Cellular Distribution of a B-Cell Specific Surface Antigen (gp54) Detected by a Monoclonal Antibody (Anti-BL4)¹

Labib Hashimi, Chang Yi Wang, Ayad Al-Katib, and Benjamin Koziner²

Lymphocyte Surface Markers Laboratory [L. H., A. A. K., B. K.] and Laboratory of Molecular Immunology [C. Y. W.], Memorial Sloan-Kettering Cancer Center, New York, New York 10021

ABSTRACT

A monoclonal antibody (anti-BL4) recognizing a previously characterized M, 54,000 glycoprotein (gp54) was developed by immunizing BALB/c mice with cells from a precursor B-cell line (Josh-7). In normal individuals, this antigenic molecule was present on tonsillar B-cells (60-80%) and on a fraction of peripheral blood B-cells (5-25%). BL4 (gp54) expression was investigated in 186 patients with a variety of hematological malignancies using indirect immunofluorescence and flow cytometric analysis. Twenty-six of 37 cases of B-cell chronic lymphocytic leukemia (CLL) and 18 of 33 cases of B-cell non-Hodgkin's lymphoma were BL4 positive. Surface expression of BL4 on reactive cases of CLL and non-Hodgkin's lymphoma was brighter than those of B1, B2, and B4. BL4 positive CLL cases expressed a higher proportion of mouse rosette forming cells and Leu-1 positive cells than the BL4 negative subgroup and were not associated with elevated serum immunoglobulin levels. Four of 7 BL4 negative CLL cases were associated with increased serum levels of immunoglobulin M. Lymphoblasts from 14 of 14 cases of non-T acute lymphoblastic leukemia and 3 of 3 pre-B lymphoid blast crisis of chronic myeloid leukemia were BL4 negative. Neoplastic cells from 2 of 3 cases of Waldenstrom's macroglobulinemia and 4 of 7 cases of hairy cell leukemia were BL4 reactive. None of 7 cases of multiple myeloma and plasma cell leukemia were BL4 positive. All 11 T acute lymphoblastic leukemia cases, 6 other T-cell malignancies, 5 cases of Hodgkin's disease, 51 cases of acute nonlymphocytic leukemia, and 9 cases of chronic myeloid leukemia in chronic phase thus far studied were BL4 negative. An in vitro induction experiment using phorbol ester on a case of B-CLL demonstrated disappearance of BL4 accompanied with further B-cell differentiation. Our study further substantiates the previous finding that gp54 is a differentiation antigen restricted to the B-cell lineage and expressed during the intermediate stage of B-cell ontogeny.

INTRODUCTION

Since the introduction of the hybridoma technology by Köhler and Milstein (1), MoAbs¹ have been utilized to supplement conventional cell surface markers in the diagnosis and classification of lymphomas and leukemias. However, the phenotypic characterization of B-cell neoplasias has been hampered by the lack of lineage specificity of most conventional surface markers (2-10), and MoAb developed against B-cells.

Among the previously reported B-cell associated MoAbs are B1A and B2A, raised against a pre-B ALL cell line (Nalm-6-M1). B1A identifies a determinant expressed primarily on PB B-lymphocytes, B-CLL, B-NHL, and the majority of non-T ALL cases (11). However, it has been also found to be expressed on granulocytes. B2A detects an antigen expressed on BM progenitor cells, 50% of slg positive CLL, 77% of non-T ALL, and 17% of T-ALL cases (12).

Other MoAb reported thus far include those reacting with B1, B2, B4, PC1, BL1, BL2, BL3, and BL7 antigens (13-18). Their diagnostic value lies predominantly in relating B-cell tumors to specific differentiative stages. Although all B-cell antigens are preferentially expressed on PB slg positive B-cells and B-CLL cells, B1 and B4 are also present on pre-B ALL blasts exhibiting immunoglobulin gene rearrangements but reported to be absent from blasts of non-B cell hematological malignancies (19). With regard to the various BL antigens (Fig. 1), previous studies have indicated that BL1 and BL2 are expressed during the early stages of B-cell development (17, 20). It was subsequently reported that 46 of 49 cases of non-T ALL were anti-BL2 and/or anti-BL1 reactive (21). In addition, both antigens were expressed on B-cell neoplasias, including CLL and NHL; however, their expression has been shown to be heterogeneous. Unlike BL1 and BL2, BL3 has been shown to be expressed primarily at the later stages (17) and BL7 at the intermediate stages of B-cell differentiation (18). Although the expression of these BL antigens is mostly restricted to the B-cell lineage, BL2 and BL7 have been reported to be present on blasts of a proportion of ANLL cases studied (60 and 20%, respectively). Among the other described BL antigens are BL5 (Pro-IM2) and BL6 (Pro-IM1), both detecting nonlymphoid associated determinants (22).

