An Antigen Common to Chronic Lymphocytic and Hairy Cell Leukemia Cells Not Shared by Normal Lymphocytes or by Other Leukemic Cells

By Julia F. Agee, Fred A. Garver, and Guy B. Faguet

Rabbit xenoantisera and mouse monoclonal antibodies prepared against human chronic lymphocytic leukemia (CLL) B cells were found to react against a single polypeptide chain with a mol wt of 69 kd found on leukemic cells of all CLL (N = 40) and B type hairy cell leukemia (HCL) patients (N = 9) examined. This common CLL-associated antigen (cCLLa) was not detectable on circulating T or B lymphocytes, thymocytes, lymph node and splenic lymphocytes, or bone marrow leukocytes from normal persons. In addition, the cCLLa was not detectable on cultured T or B lymphoblastoid cell lines or on malignant cells from other forms of lymphocytic or myelocytic leukemia. Non-Hodgkin's lymphoma cells also failed to express the antigen. Autologous cultured lymphoblastoid cell lines were established from residual normal B cells from a CLL patient whose cells were used to generate one of the antisera.

Despite the expression of normal antigens on the surface of neoplastic hematopoietic cells has facilitated the elucidation of the cellular origin and differentiation pathways of these cells. Thus, leukemias and lymphomas, traditionally grouped according to histology, cell morphology, and cytochemistry, can now be subclassified based on their membrane markers. Cells from patients with acute lymphoblastic leukemia (ALL), for example, exhibit T or B lymphocyte markers or lack both, the latter being associated with a better prognosis.

Antibody Absorption Studies

Absorption of the antibody with these cultured polyclonal B cells did not affect the anti-CLL activity, which suggests that the cCLLa is not HLA related. Unlike the T cell differentiation complex gp65-71, the cCLLa was not expressed on fetal or cord blood lymphocytes or on mitogen-stimulated normal lymphocytes and was distinct from the antigen recognized by the LEU-1 antibody in spite of their similar mol wt. The cCLLa was also determined to be unrelated to the human T cell leukemia lymphoma virus (HTLV-I). One of the monoclonal antibodies generated against the cCLLa was a complement binding IgG which exhibited highly selective cytotoxic activity against 100% of cells bearing the cCLLa. Such an antibody might prove clinically useful in early diagnosis and treatment of CLL and HCL.

MATERIALS AND METHODS

Cells. Normal and CLL lymphocytes and blasts from leukemic patients were prepared from heparinized peripheral blood by ficoll-Hypaque gradient modified to remove monocytes. CLL cells were obtained from patients with B cell lymphoproliferations with circulating lymphocyte counts >30,000/μL. Promyelocytes were obtained from the buffy coat fraction after dextran sedimentation of venous blood from patients with acute promyelocytic leukemia (APrML). Thymic, splenic, lymph node, and bone marrow cells were teased from fresh biopsy specimens. Erythrocytes were removed from bone marrow samples by hypotonic lysis. B-enriched and T-enriched lymphocyte populations were separated according to the method of Weiner and colleagues.

B-enriched cells were in some instances prepared by the HEAC rosetting technique. B-enriched and T-enriched lymphocyte preparations were consistently >80% and >90% phenotypically homogeneous, respectively. Myeloid and monocytoid cell contamination averaged <3%, respectively, as illustrated in Table 1. Lymphoid cell lines were established from peripheral blood lymphocytes of a CLL patient and of two patients with HCL as outlined by Moore.

Lymphoblastoid cell lines of B-cell (LS-1, MA-1, SA-1, KS-1, DBA-2, DBA-3, B6, B110, B411, RPMI-1788, IM9, SB, and QIK) or T cell lineage (HSB, CEM, TAR, and MOLT-4), the pluripotent K562 cell line, and a fetal lymphoid cell line were cultured in appropriate media and stored in liquid nitrogen. Target cell preparations were characterized by cytomorphology and by surface marker phenotyping prior to assay. Cell subset distribution in cell preparations is indicated in the text when appropriate.

