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B Lymphocyte Antigens in the Differential Diagnosis of Human Neoplasia

By Marshall E. Kadin and Ronald J. Billing

Human B lymphocyte antigens (HBLA) were detected with fluorescent-labeled antibodies on malignant cells of 102 patients with Hodgkin disease and other lymphomas, plasma cell myeloma, and nonlymphoreticular neoplasms including carcinomas of the breast, lung, and ovary, soft tissue sarcomas, and neuroblastoma. HBLA were present in Hodgkin disease and other lymphomas of B cell or histiocyte derivation. They were absent in plasma cell myeloma and nonlymphoreticular neoplasms. Absorption studies revealed that malignant T cells had smaller amounts of HBLA, usually not detected by immunofluorescence. Expression of HBLA was dependent on both cell differentiation and origin. Detection of HBLA enabled immunologic distinction of Reed-Sternberg and other lymphoma cells from morphologically similar cells of nonlymphoreticular origin. The rapidity, reproducibility, and economy of the immunofluorescence test make this a useful clinical tool for the differential diagnosis of lymphoma from other malignant disorders in man.

HODGKIN DISEASE (HD) and non-Hodgkin lymphomas must be distinguished from other sarcomas and tumors of epithelial origin (carcinomas) so that appropriate therapy may be given. The morphologic distinction of these disorders may be difficult or impossible when the malignant cells are not well differentiated. The Reed-Sternberg (RS) cell, which is diagnostic of HD, may be confused with morphologically similar cells found in other malignant disorders, including carcinoma of the breast, carcinoma of the lung, and malignant melanoma. To clarify the diagnosis in these difficult cases, we have developed a rapid immunofluorescence method that employs a rabbit antibody against solubilized membrane antigen from human histiocytic lymphoma cells. This antibody detects human B lymphocyte antigens (HBLA) expressed by the majority of lymphoma and leukemia cells but not by tumor cells of nonlymphoreticular origin.

We previously cited evidence that the rabbit antibody detects the same B lymphocyte antigens that react with alloantisera from multiparous women. However, the alloantisera generally do not react with all B cells and detect genetic polymorphism, whereas the rabbit antiserum appears to react with all cells detected by the entire range of human B lymphocyte alloantisera. The
rabbit antiserum is also reproducible, occurs at high titers, and does not require absorption with a source of HLA antigens before being used to detect HBLA. For these reasons, we prefer to use it rather than the alloantisera for this differential diagnosis of human neoplasia.

**MATERIALS AND METHODS**

*Patients.* Fourteen children up to 16 yr of age and 88 adults with malignant diseases who were patients at the University of California at San Francisco from July 1974 to July 1977 comprised the patient population. Table I shows the number of patients in each disease category whose malignant cells were studied for HBLA. Selection of patients was based on the availability of viable tumor cells after tissue was taken for routine pathologic examination. In several instances, cells were obtained from a portion of a second biopsy when a definitive diagnosis could not be rendered on the initial specimen. Control lymph nodes, spleens, and bone marrows were obtained from patients with nonmalignant disorders.

*Cells.* Viable tumor cell suspensions were prepared in chilled Hank's balanced salt solution without calcium and magnesium. Involved tissues (spleen, lymph nodes, and extranodal soft tissues) were scraped with a 60-gauge wire cloth, and the dispersed cells were aspirated into a 3-ml syringe through a 25-gauge needle. Usually no more than 1 cc of tumor tissue was needed to obtain the 10^6 cells required for immunofluorescence studies. Cells were obtained directly from

