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Cell Surface Differentiation Antigens of the Malignant T Cell in Sezary Syndrome and Mycosis Fungoides

BARTON F. HAYNES, PAUL BUNN, DEAN MANN, CHARLES THOMAS, GEORGE S. EISENBARTH, JOHN MINNA, and ANTHONY S. FAUCI, Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205; Immunology Branch and the National Cancer Institute-Veterans Administration Medical Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205 and Washington, D.C. 20422; Department of Medicine, Duke University School of Medicine, Durham, North Carolina 27710

ABSTRACT Using a panel of monoclonal antibodies and rabbit heteroantisera, we have studied the cell surface markers of peripheral blood (PB) Sezary cells from six patients with mycosis fungoides or Sezary syndrome, disease grouped within the spectrum of cutaneous T cell lymphomas (CTCL). Furthermore, we have studied two cell lines (Hut 78 and Hut 102) derived from malignant Sezary T cells from CTCL patients. The monoclonal antibody 3A1 defines a major human PB T cell subset (85% of PB T cells) while the antigen defined by the monoclonal antibody 4F2 is present on a subset (70%) of activated PB T cells and on circulating PB monocytes.

In contrast to normal subjects in whom 60–70% of circulating PB mononuclear cells were 3A1+ T cells, PB mononuclear cells from six CTCL patients studied had an average of only 10.6±3.2% 3A1+ T cells. Whereas 85% of E-rosetting positive cells from normal individuals were 3A1+, virtually all E-rosette positive T cells from the Sezary patients were 3A1−. Two patients with high numbers of circulating Sezary T cells had both aneuploid and diploid PB T cell populations present; after separation of PB T cells into 3A1+ and 3A1− cell suspensions, all 3A1− cells were found to be aneuploid. In contrast to normal resting PB T cells which were 4F2−, all PB Sezary cells were 4F2+, suggesting a state of activation. The 3A1 antigen was on a variety of acute lymphoblastic leukemia T cell lines (HSB-2, RPMI-8402, MOLT 4, CEM) but was absent on the Hut 78 and Hut 102 Sezary T cell lines.

Using rabbit anti-human T and anti-human Ia (p23, 30) antisera, we found that all malignant Sezary PB cells tested were killed by anti-T cell antiserum plus complement but not by anti-Ia plus complement. In contrast, Sezary cell lines Hut 78 and 102, were killed by both anti-T cell antiserum and anti-Ia plus complement.

Similar to 3A1− normal PB T cells, 3A1− Sezary PB T cells proliferated poorly to phytohemagglutinin and concanavalin A. However, 3A1+ Sezary T cells were able to provide T cell help toward pokeweed mitogen-induced in vitro B cell immunoglobulin synthesis, an immunoregulatory function limited to 3A1+ T cells in normal subjects.

Thus, the 3A1 antigen is present on 85% of normal PB T cells, and on most T-acute lymphoblastic leukemia lines tested; in contrast the 3A1 antigen is not present on the majority of circulating malignant Sezary PB T cells nor on T cell lines derived from malignant Sezary T cells. The lack of expression of the 3A1 antigen may be associated with malignant transformation of T cells in CTCL and may be an important marker for tracing the clonal origin of the malignant Sezary T cell.

INTRODUCTION

Mycosis fungoides (MF) and the Sezary syndrome (SS) are uncommon lymphoid malignancies that are

1 Abbreviations used in this paper: ALL, acute lymphoblastic leukemia; C, complement; Con A, concanavalin A; CTCL, cutaneous T cell lymphoma; E, erythrocyte; MF, mycosis fungoides; PB, peripheral blood; PHA, phytohemagglutinin; PWM, pokeweeds mitogen; sIg, surface immunoglobulin; SS, Sezary syndrome.
part of the spectrum of cutaneous T cell lymphomas (CTCL) (1, 2). Classic MF presents with a scaly eruption that progresses through a plaque stage leading to skin tumors (3). MF patients are not overtly leukemic, although 25% of cases have circulating malignant Sezary cells (4). SS is manifested by generalized exfoliative erythroderma and, as with MF, is frequently associated with circulating malignant T cells in the peripheral blood (PB) (3, 4).