In this study, we describe a new murine MoAb (anti-BL4) directed against a previously described B-cell antigen termed gp54, a glycoprotein with preferential expression on a subset of tonsillar and PB B-cells, yet distinct from slg, Ia-like antigens, C3 receptors, and Fc receptors as determined by rabbit antisera (23). Reactivity of this MoAb was found to be B-cell lineage specific and limited to distinctive stages of B-cell differentiation. Cellular distribution of BL4 in various hematological malignancies and its correlation with the surface expression of other B-cell associated antigens are subjects of this report.

MATERIALS AND METHODS

Cell Surface Iodination and Immunoprecipitation. MoAb anti-BL4, an IgGb antibody, was developed by immunization of BALB/c mice with the cell line Josh-7, a cell line established from non-T BM cells of a young patient with X-linked agammaglobulinemia, and is thought to be composed of precursors of B-lymphocytes (24). To characterize the target antigen recognized by anti-BL4, Josh-7 cells were radiolabeled externally with ¹²⁵I by the lactoperoxidase catalyzed reaction and lysed with Nonidet P-40 detergent, and the resulting solubilized surface membrane proteins were reacted with anti-BL4, anti-BL7, and control mouse IgG₃, respectively. The immunoprecipitates were analyzed by SDS-PAGE under both nondenatured and reduced conditions.

Peripheral Blood and Tissue Specimens. PB from 10 different healthy donors was studied. Tonsillar tissue was obtained from tonsillectomies specimens in 10 patients ages 3 to 35 years. Samples from PB, BM, LN, spleen, and malignant pleural fluid were obtained, either at the

¹ Received 10/8/85; revised 5/14/86; accepted 7/7/86.

2 The abbreviations used are: MoAb, monoclonal antibodies; PB, peripheral blood; BM, bone marrow; LN, lymph node; slg, surface immunoglobulin; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; NHL, non-Hodgkin's lymphoma; CML, chronic myelogenous leukemia; HCL, hairy cell leukemia, ANLL, acute nonlymphoblastic leukemia; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; WM, Waldenstrom's macroglobulinemia; MM, multiple myeloma; CALLA, common acute lymphoblastic leukemia antigen; MRFC, mouse rosette forming cells; CMFI, channel number of mean fluorescence intensity; CPF1, channel number of peak fluorescence intensity; TPA, 12-O-tetradecanoylphorbol-13-acetate.

3 To whom requests for reprints should be addressed, at Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021.

4 Supported by Grants CA-08748, CA-20194, and CA-05826 from the National Cancer Institute; Grant AI-18321 from the NIH; Grants CH-322 and PDT-246 from the American Cancer Society, and by the Norma and Rosita Winston Foundation, Inc., Dr. Burton J. Lee, and the Julie Gould Foundation for Medical Research.

5 The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

6 The abbreviations used are: MoAb, monoclonal antibodies; PB, peripheral blood; BM, bone marrow; LN, lymph node; slg, surface immunoglobulin; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; NHL, non-Hodgkin's lymphoma; CML, chronic myelogenous leukemia; HCL, hairy cell leukemia, ANLL, acute nonlymphoblastic leukemia; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; WM, Waldenstrom's macroglobulinemia; MM, multiple myeloma; CALLA, common acute lymphoblastic leukemia antigen; MRFC, mouse rosette forming cells; CMFI, channel number of mean fluorescence intensity; CPF1, channel number of peak fluorescence intensity; TPA, 12-O-tetradecanoylphorbol-13-acetate.

Downloaded from cancerres.aacjr.org on April 28, 2017. © 1986 American Association for Cancer Research.
time of diagnosis or at relapse, from 186 patients with different types of hematological neoplasias. The breakdown of cases was: ANLL, 51; B-CLL, 37; NHL, 33; T-ALL, 11; chronic phase CML, 9; non-T ALL, 14; HCL, 7; Hodgkin's disease, 5; T-cell hematological malignancies, 6; lymphoid blastic crisis of CML, 3; WM, 3; MM, 5; and plasma cell leukemia, 2. LN from 5 patients with reactive lymphadenopathy were also studied. Two of these 5 patients were diagnosed as having acquired immunodeficiency syndrome and a third one was a healthy homosexual. The remaining 2 patients were known cases of NHL with hyperplastic lymph nodes.

All patients were seen at the Memorial Sloan-Kettering Cancer Center during the period of February 1983 through April 1986. The diagnosis of leukemia or lymphoma was based on standard clinical, morphological, histochemical, and cyto genetic criteria. This was further substantiated by conventional surface marker studies and reactivities with other MoAb identifying B- or T-cell lymphoid or myeloid related antigens. The French-American-British system (25) was used for the classification of acute leukemia and the Rappaport scheme (26) for the classification of NHL. In acute leukemia cases the proportion of blasts was 80% or more. In patients with other types of neoplastic lymphoid diseases, PB or BM showed 30-100% malignant cells. In cases where pleural fluid was studied, involvement was documented by cytomorphological examination. Representative portions from histopathologically involved LNs or spleens were studied in selective cases of NHL, HCL, CLL, and Hodgkin's disease.

