A New Human B-Lymphocyte Surface Antigen (BL 2) Detectable by a Hybridoma Monoclonal Antibody: Distribution on Benign and Malignant Lymphoid Cells

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A hybridoma-derived monoclonal antibody, produced by immunization with the Burkitt's tumor-derived B-lymphoblastoid cell line, B35M, was previously shown to detect a 68,000 dalton surface membrane protein, BL2, on the surface of peripheral blood B cells, which is absent from thymocytes, T cells, and granulocytes. In this study, we investigated the expression and distribution of BL2 on benign and malignant human lymphoid cells. Indirect immunofluorescent assay with this monoclonal antibody demonstrated that BL2 is expressed by cells within the fetal liver and by a variable proportion of lymph node, tonsil, and spleen B cells, but not by T cells. The neoplastic cells isolated from 18 T-cell malignancies were BL2+. BL2 was heterogeneously expressed by a variable proportion of the malignant cells in 29/32 cases of B chronic lymphocytic leukemia and 33/38 cases of B-cell lymphomas, but appeared to be lost in the terminal stages of B-cell differentiation, as myeloma plasma cells were BL2+. BL2 expression was not limited to B cells of a particular surface immunoglobulin isotype. Immunofluorescent staining for BL2 in cryostat tissue sections demonstrated that the majority, but not all, germinal center and interfollicular Ia( non-T) cells are BL2+. These findings suggest that BL2 is a B-cell lineage-specific differentiation marker that may be useful in the study of B-cell ontogeny and in defining subgroups of the B-cell malignancies.

HUMAN LYMPHOCYTES are divisible, according to their expression of certain surface membrane characteristics, into phenotypically and functionally distinct populations. Human B lymphocytes, for example, may be detected by the expression of intrinsic surface membrane immunoglobulin, the HLA-D-coded Ia antigens, receptors for C3 and the Fc portion of IgG, and the ability to form rosettes with mouse erythrocytes. Investigators have utilized the variable expression of these phenotypic markers by normal and malignant B cells to attempt to outline schema of B-cell differentiation and to more reliably classify the B-cell-derived lymphoproliferative malignancies.

Unfortunately, use of these conventional phenotypic markers is limited, since they may also be expressed on other cell populations, e.g., certain T-cell subsets express Fc receptors and activated T cells may express Ia antigens. Recently, some investigators have turned to somatic cell hybridization techniques in an attempt to prepare monoclonal antibodies that may be useful in defining B-cell lineage-specific antigens. Certain of the antibodies prepared in this fashion appear to be detecting B-cell-specific differentiation antigens.

We recently described the preparation of a hybridoma monoclonal antibody, derived following immunization with the B35M Burkitt's B-lymphoblastoid cell line, which detects a 68,000-dalton single-chain polypeptide, referred to as BL2. Surface antigen BL2 has been shown to be a B-cell lineage-specific antigen that is expressed on normal peripheral blood B cells but not on thymocytes, peripheral blood T cells, or granulocytes. In the present report, we describe the expression and distribution of BL2 by benign human lymphoid tissue cells and by human lymphoid malignancies.

MATERIALS AND METHODS

Specimens

Representative portions of lymph nodes were freshly obtained, under sterile conditions, from the surgical specimens of patients undergoing biopsy evaluation for presumed malignant lymphoma. These lymph node biopsies were classified according to conventional histopathologic criteria as benign lymphoid hyperplasia or malignant lymphoma. The latter were further classified according to Rappaport, and Lukes and Collins. Representative portions of tonsil, spleen, and thymus were obtained under similar conditions from surgical specimens removed during the course of standard operative procedures. Portions of fetal liver were obtained during the course of postmortem dissection of spontaneously and therapeutically aborted fetuses. Samples of heparinized venous blood and aspirated bone marrow were collected from selected patients with acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myeloblastic leukemia (AML), and multiple myeloma or plasma cell leukemia.

Mononuclear Cell Isolation

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 deter-
mined by Trypan blue exclusion, were discarded. A mononuclear cell suspension of greater than 95% viability and free of contaminating erythrocytes was prepared from each tissue, peripheral blood, and bone marrow specimen by Ficoll-Hypaque density gradient centrifugation.

