Advances in cellular immunology in the past decade have resulted in the delineation of lymphocyte subpopulations with distinctive surface membrane and functional characteristics. More recently, several investigators have also attempted to characterize lymphocytes according to the presence or absence of certain hydrolytic enzymes such as acid α-naphthyl acetate esterase, B-glucuronidase, and acid and alkaline phosphatase.

Acid α-naphthyl acetate esterase has been the most intensively studied enzyme of this group. Consequently, its role in the evaluation of both normal and neoplastic lymphoid cell populations has become reasonably well defined. Unfortunately, this has not been the case for certain other of these enzymes, such as B-glucuronidase. Methodological differences and the failure to correlate cytochemical markers with the conventional immunologic markers has resulted in conflicting data and conclusions. Therefore, those cell populations expressing B-glucuronidase activity remain poorly characterized and the role of cytochemically demonstrable B-glucuronidase activity in the evaluation of benign and malignant lymphoid cell populations remains ill-defined.

This study concerned itself with the identification of normal and neoplastic human lymphoid cell populations that possess cytochemically demonstrable B-glucuronidase activity. Various human lymphoid cell populations were simultaneously analyzed for the expression of surface membrane immunoglobulin (SIg) and the ability to form nonimmune sheep erythrocyte (E) rosettes, the standard markers for B and T cells, respectively, Ia antigens, which are present on B cells and certain stem cells, and acid α-naphthyl acetate esterase activity, a marker of post-thymic T cells. The results of these studies were correlated with the expression of B-glucuronidase activity by the various lymphoid populations.

Materials and Methods

Mononuclear Cell Isolation

Representative portions of lymph node tissue were obtained freshly from the surgical biopsy specimens of patients undergoing diagnostic evaluation for malignant lymphoma. These biopsy specimens were classified according to conventional histologic criteria as benign, reactive lymphoid hyperplasia, or non-Hodgkin’s malignant lymphoma. The latter were classified according to Rappaport, and Lukes and Collins. Representative portions of thymus, tonsil, and spleen were obtained freshly from surgical specimens removed during the course of standard operative procedures. Cell suspensions were prepared by teasing apart the tissue in RPMI 1640 until the cells were separated from the connective tissue stroma. Cell suspensions with a viability less than 70%, as determined by trypan blue exclusion, were discarded. Samples of heparinized venous blood were collected from normal laboratory personnel. Samples of heparinized venous blood and aspirated bone marrow were collected from selected patients with lymphocytic leukemia at the time of diagnosis or during the course of therapy. The mononuclear cells from each tissue, peripheral blood, and bone marrow specimen were isolated by ficoll hypaque density gradient centrifugation. In each case the...
viability was found to be greater than 95% by trypan blue exclusion.

Surface Immunofluorescence

Rhodamine or fluorescein conjugated rabbit anti-human immunoglobulin F(ab')2, antibody fragments monospecific for α, β, γ, α, κ and λ determinants were prepared as previously described. A heteroantiserum directed against human Ia-like antigens (referred to here as Ia for simplicity), which are preferentially expressed on human B lymphocytes, certain stem cells and a small percentage of T lymphocytes, was prepared by immunization of a rabbit with Ia alloantigen molecules isolated from human B lymphoblastoid cell lines. Surface immunoglobulin and Ia antigens were demonstrated by direct immunofluorescence. Cytophilic uptake of IgG as a cause of nonspecific immunofluorescent staining was avoided by incubating the cells at 37°C prior to immunofluorescent staining.

E Rosette Assay and Cell Fractionation

Spontaneous rosette formation between isolated lymphoid cells and unsensitized sheep erythrocytes was assayed according to Hoffman and Kunkel using Vibrio cholerae neuraminidase treated sheep erythrocytes at 4°C. Isolated mononuclear cells were fractionated on ficoll-hypaque density gradients according to their capacity to form E rosettes.

Cell Smears

Smears were prepared by spinning 0.025 ml of each mononuclear cell suspension (2.5 x 10⁶ cells/ml) onto glass microscope slides by cytocentrifugation at 500 rpm for 5 min. (Cytospin, Shandon-Southern)

Acid α-Naphthyl Acetate Esterase (ANAE)

Cytocentrifuge smears were fixed immediately in cold buffered formol acetone, pH6.6, at 4°C for 10 min and then washed with distilled water. T lymphocyte and monocyte acid α-naphthyl acetate esterase activity was demonstrated cytochemically using α-naphthyl acetate as substrate coupled to hexazonium pararosaniline.

