Expression of Human B Cell-Associated Antigens on Leukemias and Lymphomas: A Model of Human B Cell Differentiation

By Kenneth C. Anderson, Michael P. Bates, Bruce L. Slaughenhoup, Geraldine S. Pinkus, Stuart F. Schlossman, and Lee M. Nadler

A series of monoclonal antibodies that define B cell restricted and associated antigens was utilized in an attempt to characterize tumors of B lineage and to relate these tumors to B cell differentiative stages. Antigens that were previously shown to be B cell restricted on normal B lymphocytes were similarly expressed only on B cell malignancies. In contrast, antigens that were B cell associated were also found on tumors of other lineages. Moreover, on the basis of cell surface phenotypes, tumors of B cell origin were divided into three major subgroups, which corresponded to the level of differentiation of the malignant tumor cell: pre-B cell stage (non-T acute lymphoblastic leukemia and chronic myelocytic leukemia in lymphoid blast crisis); the mid-B cell stage (chronic lymphocytic leukemia, poorly differentiated lymphomas); and secretory B cell stage (large cell lymphomas and plasma cell tumors). A hypothetical model is derived that relates the malignant B cell to its normal cellular counterpart on the basis of cell surface expression of this panel of B cell-restricted and B cell-associated antigens.

HUMAN LEUKEMIAS and lymphomas have long been recognized as heterogeneous diseases by their morphological appearance, clinical presentation, and response to therapy.1-3 Their classification has been traditionally based on cytologic appearance and cytochemical properties of the tumor4-9 and, more recently, by immunologic markers that define both lineage and state of differentiation of the tumor cell.10-18 Specifically, B cell tumors have been defined by their expression of cytoplasmic immunoglobulin (clg)10 or monoclonal surface immunoglobulin (slg).11,12 In an attempt to better characterize B cell tumors, several laboratories have developed monoclonal antibodies directed at antigens that are largely restricted to normal and malignant B cells and distinct from previously described B cell-associated markers, including Ia, slg, Fc, and C3 receptors.19-25 Nine such B cell-associated antigens have been reported from this laboratory: B1,20,21 B2,22 B4,27 and PC-1,28 which are B cell restricted within the hematopoietic system; Ia,21 CALLA,22,23 T1,23,24 T10,25,26 and PCA-1,28 which are B cell associated as they are also expressed on other lymphoid and myeloid cells.

The B cell-specific antigens B1, B2, B4, and PC-1, which have previously been shown to be phenotypically and molecularly distinct from known B cell determinants, are expressed at limited stages of B cell differentiation.20,22,27,29 These antigens differ from one another in their expression on B cells: B1 and B4 span most of B cell differentiation, whereas B2 and PC-1 are expressed at limited stages of B cell differentiation, i.e., the midstage and plasma cell stage, respectively. A second group of B cell-associated markers, including Ia, CALLA, T1, T10, and PCA-1, in contrast to the former group, are not restricted to B lymphocytes within the hematopoietic system, but may appear on granulocytes, monocytes, T lymphocytes, and null cells.29,31-38 Although the wider lineage distribution of these antigenic markers limits their utility when used alone, in combination with other markers they provide a very useful fingerprint of both the malignant and normal cell. The combination of both B cell restricted and associated antigens have clearly complemented conventional morphological and cytochemical techniques for the characterization of both normal B cell differentiation and malignant B cell categorization.40-43

In the studies to be described below, we have analyzed approximately 700 lymphomas and leukemias with a panel of monoclonal antibodies defining both B cell restricted and associated antigens. The analysis of large numbers of tumors with an extensive panel of antibodies has permitted the definition of their relationship to normal hematopoietic cells. Moreover, our studies suggest that B cell tumors fit into defined stages of normal B cell differentiation.

