Distribution of T and B Lymphocytes in Lymphoid Tissue of Infants and Children

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Normal lymphoid tissue from children undergoing elective surgery was examined for T and B lymphocyte distribution. Although established for peripheral blood and bone marrow, T and B lymphocyte distributions have not been previously reported for lymph nodes, appendix, thymus, and spleen tissues in children. Thymus-dependent T cells were determined by the sheep erythrocyte rosette technique, and thymus-independent B cells were determined by the fluorescent labeling of surface immunoglobulins A (IgA), G (IgG), and M (IgM). Fifty percent of lymph node cells were either T or B cells; 65% of these cells were T lymphocytes, whereas 58% of B cells were of the IgM subclass. Less than half of the appendix cells were either T or B cells; 47% of these were T lymphocytes, and the remainder B lymphocytes had a subclass distribution similar to that of lymph nodes but different from peripheral blood and bone marrow where B cells bearing IgG predominate. Thymus tissue contained 43% T cells and less than 1% B cells, but the spleen was composed largely of B cells, predominantly of the IgM type. Lymphoid tissue from nine children with either inflammatory or neoplastic diseases were studied and included for contrast. This paper establishes relative distribution values for T and B lymphocytes in normal lymphoid tissue and points out the potential use of this technique to quantitate deviations from normal in certain inflammatory and neoplastic diseases.

The identification of peripheral blood lymphocyte markers has become a recognized immunological technique. The thymus-dependent (T lymphocyte) population can be identified by the spontaneous formation of rosettes with sheep erythrocytes (8, 14). The B cell or thymus-independent lymphocyte carries surface immunoglobulin that can be detected by fluorescent antibody techniques (12, 13).

Using these techniques, normal peripheral blood and bone marrow lymphocyte populations have been characterized (1, 5). Deviations from normal have been observed in leukemias and in some immunodeficiency states (2, 4, 7, 9). Recently, attention has been directed to adapting these techniques to determine markers on lymphoid tissue of human fetuses (15) and adults (7).

T and B lymphocyte markers on normal lymphoid tissue in the pediatric age group have not been studied extensively. A normal control population is necessary for evaluation of subsequent studies. We examined a variety of normal lymphoid tissue from children for T and B lymphocyte distribution. Tissue was obtained from lymph node, appendix, thymus, and spleen. Lymphoid tissue specimens from a small number of children with inflammatory and neoplastic diseases were also studied.

MATERIALS AND METHODS

Fresh lymphoid tissue was obtained from 42 children, ages 3 months to 17 years, at the time of elective surgery. In every case informed consent was obtained in compliance with the Declaration of Helsinki and the Institutional Human Investigation Committee. Tissue was placed in sterile saline, and the sample was coarsely minced with scissors. Single-cell suspensions were prepared by forcing the specimen through 120-gauge stainless steel mesh with gentle pressure. Lymphoid cells were isolated by the Ficoll-Hypaque gradient technique (6). The mononuclear cell interface was collected from the gradient, washed, and adjusted to a concentration of 10⁶ cells/ml. Wright stain smears revealed all samples to contain more than a 98% concentration of lymphocytes.

T lymphocytes were determined by the sheep erythrocyte rosette technique (8, 10). Equal volumes of lymphocyte suspension and a 0.5% suspension of washed sheep erythrocytes were combined and incubated in a shaking water bath at 37 C for 5 min. The cells were centrifuged at 200 × g for 5 min and incubated on ice for 1 h. The pellet was gently resus-
pended, and the percentage of rosettes was determined. A rosette was defined as a lymphoid cell closely surrounded by three or more sheep erythrocytes.

Fluorescein isothiocyanate-conjugated goat antihuman immunoglobulin antisera (Hyland Laboratories, Los Angeles, Calif.) were used to determine the B lymphocyte population (12). Antisera specific for human immunoglobulin G (IgG), IgA, and IgM were used separately. Heavy chain-specific antisera were used to eliminate cross-reactivity between classes. The total B lymphocyte population was derived from the sum of the three above-mentioned subpopulations. Pooled antisera of IgG, IgA, and IgM agreed within 3% of the result obtained by summing the subpopulations. The antisera were diluted to a total protein concentration of 2 mg/ml and incubated in equal aliquots with the lymphocyte suspension for 30 min at room temperature. The fluorescein isothiocyanate/protein molar ratio was between 2.5 to 3.5 to 1. The washed cell pellet was examined for immunofluorescence with a Zeiss ultraviolet microscope equipped with an Osram HBD 200 mercury arc lamp. Fluorescence was observed on the cell membrane with either a capped or diffuse pattern.

RESULTS

Lymph node samples were primarily from mesenteric lymph nodes (28), with cervical (3), parabronchial (4), axillary (2), mediastinal (2), inguinal (2), and retroperitoneal (1) nodes also included. The results are shown in Table 1. The lymph node samples showed higher percentages of T than B lymphocytes, with IgM the predominant B cell immunoglobulin class. There was no difference by the Student t test in the mean percentage of T and B lymphocytes between samples from male and female patients, nor was there any difference when the values were grouped by age or by anatomic site. In particular, the pattern of immunoglobulin subtypes in the mesenteric nodes did not differ from the other nodes.

Appendix samples from nine patients showed a smaller percentage of T lymphocytes than did the lymph nodes (Table 2). The B cell distribution was similar, but slightly higher, with IgM-bearing B cells predominating. The four samples of thymic tissue demonstrated only T lymphocytes, with less than 1% of B lymphocytes detectable. The spleen contained predominantly IgM B lymphocytes in the one patient studied.

