Different Distributions of Lung and Blood Lymphocyte Subsets in Pediatric AIDS or Tuberculosis

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ABSTRACT

Pulmonary immunity has not been studied in children with acquired immunodeficiency syndrome (AIDS) or tuberculosis (TB), even though lungs of both children and adults infected with human immunodeficiency virus (HIV-1) or Mycobacterium tuberculosis are affected frequently and severely. In the present studies, the distributions of T (CD3+, CD4+, CD8+) and B (CD19+) lymphocytes in bronchoalveolar lavage fluid (BALF) and blood of children with AIDS (N = 28) and children with pulmonary TB (N = 18) were determined using direct immunofluorescence (flow microfluorimetry). The distributions of lymphocyte subsets in BALF differed dramatically from those in blood.

In pediatric AIDS, reduction of CD4/CD8 ratio was much more pronounced in BALF than in peripheral blood (0.15 ± 0.04 vs. 0.43 ± 0.11). This difference was due to selective depletion of BALF CD4+ lymphocytes, rather than to a great influx of CD8+ cells into the lung. In childhood TB, the CD4/CD8 ratio in BALF also was significantly decreased, despite its elevation in blood (1.02 ± 0.26 vs. 1.96 ± 0.32). The results show that (1) examination of peripheral blood lymphocytes does not reflect the kind and extent of changes observed in the distribution of pulmonary lymphocyte subsets, and (2) the profound decrease of the CD4/CD8 ratios in BALF of children with AIDS or TB is due to decreased percentages and absolute numbers of BALF CD4+ lymphocytes. The data suggest that analysis of BALF provides a more accurate evaluation of the patient pulmonary immune status than monitoring peripheral blood.

Introduction

Differences in the clinical courses of HIV-1 infection and pulmonary TB disease among adults and children are well documented, and frequent pulmonary involvement in pediatric AIDS has been recognized. Nevertheless, there have been only limited studies of local pulmonary immunity in pediatric AIDS and none in childhood TB.
Peripheral blood is the traditional source material for the clinical laboratory evaluation of patient immune status. Recent advances in development of the techniques of fiberoptic bronchoscopy and bronchoalveolar lavage have permitted direct sampling of lung epithelial lining fluid and lymphoid cells recovered in the bronchoalveolar lavage fluid (BALF). Studies of BALF in some diseases, such as sarcoidosis and other interstitial lung diseases, indicated that local pulmonary changes of lymphocyte subpopulations may not be accurately reflected in peripheral blood.16

In the present studies, the distributions of lymphocyte subsets in matched bronchoalveolar lavage fluid and peripheral blood, obtained from each of the 28 children with AIDS and from each of the 18 children with tuberculosis, were analyzed. The results showed that (1) examination of peripheral blood lymphocytes does not reflect the kind and extent of changes in the distribution of pulmonary lymphocyte subsets, and (2) the profoundly decreased BALF CD4/CD8 ratios in pediatric AIDS and pediatric TB are due to decreased percentages and absolute numbers of BALF CD4+ lymphocytes. These observations indicate that analysis of BALF provides a more accurate evaluation of the patient pulmonary immune status than monitoring of peripheral blood.

Materials and Methods

Patients, Bronchoalveolar Lavage Fluid, and Peripheral Blood

Specimens of BALF and peripheral blood were obtained from children with clinical AIDS and acute pulmonary disease, and from children with active pulmonary TB. Diagnostic bronchoscopy and bronchoalveolar lavage (BAL) were performed at the Children's Medical Center of Brooklyn (University Hospital or Kings County Hospital Center) after appropriate consent was obtained from a parent or guardian. Procedures were performed using an Olympus BF-type 3C4 fiberoptic bronchoscope. The patients were sedated with meperidine (two mg per kg IM), Phenergan (one mg per kg IM), and chlorpromazine (one mg per kg IM) administered one hour before the procedure. Patients were monitored with a pulse oximeter and a cardiac monitor. Lidocaine (0.1 ml of a two percent solution, topical) was used to anaesthetize the nose, hypopharynx, and larynx. Bronchoalveolar lavage was performed with a total average of 60 ml of non-bacteriostatic saline at room temperature; the average fluid return was greater than 50 percent. Blood specimens were obtained by venipuncture within two hours of the BAL procedure using ethylenediamine tetraacetic acid (EDTA) as an anti-coagulant.

