Cytochemical reactions of normal and neoplastic lymphocytes

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SUMMARY Rosetting and non-rosetting lymphocytes collected from normal individuals were stained for the presence of beta-glucuronidase, periodic-acid Schiff activity, gamma glutamyl transpeptidase, acid phosphatase, and alpha-naphthyl butyrate esterase. Lymphocytes which formed rosettes with sheep erythrocytes and non-rosette forming lymphocytes contained cytochemical reaction products for all five stains. Beta-glucuronidase (p < 0.02) and acid phosphatase (p < 0.01) were more frequently found in rosette forming lymphocytes. However, non-rosetting cells were more frequently periodic-acid Schiff positive (p < 0.001). Gamma-glutamyl transpeptidase and alpha-naphthyl butyrate esterase were present equally in rosette and non-rosette forming lymphocytes. In addition, 33 non-Hodgkin’s lymphomas were studied for cell surface markers and cytochemical reactions. In 17 of 19 B cell lymphomas, there was a paucity of lymphocytes containing beta-glucuronidase. However, in three of four T cell proliferations, there were numerous lymphoid cells positive for beta-glucuronidase. The periodic-acid Schiff and acid phosphatase reactions varied greatly within B, T, and null cell lymphomas and thus were of little diagnostic value in determining the cell of origin of these neoplastic lymphoid cells.

Lymphocytes are heterogeneous and can be separated into two subpopulations by a variety of immunological techniques (Aiuti et al., 1975). No morphological differences exist which permit the distinction of normal human thymic dependent lymphocytes (T cells) from bursa dependent lymphocytes (B cells) (Catovsky et al., 1974). Even scanning electron microscopy (Polliack and De Harven, 1975) by itself cannot be relied upon to differentiate the two lymphocyte subpopulations.

Recently, cytochemical studies in a variety of lymphoproliferative disorders suggested that beta-glucuronidase was an enzyme marker for T cells (Flandrin and Brouet, 1974; Flandrin and Daniel, 1974) and periodic-acid Schiff (PAS) a marker for B cells (Astaldi and Verga, 1957; Stein et al., 1972; Catovsky et al., 1974). Some investigators (Catovsky et al., 1974; Ritter et al., 1975) suggested that acid phosphatase was a T cell marker in acute lymphocytic leukaemia. In contrast, others (Barr and Perry, 1976) have noted negative or weak acid phosphatase activity in normal human peripheral blood T lymphocytes and moderate to strong reactivity in B and null cells. In addition, other investigators (Szmigielski et al., 1965) have shown that 75% of human peripheral blood lymphocytes and 2-10% of bone marrow lymphocytes contain gamma-glutamyl transpeptidase activity. Since T cells have a similar distribution in the blood and bone marrow, it seemed possible that gamma-glutamyl transpeptidase was also a cytochemical marker for T cells.

Li et al. (1973) demonstrated that monocytes and histiocytes, but not lymphocytes, contained non-specific esterases which stained the cytoplasm diffusely when alpha-naphthyl butyrate was employed as substrate. Recently, we have noted that a small percentage of lymphocytes may demonstrate a focal deposit of alpha-naphthyl butyrate esterase.

Thus, this report describes an analysis of the beta-glucuronidase, PAS, gamma-glutamyl transpeptidase, acid phosphatase, and alpha-naphthyl butyrate esterase cytochemical reactions in lymphocytes forming rosettes with sheep erythrocytes and in those non-rosetting lymphocytes collected from the peripheral blood of normal individuals. In addition, we describe our experience in defining the cytochemical reactions in 33 cases of non-Hodgkin’s lymphoma analysed for the presence of cell surface markers.