MATERIALS AND METHODS

Production of Monoclonal Antibodies

The techniques of immunization, somatic cell hybridization, and selection of hybridomas that were used to produce the monoclonal antibodies directed at the Ia, B4, CALLA, B1, B2, slg, T1, T10, PC-1, and PCA-1 antigens have been previously described.44-46

Reactivity on Normal Tissues

The reactivity and specificity of the monoclonal antibodies employed in this study on Ficoll-Hypaque mononuclear cells,44

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(T) cells, affinity-purified slg positive \(^{48,49}\) or immune rosette negative \(^{50}\) (B) cells, adherent monocytes, \(^{51}\) granulocytes, \(^{52}\) and erythrocytes obtained both from peripheral blood and from lymphoid tissues, including tonsil, lymph node, spleen, bone marrow, and thymus, have been previously described in detail. \(^{20-27,40}\) The expression of these antigens on B cells, as well as their cross-reactivity on cells of other lineages, is summarized in Table 1.

**Human Leukemia, Lymphoma, and Myeloma Samples**

After appropriate human protection committee validation and informed consent, human samples were obtained for study. The 661 tumor samples were obtained from the Dana-Farber Cancer Institute, as well as from the Brigham and Women’s, Beth Israel, and Massachusetts General Hospitals. Tumor cells obtained from patients with acute and chronic leukemia, non-Hodgkin’s lymphomas, and plasma cell dyscrasias, in all instances, contained >75% neoplastic cells by Wright-Giemsa morphology. These tumors were classified histopathologically, utilizing a modified Rappaport system. \(^{3}\) B lineage was defined by the presence of monoclonal slg, as determined by indirect immunofluorescence with the use of anti-\(\gamma\), \(\mu\), \(\lambda\), and \(\kappa\) monoclonal antibodies. \(^{53}\) T cell tumors were identified by reactivity with specific T cell monoclonal antibodies including anti-T\(\text{3}^{32,33,34}\) and anti-T\(\text{11}^{54,55}\). Myeloid lineage was established by standard morphological and histochemical techniques, as well as by reactivity with the anti-MY7 and MY8 \(^{56}\) and anti-Mol \(^{57}\) monoclonal antibodies. All tissue specimens were immediately placed in X, and IL, B lineage was defined by \(\text{2}^{35}\). Tumor cells obtained from patients with acute and chronic leukemia, non-Hodgkin’s lymphomas, and from the Brigham and Women’s, Beth Israel, and Massachusetts General Hospitals. Tumor cells obtained from such patients were either used fresh or cryopreserved in –196°C vapor-phase liquid nitrogen in 10% dimethyl sulfoxide (DMSO) and 20% human serum until the time of surface characterization by indirect immunofluorescence.

**Indirect Immunofluorescence**

Tumor cells were analyzed for surface phenotype by indirect immunofluorescence as previously described. \(^{36}\) In brief, \(0.5-1 \times 10^6\) viable washed cells were treated with 100 \(\mu\)l of a specific or control unreactive monoclonal antibody at saturating concentrations, incubated at 4°C for 30 min, and washed 3 times. Cells were then treated with 100 \(\mu\)l of a 1:40 dilution of goat anti-mouse IgG and goat anti-mouse IgM conjugated with fluorescein isothiocyanate (FITC) (Coulter Electronics, Hialeah, FL) and incubated at 4°C for 30 min. After washing, tumor cells were analyzed for immunofluorescence using an Epics V cell sorter (Coulter), and the percent positive cells was determined using the EASY system immunoprogram (Coulter).

In addition to measuring the presence or absence of antigen expression on tumor cells, the relative intensity of antigen expression was also assayed qualitatively utilizing flow cytometry. This was done in an effort to define heterogeneity within the histologically defined subgroups of B cell tumors, as well as to relate the malignant cells to their normal cellular counterparts. Because antigen intensity, as assessed by indirect immunofluorescence, is dependent on both the number of antigenic determinants per cell and the cell surface area, we have attempted to compare cells of similar size within each of the histologically defined subgroups of B cell leukemias and lymphomas utilizing flow cytometric gating. The intensity of antigen expression on the tumor cell surface was qualitatively assessed utilizing the scale displayed in Fig. 1 (A–D): weak immunofluorescent staining (+), moderate staining (++), strong immunofluorescence (+++), and brightest staining (+++). Although heterogeneity of individual antigen intensity was observed within each phenotypic subgroup, a common phenotype based on the presence or absence of cell surface antigens as well as intensity of antigen expression was usually evident.

