Classification of Human Leukemia by Membrane Antigen Analysis with Xenoantisera

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Abstract

Rabbit and monkey antisera after appropriate absorption were rendered specific for normal or leukemic lymphoid- and myeloid-associated antigens. Antisera defining a common peripheral blood T-cell antigen, a thymus leukemia antigen, HLADR or la-like antigen, common acute lymphoblastic leukemia antigen (CALLA), and a myeloid-monocyte (M) antigen were used in a microcytotoxicity assay to classify leukemic cells from 30 patients in a double blind study. The antisera to the M antigen reacted with adherent peripheral blood cells and polymorphonuclear leukocytes and failed to react with nonadherent mononuclear cells and enriched T-cells and chronic lymphocytic leukemia cells. The M antisera also reacted with U937, a monocytic-type cell line, and with HL60, a promyelocytic-type cell line, but failed to react with T and B lymphoblastoid cell lines. The specificities of the other antisera have been described in previous reports.

Cells from three of the patients could not be phenotyped by microcytotoxicity testing. Cells from 25 patients had a consensus morphological or histochemical diagnosis of either acute lymphoblastic leukemia or acute nonlymphocytic leukemia. The serological classification of these patients using the five types of antisera listed above were consistent with the consensus diagnosis. In addition, the lymphoid cancers were further subclassified as to T-, B-, or thymus antigen types. There was no consensus lymphoid versus myeloid diagnosis on cells from two patients. The serological classification in both cases favored a diagnosis of myeloid rather than lymphoid leukemia.

Introduction

Xenoantisera are now widely used for the detection and classification of types and subtypes of acute and chronic leukemia in humans (see reviews in Refs. 10 and 16). Some of the markers such as the CALLA initially described and characterized by Greaves et al. (11) are leukemia associated; i.e., the antigen is present on most leukemic cells of a given type or subtype and either absent or found only on a small percentage of normal cell types. However, most of the antisera define antigen(s) found on populations or subpopulations of normal cells as well as on leukemic cells. Examples of the latter type antigens, described in previous reports from our laboratory, are the human la-like antigen (1), TLAA (1, 2), and THY (5, 18). This report is a summary of recent serological studies from our laboratory utilizing antisera to CALLA and normal lymphoid- and myeloid-specific antigens for the classification of leukemia by microcytotoxicity.

Materials and Methods

Antisera. The monkey and rabbit antisera used in these studies, their distribution on normal cells, and references detailing their specificity are given in Table 1. Both monkey and rabbit antisera to HS and SB cells were appropriately absorbed to detect TLAA and la-like antigens and have been well characterized in previous publications from this laboratory (1–4). The antisera to TLAA precipitate a M.W. 170,000 glycoprotein, while the la antisera precipitate the M.W. 30,000 and 35,000 polypeptides associated with these antigens (Fig. 1). The monkey and rabbit antisera to these 2 antigens precipitate the same antigens and give similar (±20%) microcytotoxicity reactions so that only a range of lysis is given which is representative of both species of antisera. Some of the absorbed antisera (e.g., αTLAA and α-la in Fig. 1) occasionally showed additional bands with some of the labeled antigens tested. Some of these bands were noted with the normal serum or Staphylococcus protein A controls, while others represent antibodies bound by antibodies not related to the antigenic specificity defined by cytotoxicity.

The THY antisera were produced in monkeys by immunization with human thymocytes and were absorbed with erythrocytes and peripheral blood leukocytes until they no longer reacted with enriched peripheral blood T- or B-lymphocytes from normal donors but still react strongly with thymocytes and certain leukemia cells (5, 18). Two antisera were studied; one was elicited to thymocytes from a single donor and the other was elicited with thymocytes from pooled donors. Essentially similar percentages of cells were lysed by both antisera (±20%), so that a range of values is given representative of both thymocyte antisera. The THY antisera precipitate 45,000 M.W. to 48,000 M.W. antigen by RIP-PAGE (Fig. 1; Ref. 5).

The rabbit antisera to CALLA have not been reported in previous publications from this laboratory but have the same serological specificity and precipitate an antigen in the same 95,000 to 100,000 molecular-weight range (Fig. 1) as that described by Greaves et al. (10–13). The CALLA antisera were elicited to uncultured non-T, non-B ALL cells and were absorbed with peripheral blood leukocytes from normal donors and with CLL cells until they no longer reacted with enriched T- and B-cells from normal donors.