Isolation and Characterization of Cells in BALF

Bronchoalveolar lavage fluid was passed through double layers of sterile gauze to remove mucus, after which cells were isolated by centrifugation (400 × g, 10 min, 4°C) and resuspended in sterile phosphate-buffered saline (PBS) at 1 × 10⁶ cells per ml. Cell recovery was 0.05 to 0.88 × 10⁶ per ml of BALF (AIDS patients) and 0.07 to 1.67 × 10⁶ per ml of BALF (TB patients), >95 percent of which were viable as determined by trypan blue dye exclusion. Leukocyte composition was determined by differential counts of hematoxylin-eosin* stained cytocentrifuge preparations.

Immunofluorescence Assay

Lymphocyte subsets in BALF and peripheral blood were evaluated using

* Diff-Quik, Scientific Products, Edison, NJ.
Distribution of Lymphocyte Subsets in Pediatrics AIDS and Pediatric Tuberculosis

<table>
<thead>
<tr>
<th>Acquired Immunodeficiency Syndrome (n = 28)</th>
<th>Tuberculosis (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of Total Lymphocytes</td>
<td>Percent of Total Lymphocytes</td>
</tr>
<tr>
<td>CD3</td>
<td>CD19</td>
</tr>
<tr>
<td>-----</td>
<td>------</td>
</tr>
<tr>
<td>BALF</td>
<td>72.7 ± 4.2</td>
</tr>
<tr>
<td>Blood</td>
<td>57.1 ± 3.6</td>
</tr>
<tr>
<td>p =</td>
<td>0.007</td>
</tr>
</tbody>
</table>

*Lymphocyte subsets were determined using direct immunofluorescence, as described in the Materials and Methods section.

BALF = bronchoalveolar lavage fluid

Statistical Analysis

The significance of the differences observed was established using the Student's t-test and variance analysis.

Results

Clinical Status

Two groups of patients were studied.

Group 1. Children with AIDS and acute lower respiratory tract disease (N = 28, mean age 3.3 ± 0.7 years). These patients were seropositive for human immunodeficiency virus (HIV-1), with clinical and radiological evidence of acute pulmonary disease, and had clinical features belonging to class P-2 of the Center for Disease Control (CDC) case definition for AIDS in children under 13 years of age. Most of the patients with AIDS had been treated with one or more therapeutic agents. The microorganisms identified most frequently in the BALF of these patients included Streptococcus viridans, considered a commensal in immunocompetent hosts, Candida albicans, and Pneumocystis carinii.

Group 2. Children with pulmonary tuberculosis and no evidence of HIV infection (N = 18, mean age 3.9 ± 0.9 years). All TB patients had positive tuberculin (Mantoux) test and had chest X-ray findings consistent with active pulmonary disease (hilar lymphadenopathy and/or pulmonary infiltrates). Most of these patients received no treatment prior to the BAL procedure, or were treated for no more than two days. Mycobacterium tuberculosis was identified by staining for acid-fast bacteria (AFB) and confirmed by culture in eight of eighteen patients studied; ten BALF specimens were AFB negative.

Lymphocyte Subsets in BALF and Blood

In children with AIDS, the distribution of CD3+, CD4+, and CD8+ T lymphocytes, and CD19+ B lymphocytes in BALF differed significantly from those in peripheral blood (table I). In children

† Becton-Dickinson, Mountain View, CA.
with pulmonary TB, CD4+ T lymphocytes and CD19+ B lymphocytes showed significant differences between BALF and peripheral blood.

Total leukocyte numbers were significantly elevated in BALF of both pediatric AIDS and pediatric TB patients (AIDS vs. adult control, p < 0.001; TB vs. adult control, p < 0.02) (table II). However, lymphocyte numbers in BALF were not increased above adult control values and accounted for a lower percentage of total cells in BALF (table III). For obvious ethical reasons, data for BALF of control, healthy pediatric population are not available.