Received for publication 8 December 1976
Material and methods

NORMAL PERIPHERAL BLOOD
Twenty millilitres of blood from 34 normal individuals were defibrinated with glass beads, and lymphocytes were separated from other blood elements on a column composed of a solution of sodium metrizoate and FicollR (Pharmacia Chemical, Uppsala, Sweden). Lymphocytes were incubated with sheep erythrocytes in 25% fetal calf serum for 60 minutes at 37°C, centrifuged at 200 g and incubated at 4°C overnight (Bach, 1973). After gentle resuspension, a portion of the sample was counted for rosetting lymphocytes (lymphocytes with three or more attached sheep erythrocytes) in a haemocytometer. The remainder of the cell suspension was spread on glass slides either directly or after cytocentrifugation and then air-dried (Hepburn and Ritts, 1974).

The accuracy of the cytocentrifuge lymphocyte rosette preparation was documented by the following control study. A comparison of percent rosetting lymphocytes was enumerated with a haemocytometer by cytocentrifugation prepared on duplicate blood samples from five normal healthy individuals and five patients with chronic lymphocytic leukaemia indicated a coefficient of correlation of 0·9282. In addition, a paired test demonstrated that the means of the two methods were not significantly different (0·7 > p > 0·6).

Ten samples from normal individuals were stained for the presence of beta-glucuronidase (Lorbacher et al., 1967), PAS (Hayhoe and Flemans, 1969), and gamma-glutamyl transpeptidase (Szmigelski et al., 1965). In addition, 12 samples were stained for acid phosphatase (Li et al., 1970) and 12 different samples were stained for alpha-naphthyl butyrate esterase (Li et al., 1973) activity.

Two hundred and non-rosetting lymphocytes were enumerated for the presence of cytochemical reaction products. A cell was considered positive when at least one distinctly stained granule was apparent in the cytoplasm. The number of positive cells in rosetting lymphocytes were compared to those in non-rosetting lymphocytes by the use of the paired t test.

PATIENTS AND CONTROLS
Lymph nodes and tissue, appropriate to establish a diagnosis, were obtained from 33 patients ultimately shown to have non-Hodgkin's lymphoma. Lymph nodes obtained from 24 patients without haematological malignancy served as controls.

PROCESSING OF TISSUE
All tissue was processed in the fresh state, and numerous touch preparations were made from a cut surface. A portion of each lymph node was fixed in either Bouin's solution or 10% buffered formalin and processed for haematoxylin and eosin stained tissue section. The remainder of the node was used as the source of the lymphocyte suspension.

PREPARATION OF LYMPHOCYTE SUSPENSION COLLECTED FROM TISSUE
Portions of tissue were gently pressed through a fine stainless steel mesh and a cell suspension was washed three times in phosphate buffered saline solution at pH 7·0. Contaminating erythrocytes were removed by washing with Tris buffered ammonium chloride solution at pH 6·2. The cell suspension was gently centrifuged, and the sedimented cells were resuspended in Hank's balanced salt solution (Grand Island Biological Co, Grand Island, NY).

Lymphocytes were tested for their ability to form rosettes with sheep erythrocytes (Bach, 1973) as previously outlined. In addition, the presence of surface membrane immunoglobulins was measured (Papamichail et al., 1971). Fluorescein labelled monovalent antisera against y, µ, α, δ, and ε immunoglobulin heavy chains; albumin; κ and λ light chains; and fluorescein labelled polyvalent antisera against y, µ, and α heavy chains were obtained from Behring Diagnostics (Department of Hoechst Pharmaceuticals Inc, Somerville, NY 08876) and Meloy Laboratories Inc (Springfield, Virginia 22151).

Results
Blood collected from 34 normal individuals indicated that the mean concentration of leucocytes was 6·5 ± 1·7 x 10³ cells/µl, the average percent of lymphocytes was 35·3 ± 11·0 and the percent of rosette forming cells was 64·2 ± 9·6.