Cell Phenotyping. SIgs were determined by direct immunofluorescence using fluorescein-conjugated antisera specific for IgM, IgG, IgA, and IgD heavy chains and for κ and λ light chains (Meloy Laboratories, Springfield, Va). Other surface determinants were assessed by indirect immunofluorescence assay (IFA) using appropriate monoclonal antibodies commercially available: (OKT series, Ortho Diagnostics, Raritan, N J; LEU series, Becton Dickinson, Mountain View, Calif; and My4, B2, MO2, and J-5, Coulter Immunology, Hialeah, Fla).

Anti-CLL antibodies. Xenogeneic antisera were produced in New Zealand white rabbits by biweekly intravenous (IV) injections
of 1 x 10^6 viable lymphocytes each from a single patient with B cell type CLL for 10 weeks with a final booster injection 2 weeks later. The antisera were heat-inactivated: 10 μL Trasybol (Aprotinin, Mobay Chemical Corp, NY) protease inhibitor was added, and anti-ABO antibodies were removed by absorption with 5 vol of AB erythrocytes. The sera were then individually absorbed with peripheral blood lymphocytes (PBL) (2.0 x 10^7/mL of antiserum) from each of three normal individuals who shared two or more HLA antigens with the antiserum-generating CLL donor. The combined absorptions accounted for all of the HLA A, B, and C antigens with the antisera-generating CLL donor. The combined antisera were then individually absorbed with peripheral blood lymphocytes (PBL) (2.0 x 10^7/mL of antiserum) from each of three normal individuals who shared two or more HLA antigens with the antiserum-generating CLL donor.

### RESULTS

**Antibody reactivity of antiserum aCLL/aB**. Antiserum aCLL/aB was titrated against CLL and normal B lymphocyte target cells as shown in Fig 1. Increasing the antisera concentration-target cell ratio resulted in greater lysis of CLL cells than of B cells. At the maximum amount of antisera added, 50 μL, lysis of B cells reached a plateau of 60%, whereas 20 μL of antisera achieved 100% lysis of malignant CLL cells (cells bearing monoclonal IgG). These results suggested that the antiserum contained different antibodies reacting with both CLL cells and normal B cells.
Fig 1. Titration of antiserum aCLL/aB by microcytotoxicity assay with 51Cr-labeled chronic lymphocytic leukemia (CLL) (o) or normal B (△) target cells (5 x 10^6 per well). Percentage of lysis is expressed as a function of antiserum volume per well.

or that CLL cells exhibited either greater density of an antigen shared by normal B cells or greater affinity for a antibody was ascertained by 51Cr-release antibody-dependent cytotoxic-
aCLL/aB (60 minutes at 15 °C) and centrifuged; cell-bound
antiserum or that CLL cells exhibited either greater density of an
antigen shared by normal B cells or greater affinity for a

Table 2. Cytotoxic Activity of Antiserum aCLL/aB After Absorption With Enriched Normal B Lymphocytes or CLL Cells

<table>
<thead>
<tr>
<th>Absorbing Cells</th>
<th>Normal B Lymphocytes</th>
<th>CLL Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ROC</td>
<td>TA</td>
</tr>
<tr>
<td>None</td>
<td>27</td>
<td>25</td>
</tr>
<tr>
<td>Normal B Cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ROC</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TA</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>GRE</td>
<td>5</td>
<td>8</td>
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<tr>
<td>DOR</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>GRI</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>GER</td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td>ME</td>
<td>27</td>
<td>24</td>
</tr>
<tr>
<td>CLL Cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIL</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>TAY</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>BRO</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>LO</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>THO</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

CLL, chronic lymphocytic leukemia.

Normal B-enriched or CLL cells (0.5 x 10^6) were incubated with antiserum aCLL/aB (60 minutes at 15 °C) and centrifuged; cell-bound antibody was ascertained by 51Cr-release antibody-dependent cytotoxicity assay. Results are shown as percentage of lysis of triplicate determinations (SEM <2%). Values <10% are considered negative.