**RESULTS**

The expression of a panel of B cell restricted and associated antigens on tumor cells isolated from patients with lymphoid and myeloid leukemias and non-Hodgkin’s lymphomas is depicted in Table 2. A total of 661 patients were studied, consisting of 459 patients with leukemia, 165 patients with lymphoma, and 37 patients with plasma cell tumors. As shown in Table 2, the distribution of B4, B1, B2, slg, and PC-1 antigens, known to be restricted, are limited in their expression to tumors of B cell origin, including non-T

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Table 1. Reactivity of Monoclonal Antibodies

<table>
<thead>
<tr>
<th>Monoclonal Antibody</th>
<th>B Cell Reactivity</th>
<th>Cross-Reactivity</th>
<th>Molecular Weight (kD)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-(\text{ia})</td>
<td>All B cells, excluding plasma cell</td>
<td>Activated T cell, CFU-C, null cell (%), monocyte</td>
<td>29, 34</td>
<td>31</td>
</tr>
<tr>
<td>Anti-B4</td>
<td>All B cells following la^+ pre-B cell, excluding plasma cell</td>
<td>None</td>
<td>40, 80</td>
<td>27</td>
</tr>
<tr>
<td>Anti-CALLA</td>
<td>Pre-B cell</td>
<td>Granulocyte (weak)</td>
<td>100</td>
<td>21, 32–34</td>
</tr>
<tr>
<td>Anti-B1</td>
<td>All B cells following la^+ CAL-LA^+ pre-B cell, excluding plasma cell</td>
<td>None</td>
<td>35</td>
<td>20, 30</td>
</tr>
<tr>
<td>Anti-B2</td>
<td>B cells following la^+ B4^+ B1^+ cu^+ pre-B cell, lost with slgD with appearance of presecretory IgM</td>
<td>None</td>
<td>140</td>
<td>22</td>
</tr>
<tr>
<td>Anti-slg</td>
<td>All B cells following la^+ B4^+ B1^+ cu^+ pre-B cell, excluding plasma cell</td>
<td>None</td>
<td>IgG 150</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IgM 900</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IgD 150</td>
<td></td>
</tr>
<tr>
<td>Anti-T1</td>
<td>Subpopulation of B1^+ B cells</td>
<td>Mature T cell</td>
<td>67</td>
<td>35, 36</td>
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<tr>
<td>Anti-T10</td>
<td>Activated B cell and plasma cell</td>
<td>Prothymocyte and thymocyte, activated T cell</td>
<td>45</td>
<td>37, 38</td>
</tr>
<tr>
<td>Anti-PCA-1</td>
<td>Plasma cell</td>
<td>Granulocyte, monocyte (weak)</td>
<td>24</td>
<td>28</td>
</tr>
<tr>
<td>Anti-PC-1</td>
<td>Plasma cell</td>
<td>None</td>
<td>28</td>
<td>29</td>
</tr>
</tbody>
</table>
cell acute lymphoblastic leukemia (non-T ALL), lymphoid blast crisis of chronic myelocytic leukemia (CML-BC), B cell chronic lymphocytic leukemia (B-CLL), and B cell non-Hodgkin’s lymphomas. In contrast, these antigens are not expressed on 37 patients with T cell tumors, including acute lymphoblastic leukemia (T-ALL), chronic lymphocytic leukemia (T-CLL), and T lymphomas. Moreover, all samples from 154 patients with myeloid leukemias, including acute myeloblastic (AML) or myelomonoblastic leukemia (AMMOL) and chronic myelocytic leukemia in stable phase (CML), were negative.

The B4 antigen has the broadest expression of the B cell-restricted antigens, as the vast majority of B cell tumors are reactive with anti-B4. The B1 antigen displays a similar distribution to B4 except that those leukemias that appear earliest in ontogeny, i.e., non-T ALL and CML in lymphoid blast crisis, often lack B1. The B2 antigen, on the other hand, is expressed more narrowly on B cell malignancies, i.e., rarely on non-T ALL (5%), rarely if ever on lymphoid CML-BC (0%), on most B-CLL (90%), and on 68% of B cell lymphomas. Surface immunoglobulin (slg), although expressed on over 80% of B cell CLL and lymphomas, is not expressed on either the earliest non-T ALL and lymphoid CML-BC or the most differentiated tumors. The PC-I antigen, the most restricted of the B cell antigens, is wholly absent from B cell tumors, except for plasma cell tumors. These studies support the view that the B cell-restricted B4, B1, B2, slg, and PC-I antigens are expressed only on distinct subgroups of B cell tumors.