**Tissue Sections**

Representative portions of tissue, obtained as described above, were transported to the laboratory in Hank's balanced salt solution or RPMI 1640 (GIBCO, Grand Island, NY). These tissue specimens were divided into 4 x 4 x 2 mm portions and placed in OCT embedding compound (Lab-Tech, IL) in air tight Beem plastic capsules (Pelco, CA) and then snap-frozen in a mixture of isopentane and dry ice. The capsules were stored at −80°C. When needed, the bottom of the frozen capsule was cut with a razor blade and the tissue, removed without thawing, was mounted on a chuck containing OCT embedding medium. Cryostat sections, 4–6 μ in thickness, were cut on an American Optical Cryostat. These sections were fixed in acetone for 5 sec, dried briefly, and stored at −20°C until staining.

**Cell Surface and Cytoplasmic Immunoglobulin**

Rhodamine or fluorescein-conjugated rabbit anti-human immunoglobulin F(ab′)2, antibody fragments monospecific for μ, δ, γ, ε, and ζ determinants were prepared as previously described. Cell surface and cytoplasmic immunoglobulin were demonstrated by direct immunofluorescence. Cytophilic uptake of IgG as a cause of nonspecific immunofluorescent staining was avoided by incubating the cells for from 2 hr to overnight at 37°C prior to immunofluorescent staining.

**E-Rosette Assay and Fractionation**

Spontaneous sheep erythrocyte (E) rosette formation was assayed according to Hoffman and Kunkel, employing Vibrio cholerae neuraminidase (VCN type V, Sigma Chemical Co., St. Louis, MO) treated sheep erythrocytes at 4°C and non-VCN-treated sheep erythrocytes at 37°C. In selected instances, mononuclear cells were separated on a Ficoll-Hypaque density gradient according to their capacity to form E-rosettes with VCN-treated sheep erythrocytes.

**Hybridoma Monoclonal Antibodies**

Monoclonal antibody anti-BL2 was prepared by hybridization of nonsecretory myeloma cells, NS-1, with BALB/c mouse spleen cells hyperimmunized with the Burkitt's lymphoma-derived B-lymphoblastoid cell line, B35M. Hybridomas secreting antibodies reactive with human B cells were cloned by limiting dilution. One of these antibodies, belonging to the IgG1 subclass, was shown to react with greater than 90% of normal peripheral blood B cells, but not with thymocytes, T-lymphoblastoid cell lines, peripheral blood T cells, or granulocytes. This antibody was shown to detect a 68,000-dalton molecular weight surface membrane antigen, termed BL2.

A monoclonal anti-human Ia (HLA-DR) antibody was prepared in an analogous fashion. This mouse IgG, monoclonal anti-human Ia antibody has been shown by immunoprecipitation to specifically detect the human Ia antigen complex and to have reactivity similar to the previously described rabbit anti-human Ia hetero sera.

**Reactivity With Hybridoma Monoclonal Antibodies in Tissue Section**

Cell surface membrane determinants reactive with the hybridoma monoclonal antibodies were demonstrated in tissue sections by indirect immunofluorescence, employing fluorescein-conjugated F(ab′)2, antibody fragments of affinity-purified goat anti-mouse IgG as the secondary antiserum. This antibody has been shown to react with all classes of mouse immunoglobulin due to Fab specificity. An appropriate ascites control was used in each experiment.

Mononuclear cells (5 x 10⁶) were resuspended in phosphate-buffered saline (PBS) with 2% bovine serum albumin (BSA) and 0.1% azide (PBS-BSA-Azide) and were incubated for 30 min at 4°C in 10 x 75 mm plastic tubes (Falcon 2038) with 0.025 ml of the appropriately diluted monoclonal antibody. Following incubation, the cells were washed 3 times with PBS-BSA-Azide at 4°C, the supernatant removed, and 0.025 ml of the appropriately diluted fluorescein-conjugated F(ab′)2 fragments of the goat anti-mouse IgG added to the plastic tubes and the cells reincubated for 30 min at 4°C. Following incubation, the cells were washed 3 times with PBS-BSA-Azide at 4°C, the supernatant removed, and 0.025 ml of the resultant cell suspension placed on a glass slide with a Pasteur pipette, cover-slipped, and sealed with a high quality clear nail polish.