Table 3. Reactivity of the aCLL and aB Antisera After Absorption With Autologous Normal B Lymphocytes

<table>
<thead>
<tr>
<th>Target Cells</th>
<th>Unabsorbed</th>
<th>Absorbed</th>
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</thead>
<tbody>
<tr>
<td>Autologous B Lymphocytes</td>
<td>aCLL</td>
<td>aB</td>
</tr>
<tr>
<td>AG</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>TA</td>
<td>0</td>
<td>72</td>
</tr>
<tr>
<td>BR</td>
<td>0</td>
<td>56</td>
</tr>
<tr>
<td>HAM</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>MIL</td>
<td>0</td>
<td>37</td>
</tr>
<tr>
<td>CLL Cells</td>
<td></td>
<td></td>
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<tr>
<td>WA</td>
<td>74</td>
<td>0</td>
</tr>
<tr>
<td>HU</td>
<td>100</td>
<td>7</td>
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<tr>
<td>BU</td>
<td>77</td>
<td>0</td>
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<td>BR</td>
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<td>0</td>
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<td>BUR</td>
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<td>OCO</td>
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<td>81</td>
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<tr>
<td>JAV</td>
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<td>34</td>
</tr>
<tr>
<td>SEN</td>
<td>91</td>
<td>43</td>
</tr>
<tr>
<td>DU</td>
<td>96</td>
<td>44</td>
</tr>
<tr>
<td>WAT</td>
<td>100</td>
<td>50</td>
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</table>

After absorption with autologous B lymphocytes isoCLL and αB antisera were used in the 51Cr-release assay against both enriched B or CLL target cells. Data are expressed as percentage of lysis of triplicate determinations (SEM <2%).
activity of the αB serum against both normal B cells and CLL cells initially reactive with the αB antiserum.

**Specificity of the αCLL antibody.** The specificity of the antibody preparation αCLL was further studied on a large panel of donors by both microcytotoxicity and immunofluorescence assays. As illustrated in Table 4, the αCLL antibody reacted with all 40 B-CLL patients tested with a range of

