T Lymphocytic and Immature Macrophage Alveolitis in Active Pulmonary Tuberculosis


The phenotype of bronchoalveolar cells from 11 healthy subjects and from affected and unaffected lungs of 15 patients with pulmonary tuberculosis (PTB) was determined. An immature macrophage alveolitis was found in the affected lung and the unaffected lung versus controls as determined by morphology and peroxidase activity. T lymphocytic alveolitis also was found in the affected but not the unaffected tuberculous lung compared with healthy controls. The majority of alveolar lymphocytes in unaffected and affected PTB lungs were T cells expressing the αβ T cell receptor. Alveolar T cells from both unaffected and affected lungs were activated, as determined by increased expression of CD69 and HLA-DR. Interleukin-2 receptor (IL-2Re) expression was, however, unchanged on alveolar lymphocytes from affected lung and was decreased in the unaffected lung. Thus, activated T lymphocytes and immature macrophages in the tuberculous lung are basic to the local immunopathogenesis of PTB.

The most common organ affected in tuberculosis (TB) is the lungs, yet very little is known about the cell types or the immunologic events occurring at this site of disease activity. Several studies show an increase in alveolar lymphocytes (AL) [1–6], and one study reports an increase in neutrophils within the bronchoalveolar cells (BAC) of patients with pulmonary TB (PTB) obtained by bronchoalveolar lavage (BAL) [6]. HLA-DR also is increased on AL from patients with PTB [3].
in Mexico City. Each had a clinical history and chest radiograph consistent with reactivation PTB and acid-fast bacilli in their sputum or BAC (or in both). Nine subjects had positive sputum cultures for Mycobacterium tuberculosis. Four patients were treated with triple antituberculous short-course therapy for a mean duration of 9 days (range, 3–14) before BAL. Nine patients had not yet received antituberculous treatment at the time of BAL. Each patient with PTB ultimately responded well to antituberculous therapy.

Subjects were not eligible if they were seropositive for human immunodeficiency virus (HIV-1) by ELISA (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France); if they had a Karnofsky performance score <60, miliary tuberculosis, or a pulmonary disease other than active PTB; if they were being treated with immunosuppressive therapy; or if they were known to have alcoholism, diabetes mellitus, cancer, chronic renal failure, or cirrhosis of the liver. Healthy control subjects (n = 11) were recruited in Mexico City if they were HIV-1 antibody–negative, had no pulmonary disease (including asthma or upper respiratory tract infections) for 1 month, and had no chronic immunosuppressive diseases.

Preparation of cells. Peripheral blood mononuclear cells (PBMC) were prepared by centrifugation of whole heparinized blood over Ficoll-Paque (Pharmacia, Uppsala, Sweden) and BAC were obtained by BAL [7]. Saline (180 mL) was instilled into two segments of the right middle lobe in healthy controls (68% of fluid retrieved), in PTB patients, into a segment of the radiographically affected lung, and in 10, a segment of the unaffected lung (62% of the fluid retrieved). The total number of BAC retrieved from healthy subjects was divided by two to normalize to the one segment lavaged from the affected and the one from the unaffected lung of the PTB patients. Viability of cells (exclusion of trypan blue uptake) was >90% in BAC and PBMC from PTB patients and healthy control subjects.

BAC and PBMC from 12 subjects were stained and fixed in Mexico before shipment. Cells from 14 subjects were shipped at 4°C and stained and fixed within 36 h of BAL in Cleveland. There was no statistical difference in any parameter measured between cells shipped before or after fixation.

Dual staining of PBMC and BAC and flow cytometry. Cells were dual stained with saturating concentrations of phycoerythrin-conjugated primary and isotypic control monoclonal antibodies (MAbs) and fluorescein isothiocyanate–conjugated secondary MAb anti-CD3, fixed in 1% paraformaldehyde, and maintained at 4°C in the dark until flow cytometry (FACScan; Becton Dickinson, San Jose, CA), which was done within 72 h. Lymphocytes were gated both by size and by fluorescence to exclude macrophages. All antibodies were obtained from Becton Dickinson except anti-αβ and anti-γδ (T Cell Diagnostics, Woburn, MA) and IgG2b (Southern Biotechnology, Birmingham, AL). Specific binding was calculated as percentage of cells positive for a particular primary MAb minus those binding the appropriate isotypic control.