Cultured Human Cell Lines. Nineteen established human B/pre-B hematopoietic cell lines (Joss-7, RAMOS, HPB-NULL, RPMI 8226, U937, 1X1-15, B46M, NK-92, ALL-1, DG-75, Nalm-12, Nalm-17, LAZ-221, KOPN-8, REH, AUR, NAK, RPMI 1788, and RPMI 8866), and 5 non-B-cell lineage leukemic cell lines (HL-60, KE37, HS6-2, HPB-ALL, and CEM) were studied for their reactivity with anti-BL4 and the other described MoAb.

Phorbol Ester Induced Differentiation. Neoplastic B-cells derived from a case of CLL were used for induction experiments with TPA (Sigma Chemical Co., St. Louis, MO). Cells were cultured in a regular medium at 37°C in humidified atmosphere of 5% CO2, as described previously (31). TPA was stored at -20°C at 1 mg/ml and was used in a final concentration of 1.6 x 10^-8 M. Control cultures received an equal final concentration of acetone without TPA. Cultures were maintained and replenished with fresh medium daily for 6 days with viability always exceeding 80%. Aliquots of cells were harvested on days 1, 3, and 6 and analyzed for phenotypic changes.

RESULTS

Biochemical Characterization of BL4 Antigen and Its Distribution on Normal Cells. Immunoprecipitates obtained by reacting the radiolabeled and Nonidet P-40 solubilized surface membrane proteins of Joss-7 cells with both MoAb anti-BL4 and anti-BL7 were analyzed by SDS-PAGE under both nonreduced and reduced conditions. A single polypeptide with molecular weights of 54,000 under nonreduced conditions and 45,000 under reduced conditions was precipitated by anti-BL4, whereas repeated attempts to precipitate the BL7 antigen, known to be heat stable, from the cell lysate failed (Fig. 2). A glycoprotein with an identical molecular weight was precipitated by the previously described rabbit anti-gp54 serum (23). Sequential precipitation with MoAb anti-BL4 after prior precipitation with anti-gp54 was described previously (30). Monocytes were removed by a carbonyl iron ingestion method. Preparations of enriched T- and B-lymphocyte populations were made by Ficoll-Hypaque gradient separation of lymphocytes rosetted with neu raminidase-treated sheep erythrocytes. Viability always exceeded 80%, as determined by trypan blue dye exclusion test.

Direct immunofluorescent staining for sIg was carried out using fluorescein conjugated rabbit F(ab)2 fragments of antibodies directed against various human immunoglobulin fractions as reported previously (31).

Reactivity with anti-BL4 and the other described MoAb was assayed by indirect immunofluorescence with fluorescein conjugated, affinity purified goat anti-mouse IgG and analyzed on a cytofluorograph system 30L (Ortho Diagnostic Systems, Westwood, MA) utilizing the 488 nm band of a 50-mW argon laser as described previously (18). Background fluorescence was obtained using normal mouse IgG. A minimum of 10,000 cells/test were analyzed. MoAb reactivity was considered positive when 25% of the leukemic cells or more displayed immunofluorescence. CMFI or CFP1 were chosen as indicators for density of antigen expression. CMFI was calculated using a 2140 Data Handling System (Ortho).

Surface Marker Analysis and Immunofluorescence. Spontaneous rosette formation with mouse erythrocytes (MRFC) was performed as described previously (31) and with sheep erythrocytes at 4°C according to the method of Bentwich et al. (32).

Phorbol Ester Induced Differentiation. Neoplastic B-cells derived from a case of CLL were used for induction experiments with TPA (Sigma Chemical Co., St. Louis, MO). Cells were cultured in a regular medium at 37°C in humidified atmosphere of 5% CO2, as described previously (31). TPA was stored at -20°C at 1 mg/ml and was used in a final concentration of 1.6 x 10^-8 M. Control cultures received an equal amount of acetone without TPA. Cultures were maintained and replenished with new media daily for 6 days with viability always exceeding 80%. Aliquots of cells were harvested on days 1, 3, and 6 and analyzed for phenotypic changes.
rabit anti-gp54 serum failed to bring down any discernible component suggesting that MoAb anti-BL4 recognized the same B-cell specific gp54 molecule described previously.

Normal distribution of BL4 antigen was determined by testing 10 different PB samples obtained from healthy donors and 10 tonsillectomy specimens. As illustrated in Fig. 3, BL4 was found to be strongly expressed on a major subset of tonsillar B-cells (60–80%) and on a variable fraction of PB B-cells, (5–25%). On the contrary, anti-BL4 reactivity was absent from all PB T-cells, monocytes, macrophages, and granulocytes in all individuals tested. This distribution was identical to that of gp54 antigen reported earlier (23).

**BL4 Expression on Leukemias and Malignant Lymphomas.** Anti-BL4 reactivity on all malignant cases studied is summarized in Table 1. Thirty-seven patients with B-CLL were tested. Cells in all cases expressed Ia antigens and faint surface immunoglobulin (slg positive) and formed rosettes with mouse erythrocytes. Furthermore, cells in almost all CLL patients tested expressed Leu-1 (33 of 35) and BL7 (23 of 25). BL4 was found to be expressed in 26 of 37 cases with a mean percentage of positive cells of 60.7%.