<table>
<thead>
<tr>
<th>Disease</th>
<th>Histology or Primary Site</th>
<th>No. Positive* / No. Studied</th>
</tr>
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<tbody>
<tr>
<td>Hodgkin disease</td>
<td>Nodular sclerosis</td>
<td>5/5</td>
</tr>
<tr>
<td></td>
<td>Mixed cellularity</td>
<td>4/4</td>
</tr>
<tr>
<td></td>
<td>Lymphocyte depletion</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td>Lymphocyte predominance</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12/12</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>Nodular</td>
<td>15/15</td>
</tr>
<tr>
<td></td>
<td>Diffuse lymphocytic well differentiated</td>
<td>3/3</td>
</tr>
<tr>
<td></td>
<td>Diffuse lymphocytic poorly differentiated</td>
<td>13/16</td>
</tr>
<tr>
<td></td>
<td>Diffuse histiocytic</td>
<td>15/15</td>
</tr>
<tr>
<td></td>
<td>Diffuse undifferentiated (Burkitt)</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td>Mycosis fungoides (Sezary syndrome)</td>
<td>1/5</td>
</tr>
<tr>
<td></td>
<td>Malignant histiocytosis</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50/57</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>Bone marrow</td>
<td>0/8</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>Breast</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>Ovary</td>
<td>0/4</td>
</tr>
<tr>
<td></td>
<td>Lung—large cell</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td>Lung—oat cell</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>Colon</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>Skin—squamous cell</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>Unknown primary, metastatic in lung</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0/19</td>
</tr>
<tr>
<td>Sarcomas and tumors of neural crest origin</td>
<td>Sarcoma of soft tissue</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td>Neuroblastoma</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>Malignant melanoma</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0/6</td>
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</table>

* Patients whose malignant cells were fluorescent with B lymphocyte antiserum.
Table 2. Distinctive Cytologic Features of Malignant Cells Identified by Phase-Contrast Microscopy

<table>
<thead>
<tr>
<th>Disease</th>
<th>Cytologic Appearance</th>
</tr>
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<tbody>
<tr>
<td>Hodgkin disease (Fig. 1)</td>
<td>Giant cells with multiple nuclei and nuclear lobulations; huge nucleoli</td>
</tr>
<tr>
<td>Diffuse histiocytic and poorly differentiated lymphocytic lymphomas (Fig. 2)</td>
<td>Large cells with irregular nuclei and one or more prominent nucleoli</td>
</tr>
<tr>
<td>Mycosis fungoides, Sézary syndrome, nodular lymphoma (Fig. 3)</td>
<td>Convoluted and highly irregular nuclei</td>
</tr>
<tr>
<td>Burkitt lymphoma, malignant histiocytosis</td>
<td>Cytoplasmic vacuoles</td>
</tr>
<tr>
<td>Plasma cell myeloma</td>
<td>Regular and round eccentric nucleus with clumped chromatin; occasional prominent nucleoli</td>
</tr>
<tr>
<td>Carcinoma of ovary, lung, and breast (Fig. 4)</td>
<td>Frequent grouping of cells, cytoplasmic vacuoles, granulations and blebs; occasional multinucleate giant cells</td>
</tr>
<tr>
<td>Soft tissue sarcoma (Fig. 5)</td>
<td>Elongate, fusiform, and spindle shapes</td>
</tr>
<tr>
<td>Neuroblastoma, melanoma</td>
<td>Occasional cell clusters, long cell processes</td>
</tr>
<tr>
<td>Well-differentiated carcinoma of colon and ovary</td>
<td>Columnar polarized cells</td>
</tr>
</tbody>
</table>

Bone marrow aspirates and from pleural and peritoneal effusions anticoagulated with 50 mg ethylenediaminetetraacetate (EDTA). They were examined following lysis of erythrocytes with 0.83% ammonium chloride. Sézary cells were obtained from blood by centrifugation through a Ficoll-Hypaque gradient.

Phase-contrast microscopy was used to identify malignant cells by their nuclear and cytoplasmic features, cell shapes, and groupings (Table 2).

Antisera. The B lymphocyte antiserum was produced in rabbits immunized with papain digests of cell membranes from diseased spleens of patients with histiocytic lymphoma. The method of antigen solubilization and the immunization procedure were previously described. The unabsorbed immune serum was heat inactivated at 56°C and its specificity determined by its reaction with normal and leukemic cells in cytotoxicity and immunofluorescence assays. It reacted with 6%, 15%, peripheral blood mononuclear cells from normal individuals. Blast cells from 70%, of patients with acute lymphocytic leukemia, acute myelocytic leukemia, and chronic myelocytic leukemia and from nearly all patients with chronic lymphocytic leukemia were also reactive. Bone marrows from normal individuals and patients in remission from leukemia contained 0%, 5%, positive cells. Ten nonmalignant lymph nodes and 12 spleens, respectively, contained 28%, 74%, (median 33%), and 32%, 57%, (median 50%), mononuclear cells expressing HBLA. Reactive normal cells included B lymphocytes, monocytes, and histiocytes. Null lymphocytes without surface immunoglobulin or T cell characteristics were also reactive. Nonreactive cells included T lymphocytes, thymocytes, plasma cells, granulocytes, platelets, erythrocytes, and mesothelial cells.