<table>
<thead>
<tr>
<th>Target Cells</th>
<th>Tests (N)</th>
<th>Total</th>
<th>Positive</th>
<th>Reactive Cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells bearing cCLLa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLL (B phenotypes)</td>
<td>40</td>
<td>40</td>
<td>92(7)</td>
<td></td>
</tr>
<tr>
<td>CLL cell line (SeD)</td>
<td>1</td>
<td>1</td>
<td>35(4)</td>
<td></td>
</tr>
<tr>
<td>HCL (B phenotypes)</td>
<td>9</td>
<td>9</td>
<td>46(5)</td>
<td></td>
</tr>
<tr>
<td>HCL cell lines (EH and HK)</td>
<td>2</td>
<td>2</td>
<td>63(5)</td>
<td></td>
</tr>
<tr>
<td>Cells not bearing cCLLa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal lymphoid cells (N = 77)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBL</td>
<td>25</td>
<td>0</td>
<td>0-4</td>
<td></td>
</tr>
<tr>
<td>T cells (97% [SEM 4] OKT3+)</td>
<td>10</td>
<td>0</td>
<td>0-4</td>
<td></td>
</tr>
<tr>
<td>B cells (85% [SEM 4] Slg+)</td>
<td>10</td>
<td>0</td>
<td>0-4</td>
<td></td>
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<tr>
<td>Thymocytes</td>
<td>2</td>
<td>0</td>
<td>0-4</td>
<td></td>
</tr>
<tr>
<td>Splenocytes</td>
<td>2</td>
<td>0</td>
<td>0-4</td>
<td></td>
</tr>
<tr>
<td>Lymph node cells</td>
<td>2</td>
<td>0</td>
<td>0-4</td>
<td></td>
</tr>
<tr>
<td>Bone marrow interface cells</td>
<td>4</td>
<td>0</td>
<td>0-4</td>
<td></td>
</tr>
<tr>
<td>Cord blood lymphocytes</td>
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<td>0</td>
<td>0-4</td>
<td></td>
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<tr>
<td>PWM-stimulated PBL</td>
<td>4</td>
<td>0</td>
<td>0-4</td>
<td></td>
</tr>
<tr>
<td>PHA-stimulated PBL</td>
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<td>0-4</td>
<td></td>
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<tr>
<td>CON-A-stimulated PBL</td>
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<tr>
<td>Malignant cells (N = 76)</td>
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<td>Leukemias</td>
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<tr>
<td>B lymphomas</td>
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<td>0-4</td>
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<tr>
<td>ALL (adulthood)</td>
<td>9</td>
<td>0</td>
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<tr>
<td>AML</td>
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<td>0</td>
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<td></td>
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<tr>
<td>AMMol</td>
<td>4</td>
<td>0</td>
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<tr>
<td>APrML</td>
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<td>0</td>
<td>0-4</td>
<td></td>
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<tr>
<td>CML in blastic crisis</td>
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<td>0</td>
<td>0-4</td>
<td></td>
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<tr>
<td>Non-Hodgkin’s lymphoma</td>
<td>24</td>
<td>0</td>
<td>0-4</td>
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<tr>
<td>PBL</td>
<td>19</td>
<td>0</td>
<td>0-4</td>
<td></td>
</tr>
<tr>
<td>T lymph node cells</td>
<td>6</td>
<td>0</td>
<td>0-4</td>
<td></td>
</tr>
<tr>
<td>Hodgkin’s disease</td>
<td>5</td>
<td>0</td>
<td>0-4</td>
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<tr>
<td>Lymph node cells</td>
<td>3</td>
<td>0</td>
<td>0-4</td>
<td></td>
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<tr>
<td>Splenocytes</td>
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<td>0</td>
<td>0-4</td>
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<td>Cell lines</td>
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<td>0-4</td>
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<tr>
<td>Fetal lymphoblastoid cell line</td>
<td>1</td>
<td>0</td>
<td>0-4</td>
<td></td>
</tr>
<tr>
<td>B lymphoblastoid cell lines</td>
<td>14</td>
<td>0</td>
<td>0-4</td>
<td></td>
</tr>
<tr>
<td>T lymphoblastoid cell lines</td>
<td>4</td>
<td>0</td>
<td>0-4</td>
<td></td>
</tr>
<tr>
<td>Pluripotent cell line (K-562)</td>
<td>1</td>
<td>0</td>
<td>0-4</td>
<td></td>
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</table>

Reactivity of the αCLL against normal and malignant cells was assayed by both 51Cr-release cytotoxicity and immunofluorescence staining and showed similar results. Only immunofluorescence data are shown here.

CLL, chronic lymphocytic leukemia; cCLLa, common CLL-associated antigen; HCL, hairy cell leukemia; PBL, peripheral blood lymphocytes; PWM, pokeweed mitogen; PHA, phytohemagglutinin; CON-A, concanavalin-A; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; AMMol, acute myelomonoblastic leukemia; APrML, acute promyelocytic leukemia; CML, chronic myelogenous leukemia.

*Reactive cells are reported as percentage (SEM) of 600 cells counted for each assay except negative controls, which are reported as range.

lysis of 64% to 100% and a range of fluorescence of 73% to 100%. Included in this group were three patients with prolymphocytic leukemia (PLL). SeD, a cell line derived from a patient with CLL also showed reactivity with the αCLL antibody. In addition, cells from nine patients with Slg-positive HCL were positive for the cCLLa, as were cell lines EH and HK derived from two HCL patients (Faguet and Agee, manuscript in preparation). To ascertain the identity of the antigenic determinant determined by the αCLL on CLL and HCL cells, the following studies were done: Saturating concentrations of αCLL were incubated with 10^6 B-CLL cells (93% monoclonal Slg positive) before and after absorption for 60 minutes at 15 °C with CLL or HCL cells or with T-enriched (96% OKT3 positive) and B-enriched (83% Slg positive) lymphocytes from a normal donor at concentrations of 1.5 x 10^9/mL and assayed by IFA. CLL cell reactivity with unabsorbed antibody (92%) was completely abrogated by absorption with CLL (2%) or HCL cells (2%), but was unaffected by absorption with either normal T (93%) or B (92%) lymphocytes. Similar experiments using cells from two patients with HCL as target cells yielded comparable results. Neither CLL nor HCL cells with non-B phenotypes were available for testing.