B-Glucuronidase (BG)

Cytocentrifuge smears were fixed immediately in a mixture of 10% formalin and methanol (ratio 7:3) for 3 min. Washed in distilled water at room temperature and air dried for 30 min. They were then placed at −20°C for 1 hr overnight, and finally stored at 4°C for from 1 to 3 days prior to the actual staining. The incubation mixture was prepared by adding 2.4 ml of hexazonium pararosaniline to 20 ml of 0.2 M acetate buffer, pH 5.0, adjusting the pH to 5.2 and adding 20 ml of substrate solution. Hexazonium pararosaniline was prepared by combining equal volumes of two solutions: (A) freshly prepared 4% sodium nitrite in distilled water and (B) 1 g pararosaniline dissolved in 20 ml distilled water and 5 ml 12 N HCl. The substrate solution was prepared by adding 0.028 g Naphthol AS-BI-B-d-glucuronide dissolved in 1.2 ml 0.05 M NaHCO₃ to 100 ml of 0.2 M acetate buffer, pH 5.0. The incubation mixture was filtered and the slides incubated at 37°C for 2 hr, following which they were washed in distilled water at room temperature for 15 min. The slides were counterstained with 1% methyl green in acetate buffer, pH 4.2, for 1 hr, rinsed with distilled water, dehydrated in increasing concentrations of ethanol, cleared in xylene, and mounted with permount.

Microscopic Examination of Slides

The immunofluorescent slide preparations were examined by a Leitz Dialux microscope equipped with alternating phase optics, incident fluorescent illumination and a filter system appropriate for fluorochrome-stained preparations. The ANAE and BG slide preparations were examined by conventional light microscopy using an American Optical microscope equipped with a high resolution oil immersion objective.

In each case, between 200 and 400 cells were counted, depending upon the number of positive cells encountered, and the number of positive cells was indicated as a percentage of the total cells counted. These determinations were performed in duplicate. The reported value represents the mean value of these two determinations. With respect to the cytochemical staining for ANAE and BG activity, attention was paid to the percentage of positive cells, the staining patterns, and the cytomorphologic features of the positive and negative cell populations in each mononuclear cell suspension.

One of us (G.A.M.) performed and interpreted the B-glucuronidase staining without prior knowledge of the results of the cell marker analysis, which was performed by the other authors (J.P.H., D.M.K.). Thus, the percent BG+ cells was determined independently of the results of cell marker analysis.

RESULTS

Peripheral Blood

B-glucuronidase activity was demonstrable in peripheral blood lymphocytes, monocytes, and polymorphonuclear leukocytes. Both monocytes and neutrophils display weak, diffuse, agranular cytoplasmic red reaction product while the vast majority of BG+ lymphocytes display a granular cytoplasmic red reaction product. The lymphocyte BG reaction product ranges from a small solitary nodule adjacent to the cell membrane at one pole of the cell to multiple well defined, large red cytoplasmic granules (Fig. 1). Occasional BG+ lymphocytes display weaker, agranular reaction product as well. The distinctive BG staining patterns combined with the ill-defined cell borders and abundant cytoplasm of the monocytes and neutrophils allow ready distinction between the latter cells and BG+ lymphocytes in most instances.

The percentage of monocytes, as determined by BG activity, ranged between 15% and 30% in whole mononuclear cell suspensions, comparable to that determined by the presence of diffuse cytoplasmic acid α-naphthyl acetate esterase activity. The percentage of BG+ lymphocytes was comparable to the percentage of E rosette forming lymphocytes in 14 of 16 peripheral blood specimens tested (Table 1). The percentage of E+ cells exceeded the percentage of BG+ cells by greater than 10% in only 2 of these 16 patients. In no instance did the percentage of BG+ cells exceed the percentage of E+ cells by greater than 10%. The mean percentage of E+ and BG+ cells was 72% and 68%, respectively, in these 16 patients.