In most cases of MF and SS, the malignant Sezary cells have been shown to be T cells by reaction with antiserum to human T lymphocyte antigens, lack of surface immunoglobulin (sIg), and rosette formation with sheep erythrocytes (E) (5).

In contrast to many leukemic lymphoblastic T cells which are thought to be derived from immature T cells (6), the malignant T cells in SS and MF are thought to be derived from relatively mature T cells as determined by the absence of terminal deoxynucleotidyl transferase (7), by the presence of in vitro helper T cell activity for B cell immunoglobulin synthesis (8), and by the production of macrophage migration inhibitory factor (9).

We have recently characterized several lymphocyte hybrid monoclonal antibodies that identify subsets of human PB resting and activated T cells (10–13). The present study reports the characterization of the Sezary T cell using three of these reagents.

METHODS

Cell suspensions. Purified mononuclear cell suspensions were obtained from heparinized venous blood or from leukapheresis of six normal adults and six MF or SS patients by standard Hypaque-Ficol density centrifugation (14).

Cells were counted in a coulter counter, model Fn (Coulter Electronics, Hialeah, Fla.), and differential counts of mononuclear cell suspensions were performed on cyto-centrifuge preparations stained with Wright’s stain. Cell viability was determined by the trypan blue dye exclusion method. In some cases, patients’ leukapheresis PB cells were frozen in 7.5% dimethyl sulfoxide in RPMI supplemented with 10% fetal calf serum for assay at a later date.

Cell lines used. The HSB-2 T, SB B, and IMR-32 neuroblastoma cell lines were obtained from the American Type Culture Collection, Rockville, Md. (15). Hut 78 and Hut 102 T cell lines were derived from PB and lymph node T cells, respectively, from MF on SS patients (16). Hut 51, Hut 77, and NUT 128 cell lines were Epstein-Barr virus-transformed B cell lines, started from the PB cells of patients with oat cell carcinoma. Hut 69C, Hut 376, and NUT 231 lines were started from tumor cells from patients with oat cell carcinoma of the lung. All other commonly used cell lines were obtained from either Dr. Gazdar or Dr. Mann.

Identification of lymphocyte subpopulations. T cells were identified by their ability to form spontaneous rosettes with sheep E or by their ability to be lysed in the presence of complement (C) by anti-human T cell antisera prepared by the method of Anderson and Metzgar (17) or as previously described (18). B lymphocytes were identified by the presence of surface membrane immunoglobulin. Cells bearing Fe receptors for either IgG or IgM were determined as previously described (14). Cells bearing human IgA determinants were determined by their ability to be lysed by anti-p23, 30 antiserum and C (19). Cytotoxicity assays were carried out using a modification of the microcytotoxicity assay of Terasaki and McClelland (20). Less than 20% of cells were killed in the presence of C alone.

Lymphocyte hybrid monoclonal antibodies and flow cytometry. Lymphocyte hybrid monoclonal antibodies used included 3A1, 4F2, and 3F10 (11). These three monoclonal antibodies were passed as ascites tumors in BALB/c mice; ascites fluid was harvested, isoelectrically focused or ammonium sulfate precipitated, and directly fluoresceinated for use in flow cytometry (13). The 3A1 monoclonal antibody recognizes a 40,000-mol wt cell surface T cell antigen which is on >90% of human thymocytes, 30–55% of spleen cells, and 85% of PB E-rosette positive T cells. 3A1+ T cells blast transform to phytohemagglutinin (PHA) and canavalan A (Con A), induce in vitro B cell immunoglobulin synthesis, and also contain cells which can be induced by Con A to suppress immunoglobulin synthesis (13). 3A1- T cells respond submaximally to PHA, not at all to Con A, and do not induce in vitro B cell immunoglobulin synthesis but do mediate antibody-dependent cellular cytotoxicity (13). Antibody 4F2 binds to activated but not resting PB lymphocytes as well as to other types of rapidly dividing cells and to circulating PB monocytes (11, 12). Antibody 3F10 precipitates the 44,000-mol wt nonpolyclonal HLA antigen and thus binds to all human cells that express HLA determinants (11). Characteristic of the monoclonal antibodies used are summarized in Table I. Controls for background staining of cells included unstained cells and cells incubated with directly fluoresceinated IgG or IgG mouse myeloma protein which was nonreactive with human lymphoid cells (13). Flow cytometry was performed on a Becton-Dickinson FACS-II system (Becton, Dickinson & Co., Rutherford, N. J.). (23).