Rabbit antisera detecting myeloid-monocyte-associated antigen (M) were elicited to cells from a patient with AMOL and to adherent peripheral blood cells from a normal donor. The antisera were ab-

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2 Special Fellow of the Leukemia Society of America.
3 The abbreviations used are: CALLA, common acute leukemia-associated antigen; THY, thymus leukemia antigen; AMOL, acute mononuclear leukemia; RIP-PAGE, radiomunoprecipitation combined with sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; PMN, polymorphonuclear leukocytes; AMML, acute myelomonocytic leukemia; T-ALL, thymus-derived acute lymphoblastic leukemia; E-rosette, erythrocyte-forming rosette.

1 Unpublished data.
sorbed with B- and T-lymphoblastoid cell lines until they no longer reacted by microcytotoxicity with these cell lines or with enriched peripheral blood B- or T-lymphocytes. Additional data on the specificity of these 2 M antisera are given in the text. Both antisera had essentially the same titer, and the percentage of target cells lysed was ±10%. Therefore, data from both antisera are referred to collectively as α-M.

Cells. The frozen cells were obtained from Dr. David Gordon, Center for Disease Control, Atlanta, Ga., and many were obtained from patients in the Southeastern Cancer Study Group. The samples were tested by microcytotoxicity by us without prior knowledge of the diagnosis. The diagnosis of leukemia or lymphoma on these cells was made by a consensus of opinions based on morphology, cytogentetics, histochemistry, enzymatic analysis, and certain surface marker characteristics. The properties of the cells from some of these patients have been described previously (8, 9). The cells were from the peripheral blood, and only patients with 60% or more abnormal cells in the peripheral blood were studied.

The tissue culture cell lines derived from patients with leukemia and lymphoma were grown on Roswell Park Memorial Institute Medium 1640 with 10% fetal calf serum. Some membrane properties of these cell lines have been described previously (7, 17, 20).

Enriched fractions of PMN, lymphocytes, and monocytes were prepared from peripheral blood according to the procedure of English and Anderson (6). The PMN fraction was determined to be >95% PMN cells by Wright-Giemsa staining. Adherent cells containing >80% monocytes by esterase staining properties were prepared by adherence of mononuclear cells to plastic Petri dishes for 1 hr at 37° in Roswell Park Memorial Institute Medium 1640 containing 20% normal human serum. The adherent cells were removed from the Petri dishes by gentle scraping with a rubber policeman. The nonadherent cells had >2% esterase-positive cells. Enriched T-cells were prepared by removing B-lymphocytes with a goat anti-human Fab serum (14). All enriched cell fractions were >90% viable at the time of testing.

Microcytotoxicity Assay. The eosin dye exclusion microcytotoxicity assay used in these studies has been described in detail in a previous publication (1).

Cell Surface Labeling and RIP-PAGE Analysis. The procedure used for analysis of the reactivity of the antisera with cell surface molecules has been described in detail (1, 2, 5). Viable cells were labeled with 125I by the lactoperoxidase technique and solubilized with Triton X-100. Aliquots of labeled antigen (1 to 2 × 10⁵ cpm) and antisera (5 to 20 μl) were incubated for 1 hr at room temperature followed by the addition of 200 μl of 10% fixed Staphylococcus aureus (15 min at room temperature). The samples were washed 3 times, reduced in 1% sodium dodecyl sulfate and 5% β-mercaptoethanol, and electrophoresed in 5 to 15% discontinuous sodium dodecyl sulfate-polyacrylamide gradient gels. The gels were dried, and autoradiography was performed.

Results

Specificity of Xenoantisera to Myeloid-Monocyte-associated Antigens. The microcytotoxicity titers and specificity of the M antisera with normal cells and tissue culture cell lines are shown in Charts 1 and 2. The M antisera react to titers >50 with adherent peripheral blood cells and PMN and fail to react with the nonadherent cell fraction as well as enriched T-cells and CLL cells (Chart 1). The normal cell donor for the data in Chart 1 was the immunizing normal cell donor for the antisera to blood monocytes. The reactivity of the anti-M sera with established suspension-type tissue culture cell lines from leukemic donors also indicates their myeloid-monocyte specificity. The antisera react with a monocytic-macrophage-like cell line U937 and with the HL60 cell line derived from a patient with promyelocytic leukemia (7) which can be induced to terminal granulocytic differentiation (Chart 2). The antisera fail to react with lymphoid-type cell lines NALM-1, SB, MOLT, and CEM (Chart 2). Other membrane antigen properties of these cell lines can be seen in Table 2.