PBL, T cells, and B cells from normal subjects showed only background reactivity with the αCLL antibody. Likewise, normal thymus, spleen, and lymph node lymphocytes were equally unreactive, as were bone marrow aspirates from four patients with anemia of chronic illness (Table 4). To evaluate whether the cCLLa represented a B cell differentiation antigen not expressed on mature B cells, the αCLL antisera was reacted against cells likely to express such differentiation antigens, including cord blood lymphocytes, and 3-, 5-, and 7-day cultures of mitogen-stimulated normal blood lymphocytes. As shown, lymphoid cells from all ten source categories and marrow interface cells failed to express the αCLL antigen. The possible expression of the cCLLa on malignant cells of other types of leukemias was also investigated. Leukemias studied were: cALLa-positive and cALLa-negative acute lymphoblastic leukemia (ALL), acute myeloblastic leukemia (AML), acute myelomonoblastic leukemia (AMMol), acute promyelocytic leukemia (AprML), and chronic myelogenous leukemia (CML) in blast crisis. As shown, none of the 32 leukemic patients examined expressed the cCLLa. Likewise, blood and lymph node lymphocytes from 14 patients with non-Hodgkin’s lymphoma as well as lymph node lymphocytes and splenocytes from three patients with advanced Hodgkin’s disease did not express the cCLLa antigen. Last, 14 lymphoblastoid B cell lines, 4 lymphoblastic T cell lines, 1 fetal lymphoid cell line, and 1 pluripotent hematopoietic cell line also failed to express the cCLLa. Selective expression of the cCLLa described above has been confirmed by mouse monoclonal antibodies (Faguet and Agee, manuscript in preparation).

**Isolation and partial characterization of the cCLLa.** From 2.5 x 10^6 CLL cells (B phenotype), 52 mg of membrane protein were recovered with a specific antigen activity of 0.1 U/μg as judged by cytotoxicity inhibition assay expressed as percentage of inhibition of 1 μg of αCLL IgG by 1 μg of membrane protein. Of this, 2.75 mg protein
was recovered after passage through an αCLL-Sepharose affinity column. The immunoabsorbed-bound material had a specific antigen activity of 71 U/μg (710% cCLLa enrichment). Further fractionation by ion-exchange chromatography on a DEAE-cellulose column revealed three distinct peaks: Peak 1 had no activity at concentrations up to 150 μg per well; peak 2 consisted of 1.5 mg protein with cCLLa activity of 156 U/μg protein (1,560-fold enrichment). Peak 3 consisted of a small component (0.8 mg protein) with low activity (2.7 U/μg protein). The specificity of peak 2 is shown by the fact that it did not inhibit lysis of B cells by the αB antiserum at concentrations up to 1,000 times that required for complete inhibition of αCLL-induced lysis of CLL cells. SDS-PAGE gels of peak 2 revealed a major band with an estimated mol wt of 69 kd both in the presence or absence of β-mercaptoethanol and heating (Fig 2). Two experiments served as controls: (a) Using the above procedure, B cell membranes prepared from 2.5 × 10^10 B lymphocytes were applied to an αCLL-IgG-Sepharose affinity column; (b) an equivalent CLL membrane preparation was applied to an antihemoglobin A-IgG-Sepharose column equal in size to the αCLL-IgG-Sepharose affinity column. The immunoabsorbed material recovered from either column (0.56 mg and 0.84 mg of protein, respectively) showed no cCLLa activity at concentrations up to 150 μg of protein per well. In addition, further fractionation of these materials on a DEAE-cellulose column revealed only one peak (peak 1) with no cCLLa reactivity.