**Reactivity With Hybridoma Monoclonal Antibodies in Cell Suspension**

Cell surface membrane determinants reactive with the hybridoma monoclonal antibodies were demonstrated in cell suspension by indirect immunofluorescence, employing fluorescein-conjugated F(ab′)2, antibody fragments of affinity-purified goat anti-mouse IgG as the secondary antiserum. This antibody has been shown to react with all classes of mouse immunoglobulin due to Fab specificity. An appropriate ascites control was used in each experiment.

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Cell surface membrane determinants reactive with the hybridoma monoclonal antibodies were demonstrated in tissue section by the biotin-avidin horseradish peroxidase technique. At the time of staining, the cryostat sections were further fixed in acetone for 15 min at 4°C, and then removed to room temperature for all subsequent procedures. The sections were initially incubated with phosphate-buffered saline (PBS), pH 7.2, containing 2% normal horse serum. From this point on, the slides were kept moist at all times. After 15 min the excess PBS was wiped off and the sections were overlaid with 100–150 μl of the appropriately diluted monoclonal antibody and incubated at room temperature in a moist chamber for 15 min. The sections were gently rinsed with PBS and then allowed to sit overlayed with PBS for 15 min. Excess PBS was removed and the slides were overlaid with 100–150 μl of appropriately diluted biotin-conjugated affinity-purified horse anti-mouse IgG and incubated in a moist chamber at room temperature for 30 min. The sections were subsequently rinsed gently with PBS and allowed to sit overlayed with PBS for 15 min. Free avidin DH and biotin-conjugated horseradish peroxidase H were incubated together for 5 min in proportions giving a slight excess of avidin DH and forming the appropriate avidin-biotin peroxidase complexes. These complexes were added in 100-μl aliquots to the sections, which were then incubated in a moist chamber for 45 min at room temperature. This was followed by a final 15 min PBS rinse.

The sections were subsequently incubated for 5 min in a solution of 3,3′-diaminobenzidine, 0.75 mg/ml in PBS, pH 7.2, with 0.01% hydrogen peroxide. Finally, the sections were washed and incubated in PBS for 15 min, rinsed in distilled water, incubated for 5 min in 0.5% CuSO₄ in 0.9% NaCl, then rinsed again in distilled water, counterstained with 1% toluidine blue, dehydrated and mounted in Permount.

Endogenous tissue peroxidase activity was blocked following the reaction of the tissue sections with the second antibody, the biotin-coupled horse anti-mouse IgG. Blocking was accomplished by a 30-min incubation in 0.3% hydrogen peroxide in methanol, and was followed by a 15-min rinse in PBS.

All reagents were centrifuged in a Serofuge (Becton-Dickinson, Parsippany, NJ) at 1,000 rpm for 15 min prior to use to help remove aggregates. Controls included sections incubated with ascites obtained from mice injected with nonsecretory hybridoma cells, sections incubated with PBS-BSA alone, and sections incubated with peroxidase-conjugated horse anti-mouse IgG alone.
In selected instances, a double immunofluorescent staining technique was employed to demonstrate the presence of both Ia and BL2 antigens on the same cell in a single slide preparation. In this case, rhodamine-conjugated F(ab')2 rabbit anti-human Ia, monoclonal anti-BL2, and fluorescein-conjugated affinity-purified F(ab')2 goat anti-mouse IgG were overlaid sequentially, similar to the technique described above. In this system, the orange-red fluorescence of rhodamine and the apple green fluorescence of fluorescein served as indicators of the presence of Ia and BL2, respectively.

**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. Between 200 and 400 cells were counted, depending on 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, and the mean value of these two determinations represents the reported value.

The immunoperoxidase tissue sections were examined by conventional light microscopy using an American Optical microscope equipped with a high resolution oil immersion objective. Particular attention was given to the topographic distribution of the reactive and nonreactive cell populations.

**RESULTS**

**Benign Lymph Nodes, Tonsils, Spleen**

Mononuclear cell suspensions prepared from 20 benign, normal, and/or reactive lymph nodes consisted of 8%-56% (mean 33.4%) Ia + cells, 10%-50% (mean 26.9%) Slg + cells, and 6%-59% (mean 26.3%) BL2 + cells. Mononuclear cell suspensions prepared from 5 tonsils consisted of 43%-55% (mean 50.8%) Ia + cells, 40%-53% (mean 48.1%) Slg + cells, and 27%-54% (mean 47.6%) BL2 + cells. Mononuclear cell suspensions prepared from 5 spleens consisted of 34%-56% (mean 40.5%) Ia + cells, 21%-55% (mean 35.2%) Slg + cells, and 21%-55%, (mean 34.0%) BL2 + cells (Table 1).