Solid-phase radioassay to measure cell-surface antibody binding. 5 x 10^6 cells were incubated in varying dilutions of 3A1, 4F2, or 3F10 ascites fluid, washed three times in Dulbecco’s phosphate-buffered saline with 0.1% gelatin and then incubated with affinity-purified 125I-labeled right anti-mouse IgG (20.000 cpm), and counted as previously described (10, 12).

Fractionation of mononuclear cell suspensions. Purified T cells were isolated by rosetting mononuclear cells with sheep E followed by Hypaque-Ficol centrifugation (14). 3A1+ and 3A1- cell suspensions were obtained using 3A1-coated polystyrene microbeads (13). 3A1 antibodies diluted 1:300 in phosphate-buffered saline, pH 7.4, was incubated for 4 h at 4°C on a 100 x 15-mm plastic petri dish (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) followed by multiple washes in cold phosphate-buffered saline. 50 x 10^6 lymphocytes in 5 ml RPMI 1640 medium supplemented with 10% fetal calf serum were added to a 3A1-coated plate and incubated 1 h at 20°C. During this period, the nonadherent lymphocytes were gently washed with the plate containing 3A1+ (adherent) lymphocytes was washed 5 times with phosphate-buffered saline, then incubated with 5 ml of RPMI 1640 supplemented 10% fetal calf serum for 15 min at 37°C. 3A1+ cells were then removed by aspiration. 3A1- cells contained <5% contaminating 3A1- cells whereas 3A1+ cells contained <5% contaminating 3A1+ cells.

Assays for Sezary cell DNA content. For DNA content analysis Sezary and normal T cells were stained with
Calculating lymphocytes summarizes the clinical and described patients with CTCL and the Kirshan for features for described as immunoglobin production.

RESULTS

Patients and cell lines studied. Table II summarizes the clinical and laboratory data on the six CTCL patients studied. Between 64 and 99% of the circulating lymphocytes of the patients at the time of study were Sezary cells. Leukocyte counts ranged from 12,000 to 150,000 cells/mm³. Only one patient (163) had received any chemotherapy prior to study.

Assay for 3A1 antigen on Sezary cell lines. Because of our initial observation that the YT4E Sezary T cell line did not express the 3A1 antigen (13), we determined the presence of 3A1 antigen on two additional T cell lines from Sezary patients (Hut 78 and Hut 102 T cell lines [16]), on several ALL T cell lines, and on a variety of other cell lines (Table III). As we have previously reported, the HSB-2 T cell (and all other ALL cell lines thus far tested) strongly bound 3A1 antibody in the ¹²⁵I-rabbit anti-mouse IgG radioassay (10, 13). All B cell lines tested thus far have been 3A1⁻. More importantly, as with the YT4E Sezary T cell

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis and stage</th>
<th>WBC*</th>
<th>% of total lymphocytes</th>
<th>Previous systemic chemotherapy before study</th>
</tr>
</thead>
<tbody>
<tr>
<td>155</td>
<td>60</td>
<td>M</td>
<td>MF, IVB</td>
<td>37,000</td>
<td>83 2 16 80</td>
<td>None</td>
</tr>
<tr>
<td>185</td>
<td>78</td>
<td>M</td>
<td>SS, IVB</td>
<td>122,000</td>
<td>95 4 1 95</td>
<td>None</td>
</tr>
<tr>
<td>167</td>
<td>62</td>
<td>M</td>
<td>MF, IVB</td>
<td>12,000</td>
<td>42 15 33 67</td>
<td>None</td>
</tr>
<tr>
<td>163</td>
<td>72</td>
<td>F</td>
<td>SS, IVB</td>
<td>150,000</td>
<td>53 1 46 64</td>
<td>MOPP, CHOP</td>
</tr>
<tr>
<td>110</td>
<td>42</td>
<td>M</td>
<td>SS, IVB</td>
<td>130,000</td>
<td>49 4 13 96</td>
<td>None</td>
</tr>
<tr>
<td>152</td>
<td>60</td>
<td>M</td>
<td>SS, IVB</td>
<td>19,000</td>
<td>86 1 13 99</td>
<td>None</td>
</tr>
</tbody>
</table>