Further characterization of the cCLLa by monoclonal antibodies. The identity of the cCLLa was further ascertained as follows: Aliquots of 10^6 B-CLL cells enriched normal T and B lymphocytes were either preincubated with αCLL or not prior to exposure to a panel of monoclonal antibodies. Fresh cells were phenotyped as described in the Materials and Methods section. Cells preincubated with αCLL were assayed by indirect IFA using fluoresceinated goat anti-mouse or goat anti-rabbit antibodies to monitor mouse monoclonal and rabbit xenogenic antibody binding, respectively. As shown in Table 1, the phenotype of fresh CLL cells and of fresh enriched circulating normal T and B lymphocytes demonstrated homogeneity of lineage. Fresh cells from each subset exhibited only background reactivity when exposed to J-5 and HTLV-1 antibodies (a generous gift from Dr D. Bolognesi, Duke University, Durham, NC). As expected, the LEU-1 antibody reacted with 88.3% of fresh CLL cells and with 92% of fresh T cells and showed background reactivity with fresh B cells. In contrast, the CLL-1 and αCLL antibodies reacted with 85.1% and 82.5%, respectively, of fresh CLL cells but did not react with either fresh normal T or B lymphocytes. The reactivity of LEU-1 with target CLL cells (88.3%) and with normal T lymphocytes (92%) was not altered by preincubation with αCLL (83.9% and 95.3%, respectively). In contrast, CLL-1 reactivity with target CLL cells (85.1%) was completely abrogated (2.9%) by preincubation with αCLL. That competitive binding to the target antigen by αCLL accounts for the abrogation of CLL-1 reactivity is supported by the use of goat anti-rabbit second antibody. Moreover, experiments in which preincubation of the CLL-1 with purified cCLLa completely abrogated its reactivity with target CLL cells demonstrate that the target antigen CLL-1 is indeed cCLLa (manuscript in preparation). Using a large panel of normal and neoplastic cells, CLL-1 reactivity has been demonstrated to parallel that of αCLL (Faguet and Agee, manuscript in preparation). As expected from their respective specificities for antigens other than the cCLLa, reactivity of the other antibodies of the panel was not altered by preincubation with αCLL and is shown in Table 1 as controls. The above observations strongly suggest nonidentity between the cCLLa and LEU-1 antigens and clearly demonstrate that the cCLLa is the common antigenic determinant for both CLL-1 and αCLL.

DISCUSSION

The purpose of the present study was to explore the possible existence of distinct antigenic markers on CLL cells. Sequential absorption of the initial CLL antiserum with AB erythrocytes and HLA-matched peripheral blood lymphocytes resulted in a serum devoid of antibody activity against RBCs and T cells but retaining cytotoxicity against CLL cells and, to a lesser extent, B cells. Differential absorption with a panel of normal enriched B cells or CLL cells of B lineage suggested that the antiserum contained antibodies to an antigen found on 20% to 70% of B cells from ~50% of normal subjects and B-CLL patients and also antibodies against an antigen present on CLL cells but not on normal B lymphocytes. The different susceptibility of the two cell populations to antiserum αCLL/αB is attributed to two distinct antigens rather than to a different density of the same antigen on the two cell populations. This view is supported by the following observations: (a) distinct reactivity patterns after selective absorption with normal B or CLL cells; (b) persistence of anti-CLL activity after absorption with fivefold excess of B lymphocytes bearing free B antigen; (c) failure of autologous normal B lymphocytes to absorb anti-CLL activity, and (d) absence of cCLLa in normal B lymphocytes.
membrane preparations as demonstrated by chromatography studies and binding blocking experiments.

The cCLLa was expressed on most cells from all CLL patients studied but not on peripheral blood lymphocytes, enriched T and B cells from normal subjects, lymphocytes isolated from the thymus and spleen, or on other hematopoietic cells. However, these findings do not rule out the possibility that the antigen may be expressed by a minor subset of normal lymphocytes most susceptible to neoplastic transformation or that it is only transiently expressed early during differentiation, at a time when lymphocytes may be particularly susceptible to transformation and maturation arrest. This is consistent with the findings that CLL cells appear to be arrested in differentiation at an intermediate step between the pre-B and mature B lymphocyte stages.23