Immunofluorescence. Tumor cells determined to be viable by trypan blue dye exclusion were examined by indirect immunofluorescence using the rabbit B lymphocyte antiserum now available from Alpha Gamma Laboratories, Sierra Madre, Calif., and a goat anti rabbit γ globulin fluorescein isothiocyanate (GAR-FITC) conjugate (Meloy Laboratories, Springfield, Va.). Tumor cells were also examined with serum obtained from rabbits prior to immunization and the GAR-FITC conjugate. For each test, 2 x 10⁶ viable cells were cooled to 4°C and washed with 1%, bovine serum albumin in phosphate-buffered saline (PBS) with 0.2%, sodium azide added. The washed cells were incubated at 4°C for 30 min with 50 μl of the rabbit B lymphocyte antiserum. They were again washed three times and incubated for 30 min with the GAR-FITC conjugate, also with azide added. Stained cells were viewed alternately by phase-contrast and fluorescence microscopy using a Leitz Orthoplan microscope with an HBO 100-watt mercury vapor lamp and incident light fluorescence illuminator. The following filters were used: KT-490 excitation; K-530 and BG-38 suppressor; KG-1 heat; K-480 quenching; and S-525 selective for FITC. Cells were examined for
Table 3. Absorption of B Lymphocyte Antiserum with Fluorescent (HBLA-positive) and Nonfluorescent (HBLA-negative) Malignant Cells

<table>
<thead>
<tr>
<th>Absorbing Cell (Patient)</th>
<th>Target Cell of Patient:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>None</td>
<td>+++</td>
</tr>
<tr>
<td>Fluorescent (HBLA-positive) cells</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td>Nonfluorescent (HBLA-negative) cells</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>D</td>
</tr>
<tr>
<td></td>
<td>E</td>
</tr>
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Fluorescent (HBLA-positive) cells: A, nodular and diffuse histiocytic lymphoma; B, poorly differentiated lymphocytic lymphoma. Nonfluorescent (HBLA-negative) cells: C, cutaneous poorly differentiated T lymphocytic lymphoma; D, mediastinal poorly differentiated T lymphocytic lymphoma; E, carcinoma of the breast.

Fluorescence and for malignant features using the cytologic criteria summarized in Table 2. Results were recorded on Kodak Tri-X-pan film, ASA 400.

Absorption. Absorption experiments were done to determine if nonfluorescent cells had small amounts of HBLA. Aliquots (300 µl) of B lymphocyte antiserum were diluted 1:200 in PBS (two dilution steps before the endpoint for immunofluorescence). They were then absorbed for 30 min at 20°C with 10^5 fluorescent (HBLA-positive) or nonfluorescent (HBLA-negative) malignant cells. HBLA-positive cells used were from patients with nodular and diffuse histiocytic lymphoma and diffuse lymphocytic poorly differentiated lymphoma. HBLA-negative cells were from patients with cutaneous or mediastinal diffuse lymphocytic poorly differentiated lymphoma with a T cell characteristic (E rosettes) and carcinoma of the breast. HBLA-positive and HBLA-negative malignant cells from these patients were also used as target cells for immunofluorescence with each of the absorbed antisera. Fluorescence was graded from 0 (none) to ++++ (maximum) intensity and was judged without prior knowledge of the absorbing or target cell. The results of the complete absorption study are presented in Table 3.

Sheep erythrocyte (E) rosettes. Malignant cells were examined for their capacity to form E rosettes, a T cell characteristic, as previously described.

RESULTS

Positive cells showed bright ring fluorescence with the rabbit B lymphocyte antiserum and the GAR-FITC conjugate. Capping was not observed with the conditions used in these experiments. No fluorescence was seen when serum obtained from rabbits prior to immunization was used in place of the specific B lymphocyte rabbit antiserum. Some nonmalignant cells were usually present in the sample and provided an inherent control for the immunofluorescent staining of malignant cells (Figs. 1, 4, 5). Fluorescent (HBLA-positive) malignant cells are listed in Table 1.