Thus, both the Slg + and the BL2 + cell populations appeared to be entirely contained within the Ia + cell population. However, despite the similar range and mean percent of Slg + and BL2 + cells, reactivity with monoclonal anti-BL2 did not correlate directly with Slg expression. The percent BL2 + cells exceeded the percent Slg + cells in several instances and was considerably smaller than the percent Ia + Slg + cells in certain other instances. Thus, the benign lymph nodes, tonsils, and spleens studied here appeared to contain Ia + Slg + BL2 +, Ia + Slg + BL2 +, Ia - Slg + BL2 + and Ia - Slg - BL2 + cell populations. The small, but definite, Ia - Slg - cell population demonstrated here has been previously described and shown to largely represent B cells. The variable number of Ia + BL2 + cells were best demonstrated in E-rosette-depleted fractions (Table 2).

Mononuclear cell suspensions prepared from 8 lymph nodes, 5 tonsils, and 1 spleen were separated into T-cell and non-T-cell fractions on a Ficoll-Hypaque density gradient according to the cells' capacity to form E-rosettes (Table 2). E-rosette enrichment of these mononuclear cells depleted the BL2 + cell population, demonstrating that BL2 + is not generally expressed on T cells. E-rosette-depleted fractions, consisting of ±95% Ia + cells, contained 30%-95% BL2 + cells, a clear demonstration that a variable proportion of lymph nodal, tonsillar, and splenic Ia + B cells are BL2 +.

The distribution of BL2 + cells, and their topographic orientation with respect to the Ia + cell population, was studied in cryostat sections prepared from 6 lymph nodes, 5 tonsils, and 1 spleen. In each instance, the Ia + and the BL2 + cells appeared to represent overlapping cell populations. The vast majority of the germinal center cells were Ia + BL2 + and expressed high density BL2 in every germinal center encountered (Figs. 1 and 2). Scattered cells in the interfollicular zones were also shown to be Ia + BL2 + by the double staining technique described above (Fig. 2). Some of the latter Ia + BL2 + cells had ill-defined borders and

### Table 1. Comparative Percentage of Normal Human Tissue Lymphocytes that Express Ia Antigen, Surface Immunoglobulin (Slg), BL2, OKT3, and Form Sheep Erythrocyte (E) Rosettes

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No. of Specimens</th>
<th>Ia</th>
<th>Slg</th>
<th>BL2</th>
<th>E</th>
<th>OKT3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph node</td>
<td>20</td>
<td>33.4</td>
<td>26.9</td>
<td>26.3</td>
<td>69.5</td>
<td>65.1</td>
</tr>
<tr>
<td>Tonsil</td>
<td>5</td>
<td>50.8</td>
<td>48.1</td>
<td>47.6</td>
<td>46.7</td>
<td>48.4</td>
</tr>
<tr>
<td>Spleen</td>
<td>5</td>
<td>40.5</td>
<td>35.2</td>
<td>34.0</td>
<td>53.0</td>
<td>53.5</td>
</tr>
<tr>
<td>Thymus</td>
<td>7</td>
<td>±2</td>
<td>±2</td>
<td>±2</td>
<td>≥5</td>
<td>ND</td>
</tr>
</tbody>
</table>

Figures represent the percentage of cells expressing each marker. ND, not done.

### Table 2. Comparative Percentage of Lymphocytes in E-Rosette-Enriched (T-Cell) and E-Rosette-Depleted (Non-T) Fractions of Normal Human Lymphoid Tissues that Express BL2

<table>
<thead>
<tr>
<th>Tissue</th>
<th>E-Rosette-Enriched</th>
<th>E-Rosette-Depleted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ia E BL2</td>
<td>Ia E BL2</td>
</tr>
<tr>
<td>Lymph node</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>95 1</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>95 1</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>95 2</td>
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<td>4</td>
<td>2</td>
<td>95 2</td>
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<td>1</td>
<td>95 1</td>
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<tr>
<td>6</td>
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<td>95 2</td>
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<tr>
<td>7</td>
<td>1</td>
<td>95 1</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>95 2</td>
</tr>
<tr>
<td>Tonsil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>95 1</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>95 3</td>
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<td>3</td>
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</tr>
<tr>
<td>5</td>
<td>4</td>
<td>95 5</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>95 3</td>
</tr>
</tbody>
</table>

Figures represent the percentage of cells expressing each marker.
**Fig. 1.** Cryostat tissue section of normal human tonsil stained for BL2 by the avidin-biotin-complex immunoperoxidase technique. The surface tonsillar epithelium is BL2. A large circumscribed germinal center (B-cell zone) contains predominantly BL2 cells demonstrated by the numerous dark brown rings of immunoperoxidase staining. The surrounding interfollicular (T-cell zone) tissue contains only scattered BL2 cells (×81).

