Immunologic Classification of Lymphocytic Leukemias Based on Monoclonal Antibody-Defined Cell Surface Antigens

By Robert W. Schroff, Kenneth A. Foon, Ronald J. Billing, and John L. Fahey

A panel of monoclonal antibodies reactive with normal lymphocyte subsets was used to classify cases of lymphocytic leukemia on the basis of cell surface antigen expression. The antibodies employed were commercially available and included a common framework HLA-DR antibody, two pan-T antibodies (Leu-1 and OKT-3), and antibodies defining cytotoxic/suppressor (Leu-2 and OKT-8) and helper/inducer (Leu-3 and OKT-4) subpopulations of normal T lymphocytes. Cases of ALL could be subgrouped into non-T non-B, pre-T and T-ALL on the basis of reactivity with HLA-DR, Leu-1, and OKT-3 antibodies. Leukemic cells from patients with T-cell CLL could be divided into Leu-2/OKT-8 reactive and Leu-3/OKT-4 reactive subpopulations, as well as a subgroup in which the majority of cells were unreactive with either of these antibodies. With the exception of one individual, all Sézary cell leukemias expressed a phenotypic pattern similar to that of the Leu-3 subgroup of T-CLL. Malignancies of B-cell lineage (B-CLL, prolymphocytic leukemia, and lymphosarcoma) that were examined were reactive with both the HLA-DR and Leu-1 antibodies. On the contrary, normal B lymphocytes and lymphoid cell lines of B-cell origin did not express surface antigens recognized by the Leu-1 antibody.

In recent years it has become evident that various levels of lymphocyte differentiation can be defined using a number of immunologic parameters. Highly specific antisera and unique surface markers as well as intracellular enzymes have all been employed in the phenotypic analysis of normal and malignant lymphoid cells. Investigations in this area have provided important insights into lymphocyte differentiation as well as the origin of leukemias. Both murine and human T-lymphocyte populations have been demonstrated to contain several subsets that differ in function as well as in cell surface characteristics. Murine T-cell subsets can be distinguished by means of antibodies to cell surface antigens unique to particular T subsets. These cell surface antigens include the LY, Qa, and IL antigen systems. In man, attempts to develop T-subset-specific antibodies have been complicated by the outbred nature of human populations. However, the hybridization technique of Kohler and Milstein for production of monoclonal antibodies has revolutionized the development of human T-lymphocyte antibodies. Prior to the advent of monoclonal antibodies, only limited T-cell subpopulations could be defined using xenogeneic antisera or human autoimmune sera. With the advent of monoclonal reagents, a variety of T-lymphocyte antigens has been defined that is present on peripheral blood T lymphocytes as defined by their ability to rosette spontaneously with sheep erythrocytes, and are not found on peripheral blood B lymphocytes. A 65,000–69,000 dalton antigen is recognized by the Leu-1, OKT-1, and T101 antibodies. The other pan-T antigen has a molecular weight of 19,000 daltons and is recognized by the OKT-3 antibody. Two T-cell subpopulations have been described that appear to contain lymphocytes with different immunoregulatory capabilities. The T subset bearing a 70,000 dalton antigen recognized by the Leu-2a and OKT-8 antibodies reportedly includes cells mediating cytotoxic and immunosuppressive events. The T subset bearing a 62,000 dalton antigen recognized by the Leu-3a and OKT-4 antibodies includes T cells exhibiting helper and inducer...
activity. These latter antibodies define different sets of T lymphocyte differentiation antigens. The common ALL antigen (cALL) has proven to be an important aid in the identification of cases of acute lymphocytic leukemia.

Phenotypic analysis of lymphocytic leukemias by monoclonal antibodies in combination with the more conventional immunologic parameters facilitates further definition of lymphoid differentiation and investigation of the origins of lymphoid leukemias. This investigation was initiated to provide a phenotypic analysis of a variety of clinical forms of lymphoid leukemias. Monoclonal antibody analysis was employed to define distinct forms of leukemias that cannot be differentiated by conventional means of analysis. The relationship of various leukemia phenotypes to the normal pathway of lymphocyte differentiation is discussed.