Both CD4+ and CD8+ lymphocyte numbers were dramatically altered in BALF of children with AIDS: CD4+ cells showed a 4.3 fold decrease, while CD8+ cells showed a 4.1 fold increase (table II). In contrast, only the CD4+ lymphocyte population was altered in BALF of children with TB, showing a 1.75 fold decrease, while their BALF CD8+ lymphocyte count was very close to control value.

In peripheral blood, where control values are available for the pediatric as well as adult populations, age-dependent changes in the distributions of lymphocyte subsets become an important factor in evaluating and interpreting disease-related alterations. For example, the control values for peripheral blood CD4+ and CD8+ lymphocyte numbers are 2.4 and 2.6 fold higher in children two to five years of age than in adults. Therefore, while the blood CD4+ lymphocyte number in children with AIDS appeared close to the control adult level, it was 3.2 times lower than the pediatric control value (table II). For childhood TB, however, the blood CD4+ lymphocyte num-

| TABLE II |
|------------------|------------------|------------------|------------------|
| Bronchoalveolar Lavage Fluid | Blood |
| | WBC (x 10⁶/ml) | CD4 (x 10⁶/ml) | CD8 (x 10⁶/ml) | WBC (x 10⁶/ml) | CD4 (x 10⁶/ml) | CD8 (x 10⁶/ml) |
| AIDS (n = 28) | 420 ±40 | 1.61 ±0.40 | 13.05 ±3.33 | 6.95±0.70 | 0.57 ±0.17 | 0.96 ±0.15 |
| TB (n = 18) | 369 ±96 | 3.93 ± 1.85 | 3.64 ± 0.96 | 9.87 ±1.03 | 1.49 ±0.22 | 0.83 ±0.10 |
| Control (Adult) | 129 ±20b | 6.87 ±0.57 | 3.21 ±0.07 | 7.40c | 0.76d | 0.45 |
| Control (2-5 years) | NA | NA | NA | 8.50e | 1.80 | 1.18 |

a Total leukocyte numbers were determined by hemocytometer counts, and lymphocyte subsets were determined by direct immunofluorescence, as described in the Materials and Methods section.


ber appeared elevated 2.0 times as compared with the adult control value, but was slightly decreased (1.2 times) relative to the pediatric control value. Finally, the blood CD8+ lymphocyte numbers were similar in both patient groups and close to the pediatric control value. It is important to note that disease-related alterations in the absolute numbers of BALF lymphocyte subpopulations were not apparent in the patient peripheral blood.

When lymphocytes from BALF of one pediatric patient whose final diagnosis excluded AIDS and TB were examined, the following pattern was observed: BALF contained $320 \times 10^3$ leukocytes/ml, and had 54.3 percent CD3+ cells, 1.2 percent CD19+ cells, 27.5 percent CD4+ cells, and 24.2 percent CD8+ cells; the CD4/CD8 ratio in BALF was 1.14. This profile resembled the mean values observed in the group of children with TB and was different from the reported values for healthy adults (tables II–IV).

### CD4/CD8 Ratios in BALF and Blood

The changes in percentages and absolute numbers of various lymphocyte subpopulations described above resulted in profoundly altered CD4/CD8 ratios (table IV). Children with AIDS exhibited a 17.4 fold decrease of the CD4/CD8 ratio in BALF, and children with TB showed a 2.6 fold decrease; the corresponding CD4/CD8 ratios in blood were also different from the control pediatric and adult values. Most important, the differences between CD4/CD8 ratios in BALF and blood were highly significant in each patient group.

### Changes in Lymphocyte Subset Distributions Among Children with TB

In an attempt to correlate lymphocyte subset alterations with clinical disease, data for children with TB were examined taking into account the results of acid-fast staining. Six children with AFB-positive BALF were compared with six AFB-negative children who had clinical TB (table V). The differences between BALF and blood T lymphocyte subset distributions showed the same general tendencies in both groups, but reached statistical significance only among AFB-negative patients. Generally, CD19+ B cells represented a much lower percentage of total lymphocytes in BALF than in

### Table III

Lymphocytes in Bronchoalveolar Lavage Fluid of Children with AIDS and Children with Tuberculosis