A panel of monoclonal antibodies directed against B cell-associated antigens was also examined for reactivity on this same group of tumor cells (Table 2). The

**Table 2. Expression of B Lineage Antigens on Leukemias and Lymphomas**

<table>
<thead>
<tr>
<th>Leukemias</th>
<th>No. of Patients</th>
<th>Percentage of Patients Expressing Antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B Cell Restricted</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B4</td>
</tr>
<tr>
<td>B cell</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-T ALL*</td>
<td>117</td>
<td>94</td>
</tr>
<tr>
<td>CML-BC†</td>
<td>12</td>
<td>92</td>
</tr>
<tr>
<td>CLL‡</td>
<td>153</td>
<td>99</td>
</tr>
<tr>
<td>T cell</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALL</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>CLL</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Myeloid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AML/AMMOL§</td>
<td>148</td>
<td>0</td>
</tr>
<tr>
<td>CML‖</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Lymphomas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B cell</td>
<td></td>
<td>151</td>
</tr>
<tr>
<td>T cell**</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Plasma cell tumors††</td>
<td>37</td>
<td>6</td>
</tr>
</tbody>
</table>

*CALLA positive and CALLA negative non-T acute lymphoblastic leukemia.
†Chronic myelocytic leukemia in lymphoid blast crisis.
‡Chronic lymphocytic leukemia.
§Acute myeloblastic and acute myelomonoblastic leukemia.
‖Chronic myelocytic leukemia in stable phase.
¶B cell lymphomas include nodular and diffuse poorly differentiated (N and D-PDL), nodular mixed (NM), and large cell non-Hodgkin’s lymphomas.
**T cell lymphoblastic lymphoma.
††Plasma cell tumors include Waldenström’s macroglobulinemia, myeloma, plasma cell leukemia (PCL), and plasmacytoma.
‡‡Two of 40 non-T ALL patients tested.
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Ia-like antigen (Ia) is expressed on nearly all B cell leukemias and lymphomas, but, in addition, is also found on 25%–33% of T cell leukemias and lymphomas and the majority of myeloid leukemias. The CALLA antigen is expressed on most (88%) non-T ALL, but in contrast, is only expressed on a smaller subset (<10%) of other B cell leukemias and 33% of B cell lymphomas. Although not found on AML or AMMOL of myeloid derivation, the CALLA antigen is expressed on a fraction of T cell leukemias and lymphomas (10%–20%), as previously described.13 The T1 antigen, known to be expressed on mature T lymphocytes and a fraction of thymocytes,36,37 is also present in the overwhelming majority of patients with B-CLL (94%). This distribution is rather restricted, as only cells from a small fraction of B cell lymphomas (16%) are reactive. The T10 antigen is similarly present on malignancies of T lineage as well as on the B-lineage plasma cell neoplasms. Finally, the PCA-1 antigen, which is strongly expressed on plasma cell tumors, is rarely (3%) and only weakly expressed on tumor cells isolated from patients with myeloid leukemias. Although the latter group of antigens are not B-lineage restricted, they are nonetheless useful, as their expression at times is limited to distinct stages of neoplastic B cell differentiation.

Utilizing the panel of B cell restricted and associated antigens, it is possible to subdivide the non-T ALL into four subgroups. Of 117 patients with non-T ALL who were analyzed in the present study, 110 (94% patients) coexpressed Ia and B4 (Table 3) and could therefore be subdivided into the following subgroups: 11 were Ia'B4'CALLA'B1'cyto-μ' (10% patients); 33 expressed Ia'B4'CALLA'B1'cyto-μ' (30% patients); 44 bore Ia'B4'CALLA'B1'cyto-μ' (40% patients); 22 had the Ia'B4'CALLA'B1'cyto-μ' cell surface phenotype (20% patients). The latter four groups defined virtually all of the non-T ALL studied. A few patients lacking both T cell and myeloid antigens and presumably non-T had unusual phenotypes, including Ia'B4', Ia'B4', and Ia'B4'. The latter patients accounted for <5% of the total population of non-T ALL analyzed. The pattern of cell surface antigens seen on lymphoid CML-BC tumor cells is similar to that described above for non-T ALL, suggesting that this tumor also corresponds to the earliest stages of malignant B cell ontogeny.