CYTOCHEMISTRY OF ROSETTING AND NON-ROSETTING NORMAL LYMPHOCYTES
The cytochemical reactions for rosetting and non-rosette lymphocytes are listed in Table 1. Beta-glucuronidase and acid phosphatase were more frequently present in rosette than in non-rosette lymphocytes (Figs 1 and 2). In contrast, non-rosette cells were more frequently positive with the periodic-acid Schiff reagent (Fig. 3). Gamma-glutamyl transpeptidase and alpha-naphthyl butyrate esterase were present equally in rosette and non-rosette forming lymphocytes (Fig. 4).

BENIGN LYMPH NODES
The cell surface marker data from lymphocyte suspensions and the results of cytochemical studies
Cytochemical reactions of normal and neoplastic lymphocytes

Table 1  Percent of normal lymphocytes with positive cytochemical reaction

<table>
<thead>
<tr>
<th></th>
<th>Beta glucuronidase</th>
<th>Periodic-acid Schiff</th>
<th>Gamma glutamyl transpeptidase</th>
<th>Acid phosphatase</th>
<th>Alpha naphthyl butyrate esterase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RL</td>
<td>NRL</td>
<td>RL</td>
<td>NRL</td>
<td>RL</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>RL</td>
<td>75 ± 15</td>
<td>67 ± 14</td>
<td>1 ± 2</td>
<td>7 ± 3</td>
</tr>
<tr>
<td>RL v NRL*</td>
<td></td>
<td>0.01 &lt; p &lt; 0.02</td>
<td>p &lt; 0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RL—rosetting lymphocyte; NRL—non-rosetting lymphocyte.
*RL v NRL compared by paired t test.

Fig. 1  Cytocentrifuge preparation of rosette forming lymphocytes stained for the presence of beta-glucuronidase. Positive cytoplasmic granules are noted by the arrow. x 1432.

Fig. 2  Cytocentrifuge preparation of rosette forming lymphocytes stained for acid phosphatase. Two distinct acid phosphatase granules are noted in the rosetting lymphocyte while none is apparent in the non-rosetting lymphocyte. x 1432.

The average number of cells with surface membrane immunoglobulin was 21.0 ± 13. Fifty to eighty percent of lymphocytes from reactive lymph nodes demonstrated positive cytoplasmic staining with beta-glucuronidase and acid phosphatase. While most lymphocytes did not stain with PAS, 5-10% showed diffuse, finely granulated PAS positive granules. Lymphocytes were negative for alphaphosphoryl butyrate esterase. In contrast, macrophages exhibited intense cytoplasmic staining with beta-glucuronidase, acid phosphatase, and alphaphosphoryl butyrate esterase, and moderate diffuse reaction with periodic-acid Schiff reagent.

LYMPHORETICULAR MALIGNANCIES

B cell proliferations
Analysis of mononuclear surface markers on cell suspensions from neoplastic lymph nodes indicated that cases of undifferentiated lymphoma, histiocytic lymphoma, and lymphocytic lymphoma contained an increased number of cells bearing monoclonal immunoglobulin (Table 3). These lymphomas were classified as B cell proliferations.

When compared to reactive lymph nodes, neoplastic lymphoid cells observed on touch preparations from 17 of 19 cases classified as B cell malignancies showed a marked decrease in cells positive

for beta-glucuronidase. Staining for PAS indicated that most cells were unreactive; only 5-10% of the cells exhibited PAS positive granules. Acid phosphatase reactions of touch preparations demonstrated a varied pattern of positivity. Some cases were devoid of acid phosphatase-containing cells while others showed cells with only weak cytoplasmic staining. Neoplastic cells were negative for alpha-naphthylbutyrate esterase.

In contrast, the macrophages in these 19 B cell lymphomas and the remaining 14 lymphoid malignancies exhibited positive cytoplasmic staining for beta-glucuronidase, periodic-acid Schiff, acid phosphatase, and alpha-naphthyl butyrate esterase.