Table 2 illustrates the phenotypic features of the two different subgroups of B-CLL, as determined by their surface expression of BL4 antigen. Despite bright expression of Ia antigen in both subgroups, a larger number of cells expressed Leu-1 and MRFC in the BL4 positive subgroup.

Serum immunoelectrophoresis studies were available on 15 patients in the BL4 positive CLL subgroup and none had evidence of paraproteinemia. Serum IgM, IgG, and IgA levels were either normal or decreased. On the contrary, of the 7 BL4 negative CLL patients studied, 4 had increased serum IgM levels of 314–2420 mg/dl (normal, 50–280 mg/dl).

Leukemic cells from all CLL cases were surveyed for their reactivity with anti-BL7. It was shown that both BL4 positive and BL4 negative subsets of CLL express BL7 antigen.

Neoplastic cells from 33 cases of B-cell NHL (including one case of pre-B lymphoblastic lymphoma) were evaluated for their reactivity with anti-BL4. The B-cell lineage of these neoplasms was determined by the presence of Ia antigens, bright expression of monoclonal slg, and reactivity with MoAb identifying B-cell lineage, i.e., B1, B2, B4. Eighteen of 33 (54%) of these cases were reactive with anti-BL4 with a mean positive reactivity of 47.4% (Table 1).

Correlation between BL4 expression and the histological subtypes according to the Rappaport classification is shown in Table 3. Five of 6 cases of nodular poorly differentiated lymphocytic lymphoma and 7 of 9 cases (77%) of all nodular lymphomas reacted with anti-BL4, whereas only one-half of all diffuse lymphoma cases were BL4 positive, including 4 of 8 cases of diffuse poorly differentiated lymphocytic lymphoma and 6 of 12 cases of diffuse histiocytic lymphoma. Only 1 of 3 cases of Burkitt's lymphoma tested was anti-BL4 reactive (proportion of positive cells, 32%). Concomitant testing of malignant cells from NHL cases with anti-BL7 showed that almost all BL4 positive cases (15 of 16) and the majority of the BL4 nonreactive subgroup (9 of 12) were BL7 positive regardless of histological subtype.

Other B-cell hematopoietic malignancies examined included 3 cases of WM, 2 of which reacted with anti-BL4. One case had 55% abnormal lymphoid cells in the BM; these were of intermediate size and a few displayed clefted nuclei. These cells expressed the following surface phenotype: Ia*, B1*, B2* and B4*, CALLA*, BL4*, BL7*, BL3*. slg expression was of moderate intensity and of IgM* isotype. Paraproteinemia in this patient was of two different isotypes; the main component was IgMx (520 mg/dl) and in addition there was an IgG spike (2640 mg/dl). In another WM patient there was a serum IgMx paraproteinemia. slg fluorescence was intense and of IgMs isotype. Surface membrane phenotype in this case was as follows: Ia+, B1+, B2+, B4+, CALLA*, BL1+, BL2+, BL4+, BL7+, BL3+. In the remaining BL4– WM patient, 90% of BM cells were lymphoid. There were also atypical plasma cells. Malignant cells did not express slg and showed the following phenotype: Ia+, BL1–,

### Table 1 Reactivity of various hematopoietic malignancies with anti-BL4

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Cell lineage</th>
<th>Phenotype</th>
<th>No. of patients studied</th>
<th>Reactivities with anti-BL4*</th>
<th>BL4* (% of cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLL</td>
<td>B</td>
<td>Ia*, slg*, E–, MR*, Leu-1*, BL7*</td>
<td>37</td>
<td>+</td>
<td>70.0</td>
</tr>
<tr>
<td>NHL</td>
<td>B</td>
<td>Ia*, slg*, E–, MR*</td>
<td>33</td>
<td>+</td>
<td>54.0</td>
</tr>
<tr>
<td>WM</td>
<td>B</td>
<td>Ia*, slg*, E–, MR*</td>
<td>8</td>
<td>–</td>
<td>0.0</td>
</tr>
<tr>
<td>HCL</td>
<td>B</td>
<td>Ia*, slg*, E–, MR*–, Latex</td>
<td>7</td>
<td>+</td>
<td>57.0</td>
</tr>
<tr>
<td>MM/PCL</td>
<td>B</td>
<td>Ia*, slg*, E–, slg*–, E–</td>
<td>7</td>
<td>+</td>
<td>0.0</td>
</tr>
<tr>
<td>ALL</td>
<td>Null (pre-B)</td>
<td>slg*, CALLA*, slg*, E–</td>
<td>14</td>
<td>–</td>
<td>0.0</td>
</tr>
<tr>
<td>CML-lymphoblastic</td>
<td>Null (pre-B)</td>
<td>slg*, CALLA*, slg*, E–</td>
<td>3</td>
<td>–</td>
<td>0.0</td>
</tr>
<tr>
<td>ALL</td>
<td>T</td>
<td>Leu-1*, Leu-5*–, Leu-4*, slg*</td>
<td>11</td>
<td>+</td>
<td>11.0</td>
</tr>
<tr>
<td>T-cell malignancies</td>
<td>T</td>
<td>Leu-1*, Leu-5*–, Leu-4*, slg*</td>
<td>6</td>
<td>–</td>
<td>0.0</td>
</tr>
<tr>
<td>HD</td>
<td>E</td>
<td>slg*</td>
<td>5</td>
<td>–</td>
<td>0.0</td>
</tr>
<tr>
<td>ANLL</td>
<td>Myeloid/mönocytic</td>
<td>BL5* or OKM1*, IG10*, SF1*</td>
<td>51</td>
<td>–</td>
<td>50.0</td>
</tr>
<tr>
<td>CML-CP</td>
<td>Myeloid</td>
<td>BL5* or OKM1*, IG10*, SF1*</td>
<td>9</td>
<td>–</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* (pos.) = (positive), 25% positive cells or more with a medium to high mean fluorescence intensity; – (negative), less than 25% positive cells with low mean fluorescence intensity.

