positive (HLA-DR+) and Ia negative (HLA-DR-) alveolar macrophages in active pulmonary tuberculosis, inactive pulmonary tuberculosis, non-tuberculous lung disease patients and normal individuals.

Material and Methods

The patients included in this study were suspected cases of pulmonary tuberculosis, attending Tuberculosis Research Centre with respiratory symptoms and radiographic evidence of lung diseases of varying duration. All the patients had at least six sputum smears negative for M. tuberculosis by microscopy. All sputa were cultured for M. tuberculosis. Based on the culture results, the patients were classified into three groups.

(i) Active pulmonary tuberculosis: these patients had not received any previous antituberculosis chemotherapy and had grown M. tuberculosis in one or more sputum specimens in culture (10 patients).

(ii) Inactive pulmonary tuberculosis: These patients had had previous antituberculosis chemotherapy and presently their sputum showed culture negativity for M. tuberculosis (10 patients).
(iii) Non-tuberculous Lung Disease: This group included patients with lung abscesses, bacterial pneumonia and pulmonary manifestations of polycythemia vera (eight patients).

(iv) Normal subjects who had normal chest skiagram and pulmonary functions and were non-smokers (four individuals).

Fibreoptic Bronchoscopy and Broncho alveolar lavage (BAL)

An informed written consent was obtained from each subject. The procedure was done in the morning after overnight fasting. Atropine 0.6-0.8 mg (i.m.) and Diazepam 5 mg (i.v) were given as pre-medication. The upper airways and trachea were anesthetized with 4% xylocaine spray. After ensuring that there was no gag reflex, fibreoptic bronchoscope was inserted either through the mouth or the nose. The tip of the broncoscope was wedged into the third or fourth order bronchus in the affected lobe and also two other lobes, usually middle lobe and lingula. Sterile isotonic saline was then infused in five 20ml aliquots with each aliquot being immediately aspirated using a suction pressure of 50-100 mm of water. Each of the three lobes was lavaged with 100 ml saline for a total of 300 ml per subject. The recovered fluid was pooled and the total volume measured. The entire bronchoscopy procedure was done under continuous cardiac and pulse-oximeter monitoring, and oxygen was supplemented during and after the procedure.

Enumeration of total macrophage population in the lavage fluid

The total number of macrophages was enumerated using hemocytometer. The percentage of dead cells ranged from 10 to 20% as judged by trypan blue dye exclusion method. Smears of the alveolar cells were stained using modified Wright-Giemsa stain (Diff-Quick, American Scientific Products, U.S.A.). Based on the percentage of macrophages (based on differential count) and the total number of live cells, the total number of macrophages were calculated and expressed as $10^4$ macrophages per ml of lavage fluid.

Cytotoxicity assay

HLA-DR positive alveolar macrophages were enumerated using antibody and complement mediated conventional cytotoxicity assay by the method described by Whisler and team (13) Briefly, alveolar cells were incubated for 1 hr at 4°C with 1:100 final dilution of OK I a1 monoclonal antibody (Ortho diagnostic systems, Inc. U.S.A) (an anti-HLA-DR antigen frame work complex expressed on B-cells, activated T-cells and monocytes). Then the cell suspension was washed in RPMI -1640 (Rosewall Park Memorial Institute Medium) containing 5% foetal calf serum and was further incubated for 1 hr at 37°C with 1:4 final dilution of rabbit complement. The percentage of cells susceptible to OK I a1 and complement treatment was assessed using trypan blue dye exclusion method and expressed as percent cytotoxicity.

Statistical methods

The results are expressed as arithmetic mean ± Standard Error (SE). Significance was determined using the Student’s t-test(14).

Results

The total alveolar macrophage population of the active tuberculosis, inactive tuberculosis (treated cases) and non-tuberculous lung disease patients were significantly higher than the normal individuals (Fig.) (Normal vs. Active TB=p<0.01; Normal vs. inactive-TB=p<0.01; Normal vs. Non-TB= p<0.05). On the other hand, there was no difference in their macrophage numbers between the various groups of patients studied. Since, the individuals of the normal group were all non-smokers, the patients were divided into smokers and non-smokers. Smokers had an increased level of macrophage than the non-smoker group. However, the
active tuberculosis non-smoker group showed almost an equal level of macrophages when compared to the active-TB smoker group. Increased number of alveolar macrophages was observed in the active-TB non-smoker group than the inactive-TB non-smoker group and normal individuals. However, there was no difference in macrophage numbers between non-smoker group of active-TB and non-TB patients. A slightly decreased level of macrophage numbers was seen in the non-smoker group of non-TB patients than smoker group of the same patient group.