Statistical analysis. The paired t test was used to compare autologous PBMC and AL and the unpaired t test for comparison of cells from healthy and PTB patients. Statistical significance was considered to be P < .05. All data shown represent mean ± SE.

Results

Characteristics of study groups. The age of the subjects in the 2 groups was comparable: 29 ± 8 years (healthy controls) and 32 ± 12 years (PTB patients). Ten healthy and 10 PTB subjects were male. The number of smokers among PTB patients and controls was comparable: 3 of 11 healthy subjects and 4 of 15 PTB patients. Body weight (52 ± 7.4 vs. 72 ± 10 kg) and hemoglobin concentration (12.4 ± 1.3 vs. 16.8 ± 0.1 g/dL) were significantly lower and white blood cell count (10.5 ± 3 × 10⁹/L vs. 7.5 ± 0.9 × 10³/µL) significantly higher in the PTB patients than the healthy controls (P < .05).

Cellular profile of BAC from PTB patients and healthy controls. The cellular pattern of BAC in the affected and unaffected lungs of PTB patients and healthy controls was characterized using Wright's stain on cytocentrifuge preparations for nuclear morphology (figure 1A, B) and a peroxidase stain, which detects immature macrophages [8] and neutrophils (figure 1C, D). Wright's-stained preparations showed that neutrophils were increased (8%, 11%, and 25%) in BAC from affected lungs from only 3 (20%) of PTB patients but were rare (<1%) in the rest of the patients and all healthy subjects.

Alveolar macrophages (AM) from affected lungs appeared more heterogeneous in size than AM from unaffected lungs (figure 1A, B). The mean percentage of AM was significantly less in affected lungs (66.7% ± 17%) than in healthy control lungs (87.1% ± 5.3%) or unaffected PTB lungs (86% ± 5%; P < .001). However, there was a 2.4-fold increase in the mean percentage of BAC that were immature, peroxidase-positive macrophages in the affected PTB lung (22.3% ± 7%) compared with the unaffected lung (9.3% ± 3.4%; P < .01) and a 25-fold increase compared with control lungs (0.9% ± 0.7%; P < .001). (In the 3 patients with neutrophils by Wright's stain, the percentage of neutrophils was subtracted from the total percentage of peroxidase-positive cells so that data on peroxidase-positive cells reflected only immature macrophages.) The mean absolute number of immature macrophages per 10 mL of BAC fluid in the unaffected PTB lung (2 ± 1 × 10⁵) also was higher than in control lungs (0.1 ± 0.1 × 10⁵; P < .03) and higher in the affected PTB lungs (4 ± 2 × 10⁵) than in unaffected lungs (P < .03) or healthy control lungs (P < .001). The mean percentage of peroxidase-positive cells in PTB BAC also was 3-fold higher than in PBMC from healthy controls (25% ± 5.1% vs. 8% ± 3%; P < .05).

The mean percentage of AL in affected lungs (29.4% ± 14.8%) was 1.9-fold higher than in healthy control lungs (15.7% ± 7%) and 2-fold higher than in unaffected lungs (14.2% ± 5.1%; P < .001). Mean absolute numbers of AL per 10 mL of BAC fluid also were higher in affected (6 ± 2 × 10⁵) than unaffected PTB lungs (3 ± 1.3 × 10⁵; P < .05) or controls (2 ± 0.3 × 10⁵; P < .01). Together, these data indicate that the affected lung during active PTB was characterized by both a lymphocytic and an immature macrophage alveolitis; unaffected lungs also showed immature macrophage alveolitis but to lesser degree.