The percentages of E+ and BG+ cells were compar-
Table 2. Comparative Percentages of Normal Human Tissue Lymphocytes that Form Sheep Erythrocyte (E) Rosettes and Express B-Glucuronidase (BG) Activity

<table>
<thead>
<tr>
<th>Cell Source</th>
<th>E</th>
<th>BG</th>
<th>Cell Source</th>
<th>E</th>
<th>BG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph Node</td>
<td>54</td>
<td>50</td>
<td>Tonsil</td>
<td>60</td>
<td>50</td>
</tr>
<tr>
<td></td>
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<td>51</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>66</td>
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<td>Spleen</td>
<td>44</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>55</td>
<td></td>
<td>62</td>
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<td></td>
<td>37</td>
<td>87</td>
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</tbody>
</table>

Lymph Node

The percentage of BG⁺ lymphocytes was comparable to the percentage of E rosette forming cells in 9 of the 12 benign, reactive and non-neoplastic lymph nodes examined (Table 2). In 2 lymph nodes the percentage of E rosette forming cells exceeded the percentage of BG⁺ cells by 20%. Fractionation of the mononuclear cells isolated from 3 lymph nodes into E rosette enriched and E rosette depleted fractions (Table 3) clearly demonstrated that the BG⁺ lymphocytes reside within the E rosette forming (T) cell population. E rosette depletion resulted in a corresponding depletion of BG⁺ cells in each of these 3 lymph nodes (Fig. 2).

A marked divergence between the percent E⁺ and percent BG⁺ cells was seen in one lymph node. The latter lymph node consisted of 37% E⁺ and 87% BG⁺ lymphocytes. E rosette separation (Table 3) demonstrated that a large B cell subpopulation, as well as the

Table 3. Comparative Percentages of Normal Human Lymphocytes in Unseparated, E Rosette Enriched (T Cell) and E Rosette Depleted (Non-T Cell) Fractions that Form E Rosettes and Express B-Glucuronidase (BG) Activity

<table>
<thead>
<tr>
<th>Cell Source</th>
<th>Unseparated E</th>
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<th>E Enriched E</th>
<th>BG</th>
<th>E Depleted E</th>
<th>BG</th>
</tr>
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<td></td>
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<td>93</td>
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</tr>
<tr>
<td>Lymph node</td>
<td>54</td>
<td>57</td>
<td>90</td>
<td>85</td>
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<td>3</td>
</tr>
<tr>
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<td>37</td>
<td>91</td>
<td>70</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>87</td>
<td>90</td>
<td>92</td>
<td>4</td>
<td>81</td>
</tr>
<tr>
<td>Tonsil</td>
<td>—</td>
<td>—</td>
<td>90</td>
<td>94</td>
<td>4</td>
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<td>—</td>
<td>—</td>
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<td>53</td>
<td>—</td>
<td>—</td>
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<td>2</td>
</tr>
<tr>
<td>Spleen</td>
<td>58</td>
<td>57</td>
<td>85</td>
<td>87</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>
T lymphocytes, isolated from this lymph node were BG⁻.

**Tonsil, Spleen**

The percentage of E⁺ and BG⁺ lymphocytes was comparable in the 7 specimens examined. Fractionation of the mononuclear cell suspension into E rosette enriched and E rosette depleted fractions (Table 3) similarly enriched and depleted the BG⁻ lymphocytes.

**Thymus**

A mononuclear cell suspension was prepared from a portion of each of 5 thymuses. Each thymus largely consisted of E rosette forming (T) lymphocytes and contained less than 5% Slg⁺ (B) cells. In 4 of the 5 thymuses a sizable proportion of the thymocytes, 61%–86%, were BG⁺ while only 5% of the thymocytes were BG⁻ in 1 thymus (Table 2).

**Malignant Lymphomas**

The predominant cell population isolated from each tissue specimen of malignant lymphoma was considered neoplastic by cytologic criteria under phase microscopy and with the aid of cytocentrifuge smears and was identical to that viewed in the standard histopathologic sections. The cellular derivation of these 20 malignant lymphomas was assigned according to conventional definitions of lymphocyte populations. A B cell derived malignant lymphoma was defined as a neoplastic lymphoid proliferation composed of a monoclonal population of B cells bearing a single light chain class of surface membrane immunoglobulin. A T cell derived malignant lymphoma was defined as a neoplastic lymphoid proliferation in which the majority of the neoplastic cells formed E rosettes. In occasional cases the neoplastic cells did not express either marker and are referred to as non-B, non-T cell or "null cell" lymphomas.