**Fig. 2.** Higher magnification of the cryostat tissue section illustrated in Fig. 1 to show that the vast majority of germinal center (Ia⁺) B cells are BL2⁺. The majority of interfollicular (T) cells are BL2⁺. However, occasional scattered BL2⁺ cells, which are also Ia⁺, are present within the interfollicular zones (×180).
long processes, suggesting that they were tissue histiocytes.

The Ia'BL2 cell population demonstrable in E-rosette-depleted fractions was less obvious in the cryostat sections prepared from the same tissues. There did appear to be a greater number of Ia' than BL2' cells in cryostat sections of certain specimens, comparable to the results of cell suspension analysis. However, it was extremely difficult to state, even with the double immunofluorescent staining technique, whether the Ia'BL2 cell population resided entirely within the interfollicular zone or within both the follicular and the interfollicular zones. Occasional interfollicular Ia'BL2 cells were seen in some tonsil sections, suggesting that the tonsillar Ia'BL2' cell population resides primarily in the interfollicular zone.

Thymus

Mononuclear cell suspensions prepared from each of 7 thymuses consisted of equal to or less than 2% Ia' cells and equal to or greater than 95% E-rosette-forming cells. Only occasional BL2' cells (≤2%) were demonstrable in thymus (Table 1). Immunoperoxidase staining of cryostat tissue sections of 4 thymuses confirmed the absence of BL2 expression by thymocytes.

Fetal Liver

Cryostat tissue sections of 3 fetal livers (10, 15, and 20 wk gestational age) were studied for their reactivity with monoclonal anti-Ia and anti-BL2. In each case, variable numbers of Ia' and BL2' cells were found to lie singly or in small clusters in the hepatic sinusoids, corresponding to foci of developing hematopoietic cells. Double immunofluorescent staining of cryostat tissue sections of 4 thymuses confirmed the absence of BL2 expression by thymocytes.

B-Chronic Lymphocytic Leukemia (B-CLL)

Mononuclear cell suspensions prepared from peripheral blood, bone marrow, and lymph node specimens obtained from 32 patients with high count chronic lymphocytic leukemia were investigated for their expression of BL2 (Table 3). The vast majority of the cells in each of these cases were Ia'Slg'E', i.e., expressed the phenotype of the majority of normal mature B cells and were monoclonal with respect to their light chain determinants. The number of cases that expressed each Slg isotype were as follows: IgMD, (13), IgMD, (9), IgM, (4), IgM, (2), IgG, (2), and IgD, (2). Five cases had an associated monoclonal protein spike of the same light chain type as that expressed on the surface of the neoplastic cells, presumably related to in vivo maturation.

The neoplastic B-CLL cells were shown to express BL2 in 29 of the 32 cases studied, including 4 of the 5 cases with an associated monoclonal protein. However, BL2 expression, both in terms of the percent positive cells and the degree of antigenic density, as judged by the intensity of immunofluorescent staining, was found to be highly variable. Less than 50% of the neoplastic cells were BL2', and these cells generally expressed low density BL2 (8 cases). Approximately 50%–75% of the neoplastic cells were BL2' in 10 cases, with considerable intratumor heterogeneity of BL2 expression, as judged by the intensity of immunofluorescent staining. Finally, the percent BL2' cells was comparable to the percent Ia' cells and was of similarly high density in 14 cases.