Table 2  Cytochemical and immunological cell markers in 24 reactive lymph nodes

<table>
<thead>
<tr>
<th></th>
<th>Percent rosette forming cells</th>
<th>Percent cells with surface membrane immunoglobulin</th>
<th>Beta glucuronidase</th>
<th>Periodic-acid Schiff</th>
<th>Acid phosphatase</th>
<th>Alpha naphthyl butyrate esterase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td>50±8±15±0</td>
<td>21±0 ± 13±0</td>
<td>50%-80% Positive</td>
<td>5%-10% Positive</td>
<td>50%-80% Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Macrophages</td>
<td></td>
<td></td>
<td>Diffuse intense stain</td>
<td>Diffusely moderate stain</td>
<td>Diffuse moderate to intense stain</td>
<td></td>
</tr>
</tbody>
</table>

T cell proliferations
One case of diffuse histiocytic and three cases of diffuse poorly differentiated lymphocytic lymphoma demonstrated increased numbers of rosette forming lymphocytes or abnormal lymphoid cells forming rosettes with sheep erythrocytes in Wright-Giemsa stained smears. In addition, there was a decrease in the number of cells containing surface membrane immunoglobulin. These four cases were identified as T cell malignancies.

In three of the four cases, 50-80% of the lymphocytes in touch preparations were positive for beta glucuronidase. Only a rare PAS positive lymphocyte was present. In two cases lymphocytes were negative.

Table 2  Cytochemical and immunological cell markers in 24 reactive lymph nodes
### Table 3  Cytotoxicological and immunological cell markers in non-Hodgkin's lymphoma