The neoplastic cells isolated from 15 specimens obtained from 14 patients (Table 4, cases 1–14) expressed the Ia⁺Slg⁺E⁺ phenotype, i.e., the phenotype of the majority of peripheral blood and lymph nodal B cells. Analogous to normal B cells, IgM, with or without IgD, was the most commonly expressed heavy chain class; it was expressed in 12 of the 13 cases examined. In one case the neoplastic cells bore surface membrane IgG. The neoplastic B cells bore surface kappa light chains in 8 cases and surface lambda light chains in 5 cases; the surface immunoglobulin light chain class was not determined in 1 case.

Fifteen determinations of the percentage of E rosette forming cells, T pattern ANAE⁺ cells and BG⁺ cells were made on mononuclear cell suspensions from these 14 patients; the percentages were comparable in nearly every case. The percentage of E rosette-forming (T) cells ranged from 7% to 33%, mean 17.4%. The percentage of T pattern ANAE⁺ cells ranged from 1% to 28%, mean 9.8%. The percentage of BG⁺ cells ranged from 2% to 30%, mean 13.4%. In each of these 14 cases the neoplastic B cells failed to display ANAE or BG activity. In each case the ANAE⁺ and BG⁺ cells appeared cytologically to be small mature lymphocytes.

The neoplastic cells isolated from 4 malignant lymphomas (Table 4, cases 15–18) expressed the T cell phenotype, Ia⁺Slg⁺E⁻. Two patients (cases 15, 17) had cutaneous T cell lymphomas and 1 patient (case 16) had a mediastinal convoluted lymphoblastic lymphoma. The majority of the cells isolated from these 4 specimens were considered neoplastic by cyto logic criteria. However, while all of the neoplastic cells from cases 15 and 16 formed E rosettes only a subpopulation of the neoplastic cells from cases 17 and 18 formed E rosettes. The majority of the neoplastic T cells of case 15 (cutaneous T cell lymphoma) were BG⁻ while the neoplastic T cells of cases 16–18 were BG⁺. The neoplastic cells of the latter three cases were similarly ANAE⁻.

The neoplastic cells isolated from 2 patients (Table 4, cases 19, 20) were Ia⁺Slg⁻E⁻; these lesions were classified as "null cell lymphomas." The neoplastic cells in case 19 were BG⁻ while the majority of the neoplastic cells isolated from case 20 displayed weak, slightly diffused BG activity. The malignant lymphoma in the latter patient, a young woman with a mediastinal mass should be more properly labeled as lymphoblastic lymphoma, nonconvoluted type.
Table 4. Histopathologic, Immunologic, and Cytochemical Characteristics of 20 Malignant Lymphomas

<table>
<thead>
<tr>
<th>Patient</th>
<th>Rappaport</th>
<th>Lukes-Collins</th>
<th>Cell Source</th>
<th>IgM</th>
<th>Clonality</th>
<th>E</th>
<th>ANAE</th>
<th>BG</th>
<th>Cell of Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>WDL</td>
<td>SL</td>
<td>Lymph node</td>
<td>86</td>
<td>87</td>
<td>17</td>
<td>11</td>
<td>8</td>
<td>B</td>
</tr>
<tr>
<td>2</td>
<td>WDL</td>
<td>SL</td>
<td>Stomach</td>
<td>88</td>
<td>85</td>
<td>16</td>
<td>8</td>
<td>12</td>
<td>B</td>
</tr>
<tr>
<td>3</td>
<td>WDL</td>
<td>SL</td>
<td>Orbit</td>
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<td>90</td>
<td>13</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>WDL</td>
<td>SL</td>
<td>Orbit</td>
<td>94</td>
<td>92</td>
<td>7</td>
<td>11</td>
<td>12</td>
<td>B</td>
</tr>
<tr>
<td>5</td>
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<td>SL</td>
<td>Orbit</td>
<td>90</td>
<td>89</td>
<td>9</td>
<td>9</td>
<td>10</td>
<td>B</td>
</tr>
<tr>
<td>6</td>
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<td>SC</td>
<td>Lymph node</td>
<td>ND</td>
<td>60</td>
<td>20</td>
<td>16</td>
<td>21</td>
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<tr>
<td>7</td>
<td>NPDL</td>
<td>SC</td>
<td>Lymph node</td>
<td>71</td>
<td>64</td>
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<td>28</td>
<td>27</td>
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<tr>
<td>8</td>
<td>NPDL</td>
<td>SC</td>
<td>Abdominal mass</td>
<td>74</td>
<td>78</td>
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</tr>
<tr>
<td>9</td>
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<td>SC</td>
<td>Lymph node</td>
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<td>Lymph node</td>
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<td>17</td>
<td>7</td>
<td>21</td>
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<tr>
<td>13</td>
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<td>2</td>
<td>B</td>
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<td>14</td>
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<tr>
<td>15</td>
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<td>U</td>
<td>Skin</td>
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<tr>
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<td>81</td>
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</table>