Specificity of Other Xenoantisera. The molecular characteristics of the antigens, a brief summary of their distribution on normal cell types, and detailed references on the specificity of the various antisera are described in Table 1 and "Materials and Methods." A composite of RIP-PAGE analysis of the antigens detected by the antisera utilizing various 125I-labeled membrane antigen preparations is shown in Fig. 1 and is described briefly in "Materials and Methods." The cytotoxic reactivities of the TLAA, THY, CALLA, Ia, and M antisera with B, T, myeloid, and monocytic cell lines established from patients with leukemia or lymphoma are given in Table 2. The TLAA antisera react only with the 2 T-cell lines HSB and Molt, while the THY antisera react only with the Molt line. The Ia antisera react strongly with the B lymphoblastoid cell line (SB) and the NALM1 and NALM6 lines. The 30 to 40% lysis noted with the Ia antisera and HL60 cells is the maximum lysis noted with these cells on repeated testing, and the percentage of lysis varied with the degree of differentiation seen at different passage levels. The CALLA antisera reacted only with the 2 cell lines (Nalm 1 and 6) reported to have CALLA (17). The anti-M sera as previously shown in Chart 2 reacted only with the U937 and HL60 cell lines.

Leukemic Cell Reactivity of Xenoantisera. The rabbit and

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Molecular properties</th>
<th>Distribution on normal cells</th>
<th>Specificity references</th>
<th>Species of antisera</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLAA</td>
<td>M.W., 170,000</td>
<td>Thymocytes, peripheral blood</td>
<td>3, 5, 8, 9</td>
<td>Rabbit and monkey</td>
</tr>
<tr>
<td>THY</td>
<td>M.W., 45,000-48,000</td>
<td>T-lymphocytes</td>
<td>6, 7</td>
<td>Monkey</td>
</tr>
<tr>
<td>Ia-HLA-DRw</td>
<td>30,000 and 35,000 gp</td>
<td>B-Lymphocytes, CFU-C, 1-8%</td>
<td>3, 8, 9</td>
<td>Rabbit and monkey</td>
</tr>
<tr>
<td>CALLA</td>
<td>M.W., 95,000 to 100,000 gp</td>
<td>Some antisera reported to detect occasional cell in normal and regenerating marrow</td>
<td>2, 4, 10, 11</td>
<td>Rabbit</td>
</tr>
<tr>
<td>M</td>
<td>Not known</td>
<td>Granulocytes, monocytes</td>
<td>12 (and see Charts 1 and 2)</td>
<td>Rabbit</td>
</tr>
</tbody>
</table>

* gp, glycoprotein; CFU-C, myeloid cell colony-forming units; M, myeloid-monocyte antigen.
monkey antisera described in Tables 1 and 2 and Chart 2 were recently tested on a frozen peripheral blood cell panel from 30 patients with leukemia or lymphomas (see "Materials and Methods"). The microcytotoxicity analysis of these cells with the various antisera is shown in Table 3. Three of the 30 cell samples could not be tested because of poor viability of the cells after thawing or due to high sensitivity of the leukemic cells to rabbit complement. Three of the patients with lymphoid cancers were pediatric, while the remaining patients were adults. Patients 1 to 25 had a consensus diagnosis of either lymphoid or myeloid cancer. In a few instances, there was some problem of subclassifying the myeloid leukemia as acute myeloid leukemia, AMOL, or AMML (Patients 6, 12, and 15). The diagnosis for Patients 26 and 27 prior to cytotoxicity testing was not unanimous, and there were differing opinions on a diagnosis of myeloid versus lymphoid cancer.

The anti-M sera were strongly reactive (60 to 100% lysis) with cells from Patients 1 to 15, 26, and 27. The antisera to TLAA, THY, and CALLA were negative with cells from these patients. The la antisera were nonreactive with cells from Patient 1 and gave 35 to 100% lysis with the cells from the other patients in this group. On the basis of their reactivity with anti-M sera and their lack of lymphoid and CALLA antigens, the cells from Patients 1 to 15, 26, and 27 would be serologically classified as being from diseases of the myeloid lineage. Since the anti-M sera also react with normal myeloid cells, the diagnosis of leukemia would have to be made by other criteria. The cells from Patient 16 reacted with the anti-TLAA, anti-la, and anti-M sera and represent a serological reaction pattern typical of some AMML patients described in a previous report (4) showing reactivity with both TLAA and la antisera.