* Leukocyte count.
† Surface immunoglobulin.
§ Null cells are sIg⁻, E-rosette⁻ lymphocytes.
[1] Staging classification of National Workshop on Cutaneous T Cell Lymphomas (3). Stage IVB signifies the presence of lymphomatous involvement of visceral organ (3).
mononuclear cells and 85% of normal PB T cells were 3A1+. In contrast, virtually no 3A1+ cells were present in suspensions of PB mononuclear cells or purified T cells from patient 185 (Fig. 1C and D). To further investigate this observation, PB cell suspensions from five Sezary patients were separated into highly purified (>95% pure) 3A1+ and 3A1− cell suspensions on 3A1-coated plastic polystyrene plates (13). The 3A1+ and 3A1− cell suspensions were then analyzed for cell morphology on Wright’s stained cytocentrifuge preparations and for DNA content using flow cytometry, as well as analyzed for the presence of a variety of cell surface markers. In all patients, 3A1+ cells made up a small minority of the total number of PB cells; conversely, most of the cells isolated were 3A1−. Table V demonstrates that all of the 3A1− PB cells from five CTCL patients were T cells as judged by E-rosette formation and reactivity with rabbit anti-T cell antisera. The 3A1− Sezary T cells usually did not express IgG or IgM FcR and, in most cases, did not express Ia antigen, but were reactive with 4F2 antibody. In addition, 90–98% of the 3A1− T cells were Sezary cells by morphologic analysis.

In two patients (110 and 155) the Sezary cells had hyperdiploid contents which were easily distinguished from normal diploid PB cells by DNA content analysis (23). After separation of the patient’s PB cells into 3A1+ and 3A1− groups, we were able to demonstrate that all of the diploid cells were 3A1+ whereas all or nearly all of the hyperdiploid cells were 3A1− (Table VI). These studies confirmed directly that most Sezary T cells are 3A1−.

**Functional studies of Sezary T cells.** Numerous studies have previously shown that Sezary T cells can mediate in vitro immune cell function such as T cell help toward PWM-induced immunoglobulin synthesis (8) and the production of macrophage migration inhibitory factor (9). Other immune cell functions have been found lacking or diminished in Sezary T cells,
i.e. blastogenesis to mitogens and antigens as well as antibody-dependent cellular cytotoxicity (26). In order to correlate the presence or absence of the 3A1 antigen with in vitro lymphocyte function, we measured the ability of purified 3A1- Sezary T cells from representative patients to mediate help for PWM-induced intracytoplasmic immunoglobulin synthesis (patients 110, 155, and 185) and to blast transform to PHA and Con A stimulation (patients 155 and 185).

We found that the PB T cells of all three patients tested (110, 155, and 185) could help (although somewhat less than normal T cells) normal B cells in the PWM-stimulated in vitro immunoglobulin-synthesis assay (Fig. 2). This helper T cell functional capability is similar to that which has been previously reported for Sezary T cells (8). Similarly, as others have reported, Sezary T cells responded poorly to activation by PHA and Con A (26) (Fig. 3A and B). Table VII summarizes the differences in cell-surface markers between normal PB T cells, Sezary PB cells, ALL T cell lines, and Sezary T cell lines.