Rappaport terminology: WDL, well-differentiated lymphocytic lymphoma; NPDL, nodular, poorly differentiated lymphocytic lymphoma; DPDL, diffuse, poorly differentiated lymphocytic lymphoma; DH, diffuse histiocytic lymphoma; NH, nodular histiocytic lymphoma; DM, diffuse mixed lymphocytic and histiocytic.

Lukes-Collins terminology: SL, small lymphocyte; SC, small cleaved; LC, large cleaved; LNC, large Non-Cleaved; IMBS, immunoblastic sarcoma; CL, convoluted lymphocyte; U, undefined.

Lymphocytic Leukemias

Peripheral blood, bone marrow, and lymph node specimens from 15 patients with lymphocytic leukemia were examined (Table 5). The diagnosis of chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), and the Sezary syndrome were based upon standard clinical and cytomorphologic criteria. In each case the specimen was clearly involved by the leukemic process and the predominant cell population (greater than 80%) isolated was considered neoplastic by cytomorphologic criteria.

Six patients (Table 5, cases 1–6) were classified as chronic lymphocytic leukemia. In five cases the
neoplastic cells were shown to represent a monoclonal B cell proliferation expressing characteristically low density Slg.\(^{20}\) In one instance the neoplastic cells were Ia\(^+\)Slg\(^-\), as has been reported in occasional cases of CLL.\(^{20}\) In each of these 6 cases the percentage of E\(^-\) and T pattern ANAE\(^+\) cells was reasonably comparable and these cells were considered to represent benign, residual and/or reactive T cells. In each of these 6 cases a sizable proportion, 43\%-97\% of the isolated cells, including both benign T cells and neoplastic CLL B cells, expressed cytochemically demonstrable BG activity.

Seven patients (Table 5, cases 7-13) were classified as acute lymphoblastic leukemia. Six of these 7 patients (86\%) were considered to be so-called common type or non-B, non-T-ALL as the neoplastic cells were Slg\(^+\)E\(^-\). In the four cases tested the neoplastic cells expressed Ia antigens. The non-B, non-T cell ALL blasts were BG\(^+\) in 2 cases but a variable proportion of the non-B, non-T-ALL blasts were BG\(^+\) in the 4 remaining cases. The neoplastic cells of the one patient with T cell ALL were BG\(^+\). Two patients in this series (Table 5, cases 15 and 16) were diagnosed as having the Sezary syndrome. On the day of study the vast majority of the lymphoid cells in the peripheral blood of each of these patients were Sezary cells. These cells were E\(^+\)ANAE\(^-\)BG\(^+\) (Fig. 3).

**Multiple Myeloma and Plasmacytomas**

Peripheral blood, bone marrow, and tissue specimens from 7 patients with plasma cell leukemia, multiple myeloma and soft tissue plasmacytoma were studied (data not shown). In these cases the percentage of plasma cells, as determined by the presence of cytoplasmic immunoglobulin, ranged between 25\% and 95\%. In each case a variable proportion, but not all, of the plasma cells expressed BG activity. This BG activity was primarily demonstrable as large dots of red reaction product concentrated in the Golgi zone although occasional plasma cells displayed diffuse, intense BG positivity (Fig. 4).