<table>
<thead>
<tr>
<th>Case</th>
<th>Histopathological diagnosis</th>
<th>Percent rosette-forming cells</th>
<th>Percent cells with surface membrane immunoglobulin</th>
<th>Beta glucuronidase</th>
<th>Periodic-acid Schiff</th>
<th>Acid phosphatase</th>
<th>Alpha-naphthyl butyrate esterase</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B cell proliferations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>DHL</td>
<td>0</td>
<td>89-0 IgGk</td>
<td>Negative</td>
<td>10-15% Positive</td>
<td>&gt;50% Weakly positive</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>DHL</td>
<td>1-4</td>
<td>96-0 IgMk</td>
<td>Negative</td>
<td>10% Positive</td>
<td>&gt;50% Weakly positive</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
<td>DHL</td>
<td>0-1</td>
<td>94-5 IgMk</td>
<td>Negative</td>
<td>Rare positive</td>
<td>&gt;50% Weakly positive</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>DHL</td>
<td>5-5</td>
<td>82-0 IgMk</td>
<td>ND</td>
<td>Rare positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>DHL</td>
<td>6-0</td>
<td>84-0 IgM</td>
<td>&gt;50% Positive</td>
<td>Rare positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>DHL</td>
<td>17-2</td>
<td>88-0 IgMk</td>
<td>Negative</td>
<td>&gt;50% Weakly positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>7</td>
<td>UL</td>
<td>1-0</td>
<td>40-0</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>8</td>
<td>UL</td>
<td>7-0</td>
<td>81-0 IgMk</td>
<td>Negative</td>
<td>Rare positive</td>
<td>Negative</td>
<td>Rare focal positive</td>
</tr>
<tr>
<td>9</td>
<td>UL</td>
<td>29-0</td>
<td>82-0 IgMk</td>
<td>Negative</td>
<td>Rare positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>10</td>
<td>NMHLL</td>
<td>33-0</td>
<td>31-0 IgMk</td>
<td>Negative</td>
<td>Rare positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>11</td>
<td>NPDLL</td>
<td>15-5</td>
<td>74-5 IgMk</td>
<td>Negative</td>
<td>15% Positive</td>
<td>&gt;50% Weakly positive</td>
<td>Negative</td>
</tr>
<tr>
<td>12</td>
<td>NPDLL</td>
<td>2-8</td>
<td>69-0 IgMk</td>
<td>Negative</td>
<td>5% Positive</td>
<td>&gt;50% Weakly positive</td>
<td>Negative</td>
</tr>
<tr>
<td>13</td>
<td>NPDLL</td>
<td>ND</td>
<td>86-0 IgMk</td>
<td>Negative</td>
<td>Rare positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>14</td>
<td>NPDLL</td>
<td>57-0</td>
<td>20-0 IgAk</td>
<td>Negative</td>
<td>Rare positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>15</td>
<td>NPDLL</td>
<td>39-0</td>
<td>34-0 IgGk</td>
<td>Negative</td>
<td>Rare positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>16</td>
<td>NPDLL</td>
<td>35-0</td>
<td>31-0 IgMk</td>
<td>Negative</td>
<td>Rare positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>17</td>
<td>NPDLL</td>
<td>46-0</td>
<td>51-0 IgMk</td>
<td>5% Positive</td>
<td>ND</td>
<td>&gt;50% Weakly positive</td>
<td>Negative</td>
</tr>
<tr>
<td>18</td>
<td>DPDLL</td>
<td>5-5</td>
<td>88-0 IgMk</td>
<td>Negative</td>
<td>5% Positive</td>
<td>&gt;50% Weakly positive</td>
<td>Negative</td>
</tr>
<tr>
<td>19</td>
<td>DWDLL</td>
<td>28-0</td>
<td>57-0 IgAk</td>
<td>50% Positive</td>
<td>Positive</td>
<td>&gt;50% Weakly positive</td>
<td>Negative</td>
</tr>
<tr>
<td><strong>T cell proliferations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>DHL</td>
<td>79-5</td>
<td>5-0</td>
<td>50% Positive</td>
<td>Rare positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>21</td>
<td>DPDLL</td>
<td>62-0</td>
<td>0</td>
<td>Rare positive</td>
<td>&gt;50% Positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>22</td>
<td>DPDLL</td>
<td>75-0</td>
<td>4-0</td>
<td>Rare positive</td>
<td>&gt;50% Positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>23</td>
<td>DPDLL</td>
<td>42-5</td>
<td>9-0</td>
<td>85% Positive</td>
<td>Negative</td>
<td>&gt;50% Weakly positive</td>
<td>Negative</td>
</tr>
<tr>
<td><strong>Null cell proliferations</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>DHL</td>
<td>33-0</td>
<td>7-0</td>
<td>Rare positive</td>
<td>ND</td>
<td>&gt;50% Weakly positive</td>
<td>Negative</td>
</tr>
<tr>
<td>25</td>
<td>DMHLL</td>
<td>2-7</td>
<td>8-0</td>
<td>ND</td>
<td>Rare positive</td>
<td>&gt;50% Weakly positive</td>
<td>Negative</td>
</tr>
<tr>
<td>26</td>
<td>NMHLL</td>
<td>48-5</td>
<td>5-0</td>
<td>Rare positive</td>
<td>Rare positive</td>
<td>30% Moderate positive</td>
<td>Negative</td>
</tr>
<tr>
<td>27</td>
<td>NPDLL</td>
<td>57-0</td>
<td>8-0</td>
<td>Rare positive</td>
<td>Rare positive</td>
<td>30% Moderate positive</td>
<td>Negative</td>
</tr>
<tr>
<td>28</td>
<td>DPDLL</td>
<td>5-0</td>
<td>0</td>
<td>Rare positive</td>
<td>Rare positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td><strong>Lineage of proliferating cell uncertain</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>29</td>
<td>NMHLL</td>
<td>40-0</td>
<td>15-0 P</td>
<td>30% Positive</td>
<td>Rare positive</td>
<td>&gt;50% Weakly positive</td>
<td>Negative</td>
</tr>
<tr>
<td>30</td>
<td>NMHLL</td>
<td>38-0</td>
<td>15-0 P</td>
<td>Negative</td>
<td>Rare positive</td>
<td>&gt;50% Weakly positive</td>
<td>Negative</td>
</tr>
<tr>
<td>31</td>
<td>Immunoblastic sarcoma</td>
<td>73-0</td>
<td>13-5 P</td>
<td>15% Positive</td>
<td>Rare positive</td>
<td>&gt;50% Weakly positive</td>
<td>Negative</td>
</tr>
<tr>
<td>32</td>
<td>NPDLL</td>
<td>15-1</td>
<td>47-0 P</td>
<td>ND</td>
<td>Rare positive</td>
<td>&gt;50% Weakly positive</td>
<td>Negative</td>
</tr>
<tr>
<td>33</td>
<td>DWDLL</td>
<td>38-5</td>
<td>21-0 P</td>
<td>90% Positive</td>
<td>Rare positive</td>
<td>&gt;50% Weakly positive</td>
<td>Negative</td>
</tr>
</tbody>
</table>