CML-CP chronic myelocytic leukemia, chronic phase; PCL, plasma cell leukemia.

5433
Comparison of BL4 Antigen Expression with B1, B2, and B4 Antigens. Anti-BL4 reactivity on a selected number of cases with hematological malignancies was correlated for lineage specificity with MoAb detecting other B-cell specific antigens including B1, B2, and B4. Results of such comparison are shown in Table 4. The percentage of CLL cases expressing BL4 antigen was equivalent to those expressing B1 (22 versus 23 of 26 cases studied), while B2 and B4 were less frequently expressed than BL4. Similar findings were noticed in NHL. Only a minor subset of NHL cases demonstrated reactivity with anti-B2 (5 of 15 studied) and anti-B4 (7 of 18 studied). None of the 17 ALL cases studied showed BL4 reactivity, while only 3 of 14 non-T ALL cases reacted with B1 and another 3 expressed B4. Among the nonlymphoid leukemias, none of 31 cases tested reacted with anti-BL4 and anti-B2, whereas 4 of 7 cases with acute monoblastic leukemia (M5) expressed B1 and B4.

For objective estimation of the BL4 antigen density of expression on leukemic cells, CMFI was measured on representative CLL cases that expressed BL4 and correlated with that of BL7, B1, B2, and B4. In 16 BL4 positive CLL cases, the mean value of CMFI was 109.0, compared to 225.4 for BL7. Mean CMFI for B1, B2, and B4 in reactive CLL cases were 84.7, 73.3, and 70.4, respectively. Representative analysis in a case of CLL and a case of NHL illustrating such correlation is shown in Fig. 4. BL7 was the most brightly expressed antigen on both CLL and NHL (CMFI = 240.0 and 711.0, respectively), followed by BL4 (115.5 and 247.0, respectively). All B antigens (B1, B2, and B4) found to be expressed on CLL and NHL were much weakly expressed than BL7 and BL4, with B1 being the brightest (CMFI = 90.2 and 94.9, respectively).

Reactivity with Hematopoietic Cell Lines. Table 5 shows anti-BL4 reactivity with 24 well characterized cell lines including the Joss-7 from which anti-BL4 was prepared. None of the 6 pre-B ALL cell lines expressed bright reactivity with anti-BL4; however, 3 of 6 (REH, Nalm-12 KOPN-8) showed faint reactivity and the other 3 were negative. Among the B-cell hematopoietic cell lines, those from Burkitt's lymphoma either were BL4 negative (RAMOS, DG-75) or expressed faint reactivity (B46M, NK-9). Three of these 4 cell lines reacted with anti-BL3 and anti-BL7. On the other hand 2 Epstein-Barr virus transformed B-cell lines (RPMI 1788, RPMI 8666) expressed bright reactivity with anti-BL4 (CMFI = 532.0 and 447.0, respectively). Other B-cell lines tested were SK-LY-16 and U937, which are diffuse histiocytic lymphoma cell lines, and both lacked BL4 expression. The myeloma RPMI 8226 cell line was BL4 negative; also all non-B leukemic cell lines tested
BL4 (gp54) EXPRESSION ON LYMPHOID MALIGNANCIES

Background

---

Fig. 4. Immunofluorescence profiles from (A) a patient with CLL and (B) a patient with NHL, showing the differences in intensity of expression of various BL and B antigens. BL7 expression is the brightest of all on CLL and NHL (CMFI = 240.0 and 711.0, respectively). BL4 and B1 are moderately bright (CMFI for BL4, 115.5 and 247.0, respectively; CMFI for B1, 90.2 and 94.9, respectively); B2 and B4 are weak. Thickly dotted line, background fluorescence.