MATERIALS AND METHODS

Cells

Leukemic cells were obtained from patients at the UCLA Medical Center and the Wadsworth Veterans Administration Hospital. The diagnosis was established independently on the basis of clinical findings and morphological and histochemical evaluation of peripheral blood and bone marrow cells. Patients included in this study were selected for their high white blood cell counts (greater than 30,000 cells/cumm). For this reason, virtually all lymphoid cells examined can be assumed to represent leukemia cell populations, with a negligible component of nonmalignant cells. Mononuclear cell suspensions were prepared from peripheral blood by Ficoll-Hypaque density gradient centrifugation. Cells were studied the day the sample was collected or were cryopreserved in liquid nitrogen. In addition to cells available at UCLA, cells from patients with Sézary cell leukemia as well as Sézary cell line cells were obtained in a cryopreserved state from Dr. Paul A. Bunn, Jr. Along with patient samples, 13 lymphoid cell lines were studied. The cell line Mo, derived from an individual with a T-cell variant of hairy cell leukemia, was kindly supplied by Dr. David W. Golde, UCLA School of Medicine.

Indirect Immunofluorescence Assay.

All cells were washed twice before analysis, 0.5 x 10⁶ cells were used for each individual assay. Cells were incubated 45 min at 4°C in 100 μl of tissue culture medium (MEM, Irvine Scientific, Santa Ana, Calif.) containing 2% heat-inactivated newborn bovine serum (NBS) and 5 μl of monoclonal antibody solution. Cells were washed twice by centrifugation and resuspended in 100 μl of tissue culture medium containing 2% NBS and 3 μg fluorescein-conjugated goat anti-mouse IgG (TAGO, Inc., Burlingame, Calif.). Following a 45 min incubation at 4°C, the cells were washed twice in MEM containing 0.2% sodium azide and finally resuspended in one drop of 30% glycerol in phosphate-buffered saline (0.1 M PO₄, pH 7.4) containing 0.2% sodium azide. Analysis was performed immediately or following overnight incubation at 4°C. Cells were examined on a fluorescent microscope equipped with epillumination. A minimum of 200 cells were examined for surface immunofluorescence.

Monoclonal Antibodies

Leu-1, Leu-2a, Leu-3a, and HLA-DR antibodies were supplied by Becton-Dickinson & Co. Mountain View, Calif. at a concentration of 0.1 mg protein/0.5 ml buffered saline. OKT-3, OKT-4, and OKT-8 monoclonal antibodies were supplied in a lyophilized form by Ortho Pharmaceutical Corporation, Raritan, N.J. Ortho antibodies were reconstituted in phosphate-buffered saline to a concentration of 50 μg protein/ml for OKT-4 and OKT-8, and 25 μg protein/ml for OKT-3. All monoclonal antibodies were stored at 4°C prior to use. Purified mouse myeloma protein of the IgG2a class (RPC 5, Bionetics, Kensington, Md.) served as a negative control for immunofluorescence assays.

Common Acute Lymphoblastic Leukemia (cALL) Antigen Assays

Analysis for the expression of the cALL antigen was performed using a rabbit antiserum and microcytotoxicity assay as previously reported.

Surface Membrane Immunoglobulin (sMig) Assay

Analysis for sMig on leukemic cells was performed using a direct immunofluorescence technique. Fluorescein-conjugated goat anti-human immunoglobulin (TAGO, Inc., Burlingame, Calif.) was employed as the staining reagent.

Cytoplasmic Immunoglobulin (Cig) Assays.

Analysis for intracytoplasmic immunoglobulin heavy chains was performed using the immunoperoxidase technique as described by Taylor and Burns. Reagent antibodies were obtained from DAKO Corp., Santa Barbara, Calif.

Sheep Erythrocyte (E) Rosette Assay.

Cells forming spontaneous E rosettes (ERFC) were enumerated using the technique described by Jondal et al.

Terminal Deoxynucleotidyl Transferase (TdT)

Assays for TdT activity were performed using reagents available from Bethesda Research Laboratories, Bethesda, Md. The procedure was performed according to the manufacturer's directions.