Examination of the antigen intensity on each of these subgroups suggests that these antigens appear sequentially in B cell ontogeny (Table 4). The Ia antigen is strongly expressed (Fig. 1C) on all of the early B cell tumors. The B4 antigen, although varying from moderate to strong in intensity (Fig. 1, B and C), is clearly positive on nearly all non-T ALL; moreover, it is expressed on non-T ALL to a greater degree than on any other B cell tumor. The CALLA antigen is moderately to strongly expressed on most non-T ALL, whereas the B1 antigen is weakly to moderately (Fig. 1, A and B) expressed on approximately 60% of these tumors. As noted in Table 3, only 2 of 40 non-T ALL studied with anti-B2 were B2 antigen positive. Whether this represents another “late” subgroup is

### Table 3. Expression of B Lineage Antigens on B Cell Tumors

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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-T ALL*</td>
<td>117</td>
<td>100</td>
</tr>
<tr>
<td>CML-BC†</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>CLL‡</td>
<td>153</td>
<td>98</td>
</tr>
<tr>
<td>Lymphomas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-POL§</td>
<td>52</td>
<td>100</td>
</tr>
<tr>
<td>N-POL¶</td>
<td>29</td>
<td>96</td>
</tr>
<tr>
<td>NM¶</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Large cell</td>
<td>60</td>
<td>90</td>
</tr>
<tr>
<td>Waldenstrom**</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>Myeloma</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>PCL††</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Plasmacytoma</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

*CALLA positive and CALLA negative non-T acute lymphoblastic leukemia.
†Chronic myelocytic leukemia in lymphoid blast crisis.
‡Chronic lymphocytic leukemia.
§Diffuse poorly differentiated lymphocytic lymphoma.
¶Nodular poorly differentiated lymphocytic lymphoma.
‖Nodular mixed lymphoma.
**Waldenstrom’s macroglobulinemia.
††Plasma cell leukemia.
†††Two of 40 non-T ALL patients tested.
still to be defined. Surface Ig, as well as the plasma cell antigens PC-i and PCA-i, are absent on non-T ALL. Utilizing this panel of monoclonal reagents, one can therefore define heterogeneity within the histologic category of non-T ALL, i.e., Ia, B4, and CALLA have the broadest expression, B1 is expressed on fewer patients, and B2, slg, PCA-1, and PC-1 are rarely if ever positive.

In addition to the above, there is an additional level of heterogeneity that relates to intensity of antigen expression within each phenotypic subgroup. For example, within the Ia+B4+CALLA+ B1+cyto-μ - subgroup, the expression of B1 varies from barely detectable to strong. The precise meaning of this variability in antigen intensity within a histologically defined group is unclear.

The majority of B cell leukemias and lymphomas correspond to the midstages of malignant B cell differentiation, which are heralded by the acquisition and loss of new antigens, including B2 and the isotypes of slg, and in some cases, T1 and CALLA. The coexpression of Ia, B4, B1, B2, and slg defines the phenotype of most B-CLL (Table 3). Tumor cells from most patients with B-CLL express either very faint cell surface IgM or coexpress IgM and IgG and wholly lack the CALLA cell surface antigen. Of great interest is the observation that the majority of patients with B-CLL (94%) also express the T cell-associated antigen, T1. Although this represents the most common phenotype seen for B-CLL, it is clear that subgroups of B-CLL were defined that lack detectable slg and/or B2. These observations again show that, within a morphologically defined group of patients with B-CLL, subgroups can be identified based on immunologic cell surface heterogeneity. The antigen intensity on B-CLL also distinguishes these tumors from non-T ALL and B lymphomas (Table 4). As was true for the non-T ALL, the Ia antigen can be moderately to strongly expressed on B-CLL; however, the B4 antigen is more weakly expressed on B-CLL than on non-T ALL. In contrast to non-T ALL, where the B1 antigen can be weak or absent, most B-CLL moderately express the B1 antigen. The CALLA antigen is lost and the B2 antigen appears to be acquired at the B-CLL stage. Both integral surface Ig and B2, however, are invariably only weakly expressed when present. The plasma cell restricted (PC-1) and associated (PCA-1) antigens are wholly absent on these B-CLL tumors.