Cells from Patients 17 through 25 with lymphoid cancers showed no reactivity with the anti-M sera but, with the exception of cells from Patient 17, this group did react with antisera to CALLA, TLAA, or THY. Cells from the patient with B-cell lymphosarcoma (Patient 17) had surface immunoglobulins (not reported in Table 3) and la antigens. Cells from Patients 18,
19, and 20 were CALLA and la antigen positive and were phenotypically the non-T, non-B ALL type. Cells from the 4 patients (Patients 21 to 24) diagnosed as T-ALL or as an unclassified ALL (Patient 25) reacted with the TLAA antisera. Cells from 4 of the 5 T-ALL patients reacted with the THY antisera. Cells from 3 of the 4 THY-positive patients showed some E-rosette formation at 37°. Cells from Patient 24 had strong reactivity with anti-TLAA sera, formed a high percentage of E-rosettes at 4° and 37°, but lacked the THY antigen. Cells of Patient 24 provide additional evidence that THY antigen expression is independent from the E-rosette receptor and is the second example that we have seen of a cell that forms stable E-rosettes but is THY negative (5).

Cells from the 2 patients (Patients 26 and 27) with a discrepant consensus diagnosis had a serological phenotype of being M and la antigen positive and CALLA, THY, and TLAA negative. This serological phenotype would indicate that the cancers were myeloid rather than lymphoid.

**Discussion**

Since most of the antigens (TLAA, THY, la, and M) defined with xenantisera are not tumor specific, these reagents cannot be used by themselves for the diagnosis of leukemia or lymphoma. However, the use of these serologically defined antigenic makers have proven to be of value in the often-times difficult task of classification of leukemia or lymphoma. The addition of the antisera defining a myeloid-monocyte-associated antigen to our panel provided a clear-cut way to distinguish myeloid from lymphoid cancers (Table 3). All of the acute nonlymphocytic leukemias thus far tested have reacted with the anti-M sera, while none of the lymphoid leukemias or lymphomas have. The myeloid-type leukemia cells can be further subclassified by the presence of la antigens. Cells from some AMML patients can also be subclassified by their reactivity with TLAA antisera. Our anti-M sera do not distinguish myeloblasts or myeloblast-derived cells from monoblasts or monocytes-macrophages, and they show the same leukemic and normal cell specificity as do the anti-M sera described by Roberts and Greaves (19). Since the molecular nature of the antigen detected by Roberts and Greaves, anti-M serum (19), was not described and our M antigen also is not well characterized by RIP-PAGE analysis, the identity of the M antigens defined by the antisera from these 2 laboratories can be inferred only from comparison of the serological specificity. Future studies with monoclonal antibodies may define an antigen which is present on monocytes but not on granulocytes or myeloblasts.

The lymphoid cancers lack M antigen but react with various combinations of TLAA, THY, CALLA, and la antisera (Table 3). The B-cell lymphosarcoma cells of Patient 17 have surface immunoglobulin (data not reported here) and are la positive. The phenotype is thus similar to that noted for CLL cells (1). The other ALLs can then be subdivided into 2 major categories on the basis of reactivity with either CALLA or TLAA antisera. The TLAA antisera gave 100% lysis of the CALLA-negative ALL cells, whereas E-rosette analysis showed percentages of 3 to 93 for the "T" group. We have previously described examples of ALL cells which serologically would be classifed as T but which had low or intermediate E-rosette percentages and were la antigen negative (3). The T-ALL group can be further subdivided on the basis of reactivity with THY antisera.
In this report, 5 of 6 T-ALL patients reacted with the THY antisera. In a more detailed report (5), 15 of 19 T-ALL patients had >50% of their cells reactive with the THY antisera. Again, in this latter study, the best normal marker for classification of leukemic cells as T-cells was the TLAA antigen (5). There is also no direct correlation between active or stable E-rosette formation at 37° and THY antigen expression (Table 3). This was also noted with cells from some of the T-ALL patients reported elsewhere (5).

The advantages and disadvantages of microcytotoxicity as an assay for leukemic cell surface antigens have been discussed in detail previously (15). In brief, the disadvantages are sensitivity of some of the cells to complement or to antibodies in normal sera and the inability to detect minor populations of reactive cells (less than 10%). The advantages are that a large number of sera or dilutions can be tested rapidly on a few million target cells by one technician. Thus, although the fluorescent-activated cell sorters, radioimmunoassays, or enzyme-linked immunoassays have greater sensitivity in antigen detection by a nonhuman primate antiserum. Cell. Immunol., 72: 30-36, 1979.

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References


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