Reed-Sternberg (RS) and mononuclear Hodgkin disease (HD) giant cells were often partially surrounded by small lymphocytes. The HD giant cells showed bright membrane fluorescence, while most adherent lymphocytes were unstained (Fig. 1). This finding indicated that HD cells had HBLA while the surrounding lymphocytes did not. The same results were observed for each histologic type of HD and for all morphologic variants of the RS cell.

Immunofluorescent staining of non-Hodgkin lymphoma cells was dependent on the cell of origin. All those of B lymphocyte or histocyte origin stained, but most lymphomas of T cell origin did not. The latter included cells from
three patients with a prominent mediastinal mass and from four of five patients with cutaneous lymphoma (mycosis fungoides or Sézary syndrome). In the fifth case of cutaneous lymphoma, mycosis cells from a lymph node showed immunofluorescent staining and formed E rosettes.

The intensity of staining of malignant B lymphocytes and histiocytes was uniform and independent of their morphologic differentiation and functional characteristics (surface immunoglobulin, Fc, and complement receptors). Similar staining was observed with nodular, well, and poorly differentiated lymphocytic, histiocytic, and undifferentiated (Burkitt) lymphomas (Figs. 2 and 3).

Among the nonfluorescent HBLA-negative cases were eight examples of plasma cell myeloma and a variety of nonlymphoreticular malignancies. These included carcinomas of the breast (Fig. 4), lung, ovary, skin, and colon. Also negative was a poorly differentiated tumor in the lung with ultrastructural features suggestive of an epithelial origin. This tumor was thought to be metastatic in the lung, since the patient had had a similar tumor in the maxillary sinus 3 yr earlier.

Several tumors of nonepithelial or neural crest origin were also nonfluorescent. These were two soft-tissue sarcomas (Fig. 5), three neuroblastomas, and a malignant melanoma metastatic in a lymph node.

Absorption studies (Table 3) showed that HBLA-positive cells from each of two lymphoma patients (A and B) selected randomly from the antigen-positive group absorbed all or nearly all immunofluorescent activity of the B lymphocyte antiserum. Conversely, there was no decreased fluorescence when cells of these HBLA-positive patients were used as target cells for B lymphocyte antiserum absorbed with HBLA-negative cells from a T cell lymphoma in the mediastinum of patient D or malignant cells from patient E with carcinoma of
the breast. Interestingly, however, there was a partial but definite decrease in fluorescence of HBLA-positive lymphoma target cells from patients A and B when tested with antiserum absorbed by "antigen-negative" cells from a cutaneous T cell lymphoma of patient C. This observation indicated that these cells actually had small amounts of HBLA that could not be detected by immunofluorescence.

Fig. 2. Viable cells from diffuse lymphocytic poorly differentiated lymphoma shown by (A) phase-contrast microscopy and (B) immunofluorescence. Lymphoma cells have large nuclei and one or more prominent dark nucleoli. Cell membranes show bright fluorescent staining of HBLA. × 510.

Fig. 3. Malignant histiocyte with multiple cytoplasmic vacuoles (A) from a case of malignant histiocytosis shows uniform membrane fluorescent staining of HBLA (B). × 1090.
Clinical applications of these results are illustrated in the following four cases:

Case 1
A 20-yr-old female with a 3-cm nodule in the right groin had been treated with antibiotics for 3 mo with no response. A biopsy then showed a malignant undifferentiated tumor; there were no...
recognizable lymph node structures. Although some pathologists favored a diagnosis of soft-tissue sarcoma, a consultant hematopathologist preferred the diagnosis of lymphoma (histiocytic, large noncleaved follicular center cell, or immunoblastic sarcoma). The tumor cells did not react with the rabbit B lymphocyte antiserum (Fig. 5), supporting the diagnosis of sarcoma rather than lymphoma.

Case 2

A 32-yr-old male had a needle biopsy of a radiodense lesion in the lung. Results of this biopsy indicated that the mass could be either a histiocytic lymphoma or a large-cell carcinoma of the lung. Computerized axial tomography showed hilar, mediastinal, and periarticular lymphadenopathy, and the clinical diagnosis was “probable lymphoma.” A second biopsy was performed by mediastinoscopy and tissue was submitted for both routine and immunologic studies. Because cells from this biopsy showed no immunofluorescent reactivity with the rabbit B lymphocyte antiserum, a diagnosis of large-cell carcinoma of the lung could be made.