The HLA-DR positive alveolar macrophages were similar irrespective of the patient groups studied (smokers + non-smokers). When the patients were divided into smokers and non-smokers an increased number of HLA-DR positive alveolar macrophages was found in smokers than the non-smokers. A slightly decreased level of HLA-DR positive macrophages was observed in non-smoker group of active tuberculosis patients than the normal subjects. On the other hand, the HLA-DR positive macrophages in the non-smoker of inactive-TB and non-TB patient groups were similar to that of normal subjects (Table).

### TABLE
PERCENTAGE HLA-DR POSITIVE ALVEOLAR MACROPHAGES IN VARIOUS GROUPS OF PATIENTS AND NORMAL INDIVIDUALS

<table>
<thead>
<tr>
<th></th>
<th>% Cytotoxicity*</th>
<th>Mean ± S.E.</th>
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<tbody>
<tr>
<td></td>
<td>Smokers + Non-smokers</td>
<td>Non-smokers</td>
</tr>
<tr>
<td>1. Active-TB</td>
<td>46.5 ± 8.5 (8)</td>
<td>38.9 ± 11.3 (4)</td>
</tr>
<tr>
<td>2. Inactive-TB</td>
<td>46.2 ± 3.9 (9)</td>
<td>44.4 ± 6.0 (4)</td>
</tr>
<tr>
<td>3. Non-TB</td>
<td>59.0 ± 5.7 (6)</td>
<td>47.0 (2)</td>
</tr>
<tr>
<td>4. Normal</td>
<td>–</td>
<td>49.1 ± 5.6 (5)</td>
</tr>
</tbody>
</table>

* Percent alveolar macrophages susceptible to Okla1 monoclonal antibody and rabbit complement.

Numbers in the parantheses represent the number of subjects studied.

**Discussion**

In the present study, the patient groups (smoker + non-smoker group) had a higher number of alveolar macrophages than normal individuals. Further, the smokers also showed an increased level of total alveolar macrophages. It is well established that inflammation due to infection or smoking will lead to increased number of macrophages. (15).

Among the non-smoker groups studied, patients with active-TB showed an increased number of macrophages than normal individuals and patients with inactive-TB. This suggests that the increased macrophage population in the non-smoker group of active-TB could be due to *Mycobacterium tuberculosis* infection in the active state of the disease. In the inactive tuberculosis group, the macrophage population was equal to that of normal individuals. These findings suggest that *M. tuberculosis* infection in the lung increases the accumulation of macrophages.

It is well established that smoking or infection due to any pathogen activates the macrophages (16,17,18) and enhances the expression of Ia antigens on the surface and alters other functions of the macrophages (9,19,20) Such an increase of Ia positive cells has been shown in sarcoidosis(21) and other lung diseases(22).

In the present study, a slightly increased number of Ia positive (HLA-DR positive) alveolar macrophages were seen in the smokers, irrespective of the patient groups studied. However, non-smokers showed no difference. In the active TB non-smoker group, there was a higher macrophage population than the inactive-TB patients and normal individuals; but, slightly reduced level of Ia positive macrophages were seen in the non-smoker group of active-TB patients (Table). This could be due to the bacillary load in the lung, which is probably not enough to activate the macrophages to express more HLA-DR antigens. The second possibility is that the bacilli might have down-regulated the expression of -DR antigens on the macrophages; it has been suggested that *M. leprae* alters the membrane expression of HLA-DR antigens on the macrophages of leprosy patients(23). Further, under *in vitro* culture conditions, *M. microti* inhibits the expression of Ia antigens on the surface of the mouse peritoneal macrophages(24).

The other explanation for this decrease in DR positive alveolar macrophages in active TB non-smoker group is that there are more newly recruited cells of the monocyte-macrophage lineage which do not express a detectable level of HLA-DR antigen. The recent data from our laboratory suggest that an alveolar macrophage population resistant to OKla1 monoclonal antibody treatment (HLA-DR negative alveolar macrophages) produced more hydrogen peroxide. In a study carried out in the blood monocytes...
of tuberculosis patients, an increased level of Ia negative monocytes has been shown. The same study also suggested that HLA-DR negative and -DR positive phenotypes represent different stages of maturation(25).

The present study reveals that the increased macrophage population is not specific to active pulmonary tuberculosis. Infection by any pathogen and smoking can cause an increase in the macrophage population and other cells. It will be useful to study the role of these HLA-DR positive and negative alveolar macrophages in their microbiidal and the immunological functions to intracellular pathogens such as M. tuberculosis. Work is progressing in this direction.

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REFERENCES


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