RESULTS

Acute Lymphoblastic Leukemia (ALL)

With the use of three markers (sMig, Cig, and E rosettes), the clinical entity of ALL can be divided into four major subgroups. These subgroups include non-T, non-B ALL (sometimes referred to as "null" ALL), T-ALL, and pre-B-ALL. With the use of monoclonal antibodies, the four subgroups of ALL can be further divided. Analysis of a number of sMig negative, E rosette negative ALL cells revealed two distinct phenotypes within this subgroup. Eight of 12 cases demonstrated HLA-DR framework (DR) antigens and the common ALL antigen on the cell surface in the absence of any additional antigens studied (Table 1). A second subgroup composed of two cases displayed high percentages of Leu-1 positive cells. These cells did not stain with the DR antibody. Thus, the DR+ and the Leu-1+ phenotypes appear to represent distinct subclasses of ALL. The Leu-1 ALL subgroup is similar to the T-ALL subgroup, which has been previously described by Foon et al. on the basis of
reactivity with a rabbit antihuman thymocyte serum.\textsuperscript{11}

Those ALL cells that are DR negative, Leu-1 positive, but do not form E rosettes, are difficult to classify (cases 9 and 10). Both of these patients are male teenagers and exhibit a thymic mass. These clinical findings are similar to those reported for patients with E-rosetting T-ALL cells,\textsuperscript{33} and we prefer to classify this group as pre-T-ALL. It must be emphasized, however, that expression of the Leu-1 antigen alone is not evidence of T-cell lineage. The Leu-1 antigen is also expressed by certain malignant cells of B-cell lineage (see below).

Only one of the cases studied (case 12) exhibited the classical phenotype of T-ALL (Table 1). As expected, these cells displayed a variety of T antigens as defined by the panel of monoclonal antibodies. Cells from case 11 lacked the ability to form E rosettes, but did exhibit several T antigens. These cells did not express receptors for sheep erythrocytes, but otherwise had T-cell features, and we therefore include this case under the subgrouping of T-ALL.

Analysis of cALL antigen expression indicated that both the non-T, non-B and the pre-T subgroups were cALL positive. Both cases of T-ALL were found to be cALL negative.

As would be expected, all cases of ALL examined were TdT positive.

\textbf{Chronic Lymphocytic Leukemia (CLL)}

Two major subgroups of CLL can be defined using E rosette formation and sMlg and Clg markers: B-CLL and T-CLL. During the course of this study, cells from 15 CLL patients were analyzed for cell surface markers. As is evident from Table 2, both subgroups of CLL were represented in our patient population. Patients diagnosed as having B-CLL, were considered typical cases\textsuperscript{40} by the clinicians. Morphologically, they were either normal appearing lymphocytes or somewhat enlarged with clumped nuclear chromatin and
were monotonously similar in the blood of any given patient. The lymphocyte counts ranged from 50 x 10^9/liter to 150 x 10^9/liter. In addition, one patient with the clinical presentation described by Galton as prolymphocytic leukemia (case 21) was included. This patient presented with greater than 100 x 10^9/liter prolymphocytes with large nuclei, large nucleoli, and abundant cytoplasm. Clinically the patient had massive splenomegaly without lymphadenopathy.

Cells with Clg in the absence of sMIg and cells bearing sMIg all displayed similar staining patterns with the panel of monoclonal antibodies. The inability to demonstrate sMIg on Clg+ CLL cells is probably due to very faint expression of sMIg by these cells. With the possible exception of case 17, cells within this group reacted with Leu-1 and DR antibodies, but not with the other antibodies in the panel (Table 2). The prolymphocytic cells displayed a similar phenotype, but stained more intensely for SM1g.

The E-rosette-forming CLL cells (T-CLL) could be divided into three subgroups on the basis of reactivity with monoclonal antibodies. The cells in the first subgroup (T-CLL, cases 22 and 23) displayed a mixed staining pattern with the Leu-2 and Leu-3 antibodies. Case 23 was unique in that all the T leukemia cells tested reacted with the OKT-3 and not the Leu-1 antibody. Case 22 was also interesting in that while 72% of the cells formed E rosettes, no more than 21% of the cells reacted with any T-cell antibody.