Case 3

A 42-yr-old female had a large gastric ulcer with raised margins thought to be a carcinoma. Histologically, the tumor cells were undifferentiated and appeared to infiltrate the mucosa secondarily. The malignant cells were reactive with the rabbit B lymphocyte antiserum. A diagnosis of diffuse histiocytic lymphoma in the stomach was established.

Case 4

A 16-yr-old female with both cervical and axillary lymphadenopathy had a cervical lymph node biopsy. The disease process was diagnosed as benign by one consultant hematopathologist and malignant by another. Later, a second surgical biopsy was performed and two axillary lymph nodes were removed. One node was morphologically normal, and 40% of the cells were reactive with the B lymphocyte antiserum. Nearly 40% of the cells also had surface immunoglobulin, and 20% had receptors for complement as determined by EAC rosettes.2 The remaining 60% of the cells were determined to be T cells forming E rosettes. The other axillary lymph node was abnormal and morphologically resembled the cervical lymph node. More than 95% of the cells from this node were reactive with the rabbit B lymphocyte antiserum but were negative for other lymphocyte surface markers. This lymph node contained diffuse histiocytic lymphoma.

DISCUSSION

As therapy for Hodgkin disease (HD) and other lymphomas becomes more specific and thus more effective, it is increasingly important that these diseases be diagnosed correctly. If they are confused with nonlymphoreticular malignancies, patients with HD and lymphoma might be denied appropriate therapy, while those with nonlymphoreticular malignancies might experience unwarranted drug and radiation toxicity without optimal tumoricidal effect. In this study we found that HD and most other lymphomas could be identified immunologically by the presence of human B lymphocyte antigens (HBLA), which are absent in nonlymphoreticular neoplasms.

Examination for HBLA was carried out on malignant cells of 102 patients with lymphoma and other solid tumors. Patients could be divided into two groups based on the presence or absence of HBLA. Those with HBLA were patients with lymphomas of B lymphocyte or histiocytic derivation, including HD. Those without HBLA included all patients with malignancies of nonlymphoreticular origin or with multiple myeloma and most patients with lymphomas having T cell characteristics.

Detection of HBLA allowed distinction of lymphoma from morphologically similar tumors that appeared at an unusual primary site or as a metastasis from
an occult primary tumor. We described patients with undifferentiated sarcoma (case 1) and carcinoma (case 2) who might have been treated for lymphoma because of the clinical characteristics of their disease and the morphologic appearance of their biopsy specimens. The absence of HBLA on their malignant cells indicated a different diagnosis, requiring a new therapeutic approach. In other cases, when the appearance of the tumor had led to an earlier diagnosis of carcinoma (case 3) or a benign disorder (case 4), HBLA on malignant cells with no other lymphocyte surface markers helped to establish the correct diagnosis of lymphoma.

The importance of HBLA is also apparent in the diagnosis of HD, which depends on the demonstration of RS cells, tumor giant cells with multiple nuclei, or nuclear lobulations and huge prominent nucleoli. The nature and origin of the RS cell remains uncertain, and morphologically identical cells have been demonstrated in a variety of malignant and benign disorders.1 We found that recognition of HBLA on true RS cells provides a means of distinguishing them immunologically from morphologically similar cells in malignant melanoma and carcinomas of the breast and lung. Furthermore, the distribution of HBLA in normal and malignant tissues is consistent with other evidence that the HBLA-positive RS cell is derived from a B lymphocyte or histiocyte.7

Plasma cell myeloma is readily diagnosed in typical cases with bone marrow infiltration and monoclonal immunoglobulin in the serum, urine, or both. However, patients with plasmacytoma who present with a soft-tissue mass may be incorrectly diagnosed as having lymphoma. In these cases, HBLA should be absent; presence of HBLA would favor a diagnosis of lymphoma. Our studies showed that HBLA are present on normal B lymphocytes and on lymphoblasts in lymphoma and leukemia and are absent on malignant and also normal plasma cells.