DHL = diffuse histiocytic lymphoma; UL = undifferentiated lymphoma; NMHLL = nodular mixed histiocytic lymphocytic lymphoma; NPDLL = nodular poorly differentiated lymphocytic lymphoma; DPDLL = diffuse poorly differentiated lymphocytic lymphoma; DWDLL = diffuse well differentiated lymphocytic lymphoma; ND = not done.
for acid phosphatase, and two cases showed 30-70% of lymphocytes positive for acid phosphatase. Alpha-naphthyl butyrate esterase was negative in the proliferating lymphoid cells.

**Null cell proliferations**

Five cases were listed as null cell lymphomas. These neoplastic cells had either a marked decrease in all surface markers or the abnormal lymphoid cells were not observed to rosette with sheep erythrocytes on Wright-Giemsa smears concurrent with a marked decrease in cells with membrane immunoglobulin.

The cytochemical reactions in touch preparations from this group of lymphomas showed that the neoplastic lymphocytes were rarely positive for beta-glucuronidase and PAS, occasionally reactive with acid phosphatase, and negative for alpha-naphthyl butyrate esterase.

**Neoplasms of uncertain lineage**

Five cases are listed as lymphoma of uncertain lineage. In these lymphoreticular malignancies the distribution of cell surface markers was not significantly different from those observed in cells from non-neoplastic lymph nodes.

The reaction product in lymphoid cells for beta-glucuronidase and acid phosphatase varied greatly among the five cases. Lymphoid cells appeared negative for PAS reagent and alpha-naphthyl butyrate esterase.

**Discussion**

The concentration of leucocytes, percent of lymphocytes (Davidsohn and Henry, 1974), and percent of rosetting lymphocytes (Bach, 1973) in this study are similar to other normal published values.

Beta-glucuronidase, a lysosomal enzyme, has been demonstrated in granulocytes, erythroid cells, platelets, macrophages, and lymphocytes (Lorbacher et al., 1967). Tamaoki and Essner (1969) found beta-glucuronidase localisation in the diffuse lymphatic tissue of the human lymph node cortex and around the central arteriole of the splenic white pulp.

Flandrin and Daniel (1974) found that beta-glucuronidase activity was low in lymphocytes from patients with chronic lymphocytic leukaemia (a B-cell proliferation) and had increased activity in lymphocytes from patients with Sézary syndrome (a T-cell proliferation). In contrast, Brouet et al. (1976) could not substantiate an increase of beta-glucuronidase in cells from T-derived acute lymphocytic leukaemia. However, Barr and Perry (1976), using normal T-cell enriched populations, noted large blocks of cytoplasmic stain with beta-glucuronidase in more than 90% of T cells. The B cell fraction showed a variable pattern of multiple smaller positive reactions ranging from 10 to 76% of cells. Data presented in this current study demonstrated beta-glucuronidase in both rosette and non-rosette forming normal lymphocytes and confirmed the observation that beta-glucuronidase is more frequently present in T cells. In addition, there was a marked decrease in the number of beta-glucuronidase positive cells in 17 of 19 B cell lymphoid malignancies and a preservation of beta-glucuronidase positive cells in three of four T cell tumours.