Phenotypic analysis of AL and blood lymphocytes in PTB patients and healthy controls. T lymphocytes (CD3⁺) were the predominant lymphocyte population in AL from control
Figure 1. Bronchoalveolar cells (BAC) from unaffected and affected lungs in PTB. Cytospin preparations of BAC (original magnification, ×160). Wright’s stain of BAC from unaffected (A) and affected (B) lung of PTB patient shows increase in alveolar lymphocytes in affected lung (arrow); occasional neutrophil also can be seen.
Figure 1, Continued. Peroxidase stain of BAC from unaffected (C) and affected (D) lung of PTB patient.
Table 1. Characterization of alveolar lymphocytes (AL) in PTB.

<table>
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<tr>
<th>Marker*</th>
<th>Control lung</th>
<th>Unaffected lung</th>
<th>Affected lung</th>
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<tbody>
<tr>
<td>CD3</td>
<td>82 ± 3</td>
<td>91 ± 1</td>
<td>93 ± 1</td>
</tr>
<tr>
<td>CD19</td>
<td>15 ± 3</td>
<td>5 ± 1*</td>
<td>3 ± 1*</td>
</tr>
<tr>
<td>CD56*, CD16*</td>
<td>4 ± 1</td>
<td>3 ± 1</td>
<td>4 ± 1</td>
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<tr>
<td>CD4</td>
<td>50 ± 5</td>
<td>54 ± 4</td>
<td>59 ± 9</td>
</tr>
<tr>
<td>CD8</td>
<td>35 ± 5</td>
<td>37 ± 5</td>
<td>30 ± 10</td>
</tr>
<tr>
<td>αβ-TCR</td>
<td>80 ± 3</td>
<td>80 ± 3</td>
<td>86 ± 6</td>
</tr>
<tr>
<td>γδ-TCR</td>
<td>7 ± 1</td>
<td>5 ± 1</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>CD45RO</td>
<td>83 ± 3</td>
<td>84 ± 5</td>
<td>87 ± 5</td>
</tr>
<tr>
<td>CD69</td>
<td>80 ± 6</td>
<td>91 ± 5*</td>
<td>86 ± 8†</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>68 ± 14</td>
<td>83 ± 9†</td>
<td>86 ± 11†</td>
</tr>
<tr>
<td>Transferrin-R</td>
<td>7 ± 3</td>
<td>13 ± 4*</td>
<td>9 ± 5</td>
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<tr>
<td>IL-2Ra</td>
<td>16 ± 13</td>
<td>4 ± 2*</td>
<td>10 ± 13</td>
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NOTE. TCR, T cell receptor; R, receptor; IL, interleukin.
* Anti-CD19 and its isotypic control (IgG1) were used without anti-CD3 as second antibody. All other markers are given as % of CD3+ cells.
Significant vs. control AL: †P < .05; ‡P = .056.

and from unaffected and affected lungs of PTB patients (table 1). Although there was a trend toward an increase in CD3+ cells in PTB patients, it was not statistically significant. The percentage of B lymphocytes (CD19+) in AL from unaffected and affected lungs was 3- and 5-fold lower, respectively, than in AL from healthy controls (P < .05). NK cells (CD56+CD16+CD3−) accounted for <5% of AL from healthy controls and PTB patients.

In PBMC from controls and PTB patients, the percentages of CD3+ lymphocytes were comparable. The percentage of NK cells was 1.8-fold lower in PTB PBMC (15.4% ± 3%) than in control PBMC (28.5% ± 4%; P < .05). The proportion of B lymphocytes in PTB PBMC (11.3% ± 2%) was 2.7-fold higher than in control PBMC (4.1% ± 0.8%; P < .01). A reciprocal decrease in B lymphocytes was found in the affected and unaffected PTB lung compared with PTB PBMC (P < .001 and P < .01, respectively).

Subpopulations and markers of activation on T lymphocytes in PTB. The percentages of CD4+, CD8+, αβ− and γδ− T cell receptor (TCR)+, and CD45RO+ (memory cells) AL from affected and unaffected lungs of PTB patients were similar to those in control lungs (table 1). Proportions of CD4+ and αβ− and γδ−TCR+ lymphocytes in PBMC from PTB and healthy controls also were comparable (data not shown). The percentage of CD8+ lymphocytes in PBMC from PTB (22% ± 2.5%) was lower than that in control PBMC (32% ± 3%; P < .05). CD45RO+ T lymphocytes also were less numerous in PTB PBMC (40% ± 11%) than in control PBMC (56% ± 5%; P < .05).