Eighteen lymph node samples were also obtained from nine patients with inflammatory or neoplastic disease (Table 3). As a group, these samples showed decreased percentages of T lymphocytes and increased percentages of B lymphocytes. The B lymphocytes were primarily IgM-bearing cells. Five mesenteric lymph node samples from two teen-age patients with inflammatory bowel disease did not differ from normal lymph node samples in their distribution of T and B lymphocyte markers. Two patients with acute myelogenous leukemia showed a considerable decrease in both T and B lymphocytes, whereas a patient with histoplasmosis showed only a decrease in the percentages of B cell markers. Involved lymph nodes

### Table 1. Distribution of T and B lymphocytes on normal lymph node and peripheral blood samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>T cells (%)</th>
<th>B cell immunoglobulin subclasses</th>
<th>Total B cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgG</td>
<td>IgA</td>
</tr>
<tr>
<td>Lymph node</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients (42)</td>
<td>32.4 ± 2.7</td>
<td>5.0 ± 0.6</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>Male (30)</td>
<td>33.2 ± 3.2</td>
<td>4.9 ± 0.7</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>Female (12)</td>
<td>30.3 ± 5.5</td>
<td>5.3 ± 1.7</td>
<td>3.3 ± 0.9</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult (51)</td>
<td>46.3 ± 1.8</td>
<td>16.1 ± 0.9</td>
<td>5.2 ± 0.4</td>
</tr>
<tr>
<td>Children (26)</td>
<td>44.0 ± 4.2</td>
<td>14.9 ± 3.1</td>
<td>3.3 ± 2.3</td>
</tr>
</tbody>
</table>

a Results are expressed as mean ± standard error of the mean.

### Table 2. Distribution of T and B lymphocytes on normal appendix, thymus, and spleen

<table>
<thead>
<tr>
<th>Sample (no.)</th>
<th>T cells (%)</th>
<th>B cell immunoglobulin subclasses</th>
<th>Total B cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgG</td>
<td>IgA</td>
</tr>
<tr>
<td>Appendix (9)</td>
<td>19.4 ± 3.5a</td>
<td>6.0 ± 2.7</td>
<td>2.1 ± 0.9</td>
</tr>
<tr>
<td>Thymus (4)</td>
<td>42.4b</td>
<td>0.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Spleen (1)</td>
<td>16</td>
<td>15</td>
<td>6</td>
</tr>
</tbody>
</table>

a Mean ± standard error of the mean.
b Mean.
from a 12-year-old female with Hodgkin's disease showed a decreased percentage of T lymphocytes and greatly increased percentages of B lymphocytes. Two uninvolved nodes from the same patient showed normal T and B lymphocyte populations.

**DISCUSSION**

The techniques for T and B lymphocyte identification have proven to be valuable immunological tools. Determination of the normal T and B lymphocyte distribution in peripheral blood and bone marrow (1, 5) was followed by the application of these techniques to the lymphocyte in immune deficiency disorders (2). Thus, it became possible to distinguish between an absent or diminished lymphocyte population and altered distribution as factors in immune dysfunction. These techniques were also applied to the study of the lymphocytic cell population in leukemia. Chronic lymphocytic leukemia was recognized as primarily a B cell disorder (7), whereas acute lymphoblastic leukemia of childhood presented as either an unmarked or T cell disorder (4, 9) and only rarely as a B cell disorder (14).

More recently, lymphoid tissue has been examined for relative distribution of T and B lymphocytes. Early studies investigated primarily pathological tissue. Aisenberg and Long studied lymphocyte surface characteristics in a group of adult patients with malignant lymphomas and found differences in both T and B lymphocyte distribution between poorly and well differentiated lymphomas (3). Lymphoid tissue markers from adults with Hodgkin's disease did not differ greatly from normal, but non-Hodgkin's lymphoma tissue had predominantly B cell lymphoid cells. Mavligit et al. examined gut-associated lymphoid tissue in two adult patients in an attempt to define the relationship between this tissue and the human bursal equivalent, but T cells in addition to B cells were found in substantial numbers (11).

Our study examined normal lymphoid tissues defining a control population in a group of children from 3 months to 17 years of age. T lymphocytes predominated in lymph nodes, whereas IgM was the most frequent B lymphocyte subclass observed. In the appendix the lymphocyte population was approximately one half the lymph node value, whereas the B lymphocyte distribution was similar to that of the lymph node. The B lymphocyte distribution in the tissues contrasts to that of the peripheral blood and bone marrow, where IgG is the predominant subclass. As anticipated, the thymus samples demonstrated only T lymphocytes, with virtually no B lymphocytes detectable. There were no differences between values obtained from male and female patients, nor were age-related differences observed among these samples.

Lymphoid tissue markers from a 12-year-old female with Hodgkin's disease provide an example of the change in distribution of T and B lymphocytes with malignancy. Two involved lymph nodes, one cervical and one axillary, showed a distinct change in the relative distribution of lymphocytes, whereas two uninvolved mesenteric lymph node specimens demonstrated values well within the control values established here.

It is not possible, at this time, to calculate an absolute tissue lymphocyte count on the basis of tissue sampling as can be obtained from a blood specimen and, thus, extrapolate absolute T and B lymphocyte numbers. All normal classification must be made on the basis of percentage of lymphocytes bearing these surface characteristics and subsequent abnormal states defined by relative changes in distribution.

This study establishes values for relative distribution of T and B lymphocytes from a control pediatric population and provides the first step
in the evaluation of potential changes in the tissue lymphocyte subpopulations in inflammatory and neoplastic disorders. A continuing study of these cells in disease states may then provide further clues to the etiology and pathophysiology of a variety of conditions.

ACKNOWLEDGMENTS

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LITERATURE CITED