B-Cell Malignant Lymphomas

Lymph node, peripheral blood, and other tissue specimens obtained from 38 patients were shown to be involved by malignant lymphoma by histopathologic criteria (Table 3). The neoplastic cells in these cases expressed the Ia'Slg'E' phenotype, i.e., the phenotype expressed by the majority of normal mature B cells, and were monoclonal with respect to their light chain determinants. Hence, these 38 cases were classified as B-cell-derived malignant lymphomas. The number of cases that expressed each surface immunoglobulin isotype was as follows: IgM, (10), IgMD, (9), IgMD, (5), IgG, (4), IgM, (4), IgG, (1), and IgD, (1). The heavy chain type was not determined in 4 cases. A serum monoclonal protein spike of the same light chain class as that expressed on the surface of the neoplastic cells was present in 3 cases.

These 38 cases were subclassified according to Rap-
paport17 as follows: well differentiated lymphocytic (WDL), 10; intermediate differentiation (IL), 5; nodular, poorly differentiated lymphocytic (NPDL), 10; diffuse, poorly differentiated lymphocytic (DPDL), 7; diffuse mixed lymphocytic and histiocytic (DMLH), 1; nodular histiocytic (NH), 1; and diffuse histiocytic (DH), 4.

A variable percentage of the neoplastic cells isolated from 33 of the 38 cases of B-cell-derived malignant lymphoma expressed BL2. This included 9 of 10 cases of WDL, 5 of 5 cases of IL, 7 of 7 cases of DPDL, 6 of 10 cases of NPDL, 1 of 1 case of DMLH, 4 of 4 cases of DH, and 1 of 1 case of NH. All 3 B-cell lymphomas with an associated serum monoclonal protein expressed BL2. Malignant lymphoma cells expressing each surface immunoglobulin isotype were BL2+; BL2 expression was not limited to a particular B-lymphocyte class.

Despite the fact that the majority of B-cell lymphomas studied were found to be BL2+, there was marked inter- and intratumor heterogeneity of BL2 expression. First, there was marked case-to-case variability in terms of the percentage of lymphoma cells that expressed BL2. For example, while the percent Ia+, Slg+, and BL2+ cells was comparable in some cases, the percent Ia+ and Sig+ cells greatly exceeded the percent BL2+ cells in other cases. Thus, the vast majority of the lymphoma cells were Ia+Slg+BL2+ in some cases, while large numbers of both Ia+BL2+ and Ia+BL2- cells were present in other cases. Second, there was marked inter- and intratumor variability with respect to antigenic density, as judged by immunofluorescent staining intensity. In some instances, the lymphoma cells were uniformly either weakly or strongly reactive with anti-BL2, while in other instances, the neoplastic cells isolated from a given lymphoma ranged from faintly to strongly reactive with anti-BL2.

Multiple Myeloma

The vast majority of the mononuclear cells isolated from each of 4 specimens obtained from patients with multiple myeloma or plasma cell leukemia were immunoglobulin-containing plasma cells (Table 3). These myeloma cells were found to be Ia-, as has been previously described.31 In no instance did any of the myeloma plasma cells express BL2. The only BL2- cells present in these cases were occasional small benign-appearing lymphocytes, which probably represent benign residual B cells.

T-Cell Malignancies

Mononuclear cell suspensions were prepared from peripheral blood, lymph node, and other tissue specimens obtained from 18 patients with Sézary's syndrome, cutaneous lymphoma, and acute lymphoblastic leukemia (Table 3). In each case, the majority of the isolated cells were identifiably malignant by cytomorphological and histopathologic criteria and were shown to express the Ia Slg E+ phenotype, i.e., the phenotype of the majority of normal resting T cells. Hence, these 18 malignancies were considered to be of T-cell origin.

In no instance did the neoplastic T cells express BL2. In each case only a small percentage of BL2+ cells could be identified, and this percentage corresponded to the small numbers of Ia+ and Slg- cells. These Ia-, Slg-, and BL2+ cells were recognizable under phase microscopy as small benign-appearing lymphocytes and probably represent residual B cells.

Common Type Acute Lymphoblastic Leukemia

Approximately 80% or more of the mononuclear cells isolated from specimens obtained from 19 patients with common type acute lymphoblastic leukemia (ALL) were identifiably malignant by cytomorphological criteria and were shown to express the Ia-Slg E- phenotype (Table 3). The neoplastic ALL cells in 16 of these 19 cases expressed BL2. However, as in the case of the B-cell lymphomas and B-CLL, the expression of BL2 was variable both with respect to percent positive cells and antigenic density.