Expression of CD69, an early marker appearing within hours of stimulation [9], and of HLA-DR, which is expressed on T cells within 1–2 days of stimulation [10, 11], was more frequent on affected and unaffected alveolar T cells from PTB patients than on cells from controls (table 1). CD69 and HLA-DR also were more frequently expressed on the CD4 and CD8 subsets of alveolar T cells from affected lung than on those subsets from controls in 2 patients studied (data not shown). The transferrin receptor was expressed more frequently on AL from unaffected but not affected lung than on AL from controls. Expression of IL-2Rα was significantly decreased on AL from unaffected but not affected PTB lungs. Expression of all markers of activation examined were comparable on PBMC from healthy subjects and PTB patients (data not shown).

Discussion

This study demonstrated lymphocytic and immature macrophage alveolitis in the radiographically affected lung in PTB. In 20% of subjects, there was also a neutrophilic alveolitis. The radiographically unaffected lung demonstrated immature macrophage alveolitis but no increase in AL. In both unaffected and affected lungs, however, the AL were activated, as determined by expression of CD69, and HLA-DR and most of the lymphocytes were αβ-TCR+ cells.

Detection of higher numbers of peroxidase-positive immature macrophages in PTB BAC was an unexpected and intriguing finding. Macrophages are major cellular constituents of granulomas and ultimately become epithelioid cells and multinucleated giant cells. The finding of immature macrophages in the affected PTB lung therefore suggests that such cells either are being actively recruited from the blood or are proliferating locally as they become participants in the granulomatous process. However, macrophages are generally nonreplicating cells. Furthermore, the concurrent finding of increased percentages of monocytes in the blood from PTB patients in our study as well as those of others [12, 13] supports the likelihood that immature macrophages at the site of disease in the tuberculous lung are recruited from the blood.'

Zhang et al. [6] found neutrophilic alveolitis in ~70% of their PTB patients (mean, 37% neutrophils in BAC from the total group). We also observed an increase in neutrophils in BAC in PTB but only in 20% of the patients (mean, 15% among the 3 of 15 subjects with neutrophils in BAC). The reason why few of our patients had neutrophilic alveolitis is unclear. All of our subjects, however, had reactivation PTB determined clinically. It is possible that neutrophilic alveolitis may be more frequent in progressive primary disease, in which an acute pneumatic process is more likely. We cannot exclude the possibility of preferential lysis of neutrophils during shipment of cells that were shipped before fixation, but most BAC preparations from PTB patients had rare neutrophils regardless of whether they were fixed before or after shipment.

In the affected PTB lung, AL not only were increased in number, but the cells expressed CD69 and HLA-DR more frequently than did AL from healthy subjects, indicating that the cells were activated. In the unaffected lung, the number of AL was not increased but, in addition to increased expression...
of CD69 and HLA-DR, the percentage of alveolar T cells expressing transferrin receptors also was increased. The basis for immature macrophage alveolitis and activation of T cells in unaffected lungs is unclear; however, it could relate to subradiographic disease resulting in increases in inflammatory mediators locally or systemic activation with circulating cytokines, other mediators of inflammation, or immune complexes.

Of interest, there was a decrease in alveolar T cells from unaffected tuberculous lungs expressing IL-2Ra and a trend toward a decrease in the affected lung. The finding of activated T cells in tuberculous lungs with either normal (affected) or decreased (unaffected) IL-2Ra expression might reflect an influx of unactivated lymphocytes from the blood that are activated polyclonally by inflammatory cytokines in the lung milieu before activation by specific antigens to express IL-2Ra.

In summary, the current study suggests a primary role for activated $\alpha\beta$-TCR$^+$ T cells and immature as well as mature macrophages in the host immune response to $M. tuberculosi$s in the lung. The functional consequences of these findings are under investigation.

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References