The cCLLa was not detected on adult or fetal lymphoid cells or on cultured lymphoblasticoid cell lines, in contrast to other xenoantisera generated against CLL.6,22 In addition, retention of anti-CLL reactivity after absorption of αCLL with autologous residual normal B lymphocytes (fully histocompatible with respect to the normal antigens on the patient’s leukemic cells) provides additional experimental evidence that CLL B cells express an antigen not detectable on autologous mature normal B cells. Unlike known differentiation antigens, this antigen was likewise not expressed on cord blood lymphocytes or during in vitro transformation of T and B lymphocytes using appropriate mitogens. This, and the fact that the cCLLa and LEU-1 are antigenically distinct, suggest that unlike the T cell differentiation antigen complex gp 65–71,24–28 the 69-kd mol wt cCLLa may be a reflection of the disease than a normal marker of lymphocyte differentiation unless the event occurs early at a stem-cell level. This is unlikely, however, because lymphoblasts from childhood and adult ALL failed to express the cCLLa. Moreover, because of the apparent common cell origin of B lymphocytes and myeloid cells in CML as judged by cytoge genetics and enzyme markers,27 some myeloid cells would be expected to express the cCLLa. Although such a predicted reactivity pattern has been observed with certain reported xenogeneic anti-CLL antisera that reacted with CML and AML cells,12,13 none of the relatively few (N = 18) myeloid leukemias studied thus far expressed the cCLLa, nor did K562 cells, a pluripotent cell line derived from a patient with CMLBC.28 The observation that lymphoma cells failed to express the cCLLa was unexpected, given the biologic and phenotypic similarities between these tissues and CLL. However, the number of cases studied thus far is small and both Hodgkin’s tissues and non-Hodgkin’s PBL are expected to have low if any cCLLa reactivity. Thus, recognition of a small subset of lymphoma patients with a cCLLa-positive clone requires further accrual of cases to our studies.

The clear demonstration of the cCLLa on malignant cells from nine patients with B-type HCL and three patients with PLL supports the view that CLL, PLL, and HCL are intermediary forms of lineage-related disorders4 and suggests a common etiology for cCLLa-positive CLL, PLL, and HCL. In studies similar to those shown in Table 1, it was demonstrated that cCLLa is distinct from the HCL antigen recognized by monoclonal anti-HCL antibodies αHC1 and αHC22 (a gift from Dr D. Posnett, Rockefeller University, NY). Other monoclonal antibodies have been developed against normal and malignant T and B lymphocytes.29–33 However, these antibodies also react with surface determinants shared by malignant and normal lymphoid cells, contrary to the restricted expression of the cCLLa recognized by αCLL and PLL-1 antibodies. The presence of HTLV-1 on CLL cells and its potential relationship to cCLLa were studied by binding and by cross-competition binding, respectively, using specific monoclonal antibodies against the major core proteins P1944 and P2435 and cCLLa as blocking antibody in an IFA. Similar results were obtained using HCL cells as target cells (not shown). These findings and the observations that: (a) primary and cultured tumor cells of HTLV-1 positive malignancies are mature T cells16,17; (b) human cord blood exposed to HTLV-1 isolates infect T lymphocytes45; and (c) HTLV-1 sequences were absent in autologous B cells of patients whose neoplastic T cells contained HTLV-1 proviral sequences,39 suggest that cCLLa-positive CLL, PLL, and HCL are not etiologically related to HTLV-1–induced T cell malignancies. However, our studies do not exclude an etiologic role for other retroviruses in B-lymphoid malignancies.

Monoclonal antibodies specific for the cCLLa and B cell lines expressing this antigen may be of clinical value: Detection of the cCLLa facilitates the diagnosis of a malignant lymphoid clone (manuscript in preparation) in a preclinical and potentially eradicable phase of its development. In addition, cCLLa-positive cell lines can be used to develop xenogeneic transplantation models (Faguet and Agee, manuscript in preparation) to ascertain the potential in vivo usefulness of cytolytic anti-CLL monoclonal antibodies and of their cytotoxic conjugates as immunotherapeutic agents.

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