In normal lymphocytes a positive PAS reaction was noted more often in non-rosette forming lymphocytes than in rosette forming lymphocytes. However, when studying malignant lymphoid cells the PAS reaction was not sufficiently sensitive reliably to differentiate B from T cell neoplasms. Data from other investigators portrayed no uniform pattern. While some described a correlation between PAS reactivity and B cell lymphomas and leukaemias (Stein et al., 1972; Catovsky et al., 1974), others have noted a positive PAS reaction in Sézary cells, a T cell proliferation (Taswell and Winkelmann, 1961; Flandrin and Brouet, 1974). In addition, intense PAS reactivity was observed in phytohaemagglutinin stimulated lymphocytes, suggesting the presence of PAS positive material in T cell blasts (Quaglino et al., 1962; Stathopoulos et al., 1974).

Szmigielski et al. (1965) demonstrated that gamma-glutamyl transpeptidase is present in 75% of peripheral blood lymphocytes, a small percent of bone marrow lymphocytes, 60% of plasma cells, granulocytic cells, and normoblasts. Data reported in this study indicate that gamma-glutamyl transpeptidase is present equally in normal rosette forming and non-rosette forming lymphocytes. Since normal B cells could not be differentiated from normal T cells using the cytochemical reaction for gamma-glutamyl transpeptidase, it was elected not to study its presence in the malignant lymphoproliferative disorders.

In normal lymphocytes acid phosphatase was noted more frequently in rosetting lymphocytes than in non-rosetting lymphocytes. This finding is in contrast to that of Barr and Perry (1976), who demonstrated negative or weak acid phosphatase reaction in normal T cells and moderate to strong staining with normal B and null cells. The discordance between the two observations regarding acid phosphatase activity in normal lymphocytes cannot as yet be resolved. In the current study, the presence of acid phosphatase in lymphocytes collected from lymphoid tumours varied greatly and was interpreted not to be a reliable stain in differentiating B from T cell malignancies. Other reports indicated that lymphocyte acid phosphatase activity
Cytochemical reactions of normal and neoplastic lymphocytes

in a variety of pathological conditions showed inconstant findings. Increased acid phosphatase activity has been observed presumably in T cells of acute lymphocytic leukaemias (Catovsky et al., 1974; Brouet et al., 1976) and in the atypical lymphocytes of infectious mononucleosis (Li et al., 1970). Increased activity has also been demonstrated in cells of certain B cell proliferations, including macro-globulinaemia and multiple myelomas (Li et al., 1970). However, decreased acid phosphatase levels have been measured in chronic lymphocytic leukaemia (Douglas et al., 1973).

Alpha-naphthyl butyrate esterase was noted focally in only a small percentage of normal lymphocytes and was not observed to be present in significantly different levels in the two lymphocyte subpopulations. The great utility of this cytochemical reaction was in its ability to differentiate lymphocytes from macrophages (Li et al., 1973). Macrophages demonstrated intense diffuse cytoplasmic staining whereas only a small number of lymphocytes exhibited a focal small granular deposit. The lack of alpha-naphthyl butyrate esterase in the neoplastic cells collected from 33 cases of non-Hodgkin's lymphoma suggested that the proliferating cells were derived from lymphocytes and not from macrophages.

In summary, each cytochemical reaction was observed in rosette forming and non-rosette forming lymphocytes collected from normal individuals. However, beta-glucuronidase and acid phosphatase granules were present more frequently in rosette forming than in non-rosette forming normal lymphocytes. In contrast, a positive PAS reaction was more often observed in non-rosette forming lymphocytes. Gamma-glutamyl transpeptidase and alpha-naphthyl butyrate esterase were present equally in rosette and non-rosette forming lymphocytes. In neoplastic disorders, the beta-glucuronidase activity was decreased in 17 of 19 B cell proliferations and present in a normal number of lymphocytes from three of four T cell tumours. The PAS and acid phosphatase reactions varied greatly and appeared not to be reliable markers in determining the lineage of neoplastic lymphoid cells.

References


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