A second subgroup of T-CLL (Leu-2 T-CLL, cases 24 and 25) could be defined on the basis of reactivity with the Leu-2 antibody in the absence of Leu-3 reactivity. Likewise, cases 26 and 27 were distinct in that the cells reacted predominately with Leu-3 and not Leu-2 antibodies. These cases were termed Leu-3 T-CLL.

These findings demonstrate the heterogeneity of T-CLL with respect to surface antigen phenotype. Nonetheless, T-CLL can be divided into three subgroups on the basis of staining patterns with the Leu-2/OKT-8 and Leu-3/OKT-4 antibodies.

**Lymphosarcoma Cell Leukemia**

The majority of lymphomas and lymphosarcomas are of B-cell origin. We examined the peripheral blood cells of two patients with lymphosarcoma of B-cell origin (cases 28 and 29, Table 3). Both of these patients had poorly differentiated lymphomas that entered a leukemic phase. These cells were typical lymphosarcoma cells with large, deeply cleaved nuclei with well delineated nucleoli. These cells were similar to B-CLL cells in that they displayed a high percentage of binding with the Leu-1 and DR antibodies (Table 3). These cells did not react with the other monoclonal antibodies on the panel to any significant degree.

**Sézary Cell Leukemia**

Sézary's syndrome is a form of cutaneous lymphoma characterized by diffuse skin infiltraton, lymphadenopathy, and hepatosplenomegaly. In the leukemic phase of the disease, Sézary cells are identified by the distinctive cerebriform morphology of their nuclei and appear to be T lymphocytes in that they form E rosettes and react with T antisera. Broder et al. have reported that Sézary cells play the role of helper T cells in supporting in vitro immunoglobulin synthesis by B cells. All of the patients reported in this study had Sézary's syndrome with a high circulating count of Sézary cells confirmed by light and electron microscopy.

Our study indicated that two subgroups of Sézary cells could be defined on the basis of surface antigen phenotype (Table 4). The majority of Sézary cells were similar to subgroup 3 (cases 26 and 27) of T-CLL. Five of the six Sézary cells tested reacted with the Leu-1, OKT-3, and Leu-3/OKT-4 antibodies, but did not react with the Leu-2 antibody, thus demonstrating similar phenotype to the Leu-3 T-CLL subgroup. On the other hand, the sixth Sézary cell (case 35) exhibited relatively few Leu-3 positive cells with a larger percentage of Leu-2 positive cells. This Sézary cell was therefore similar to the cells in the Leu-2 subgroup of T-CLL. While the majority of cases of Sézary cell leukemia were similar to the Leu-3 positive subgroup of T-CLL with respect to expression of the Leu-3 antigen, these two groups of patients did not share similar clinical features. Likewise, the patient with Leu-2 reactive Sézary cell leukemia did

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**Table 3. Reactivity of Peripheral Blood Cells in Cases of Lymphosarcoma Cell Leukemia With Selected Monoclonal Antibodies**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>sMig</th>
<th>DR</th>
<th>ERFC</th>
<th>Leu-1</th>
<th>OKT-3</th>
<th>Leu-2/OKT-8</th>
<th>Leu-3/OKT-4</th>
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<tr>
<td>28</td>
<td>22</td>
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<td>96</td>
<td>1</td>
<td>98</td>
<td>3</td>
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</tbody>
</table>

**Table 4. Reactivity of Peripheral Blood Cells in Cases of Sézary Cell Leukemia With Selected Monoclonal Antibodies**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>sMig</th>
<th>DR</th>
<th>ERFC</th>
<th>Leu-1</th>
<th>OKT-3</th>
<th>Leu-2/OKT-8</th>
<th>Leu-3/OKT-4</th>
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<td>30</td>
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<td>97</td>
</tr>
<tr>
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<td>80</td>
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</tr>
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</tr>
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<td>68</td>
<td>88</td>
<td>85</td>
<td>5</td>
<td>88</td>
</tr>
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<td>41</td>
<td>81</td>
<td>83</td>
<td>89</td>
<td>58</td>
<td>33</td>
</tr>
</tbody>
</table>
not have clinical features in common with the patients with Leu-2 positive T-CLL.