**DISCUSSION**

The methodology employed in the cytochemical demonstration of leukocyte B-glucuronidase (BG) activity has primarily been that worked out by Hayashi et al.,\(^{21}\) and Lorbacher et al.,\(^{22}\) utilizing naphthol AS-BI glucuronide coupled to hexazonium pararosaniline. Employing this technique, BG is cytochemically demonstrable in neutrophils, eosinophils, lymphocytes, monocytes, and plasma cells.\(^{22}\) As in the case of acid \(\alpha\)-naphthyl acetate esterase,\(^{1}\) lymphocyte BG activity is expressed as a focal granular reaction product while monocyte and myeloid BG activity is primarily diffuse and agranular.

Several investigators, prior to our present understanding of B and T lymphocyte populations, had determined that BG\(^+\) lymphocytes are preferentially localized in the paracortical region (T cell zones) of lymph nodes, and are absent from germinal centers (B cell zones).\(^{23}\) and that BG activity is decreased or absent from certain neoplastic lymphoid cells.\(^{24\text{-}26}\) Unfortunately, in the latter peripheral blood studies\(^{24\text{-}26}\) the actual percentage of BG\(^+\) cells was not calculated; BG activity was simply scored from 1-4\(^+\) or determined biochemically.

Flandrin\(^{27\text{-}29}\) extended some of these earlier studies. Flandrin and Brouet\(^{28}\) described a marked decrease in
human lymphoid BG activity

the percentage of BG⁺ cells in the peripheral blood of 52 of 66 (79%) patients with B cell CLL and that from 85%–99% of the neoplastic cells in each of 7 patients with the Sezary syndrome and 11 patients with T cell CLL (malignancies of mature, post-thymic T cells) exhibited prominent paranuclear granular BG activity.23–26 Their examination of the peripheral blood of 20 normal healthy volunteers showed that the percentage of BG⁺ lymphocytes ranged from 60%–80%, mean 70%.28 These figures closely correspond, of course, to the percentage of T cells in normal peripheral blood as determined by E rosette formation.

All of these studies strongly suggest that both normal and neoplastic B cell populations are predominantly BG⁻ while both normal and neoplastic T cells are BG⁺. However, more recent immunologic studies (reviewed by Mann30) have demonstrated the wide range of phenotypic heterogeneity displayed by the lymphoproliferative malignancies, which was not appreciated at the time of the earlier investigations of B-glucuronidase expression. Moreover, several recent reports by other investigators4,5,31,32 have presented conflicting data and conclusions concerning BG activity in human lymphoid cells. This has served to engender confusion concerning the immunologic characterization of BG⁺ lymphoid populations and the use of BG as a lymphocyte marker. Therefore, this study concerned itself with the immunologic characterization of normal and neoplastic BG⁺ cell populations, in light of recent concepts of B and T cell differentiation.

In the studies described here, lymphocyte B-glucuronidase activity was demonstrated cytochemically by a modification of the techniques originally outlined by Hayashi et al.21 and Lorbacher et al.22 utilizing naphthol AS-BI glucuronide coupled to hexazonium pararosaniline. These modifications and certain other technical aspects of the assay deserve brief comment.

In our experience, optimal results are attained with uncrowded, well fixed cytocentrifuge smears prepared from viable mononuclear cell suspensions obtained by ficoll hypaque density gradient centrifugation. This is best achieved by cytocentrifuging 0.025 ml of a dilute suspension (2–5 × 10⁶ cells/ml) for 5 min at 500 rpm and immediately fixing the slides in formol-methanol (7:3) for a period of time not exceeding 3 min; fixation for shorter or longer periods of time does not enhance either BG staining or cytomorphologic detail. BG activity may also be demonstrated in touch preparations. However, such preparations are generally suboptimal because of the presence of dead cells, proteinaceous and cellular debris, and contaminant erythrocytes.

We found, as did Lorbacher et al.,22 that freezing and thawing the fixed smears definitely enhanced BG staining. In our hands, storage of the fixed and air dried smears at −20°C for periods ranging from 1 to 16 hr and subsequent storage for from 24 to 48 hr at 4°C prior to the actual staining gave optimal results. If necessary, however, the smears can be maintained at 4°C for up to 5 days without a significant loss in BG activity. Granular lymphocyte staining appeared to be maximal when the incubation was performed at pH 5.2 at 37°C for 2 hr. The reagents should be freshly prepared except for the pararosaniline, which may be stored at 4°C in the dark for several days and the substrate stock solution which may be stored at room temperature for a few weeks.