Acute Myeloblastic Leukemia

Mononuclear cell suspensions were prepared from bone marrow and/or peripheral blood specimens obtained from 9 patients with acute myeloblastic leukemia. The isolated cells displayed the cytomorphological and cytochemical features characteristic of AML. These malignant cells were variably Ia+, but were uniformly terminal deoxynucleotidyl transferase negative (TdT-) and common ALL antigen negative (CALLA-). Approximately 20% of the malignant cells in 2 of these 9 cases of AML expressed BL2 (Table 3).

DISCUSSION

The studies presented here define the distribution of a 68,000-dalton B-cell surface antigen (BL2) on benign and malignant human lymphoid cells. Surface antigen BL2 is expressed by a large population of hematopoietic cells (presumably pre-B-cells) within fetal liver, by a variable proportion of lymph nodal, tonsillar, and splenic B cells, and is lost during the terminal stages of B-cell differentiation, such that myeloma plasma cells are BL2+. Surface antigen BL2 was also shown to be variably expressed by 62 of 70 B-cell malignancies. Thymocytes, tonsillar, nodal, and
spleen T cells, and the neoplastic T cells from 18 T-cell malignancies were BL2. Previous studies have demonstrated that BL2 is expressed by B-lymphoblastoid cell lines but is absent from peripheral blood T cells, T-lymphoblastoid cell lines, and granulocytes. All of these findings suggest that BL2 represents a B-cell lineage-specific differentiation antigen.

Interestingly, the distribution of BL2 superficially resembles that of the Ia antigens. Both BL2 and Ia are expressed by hematopoietic cells within the fetal liver, by mature circulating and lymphoid tissue B cells, and are lost during plasma cell differentiation. Like the Ia antigens, BL2 is expressed by B cells expressing each heavy chain isotype of surface immunoglobulin. Nonetheless, BL2 is clearly distinct from the HLA-D-coded Ia antigens. First, monoclonal anti-BL2 precipitates a 68,000-dalton single polypeptide chain, while monoclonal anti-Ia precipitates a bimolecular complex of 29,000 and 34,000 daltons. Second, it is clear from the studies described here that a variable, and sometimes sizable, proportion of normal lymph nodal, tonsillar, and splenic Ia+ cells are BL2. Indeed, specimen-to-specimen variability of Ia, BL2, and surface immunoglobulin expression was considerable, suggesting the presence of Ia 'SLg'BL2', Ia 'SLg'BL2', Ia 'SLg'BL2', and Ia 'SLg'BL2' cells. The often sizable percentage of Ia 'BL2' cells was best appreciated in E-rosette-depleted fractions.

The distinction between the Ia and BL2 antigens became even more obvious during our investigation of B-cell malignancies. As anticipated from previous studies, the vast majority, if not all, of the neoplastic cells in each of the 32 cases of B-CLL studied here were Ia+. However, only a variable percentage of the neoplastic cells in 29 of these 32 cases were BL2+. Unlike the comparatively homogeneous expression of Ia antigens, BL2 expression was heterogeneous, both in terms of the percent positive cells and the intensity of immunofluorescent staining.

The B-cell-derived malignant lymphomas also showed considerable inter- and intratumor heterogeneity of expression of BL2. First, unlike the uniform expression of Ia by all 38 B-cell lymphomas studied here, a variable percentage of the neoplastic cells in only 33 of the 38 B-cell lymphomas were BL2+. Second, also unlike Ia, there was marked case-to-case variability among the BL2+ cases in terms of the percentage of BL2+ tumor cells. For example, the vast majority of the lymphoma cells were Ia 'SLg'BL2' in some cases, while large numbers of both Ia 'BL2' and Ia 'BL2' cells were present in other cases. Third, there was marked inter- and intratumor variability with respect to antigenic density, as judged by immunofluorescent staining intensity.

BL2 expression by the B-cell lymphomas crossed all conventional histopathologic lines. Examples of both diffuse and follicular lymphomas of each Rappaportian cytologic type were BL2+. Interestingly, however, 4 of 10 cases of NPDL lymphomas were BL2+, while 27 of 28 B-cell lymphomas of all other histopathologic types, including DPDL, were BL2+. Whether these BL2+ cases of NPDL represent a distinctive B-cell lymphoma arising from an Ia 'BL2' follicular center cell subset, or whether they denote the neoplastic analogue of a precise stage of B-cell differentiation, is not known at this time.