**Lymphoid Cell Lines**

A variety of lymphoid cell lines were analyzed for cell surface antigens using the panel of monoclonal antibodies. The cell lines that were studied included lines that were neither E rosette forming cells nor sMlg positive, as well as cell lines expressing the surface marker phenotypes of T and B lymphocytes.

All cell lines of T-ALL origin, except CCRF-HSB-2, expressed the Leu-1 antigen. None of these lines expressed the receptor for sheep erythrocytes (Table 5). CEM was the only line of T-ALL origin capable of expressing more mature T-cell differentiation antigens (OKT-3, Leu-3/OKT-4). The T-ALL cell lines, as was true of the T-ALL leukemic cells, did not express the cALL antigen. The T hairy cell line (Mo) and the Sézary cell leukemia cell line (Hut 155) formed E rosettes and possessed the more mature T antigens OKT-3 and Leu-3/OKT-4 in addition to Leu-1. Interestingly, both of these lines reacted with the DR antibody.

The non-T, non-B ALL and Burkitt's lymphoma cell lines did not bear sMlg, but did react with the DR antibody (Table 5). The B-lymphoblastic cell lines expressed both sMlg and DR antigens. In contrast to the B-CLL, prolymphocytic, and lymphosarcoma leukemia cells, none of the cell lines of B-cell origin expressed the Leu-1 antigen.

**DISCUSSION**

Lymphocytic leukemia occurs in a variety of clinical forms. These various clinical entities have been and continue to be distinguished on the basis of cell morphology, histochemical evaluation, and clinical findings. The purpose of this study was to explore how the various forms of lymphocytic leukemia can be subgrouped by surface antigen phenotype using a panel of commercially available monoclonal antibody reagents. The present investigation confirms and extends recent reports in the literature dealing with this subject by examining a large variety of lymphoid leukemias and cell lines. A large panel of monoclonal antibodies was employed in this study. The results obtained with the five most relevant antibodies are reported here. While heterologous antisera have been employed in the analysis of leukemic cell populations, we feel that monoclonal reagents are preferable to those antibodies in that their use facilitates standardization of testing between laboratories, and in the case of the reagents utilized in this investigation, are commercially available.

Using the conventional surface markers of E rosette formation and sMlg, the cases of ALL that were studied could be divided into non-I, non-B ALL and T-ALL. B-ALL, which occurs in less than 5% of all cases, was unavailable for study. Testing for cytoplasmic \( \mu \) chains to distinguish pre-B-ALL from non-I, non-B ALL was performed to verify that these cells were not of apparent B-cell lineage.

With the use of the Leu-1 and HLA-DR monoclonal antibodies, the non-E-rosetting group of ALL in this study could be subdivided into two distinct subgroups, non-B, non-T ALL and pre-T-ALL. Clinically, these 2 groups of patients could be differentiated in that both patients with pre-T-ALL (Leu-1 +) were teenage boys who presented with high blast counts and a mediastinal mass on chest x-ray. While the B-cell malignancies examined in this study reacted with the Leu-1 antibody, reactivity of the pre-T-ALL subgroup with this antibody does not imply a B-cell lineage. Leukemic cells from the two cases of pre-T-ALL failed to react with the B-cell-related antibody BA-1 and did not possess cytoplasmic immunoglobulin (data not shown). Both the non-B, non-T and the pre-T