Utilizing the above described methodology, the percentage of BG⁺ lymphocytes was found to be comparable, within 10%, to the percentage of E rosette forming lymphocytes, in the majority of normal, non-neoplastic peripheral blood, tonsil, spleen, and lymph node specimens examined. For example, the mean percentage of E⁺ and BG⁺ cells was 72% and 68%, respectively, in the peripheral blood samples obtained from 16 normal healthy volunteers and selected patients. These figures are analogous to the mean percentage of BG⁺ peripheral blood lymphocytes, 70%, as determined by Flandrin and Brouet.28

E rosette enrichment and E rosette depletion similarly enriched and depleted the BG⁺ lymphocyte population in 11 of 12 specimens examined. Thus, in the case of normal, non-neoplastic peripheral blood and lymphoid tissue, the BG⁺ lymphocyte population appears to be entirely encompassed within the E rosette forming (T) cell population in most instances. The non-E rosetting (non-T) cells consistently failed to express BG activity in these 11 specimens.

Divergence between the percentage of E⁺ and BG⁺ lymphocytes was occasionally found in non-neoplastic lymphoid tissues. The percentage of E⁺ cells exceeded the percentage of BG⁺ cells by 20% or more in 2 of 16 peripheral blood, 2 of 12 lymph node, and in 1 of 3 tonsillar specimens, suggesting the existence of an E⁺BG⁻ T cell subpopulation. A marked divergence between the percentage of E⁺ and BG⁺ lymphocytes was only seen in 1 lymph node. In that instance E rosette fractionation clearly demonstrated that a large proportion of the B cells, as well as the majority of the T cells, were BG⁺. However, the presence of BG⁻ non-neoplastic B cells was distinctly uncommon in the present study, being limited to this one lymph node. The latter lymph node was considered to be consistent with toxoplasmic lymphadenitis by histologic criteria. It is of interest in this regard that the only lymph node with a similarly high percentage of BG⁺ cells (82%)
reported by Pengalis et al. was involved by leishmaniasis, another protozoan organism.

In the studies described here 20 malignant lymphomas and 15 lymphocytic leukemias were characterized immunologically and the cellular origin of the neoplastic proliferation determined. The results of these studies were correlated with the expression of BG activity by the lymphoid cell populations. In each of 14 $\text{Ia}^{-}\text{Slg}^{-}$ (B cell) malignant lymphomas the neoplastic B cells failed to display BG activity, irregardless of the surface heavy chain class expressed or the histopathologic classification of the lymphoma. Actually, the percentage of $\text{BG}^{-}$ cells was comparable to the percentage of $\text{E}^{-}$ cells in 12 of these 14 cases. In 2 cases the percentage of $\text{E}^{-}$ cells exceeded the percentage of $\text{BG}^{-}$ cells suggesting once again the presence of an $\text{E}^{-}\text{BG}^{-}$ T cell subpopulation. In these 14 cases, the $\text{E}^{-}\text{BG}^{-}$ cells were consistently identified in cytocentrifuge smears as small, cytologically mature lymphocytes and appeared to represent residual and/or reactive T cells. These results would appear to confirm previous reports that the vast majority of B cell lymphomas are $\text{BG}^{-}$.4,5

In contradistinction to the uniform lack of BG expression by the 14 B cell lymphomas, all 6 B cell CLL's investigated in the present series showed a high proportion of $\text{BG}^{-}$ cells. The percent B cells was approximately 80% or greater in each of these 6 cases and the percent $\text{E}^{-}$ (T) cells ranged from 3%-17%. The percent $\text{BG}^{-}$ cells ranged from 43%-97% in these 6 cases, indicating that a sizable, albeit variable, proportion of CLL B cells expressed BG activity in each case. Moreover, a variable population of terminally differentiated B cells (plasma cells) also expressed activity in each of 7 cases of multiple myeloma and plasmacytoma studied.