Table 5 Expression of BL4 on cultured human cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Origin</th>
<th>Type of cell</th>
<th>Reactivity with anti-BL4*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Josh-7KOPN-8</td>
<td>X-linked agammaglobulinemia</td>
<td>Pre-B</td>
<td>+</td>
</tr>
<tr>
<td>KOPN-8</td>
<td>ALL</td>
<td>Pre-B</td>
<td>±</td>
</tr>
<tr>
<td>REH</td>
<td>ALL</td>
<td>Pre-B</td>
<td>±</td>
</tr>
<tr>
<td>Nalm-12</td>
<td>ALL</td>
<td>Pre-B</td>
<td>±</td>
</tr>
<tr>
<td>HPB-NULL</td>
<td>ALL</td>
<td>Pre-B</td>
<td>–</td>
</tr>
<tr>
<td>LAZ-221</td>
<td>ALL</td>
<td>Pre-B</td>
<td>–</td>
</tr>
<tr>
<td>Nalm-17</td>
<td>ALL</td>
<td>Pre-B</td>
<td>–</td>
</tr>
<tr>
<td>BALL-1</td>
<td>ALL</td>
<td>B</td>
<td>±</td>
</tr>
<tr>
<td>B46M</td>
<td>BL*</td>
<td>B</td>
<td>±</td>
</tr>
<tr>
<td>NK-9</td>
<td>BL</td>
<td>B</td>
<td>±</td>
</tr>
<tr>
<td>RAMOS</td>
<td>BL</td>
<td>B</td>
<td>–</td>
</tr>
<tr>
<td>DG-75</td>
<td>DHL</td>
<td>B</td>
<td>–</td>
</tr>
<tr>
<td>SK-LY-16</td>
<td>DHL</td>
<td>B</td>
<td>–</td>
</tr>
<tr>
<td>U937</td>
<td>DHL</td>
<td>B</td>
<td>–</td>
</tr>
<tr>
<td>RPMI 1788</td>
<td>EBV transformed</td>
<td>B</td>
<td>+</td>
</tr>
<tr>
<td>RPMI 8866</td>
<td>EBV transformed</td>
<td>B</td>
<td>+</td>
</tr>
<tr>
<td>AJUR</td>
<td>EBV transformed</td>
<td>B</td>
<td>–</td>
</tr>
<tr>
<td>NAK</td>
<td>EBV transformed</td>
<td>B</td>
<td>–</td>
</tr>
<tr>
<td>RPMI 8226</td>
<td>MM</td>
<td>B</td>
<td>–</td>
</tr>
<tr>
<td>HL-60</td>
<td>APL</td>
<td>Myelomonocytic</td>
<td>–</td>
</tr>
<tr>
<td>KE37</td>
<td>ALL</td>
<td>T</td>
<td>–</td>
</tr>
<tr>
<td>CEM</td>
<td>ALL</td>
<td>T</td>
<td>–</td>
</tr>
<tr>
<td>HSB-2</td>
<td>ALL</td>
<td>T</td>
<td>–</td>
</tr>
<tr>
<td>HPB-ALL</td>
<td>ALL</td>
<td>T</td>
<td>–</td>
</tr>
</tbody>
</table>

*+, more than 25% positive cells; –, less than 10% positive cells; ±, between 10 and 25% positive cells.

Thus far (HL-60, KE-37, CEM, HSB-2, and HPB-ALL) lacked BL4 expression. However, the latter cell line, HPB-ALL, which is a T-ALL cell line, was B2 positive.

Disappearance of BL4 upon TPA Induction of B-Cell Differentiation. Fresh cells from one patient with B-CLL were subjected to TPA induction experiments and monitored for phenotypic changes as detected by anti-BL4 and other MoAb.

Prior to TPA culture, only 40% of cells expressed low density BL4 antigen (CPFI = 100.0), whereas 75% of the cells brightly expressed BL7 (CPFI = 220.0). As shown in Fig. 5, TPA induced disappearance of BL4 expression after 3 days of culture. Comitantly, the proportion of BL7 positive cells decreased sharply in a fashion parallel to that of BL4. However, the overall BL7 reactivity remained positive. On the contrary, BL3 expression was low on day 0 and increased progressively from 10% to a peak of 91.0% on day 3 accompanied by an increase in the CPFI from 90.0 to 125.0. Other phenotypic changes noticed during this culture period included a 20% increase in Leu-1 expression on day 1, reaching a plateau on day 3 to decline thereafter but remaining strongly positive. B1 and B2 were expressed on day 0. Their expression increased on day 1 to decline thereafter. On day 6 of TPA culture, B1 expression disappeared completely, while B2 showed only marginal expression (33.0%).

DISCUSSION

This paper describes the reactivity of a monoclonal antibody (anti-BL4, IgG,b) directed at a previously defined glycoprotein with a molecular weight of 54,000 on normal and malignant human B-lymphoid cells. This murine MoAb was derived by immunization of BALB/c mice with cells from cell line (Josh-7) from which another monoclonal antibody (anti-BL7) was developed. Like BL7, BL4 antigen is expressed on a subset of tonsillar and PB B-cells, but not on earlier lymphoid precursors, pre-B cells, or more terminally differentiated elements, i.e., plasma cells.