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**Table 5. Reactivity of Lymphoid Cell Lines With Selected Monoclonal Antibodies**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Origin</th>
<th>sMlg</th>
<th>DR</th>
<th>ERF</th>
<th>Leu-1</th>
<th>OKT-3</th>
<th>Leu-2/OKT-8</th>
<th>Leu-3/OKT-4</th>
<th>cALL</th>
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<tbody>
<tr>
<td>CCRF-HSB-2</td>
<td>T-ALL</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>Neg</td>
</tr>
<tr>
<td>MOLT-4</td>
<td>T-ALL</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11</td>
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<tr>
<td>HPB-MLT</td>
<td>T-ALL</td>
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<td>0</td>
<td>0</td>
<td>99</td>
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<td>0</td>
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</tr>
<tr>
<td>RPMI-8402</td>
<td>T-ALL</td>
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<td>0</td>
<td>0</td>
<td>79</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Neg</td>
</tr>
<tr>
<td>CCRF-CM</td>
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<td>1</td>
<td>1</td>
<td>95</td>
<td>42</td>
<td>0</td>
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</tr>
<tr>
<td>Mo</td>
<td>HC</td>
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<td>5</td>
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</tr>
<tr>
<td>REH</td>
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<td>98</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Pos</td>
</tr>
<tr>
<td>P3-HR1</td>
<td>Bur L</td>
<td>0</td>
<td>7</td>
<td>0</td>
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<td>0</td>
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<td>RAJ</td>
<td>Bur L</td>
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<tr>
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</tr>
</tbody>
</table>

Bur L, Burkitt's lymphoma; N ALL, non-T, non-B ALL; T HC, T hairy cell leukemia; SS, Sézary's syndrome; B LCL, B-lymphoblastoid cell line.
subgroups of ALL expressed the common ALL antigen. A similar classification of ALL has been made previously using rabbit antisera. In that study, additional subgroups of non-E rosetting ALL cells were identified that did not express the cALL antigen. Our study did not include patients from this group.

Only one case of ALL presented with a significant number of E-rosette-forming cells. However, case 11 reacted with the OKT-3 and Leu-2 antibodies in addition to Leu-1. Clinically, this individual presented as a typical T-ALL in that he was a teenage male with a mediastinal mass and high blast count. We therefore believe that these cells represent non-E-rosette-forming T cells that would not have been identified as such without the aid of T-cell-specific antibodies (i.e., OKT-3). The ability to identify such cases is important in that T-ALL has a poorer prognosis than does non-B, non-T ALL. Indeed, as has been demonstrated using heterologous antisera, testing with a T-cell-restricted antibody such as OKT-3 is superior to the E-rosette assay in distinguishing cases of T-ALL.

Phenotypic analysis for the ALL subgroups we have defined is possible with a panel of three monoclonal reagents (DR, Leu-1, OKT-3) in combination with an antisurface membrane immunoglobulin reagent. In addition, testing for the presence of the cALL antigen appears to be of prognostic value. Sallan et al. report a better prognosis with cALL-positive cases as compared to the cALL-negative cases of non-B, non-T ALL.

A recent report by Reinherz et al. has indicated that the monoclonal antibodies OKT-9 and OKT-10, which react with thymic subpopulations as well as other hematopoietic cells, identify different subclasses to T-ALL. Our preliminary data support these findings. In this same report, Reinherz et al. reported nonreactivity in a majority of cases of T-ALL with the OKT-1 antibody. On the contrary, we found that all cases of pre-T and T-ALL we examined reacted with the Leu-1 antibody. While this antibody exhibits very similar staining characteristics to that of OKT-1, it remains to be proven whether OKT-1 and Leu-1 identify the same antigen.

Three of the nine B-CLL cells did not have detectable sM1g, but did have cytoplasmic Ig. Typical cases of B-CLL, including cases 16–21, exhibit low intensity staining for sM1g because of low Ig density on the surface membrane. While cases 13–15 did not have detectable sM1g with the reagent used, they did have Clg, and clinically and morphologically they were not unique.

All leukemias of B-cell origin that were tested expressed the same antigenic phenotype, i.e., Leu-1 and DR positive. While the presence of DR antigens on B-cell leukemias is not unexpected, the expression of the Leu-1 antigen on these cells is quite intriguing. Foon et al. reported a T-cell antigen using a xenoreantisemur found on a panel of sM1g-positive B cells. Other investigators have reported similar observations with monoclonal antibodies. While the Leu-1 antigen is not present on mature normal B cells, it is present on mature normal T cells. The expression of the Leu-1 antigen on malignant cells of B lineage may represent gene derepression, or may be attributable to cross-reactivity with tumor-associated antigens. Whatever the explanation, it is clear that the antigens reacting with Leu-1 monoclonal antibody are not restricted to T cells.