We attempted to partially answer this question by defining the topographic distribution of the Ia+ and BL2+ cell populations in cryostat tissue sections of lymph nodes, tonsils, and spleens. In each of these tissues, in every instance studied, the Ia+ and BL2+ cells appeared to largely represent overlapping cell populations. Nearly all germinal center (B) cells were Ia 'BL2+'; Ia 'BL2' cells could not be identified with certainty in germinal centers. Scattered interfollicular Ia 'BL2' cells were also demonstrated using double immunofluorescent staining. Unfortunately, it was very difficult to precisely localize, in tissue sections, the Ia 'BL2' cell population that was so readily demonstrable in E-rosette-depleted fractions. However, it appeared by double immunofluorescent staining, in at least some instances, that the Ia 'BL2' cell population is primarily interfollicular. These findings are somewhat surprising in view of the fact that 4 of 10 NPDL lymphomas were BL2+ and that all other lymphoma types were BL2+. However, it is possible that the interfollicular Ia 'BL2' cell population represents variable loss of Ia and BL2 antigen expression by cells differentiating along the plasma cell pathway in the interfollicular zones.

Other hybridoma monoclonal antibodies that detect B-cell differentiation antigens have recently been described. Nadler and his colleagues, for example, have reported monoclonal anti-B1 and anti-B2. Monoclonal anti-B1 detects a 30,000-dalton glycoprotein on the surface of the majority of normal peripheral blood and lymphoid tissue B cells and on all Ia 'SLg' B-cell lymphomas. Monoclonal anti-B2 detects a 120,000-dalton glycoprotein (under nonreducing conditions) on the surface of a subpopulation of normal mature B cells, on B-CLL cells and on the cells from about one-half of NPDL and DPDL lymphomas, but not lymphomas of other histopathologic type. Abramson et al. have described monoclonal anti-B1, which reacts with peripheral blood B cells, B-CLL cells, common type ALL cells, and most non-Hodgkin's lymphomas and with granulocytes. It is clear from the studies described here that monoclonal
anti-BL2 reacts with a B-cell differentiation antigen of different molecular weight and distribution than that of B1, B2, and BA1 and is distinct from them. The description of still another B-cell differentiation antigen supports the hypothesis that there exists a B-cell differentiation antigenic system analogous to the OKT series described for T cells.36

Finally, the neoplastic cells isolated from 16 of 19 cases of common type ALL expressed BL2. The demonstration that about 20%-30% of these cases express the pre-B-cell phenotype, cytoplasmic μ heavy chains in the absence of surface and cytoplasmic light chain, has prompted some investigators to suggest that these cases of ALL have a B-cell origin.37,38 More recently, Nadler et al.34 have demonstrated that about 50% of non-T-ALL and the majority of the CALLA+, but not the CALLA-, cases express a differentiation antigen detectable by monoclonal anti-B1. The presence of still another distinctive B-cell differentiation antigen, BL2, on the surface of these non-T-ALL cells further supports the contention that many of these cases are of B-cell origin. Additional studies correlating cytoplasmic μ heavy chain, CALLA, and BL2 expression are necessary to more completely characterize this heterogeneous group of neoplasms.

The development of a series of monoclonal antibodies that reacts with antigens expressed at distinct stages of T-cell differentiation and on functionally distinct T-cell subsets38 has greatly improved our comprehension of normal human T-cell ontogeny and T-cell heterogeneity. The delineation of T-cell phenotypic heterogeneity has in turn fostered our understanding of T-cell functional heterogeneity.39 Both accomplishments have proceeded in parallel with our improved understanding of the T-cell malignancies as phenotypic and functional neoplastic analogues of discrete stages of normal T-cell differentiation.40,41 Unfortunately, our understanding of B-cell differentiation and heterogeneity has lagged behind that of T cells. However, with the development of a series of monoclonal antibodies that detects distinctive B-cell differentiation antigens, e.g., BL2, B1,42 B2,15 BA1,35 we should soon be able to better depict B-cell ontogeny. The application of these monoclonal antibodies to the study of B-cell lymphoproliferative malignancies should aid in the recognition of therapeutically and prognostically important subgroups that have not been identifiable by conventional histopathologic and immunologic approaches.

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B-LYMPHOCYTE SURFACE ANTIGEN BL2


A new human B-lymphocyte surface antigen (BL 2) detectable by a hybridoma monoclonal antibody: distribution on benign and malignant lymphoid cells

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