Previous immunoprecipitation studies using surface iodination and SDS-PAGE analysis have shown that the BL4 (gp54) antigen comigrates with the HLA heavy chain under reduced conditions, however, which resolved into a distinct molecule with a molecular weight of 54,000 under nonreduced conditions (23). In addition, previous studies utilizing direct surface immunofluorescence on fresh leukemic cells and leukemic cell lines showed that gp54 positive cells have a characteristic speckled and patchy appearance and simultaneously revealed marked cell to cell variation in the staining intensity of these positive cells suggesting extreme heterogeneity of the gp54 antigen expression.

In the present study we further confirm that BL4 is a B-lineage specific antigen. Expression of BL4 antigen on leukemias and lymphomas as detected by indirect immunofluorescence with anti-BL4 was shown to be absent in all T-cell and myeloid leukemias and leukemia cell lines. In comparison with the other BL antigens, BL4 expression is mostly restricted to the slg-bearing intermediate stages of B-cell differentiation as shown in Fig. 1. These include CLL, HCL, and the NHL spectrum of lymphoproliferative neoplasias. All non-T ALL cases studied, including pre-B-cell leukemias defined by expression of cytoplasmic immunoglobulin μ heavy chain, were BL4 negative. However, 3 of 6 non-T ALL cell lines studied (REH,
and Bl. These antigens were present in approximately one-half cases was consistent with M5 by morphology.

reported by Anderson et al. (19); however, none of these ANLL not detected on leukemic blasts from all 148 cases of ANLL reveal any evidence of biphenotypic leukemia. Bl and B4 were pressed Bl and B4, in addition to strong expression of nonlym-

cate that BL4 expression is more restricted than BL7 at the studied). In CLL, NHL, and other B-lymphoid malignancies the majority reacted with anti-BL4 and expressed Bl (13 of 18 cases tested). Furthermore, among the B-antigens, Bl, al-

expression in a small proportion of these leukemia. Unlike BL3, which is expressed at later stages of B-cell differentiation, BL7 is present on blasts of 20% of ANLL cases, while none reacted with anti-BL4. In contrast to BL7, BL4 expression in CLL and NHL was variable. Only 70% of CLL and 54% of NHL cases were BL4 positive with mean reactivities of 60.7 and 47.4%, respectively. Al-Katib et al. (18) previously reported that all CLL (63 of 63) and the overwhelming majority of NHL (22 of 28) expressed BL7. In addition, although both BL4 and BL7 were among the most densely coexpressed antigens on the surface of B-CLL and NHL cells, the CMFI for anti-BL7 was significantly higher than that of anti-BL4 in the majority of the cases studied (240 and 711.0 versus 115.5 and 247.0, respectively), as exemplified in Fig. 4. These data indicate that BL4 expression is more restricted than BL7 at the intermediate stages of B-cell differentiation. Furthermore, anti-

BL4 apparently identifies a distinctive phenotypic subgroup in Cll, since the proportion of MRFC and Leu-1 positive cells were higher in the BL4 reactive than the BL4 nonreactive cases. Additionally, it was noticed that the cases studied in this subgroup were not secretory of immunoglobulins, while a proportion (4 of 7) of the BL4 negative subgroup were associated with elevated serum levels of IgM.

In comparison with B1, B2, and B4 antigen expression, BL4 resembled B1 regarding their frequency of expression in Cll and NHL. Both antigens were present on an equivalent number of cases tested. Furthermore, among the B-antigens, B1, al-

though weaker (mean CMFI for reactive Cll cases, 84.7), was the closest in surface density of expression to BL4. However, these two molecules are structurally different, since B1 was described by immunoprecipitation to be a nonglycosylated phosphoprotein with a molecular weight of 35,000 (35). Addition-

ally, B1 was present on a proportion of the non-T ALL (3 of 14 cases studied), yet none expressed BL4. As far as lineage specificity, both B1 and BL4 seem to be B-cell restricted. However, in several instances in our patient population (4 of 7), leukemic cells from acute monoblastic leukemia (M5) expressed B1 and B4, in addition to strong expression of nonlym-

phoid associated antigens as detected by monoclonal antibodies anti-BL5 and OKM1 (22, 28). In these B1 and B4 positive ANLL cases, BL4 was negative. Histochemical and cytofluoro-
matic analysis of leukemic cells in the these patients did not reveal any evidence of biphenotypic leukemia. B1 and B4 were not detected on leukemic blasts from all 148 cases of ANLL reported by Anderson et al. (19); however, none of these ANLL cases was consistent with M5 by morphology.