<table>
<thead>
<tr>
<th></th>
<th>Total Number x 10^3 / ml</th>
<th>Percent of Total Cells</th>
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</thead>
<tbody>
<tr>
<td>AIDS (n = 28)</td>
<td>20.6</td>
<td>4.9 ± 1.0</td>
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<tr>
<td>TB (n = 18)</td>
<td>12.9</td>
<td>3.5 ± 0.8</td>
</tr>
<tr>
<td>Control (Adult)</td>
<td>14.9*</td>
<td>11.8 ± 1.1</td>
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</table>


### Table IV

CD4/CD8 Ratios in Pediatric Acquired Immunodeficiency Syndrome and Pediatric Tuberculosis

<table>
<thead>
<tr>
<th></th>
<th>AIDS</th>
<th>TB</th>
<th>Control (Adult)</th>
<th>Control (2-5 yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALF</td>
<td>0.15 ± 0.04</td>
<td>1.02 ± 0.26</td>
<td>2.61 ± 0.29</td>
<td>NA</td>
</tr>
<tr>
<td>Blood</td>
<td>0.43 ± 0.11</td>
<td>1.96 ± 0.32</td>
<td>1.82 ± 0.14</td>
<td>1.44b</td>
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<td>P =</td>
<td>0.020</td>
<td>0.029</td>
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</tbody>
</table>


BALF = bronchoalveolar lavage fluid

AIDS = acquired immunodeficiency syndrome
TB = tuberculosis

In an attempt to correlate lymphocyte subset alterations with clinical disease, data for children with TB were examined taking into account the results of acid-fast staining. Six children with AFB-positive BALF were compared with six AFB-negative children who had clinical TB (table V). The differences between BALF and blood T lymphocyte subset distributions showed the same general tendencies in both groups, but reached statistical significance only among AFB-negative patients. Generally, CD19+ B cells represented a much lower percentage of total lymphocytes in BALF than in
TABLE V

Comparison of Lymphocyte Subsets in Acid Fast Bacillus+ and Acid Fast Bacillus- Pediatric Tuberculosis

<table>
<thead>
<tr>
<th></th>
<th>Acid Fast Bacillus+ Tuberculosis (n = 6)</th>
<th>Acid Fast Bacillus- TB (n = 6)</th>
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<tbody>
<tr>
<td></td>
<td>Percent of Total Lymphocytes CD3 CD19 CD4 CD8</td>
<td>Percent of Total Lymphocytes CD3 CD19 CD4 CD8</td>
</tr>
<tr>
<td></td>
<td>BALF</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>55.0 ± 13.3 3.7 ± 0.9 27.5 ± 12.8 21.3 ± 5.9</td>
<td>70.1 ± 6.3 9.8 ± 2.8 23.0 ± 3.8 34.4 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>BALF</td>
<td>Blood</td>
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<td></td>
<td>BALF</td>
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<td></td>
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<td></td>
<td>BALF</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>70.1 ± 6.3 9.8 ± 2.8 23.0 ± 3.8 34.4 ± 3.9</td>
<td>60.3 ± 2.4 23.3 ± 2.7 35.1 ± 2.5 25.3 ± 1.9</td>
</tr>
<tr>
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<td>0.730 0.001 0.037 0.790</td>
<td>0.165 0.003 0.017 0.050</td>
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BALF = bronchoalveolar lavage fluid

blood; CD4⁺ lymphocytes were lower, and CD8⁺ lymphocytes were higher in BALF than in blood. As a result, the CD4/CD8 ratios were quite different in each TB patient group (table VI). The CD4/CD8 ratios in patient BALF were lower than adult control BALF value (table IV) and showed a much greater (3.6 fold) decrease among AFB-negative children with TB. In contrast, CD4/CD8 ratio in blood of the AFB-negative group was normal (for age) and clearly elevated in the AFB-positive group.

Discussion

Several new and important observations have emerged from the present studies of lymphocyte subset distributions in BALF and blood of children with AIDS and children with pulmonary TB.

First, peripheral blood distributions of T and B lymphocytes do not reflect the dramatic changes observed among lymphocyte subpopulations in BALF, strongly suggesting that BALF, rather than blood, should be tested to obtain a more accurate evaluation of pulmonary immunity in pediatric diseases affecting the lungs.