B cell diffuse poorly differentiated lymphocytic lymphomas of both the nodular (NPDL) and diffuse (DPDL) subtypes also correspond to the midstages of malignant B cell differentiation (Table 3). Like the earlier B-CLL, the overwhelming majority of these tumors coexpress Ia, B4, B1, B2, and slg. In contrast to B-CLL, the nodular PDL (69%) also express the CALLA antigen, and relatively few (10%) express the T1 antigen. CALLA, it should be noted, is rather unique in that it is expressed on the non-T ALL, disappears at the stage of B cell CLL, and reappears in a significant proportion of B cell lymphomas. Within PDL, the diffuse can be distinguished from the nodular subtypes by their lack of cell surface CALLA expression. The intensity of expression of these antigens on the cell surface can be of further differential value (Table 4). Although B-CLL and DPDL strongly express Ia and lack CALLA, PCA-1, and PC-1, they differ in that the expression of B4 and T1 is of lesser intensity and the expression of B1, B2, and slg antigens of greater intensity on DPDL than on B-CLL. The nodular mixed (NM) lymphomas are phenotypically identical to NPDL, including the expression of cell
surface CALLA antigen. The moderate to strong expression of B2 on NPDL and NM lymphomas places these tumors at the mid-range of neoplastic B cell differentiation.

The terminal stage of B cell differentiation appears to begin with the transformed (B lymphoblast) B cell and proceeds to the final step of B cell differentiation, the plasma cell (Table 3). The large cell lymphomas coexpress Ia, B4, B1, and slg, but lack CALLA and B2. Also absent are the plasma cell antigens PCA-1 and PC-1. Although the intensity of Ia and B1 expression remains strong, that of B4 and slg is weaker on these tumors than on the above-mentioned NPDL, DPDL, and NM B cell tumors (Table 4). Waldenstrom cells demonstrate coexpression of Ia, B4, and B1, with only 50% of these cells expressing slg and none B2 or CALLA cell surface antigens. The Waldenstrom cell is the first B lineage tumor to express either the plasma cell-associated antigens T10 and PCA-1 or the plasma cell-restricted antigen PC-1. The intensity of the Ia, B4, B1, and slg antigens is less on the Waldenstrom cell than on large cell lymphomas. Nonetheless, the coexpression of the B cell (Ia, B4, B1, slg) and plasma cell (T10, PCA-1, PC-1) antigens supports the notion that the Waldenstrom tumor cell is in "transition" from a malignant B cell to a malignant plasma cell. Lastly, all myelomas, whether obtained as tumor cells from the bone marrow, as plasma cell leukemias, or as solitary plasmacytomas, lack most B cell-restricted and B cell-associated antigens. These terminally differentiated B cells uniformly express T10, PCA-1, and PC-1. Both PCA-1 and PC-1 are moderately to strongly expressed on plasma cell tumors (Table 4) and represent the most discrete distribution of any of the antigens.

**DISCUSSION**

In the present report, we have characterized approximately 700 leukemias and lymphomas with a panel of monoclonal antibodies that defines B cell-associated and B cell-restricted antigens in an attempt to define a broad overview of their distribution on normal tissues, and more importantly, to relate B cell tumors to normal B cell differentiation. The present report is the first comparison of recently described antigens, i.e., B4, PCA-1, and PC-1, with other previously described antigens (B1, B2, etc.) on a large number of malignancies and allows an appreciation of B cell tumor heterogeneity that could not be obtained with either single reagents or small numbers of patients. Antigens that were previously shown to be restricted to normal B lymphocytes were similarly expressed only on B cell malignancies. In contrast, antigens that were B cell associated were also found on tumors of other lineages. The expression of these B cell restricted and associated antigens on B cell leukemias and lymphomas revealed distinct patterns of antigen expression within each group of histologically defined tumors. Tumor cells of B cell origin are capable of being divided into three major subgroups that correspond to the level of differentiation of the malignant tumor cell. These subgroups include the pre-B cell stage, the mid-B cell stage, and the secretary B cell stage. A hypothetical model relating the malignant B cell to its normal cellular counterpart on the basis of cell surface phenotype is depicted in Fig. 2.