Thus, it appears that both normal and neoplastic $\text{BG}^{+}$ and $\text{BG}^{-}$ lymphocyte populations exist. The majority of normal, mature B cells isolated from peripheral blood, spleen, tonsil, and lymph node are $\text{BG}^{-}$; non-neoplastic $\text{BG}^{-}$ cells are occasionally present in certain activated lymph nodes. Also, the vast majority of neoplastic B cells isolated from malignant lymphomas are $\text{BG}^{-}$. However, terminally differentiated B cells (plasma cells), benign and malignant, express BG activity. This may help to explain the variable expression of BG activity by B cell CLL cells. The latter are known to exist at varying stages of differentiation and occasionally display in vivo maturation to plasma cells.33

The neoplastic cells isolated from 6 patients with non-B, non-T cell acute lymphoblastic leukemia (ALL) and 2 patients with "null cell" lymphoma showed variable BG expression. In 3 cases the blasts were $\text{BG}^{-}$ while in 5 cases a variable proportion of the blasts expressed BG activity. This of course confirms an earlier finding by Brouet et al.,4,5 that some or all of the blast cells in 58% of cases of non-B, non-T cell ALL are $\text{BG}^{-}$.

The neoplastic cells isolated from 7 patients were $\text{Ia}^{-}\text{Slg}^{-} \text{E}^{-}$ and were, therefore, considered to represent T cell derived malignancies. In each of these cases only a small number, less than 5%, of Slg$^{+}$ cells were present, and these are believed to represent normal, mature B lymphocytes. BG expression by these T cell derived malignancies was variable. In 1 patient with cutaneous T cell lymphoma (Table 4, case 15) and 2 patients with the Sézary syndrome (Table 5, cases 14 and 15) the majority of the neoplastic cells expressed BG. The neoplastic cells isolated from 3 T cell lymphomas, including a large tumor mass from a patient with cutaneous T cell lymphoma, but late in the course of the disease, were $\text{BG}^{-}$. The neoplastic cells isolated from one patient with T cell ALL were $\text{BG}^{-}$ while the neoplastic cells isolated from a patient with T cell derived lymphoblastic lymphoma in leukemic phase (Table 5, case 13) were $\text{BG}^{-}$.

The variable expression of BG activity by T cell derived malignancies is particularly interesting when considered with respect to normal T cell differentiation. Recently, another hydrolytic lysosomal enzyme, acid $\alpha$-naphthyl acetate esterase, also present in the vast majority of normal T cells,23 has been shown to be variably expressed by T cell malignancies.12 It has been suggested that the expression of ANAE activity by T cell malignancies may be a parallel expression of ANAE activity in normal T cell differentiation.12 A similar situation may exist with respect to BG activity. First, as demonstrated here, both $\text{E}^{+}\text{BG}^{+}$ and $\text{E}^{+}\text{BG}^{-}$ populations exist in the normal thymus. Second, there is evidence from the studies presented here to suggest the existence of an $\text{E}^{+}\text{BG}^{-}$ T cell population in normal peripheral blood and lymphoid tissue. Third, PHA and PWM stimulated T cell blasts are $\text{BG}^{-}$ (unpublished observations). Presumably, each of these normal T cell populations could represent the normal cellular analogue for corresponding $\text{E}^{+}\text{BG}^{+}$ and $\text{E}^{+}\text{BG}^{-}$ T cell malignancies.

There are several drawbacks to the use of cytochemically demonstrable BG activity as a routine T cell marker. First, although the $\text{BG}^{-}$ cells of normal peripheral blood, lymph node, spleen, and tonsil are contained within the E rosette forming (T) cell population, certain activated B cells are also $\text{BG}^{-}$. Second, the variable expression of BG activity by the neoplastic cells of CLL, ALL, and the T cell derived malignancies limit its usefulness in distinguishing between these different hematologic malignancies. Third, the
length of time of the assay, and the less than optimal
cytomorphological detail rendered by the requisite
fixation, air drying, and freeze-thawing of the cytocaly-
trifuge preparations leave it an impractical alternative
to the E rosette assay and/or cytochemically demon-
strable ANAE activity. Nonetheless, the variable
expression of BG activity by certain activated B cells
and B cell malignancies deserves continued study.

Furthermore, investigation of BG expression by T cell
derived malignancies may prove useful in sorting out T
cell phenotypic heterogeneity.

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