Secondly, the profound decreases of the CD4/CD8 ratios observed in BALF of children with AIDS and children with TB are primarily due to decreased percentages and absolute numbers of CD4⁺ cells in BALF. The mechanism(s) responsible for this selective change of CD4⁺ lymphocyte population in BALF is not known. One of the possibilities to be considered is that CD4⁺ cells in the circulating pool may be selectively excluded from entering the bronchoalveolar space. Selective lymphocyte circulation (homing) into the bronchoalveolar space may be accomplished by differential expression of specific surface adhesion molecules on the mucosa and/or by altered lymphocyte homing receptors, regulated by inflammatory mediators such as γ-interferon (γ-IFN), interleukin-1 (IL-1) and tumor necrosis factor (TNF), and other cytokines.26 Alternatively, these cells circulate through the lung but are destroyed before they enter the lung epithelial lining fluid sampled in the BAL procedures. Destruction of pulmonary CD4⁺ lymphocytes in pediatric AIDS might result from the cytopathic
effects of HIV-1, which is known to be present in the lungs of infected individuals.\textsuperscript{17} In childhood TB, however, other mechanisms have to be invoked to explain the lower numbers of CD4\textsuperscript+ lymphocytes in BALF. Another possible mechanism is the modulated expression of the surface marker itself.\textsuperscript{5,15}

The present studies have also demonstrated a consistent lack of lymphocytosis in BALF of both AIDS and TB patients studied. This was in sharp contrast with the frequent and significant increase of lymphocyte counts reported for the BALF of adults with TB\textsuperscript{25} or with AIDS,\textsuperscript{9} and underscores the importance of studying pediatric populations.

Our findings may be attributable to the possible age-related differences in the distribution of BALF lymphocyte subsets. Since data for lymphocyte subsets in BALF of healthy children have not previously been reported, our results could only be compared with reference data for lymphocyte subsets in BALF of healthy adults.\textsuperscript{4} The lymphocyte subset distribution observed in BALF of one pediatric patient who did not have AIDS or TB revealed a pattern similar to that seen in BALF of children with TB (see Results section). It should be remembered, however, that the patient had pulmonary pathology. Therefore, the data may reflect changes accompanying lung inflammation.

This report is the first analysis of the immunological changes in BALF of pediatric TB patients. Our results differ dramatically from the changes in BALF described for adults with TB. For example, a recent study of pulmonary immunologic abnormalities in adult patients with miliary TB reported significant increases in BALF CD4\textsuperscript+ lymphocytes and BALF CD4/CD8 ratios.\textsuperscript{25} Earlier studies of tuberculous adults reported a number of different, sometimes contradictory, findings in the lung.\textsuperscript{1,2,22,27} Two studies found elevated BALF T cells and "normal" CD4/CD8 ratios, although a subgroup of patients with nonreactive forms of tuberculosis showed a significant decrease in total BALF T cells and a decreased CD4/CD8 ratio.\textsuperscript{2,27} Another report indicated decreased CD4/CD8 ratios in BALF but normal values in peripheral blood of adult patients with localized pulmonary TB.\textsuperscript{1} However, inverted CD4/CD8 ratios were found in this study for BALF and blood of patients with disseminated TB. The distributions of lymphocyte subsets were also examined in adults with tuberculous pleuritis.\textsuperscript{3,10} While both studies reported normal CD4/CD8 ratios in peripheral blood of such patients, the CD4/CD8 ratios in pleural effusions were reported to be higher\textsuperscript{3} or lower\textsuperscript{10} than normal. Thus, the lymphocyte subset changes observed in children with pulmonary TB, presumably representing primary tuberculosis, exhibit a pattern that is clearly distinct from the lymphocyte profiles in adults.

In summary, the findings described here are significant because they demonstrate the value of directly examining the state of local pulmonary immunity by phenotypic analysis of BALF lymphocytes. The different distributions of lymphocyte subsets in BALF and blood of children with AIDS and children with pulmonary TB point to the local immunoregulatory mechanisms as important elements in host defense against infection, in addition to the systemic changes as detected in peripheral blood.

Acknowledgments

Thanks are extended to George Joseph for excellent technical assistance.

References

1. AINSLIE, G. M., BATEMAN, E. D., and SOLOMON, J. A.: Variation of T lymphocyte numbers and subsets in different forms and