The non-T ALL examined in this study appear to correspond to the pre-B cell stage of differentiation. Although morphologically identical, tumor cells from patients with non-T ALL could be subdivided into phenotypically defined subgroups on the basis of Ia, B4, CALLA, B1, and cyto-μ. The B cell origin of these tumors is now based on several lines of evidence. Initially, investigators demonstrated that tumor cells from approximately 15% of patients with non-T ALL contain μ-chains without light chains and correspond phenotypically to pre-B cells. Further studies demonstrated that approximately 50% of non-T ALL expressed the B1 antigen, suggesting that they were of B cell lineage. The Ia"CALLA"B1- non-T ALL were next shown to be of B cell origin, as they could be induced by tumor promoters or conditioned media to express B1 and cyto-μ. The more recent observation that almost all non-T ALL expressed the B cell-restricted B4 antigen provided additional evidence that...
all of these tumors were of B cell origin. Recent data demonstrating that all non-T ALL studied exhibited immunoglobulin gene rearrangements characteristic of B cells, provided additional evidence that virtually all non-T ALL were of B cell lineage.

The orderly acquisition of antigens on non-T ALL supports the view that these leukemic cells provide a model of normal pre-B cell differentiation. The observation that all non-T ALL strongly express the Ia antigen indicates that Ia develops very early on B cell precursors. B4 is most strongly expressed on the non-T cell ALL and CML in lymphoid blast crisis and decreases in intensity on more mature tumors. The CALLA antigen is expressed on slightly fewer non-T ALL than B4. It appears to follow B4 in B cell ontogeny. The B1 antigen follows the appearance of CALLA and is expressed on only 50% of non-T ALL. Hokland et al. have recently demonstrated that approximately half of CALLA + cells isolated from fetal lymphoid organs and normal bone marrow coexpress B1, and only 20% of these cells are cyto-μ. This observation, combined with earlier studies demonstrating that CALLA Ia - non-T ALL can be driven to express both B1 and cyto-μ, suggests that these are pre-B cells that precede the conventional cyto-μ pre-B cell stage in normal B cell ontogeny. The B1 antigen appears to be relatively weak on these tumor cells; it is strongly expressed only on the most mature non-T ALL, concomitant with the acquisition of cyto-μ chains. Thus, several B cell-restricted antigens appear prior to the conventional cyto-μ pre-B cell and develop in a sequential fashion. The quantitation of antigen intensity for each of these markers may provide a model for their appearance in normal B cell ontogeny.

The tumors corresponding to the midstages of B cell differentiation are heralded by the appearance of new antigens and more mature morphology. The tumors included in these stages of B cell differentiation include B cell CLL and poorly differentiated lymphomas (Fig. 2). Multiple investigators have demonstrated the presence of T1 on B-CLL. In our experience, B cell CLL phenotypically correspond to an immature B cell, which coexpresses Ia, B4, B1, B2, slg, and T1. Although normal B cells have not been thought to express the T1 antigen Caligaris-Cappio et al. have isolated cells that are surface immunoglobulin positive (slg +) and mouse red blood cell receptor positive (MRBC +) from normal lymph node and tonsil, but not from peripheral blood. In contrast, using dual fluorescent techniques, we can identify a small subset of circulating peripheral blood B cells and B cells isolated from tonsil and spleen that coexpress the B1 and T1 antigens. Although these cells coexpress slg, B2, and B4, they differ from peripheral blood cells because they do not express the C3 B receptor. The B-CLL cell, therefore, may be the neoplastic counterpart of this small subpopulation of normal B lymphocytes and may not be reflective of the majority of circulating B cells. Moreover, although virtually all B-CLL also express Ia, B4, and B1, small numbers do lack slg, B2, and T1. Whether this defines a degree of heterogeneity within this histologically comparable group is not clear. In addition, whereas CLL appears to be a relatively homogeneous clinical and morphological entity, significant heterogeneity of age at presentation, organ localization, and disease course is seen, which may relate to its phenotypic diversity.