HBLA closely resemble the Ia antigens (immune response gene-associated antigens) of the mouse with respect to cell differentiation and tissue distribution. Both the human and mouse antigens may be absent or difficult to detect on cells that have become fully differentiated. Human plasma cells, which are highly organized for the production of immunoglobulins, lack the HBLA present on B lymphocytes from which they are derived. In the mouse, Ia antigens expressed by primitive teratocarcinoma cells can no longer be detected after differentiation of these cells in tissue culture.14

HBLA and Ia antigens are expressed predominantly by B lymphocytes and macrophages but also may be present in smaller amounts on selected populations of T lymphocytes. Very low but significant quantities of the Ia antigens have been detected on 30%, 50%, of thymocytes using a fluorescence-activated cell sorter15 and on some T lymphocytes that have undergone blastic transformation in response to stimulation by concanavalin A.16 Another study showed Ia antigens in a mouse T cell lymphoma, providing evidence of the expression of Ia on malignant T cells.17

In our studies of human cells, we found that HBLA were absent on normal and most malignant T cells but could be detected by immunofluorescence on a minority of T cells that had undergone neoplastic transformation. This phenomenon was observed in the cells of one of our patients with mycosis fungoides. Smaller amounts of HBLA not detected by our immunofluorescence
method probably accounted for the absorption of a significant amount of antibody activity from the B lymphocyte antiserum by “antigen-negative” malignant T cells of patient C in Table 3. The absorbed antiserum showed diminished fluorescence with HBLA-positive lymphoma cells from other patients. In other cases of T cell lymphoma (patient D, Table 3), and in T cell ALL, HBLA could not be detected by immunofluorescence or absorption, and in this respect these T cell malignancies resembled carcinoma of the breast (patient E, Table 3) and other nonlymphoreticular neoplasms.

A major advantage of the B lymphocyte antiserum is its decisive reactivity with lymphoma cells that cannot be identified morphologically (poorly differentiated or undifferentiated). This reaction is independent of the presence of surface immunoglobulin, Fc, and complement receptors and therefore permits positive identification of so-called null cells. From the present study it appears that a positive reaction of malignant cells with the B lymphocyte antiserum should favor a diagnosis of lymphoma (or leukemia) rather than a nonlymphoreticular neoplasm. A negative reaction should instead lead one to suspect a nonlymphoreticular neoplasm or, less commonly, a T cell lymphoma. Only a minority of malignant T cells can be detected by immunofluorescence with the B lymphocyte antiserum, but these are usually readily identified by their capacity to form E rosettes or by their reaction with antithymocyte antiserum. These T cell disorders may also be recognized clinically by their prominent infiltration of the skin or mediastinum.

The unpredictable behavior and therapeutic responsiveness of some morphologically similar diffuse lymphomas may be due to inherent differences in the immunologic properties of the neoplastic cells. As in ALL and other lymphocyte surface markers may identify subgroups of diffuse lymphoma that have significant differences in survival. Patients with diffuse lymphoma whose malignant cells have surface immunoglobulin, receptors for complement, or both appear to have better survival chances than those whose malignant cells have no B or T cell markers (null cells). Our study indicates that lymphoma cells from the poor-risk “null” cell group can be positively identified with the B lymphocyte antiserum.

It is unlikely that immunofluorescent staining of HBLA-positive cells occurs through nonspecific attachment of the rabbit antibody to an Fc receptor. Granulocytes have an Fc receptor and do not stain. There is no staining of HBLA-positive cells when the specific antibody is replaced by serum obtained from rabbits prior to immunization. Furthermore, similar results are obtained when F(ab')2 fragments are used in place of the entire antibody.

The rapidity of the immunofluorescent method used to detect HBLA provides clinically relevant information within a few hours. Moreover, the technique is highly reproducible, as shown by analyses of fresh and cryopreserved cells at different times and in separate laboratories. The antiserum is readily obtainable at low cost. At the present time, the application of the technique is limited only by the proper handling of the tissues to ensure the viability of the tumor cell suspensions and the ability of the viewer to identify the malignant cells by phase-contrast fluorescence microscopy. Efforts are under way to apply this immunostaining method to frozen and fixed tissues so that its use may become more general and can perhaps be applied to retrospective studies.
REFERENCES


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