**DISCUSSION**

The present study has used a panel of monoclonal antibodies and heteroantisera to study the cell-surface markers of the malignant Sezary T cell. While
Table V

Characteristics of PB 3A1- Lymphocyte Suspensions from MF or SS Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>E-rosette*</th>
<th>HTLA+</th>
<th>Ia+</th>
<th>3A1+</th>
<th>4F2+</th>
<th>FcR*</th>
<th>Sezary cell morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>110</td>
<td>86</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>30 95</td>
</tr>
<tr>
<td>155</td>
<td>95</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>1</td>
<td>10 97</td>
</tr>
<tr>
<td>163</td>
<td>60</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>21</td>
<td>0 98</td>
</tr>
<tr>
<td>167</td>
<td>65</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>ND*</td>
<td>ND 90</td>
</tr>
<tr>
<td>185</td>
<td>99</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>7</td>
<td>8 97</td>
</tr>
</tbody>
</table>

* FcR, Fc receptors.
† Determined by microcytotoxicity assay using either rabbit anti-T cell antiserum (17, 18) or rabbit p23,30 antiserum (19) and C for T cell antigens and Ia antigens, respectively. HTLA, human T lymphocyte antigen.
‡ Determined by binding of directly fluoresceinated monoclonal antibody as measured by flow cytofluorometry.
§ Not determined.

possessing many of the characteristics of well-differentiated normal T cells (lack of terminal deoxynucleotidyl transferase, expression of helper T cell activity, and production of macrophage migration inhibitory factor), the Sezary T cell does not express a surface antigen (detected by 3A1 monoclonal antibody) that is readily detected on 85% of normal PB E-rosetting cells and most normal thymocytes. Furthermore, the antigen is not found on any of the three Sezary T cell lines tested (YT4E, Hut 102, and Hut 78). There are several potentially important implications of this observation for our understanding of the relationship between stable cell-surface antigens and functional capabilities, as well as between the expression of cell-surface antigens and malignant transformation of lymphoid cells.

The lack of detectable expression of the cell-surface antigen reactive with 3A1 antibody by Sezary T cells can be potentially explained in a number of ways. (a) 3A1+ and 3A1- cells may both represent progeny of T cells originally contained in the thymus. Since most, but not all thymocytes are 3A1+, it is possible that both 3A1+ and 3A1- cells leave the thymus to make up the PB T cells with the 3A1+ T cells comprising the major proportion (85%) of PB T cells. If this were the case, then the Sezary T cell may represent a malignant clonal expansion of the 3A1- T cells that are at least from a post-thymic standpoint, clonally distinct from the 3A1+ T cells. The functional studies argue against, but do not completely rule out, this possibility. Since helper T cell function in normal PB T cells is contained within the 3A1+ T cell subset, and since the Sezary PB T cells (which are 3A1-) express helper T cell function, then the Sezary T cells would have to malignantly transform while assuming a functional capability of a clonally distinct subset (3A1+ helper T

Table VI

Characterization of Diploid and Hyperdiploid Sezary PB Lymphocytes by Separation into 3A1+ and 3A1- Subpopulations

<table>
<thead>
<tr>
<th>Patient</th>
<th>155</th>
<th>110</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total PB lymphocytes, cells/mm³</td>
<td>27,750</td>
<td>123,500</td>
</tr>
<tr>
<td>3A1+ Diploid lymphocytes, %</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>3A1+ Hyperdiploid lymphocytes, %</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>3A1- Diploid lymphocytes, %</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3A1- Hyperdiploid lymphocytes, %</td>
<td>99</td>
<td>82</td>
</tr>
</tbody>
</table>

Figure 2. Ability of normal T cells and Sezary T cells to induce PWM-driven intracytoplasmic immunoglobulin in B cells in vitro. Normal or Sezary T cells were cultured alone (10⁶ cells/culture) or were added to purified normal allogeneic B cells (containing 40% sIg+ B cells, 45% monocytes and 15% null cells) in a 1:1 ratio (0.5 x 10⁶ cells to 0.5 x 10⁶ B cell). After stimulation with PWM for 7 d, the number of viable cells was counted, the cells fixed in acetone-alcohol, stained with fluoresceinated rabbit anti-human IgG (18), and counted for the number of intracytoplasmic (ICP) immunoglobulin* cells.