The heterogeneity of T lymphocytes was reflected in the variety of T-cell leukemias studied. Cases of T-CLL could be divided into those demonstrating a predominance of cells reactive with either the Leu-2 or Leu-3 antibodies and those cases in which neither of these two antigens appeared to prevail. Both cases of Leu-2 positive T-CLL expressed a high percentage of cells reactive with the HLA-DR antibody. In view of reports that activated T cells express HLA-DR antigens, these T-CLL leukemias may represent malignant counterparts of activated T cells.

The cases of T-CLL we studied were all clinically unique, but generally had a much worse prognosis than did the typical case of B-CLL. The relatively small number of patients that fell into each subgroup prohibited meaningful clinical comparisons.

Cases of Sézary cell leukemia could be classified in a manner similar to the subgrouping of T-CLL, using the Leu-2 and Leu-3 antibodies. All Sézary cases except one expressed a predominance of Leu-3-reactive cells. The one remaining case was comprised of both Leu-2 and Leu-3-reactive cells. The heterogeneity in phenotype of Sézary cell leukemias is paralleled by the functional heterogeneity of this form of leukemia. While leukemic cells from the majority of Sézary patients express helper function, there has been one reported case of a suppressor Sézary cell. Similarly, patient 35 had Sézary cells with a suppressor phenotype. Functional characteristics will be presented in a separate paper.

Cell lines provide a valuable tool in the analysis of leukemic and normal lymphoid antigens in that a large quantity of cells can be obtained and stock cultures maintained as a ready source of reference. Thirteen leukemic cell lines were characterized as part of this investigation. Cell lines with similar phenotypes to those of the major ALL subgroups were demonstrated, as well as cell lines with phenotypes similar to those T-CLL and Sézary cell leukemias that were examined.
It is quite interesting to note that while all cases of B-cell leukemias studied displayed both Leu-1 and DR antigens, none of the Burkitt's lymphoma or B-lymphoblastoid cell lines reacted with the Leu-1 antibody.

The cell lines and the leukemic cell populations are heterogeneous in antigen expression. Reactivity with an antibody is rarely 100% positive. A good example is the cell line CCRF-CEM, in which 55% of the cells were reactive with the Leu-3 antibody. These differences in antigen expression may represent different stages of maturation within the leukemic cell population or may relate to phases of the growth cycle.

Leukemia cells generally reflect the phenotype of normal cells. Analysis of leukemic cell populations, therefore, can serve as a tool for studying the normal pathway of differentiation. Table 6 indicates the forms of lymphoid leukemias that were included in this study and their normal lymphoid counterpart. The monoclonal antibodies useful in the classification of these leukemias are indicated.

ACKNOWLEDGMENT

The authors wish to acknowledge the excellent technical assistance of Loretta Chai and Mike D'Almeida, as well as the staff of the UCLA Clinical Immunology Research Laboratory: Diane Smith, Pablo Villanueva, Kendra Schwartz, and Daisy Wang. The generous gift of leukemic cells from patients with Sézary cell leukemia provided by Dr. Paul A. Bunn, Jr. is gratefully acknowledged. Monoclonal antibodies employed in this investigation were the gift of Becton-Dickenson and Co. and Ortho Pharmaceutical Corporation.

REFERENCES


22. Royston I, Majda JA, Baird SM, Meserve BL, Griffiths JC: Human T cell antigens defined by monoclonal antibodies. The 65,000 dalton antigen of T cells (T65) is also found on chronic lymphocytic leukemia cells bearing surface immunoglobulin. J Immunol 125:725-731, 1980


51. Yu DTY, Winchester RJ, Fu SM, Gibofsky A, Ko, HS,


Immunologic classification of lymphocytic leukemias based on monoclonal antibody-defined cell surface antigens

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