B2 and B4 were less frequently expressed in Cll than BL4 and B1. These antigens were present in approximately one-half of the cases tested. In NHL, only a minor subset of cases expressed B2 and B4 (5 of 15 and 7 of 18, respectively), while the majority reacted with anti-BL4 and expressed B1 (13 of 18 studied). In Cll, NHL, and other B-lymphoid malignancies when B2 and B4 molecules were present on the surface of malignant cells, their expression was usually weaker as com-

pared to BL4 (average CMFIs for B2 and B4 on reactive CLL cases, 73.3 and 70.4, respectively). These differences in density of expression are exemplified in Fig. 4.

An attempt was made to correlate anti-BL4 reactivity with specific histological patterns of malignant lymphoma. A trend was noted in which BL4 was detected in LN suspensions from the majority of nodular lymphomas (77.0%) but in only 50% of diffuse lymphomas regardless of cellular subtype. Since most pathologists attribute the cellular origin of nodular lymphoma to follicular center B-cells (36, 37), it is reasonable to assume that BL4 bearing cells are more prevalent at the germinal centers than in the other anatomical and functional compartments of LN. Unlike nodular lymphomas, diffuse lymphomas are a heterogeneous group of tumors that encompass wide range of morphological and immunological diversity (38) and there-

fore tend to variably express BL4 antigen. Another noteworthy finding was the relative absence of BL4 expression in fresh Burkitt's lymphoma cells and in cultured Burkitt's lymphoma cell lines.

It should be emphasized here that, although the malignant B-cells of WM are lymphoplasmacytoid in morphology and secretory in function (features of advanced B-cell differentiation), they most often express a cell membrane immunoglobulin isotype similar to that of Cll, i.e., IgM (39, 40). The exact derivation of these cells from the proposed stages of B-cell differentiation is a subject of controversy. For example, Anderson et al. (19) considered the lymphoid cells of WM as transformed B-cells at a pre-plasma cell level, whereas Jansen et al. (34) related lymphoplasmacytoid leukemias phenotypically to a post-CLL stage. Our analysis has shown that this group of lymphoproliferative disorders is morphologically and phen-

typically heterogeneous. Neoplastic cells in this condition may express intermediate stage phenotype (sIgM+, BL4+, BL3+) and secrete variable amounts of paraprotein or express a plasma cell phenotype (sIgM+, BL4+, BL3+) and secrete large amounts of immunoglobulin.

In this study, it was also noticed that BL4 was least frequently expressed by reactive LN. Only 1 of 5 LN showed faint (±) reactivity and the remaining were negative, while almost all tested LNs expressed either positive or faint (±) reactivity with any one of the BL antibodies (B1, B2, B7) and with B1 and B2. Therefore, this study suggests that anti-BL4 is least likely to cross-react with reactive LNs as compared with the other BL antibodies, when the examined LNs did not show evidence of slg monoclonality.

The relationship of BL4 antigen expression to B-cell differ-

entiation was further investigated by in vitro TPA induction studies. It has been reported that TPA has the capacity to induce B-CLL cells to differentiate into more mature cyto-
mplasmic immunoglobulin-containing B-cells (41). In this study, results of TPA induction in a case of B-CLL have shown that the expression of both BL4 and BL7 decreased with further B-

cell differentiation while there was a concomitant increase in reactivity with anti-BL3. The disappearance of BL4 was accom-

panied by decrease in BL7 expression, both substantiating the conclusions which were drawn from the studies on fresh cells about the sequence of BL antigen expression in B-cell neo-

plasms and on their normal counterparts. This indicates that BL4 and BL7 are both expressed in B-cell malignancies and during normal B-cell differentiation in a sequential fashion. BL7 disappearance usually follows that of BL4 but in an orderly manner.

In addition to TPA induction experiments, preliminary stud-
ies using purified F(ab')2 fragments of gp54 rabbit antiserum or the whole antiserum and thymidine labeling of B-lymphocytes revealed that these fragments can induce T-cell independent B-cell proliferative response but cannot induce immunoglobulin synthesis (23). These studies suggested that B-cell activation induced by the anti-gp54 serum was probably related to the binding of these antibodies to the BL4 surface antigen. Parallel studies using monovalent Fab fragments of the anti-gp54 reagent demonstrated marked inhibition of T-cell dependent B-cell differentiation. In vitro experiments with anti-BL4 on various mitogen stimulated cultured lymphocytes are under way to substantiate the regulatory role of this antigenic molecule in B-cell proliferation and differentiation.

Collective analysis of the presented data suggests that anti-BL4 is B-cell lineage specific MoAb, limited in reactivity to the sIg positive intermediate stages of B-cell differentiation and of clinical usefulness in the diagnosis and classification of B-lymphoid malignancies.

ACKNOWLEDGMENTS

The authors are most grateful to Dr. Jun Minowada of the Veterans Administration Hospital, Hines, IL, for providing the leukemic cell lines; to Patricia Black for her kind assistance in data gathering; and to Mary Lilly for typing the manuscript.

REFERENCES


5437
Cellular Distribution of a B-Cell Specific Surface Antigen (gp54) Detected by a Monoclonal Antibody (Anti-BL4)

Labib Hashimi, Chang Yi Wang, Ayad Al-Katib, et al.


Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/46/10/5431

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.