Haynes, Bunn, Mann, Thomas, Eisenbarth, Minna, and Fauci
cells). (b) An alternative explanation is that the Sezary T cell is derived from a 3A1+ T cell and maintains helper T cell functional capability but has lost the ability to express the 3A1 antigen in association with malignant transformation. Because helper T cell function is contained virtually exclusively within the normal 3A1+ T cell subset, this observation represents a clear-cut discordance between the expression of the 3A1 antigen and helper T cell function. (c) Related to this latter explanation are the possibilities that the 3A1 antigen could still be synthesized by the Sezary T cell but not expressed on the cell surface, or that the Sezary T cell could express the 3A1 antigen but in an altered form that is not recognized by the 3A1 monoclonal antibody. We cannot rule out any of these possibilities from the data presented, nor can we rule out a fourth possibility, (d), that there are subsets of 3A1- T cells, some of which can help in vitro immunoglobulin induction, and the Sezary T cell originates from the malignant transformation of a subset of 3A1- helper T cells.

It is of interest that unlike some ALL PB cells (19), Sezary PB cells do not express Ia determinants detected by standard reagents (Table V). However, Sezary PB T cells are positive for 4F2 antigen, a 120,000-mol wt antigen which is not found on resting lymphocytes but is found on Con A and mixed lymphocyte reaction-activated PB T cells, all dividing cell lines, and on PB monocytes (12). The significance of this differential expression of the 4F2 antigen on normal and Sezary PB T cells is at present unknown. However, since the 4F2 antigen is present on normal activated T cells, it may reflect a certain relationship between malignant transformation and markers of cell activation.

It has previously been shown that anti-Sezary T cell heteroantisera reacted with different T cell-specific cell-surface antigens than did anti-monkey thymocyte antisera (27) or antisera raised against other leukemic cells or normal human thymocytes (28). These data suggested that Sezary T cells express different cell-surface T cell antigens than do other types of normal or malignant T cells. Key to the further study of the clonal

**TABLE VII**

Comparison of Cell-membrane Markers of PB Sezary Cells, Sezary and ALL Cell Lines, and Normal PB T Cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>E-rosette</th>
<th>lg</th>
<th>IgM</th>
<th>IgG</th>
<th>la1</th>
<th>HTLAI</th>
<th>3A1§</th>
<th>4F2§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal PB T cells**</td>
<td>100</td>
<td>0</td>
<td>55–70</td>
<td>5–15</td>
<td>0–5</td>
<td>100</td>
<td>75–90</td>
<td>0£</td>
</tr>
<tr>
<td>ALL T cell lines**</td>
<td>40–70</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>PB Sezary cells</td>
<td>50–90</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Hut 78 and Hut 102 Sezary cell lines**</td>
<td>40–80</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

* Fc receptors.
† Determined by microcytotoxicity assay using either rabbit anti-P23,30 antiserum (19), a rabbit anti-T cell antiserum (17, 18) + C. HTLA, human T lymphocyte antigen.
§ Determined by binding of directly fluoresceinated monoclonal antibody as measured by flow cytofluorometry.
‡ Range of values from studies of six normal PB T cells suspensions.
¶ 4F2 antigen is present in negligible amounts on resting normal PB T cells, but is expressed on a subpopulation of activated normal T cells.
** Tissue-culture cell lines.

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origin of Sezary T cells will be the development of monoclonal antisera which differentially bind to and positively select the Sezary T cell.

Thus, using monoclonal reagents that define cell-surface antigens that are relevant to normal immune cell differentiation events, we have observed that the great majority of malignant Sezary T cells do not bind the human T cell-specific 3A1 monoclonal antibody. Study of the genetic events associated with the lack of normal expression of the 3A1 T cell antigen should provide insight into the events associated with malignant transformation and clonal origin of Sezary T cells.

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The authors would like to thank Dr. A. Gazdar for the gift of small cell, B cell, and Sezary T cell lines; Mr. Howard Mostowski for expert technical assistance; and Mrs. Betty Sylvester and Mrs. Joan Barnhart for expert editorial assistance.

REFERENCES