The diffuse and nodular PDL also correspond to stages within the mid-range of B cell differentiation. These cells appear to correspond to the virgin B cell, as evidenced by their coexpression of Ia, B4, B1, B2, IgM, and IgG. In contrast to B-CLL cells, the intensity of immunoglobulin is considerably stronger, as is the intensity of B1 expression. The B2 and B4 antigens, although slightly less intense than on B-CLL cells, do not define significant heterogeneity. Nodular PDLs appear to uniformly express the CALLA antigen, whereas DPDL does not. These observations suggest that nodular and diffuse PDL may be derived from distinct populations of B cells. Moreover, previous experiments in our laboratory have shown that the CALLA antigen may be very weakly expressed on the germinal center cell within the secondary follicle of normal lymph node, providing further evidence that nodular PDLs are of germinal center cell origin. It is important to note that there is clear-cut heterogeneity of antigen expression within the poorly differentiated lymphomas defined by the presence or absence of slg and/or B2. For example, the majority of NPDL coexpress slg and B2, but there are those subgroups that lack each of the antigens. These observations on tumors corresponding to the stages of mid-B cell differentiation complement the observations of Warnke and Link, namely, that histologically defined tumors are in fact phenotypically heterogeneous. Moreover, the heterogeneity of antigen expression suggests that histologically comparable B cell tumors may be derived from both unique and distinct subpopulations of B lymphocytes. Clearly, an understanding of the function of many of these cell surface antigens will go far in examining some of the heterogeneity noted.

The third and final stage of B cell differentiation includes the secretory cells of B lineage. Tumors corresponding to this stage include diffuse and nodular large cell lymphomas, Waldenstrom's macroglobulin-
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strom's tumors, in contrast, coexpress Bi and B4, as to express little or no B2 and large amounts of slg and B1. This phenotypic pattern is similar to the "transformed" B lymphoblast obtained by treating normal B cells with pokeweed mitogen (PWM). Waldenstrom's tumors, in contrast, coexpress B1 and B4, as well as the plasma cell-associated antigens PCA-1 and PC-1. The observed phenotype supports the notion that this cell represents a "transition" from the B lymphocyte to the lymphoplasmacytoid cell. The loss of B1, B2, and B4, coupled with the acquisition of PCA-1 and PC-1 on plasma cell dyscrasias, correlates with the in vitro pokeweed mitogen driven induction of normal B cell differentiation, in that loss of the B1 and B4 antigens occurs concomitant with the acquisition of clg, surface T10, PCA-1, and PC-1 as well as plasmacytoid morphology. This stage of B cell differentiation appears to be much more homogeneous within each of the histologically defined subgroups than has been seen at the early and mid-stages of B cell differentiation.

In the present study, we have utilized single cell suspensions isolated from patients with leukemias and lymphomas in an attempt to phenotypically correlate the malignant B cell with its normal B cell differentiative counterpart. In contrast to the immunoperoxidase technique, which preserves tissue architecture but does not permit accurate assessment of either antigen intensity or localization on the cell surface, flow cytometry of single cell suspensions of tumor cells allows one to both determine the cell surface phenotype and assess antigen intensity. One pitfall of indirect immunofluorescence and flow cytometric analysis is that small amounts of antigen may be present on individual tumor cell surfaces that are not detected by this technique. In addition, the antigen intensity on the tumor cell surface depends on both the number of antigenic determinants per cell and the size of the tumor cell, requiring one to either compare tumor cells of identical size or to calculate mean fluorescence density. In the present report, considering the large number of patients studied, we have attempted to assess antigen density on tumor cells of similar size by selected cell size gating on the fluorescence activated cell sorter. Attempts have also been made to examine cells visually with a fluorescent microscope in order to confirm our flow cytometric observations on cell antigen density. We clearly believe that the examination of tumor cells in single suspension and in tissue section by immunologic techniques will complement one another and existing techniques. New insight into diagnoses and biologic behavior of these B cell leukemias and lymphomas is likely to occur through a combination of approaches. The utilization of cell surface markers as defined above should permit us to not only define these tumors as cells of B lineage, but also to accurately identify their state of differentiation and their relation to normal B cell subpopulations.

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