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Research Article

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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-rosette 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 […]

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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 $10.6\pm3.2\%$ $3A1^+$ T cells. Whereas 85% of E-rosette 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 towards 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)[1] and the Sezary syndrome (SS) are uncommon lymphoid malignancies that are

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[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, pokeweed 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, frequently is 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 antisera 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-Ficoll 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 cytocentrifuge 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 immunoglobin. Cells bearing Fc receptors for either IgG or IgM were determined as previously described (14). Cells bearing human Ia 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 cytofluorometry. Lymphocyte hybrid 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 fluorsceinated for use in flow cytofluorometry (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 concanavalin 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 nonpolymorphic HLA antigen and thus binds to all human cells that express HLA-determinants (11). Characteristics 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_1 or IgG_{2a} mouse myeloma protein which was nonreactive with human lymphoid cells (13). Flow cytofluorometry 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. 5×10^5 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 ^{125}I-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-Ficoll centrifugation (14). $3A1^+$ and $3A1^-$ cell suspensions were obtained using 3A1-coated polystyrene plates (13). 3A1 ascites fluid diluted 1:300 in phosphate-buffered saline, pH 7.4, was incubated for 4 h at 4°C on a 100 × 15-mm plastic petri dish (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) followed by multiple washes in cold phosphate-buffered saline. 50×10^6 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 incubation, the nonadherent lymphocytes were gently resuspended by swirling the plate once after 30 min. After 1 h, the $3A1^-$ (nonadherent) cells were gently poured off, and 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

TABLE I
Characteristics of Monoclonal Antibodies used to Phenotype Sezary T Cells

	Monoclonal antibody		
	3A1	4F2	3F10
Murine immunoglobulin subclass	IgG_1	IgG_{2a}	IgG_{2a}
Ability to bind C and Staphylococcal protein A	Yes	Yes	Yes
Binding to normal PB mononuclear cells	Binds 85% of PB T cells	Binds PB monocytes and a subset of activated T cells	Binds all PB mononuclear cells
Antigen characterization	40,000-mol wt glycoprotein	120,000-mol wt glycoprotein*	44,000-mol wt HLA

* Antigen size determined under nonreducing conditions.

hypotonic propidium iodide according to the method of Kirshan (22), and the DNA content of the cells measured in a Coulter TPS-1 cell sorter (Coulter Electronics) as previously described in detail (23).

Lymphocyte functional assay. In vitro lymphocyte cultures for the determination of blast transformation to mitogen stimulation, as measured by tritiated thymidine incorporation, or for pokeweed mitogen (PWM)-driven intracytoplasmic immunoglobin production were set up and assayed as previously described (18).

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/mm^3. 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 ^{125}I-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 II
Hematologic Profile of MF or SS Patients at the Time of Study

Patient	Age	Sex	Diagnosis and stage	WBC*	% of total lymphocytes				Previous systemic chemotherapy before study
					E-rosette	sIg‡	Null§	Sezary morphology	
	yr								
155	60	M	MF, IVB‖	37,000	83	2	16	80	None
185	78	M	SS, IVB	122,000	95	4	1	95	None
167	62	M	MF, IVB	12,000	42	15	33	67	None
163	72	F	SS, IVB	150,000	53	1	46	64	MOPP, CHOP¶
110	42	M	SS, IVB	130,000	49	4	13	96	None
152	60	M	SS, IVB	19,000	86	1	13	99	None

* Leukocyte count.
‡ Surface immunoglobulin.
§ Null cells are sIg^-, $E\text{-rosette}^-$ lymphocytes.
‖ Staging classification of National Workshop on Cutaneous T Cell Lymphomas (3). Stage IVB signifies the presence of lymphomatous involvement of visceral organ (3).
¶ MOPP designates nitrogen mustard, vincristine, procarbazine, and prednisone (23). CHOP designates doxorubricin, prednisone, vincristine, and cyclophosphamide (24).

TABLE III

Reactivity of ALL T Cell Lines, Sezary T Cell Lines, B Cell Lines, and Various Other Cell Lines with Monoclonal Antibodies 3A1, 3F10, and 4F2

Cell line	Type	3A1	3F10	4F2
T cell lines				
CEM	T-ALL	+*	+	+
RPMI-8402	T-ALL	+	+	+
HSB-2	T-ALL	+	+	+
MOLT 4	T-ALL	+	+	+
YT4E	Sezary	−‡	+	+
Hut 78	Sezary	−	+	+
Hut 102	Sezary	−	+	+
B cell lines				
Hut 51	B cell	−	+	+
Hut 77	B cell	−	+	+
Hut 128BL	B cell	−	+	+
SB	B cell	−	+	+
RPMI-8392	B cell	−	+	+
Others				
Hut 69C	Oat cell carcinoma	−	+	+
Nut 231	Oat cell carcinoma	−	+	+
Nut 376	Oat cell carcinoma	−	+	+
IMR-32	Neuroblastoma	−	−	+

* +, Significant binding (counts per minute greater than threefold background counts per minute) over background counts of the monoclonal antibody to the cell line as measured in the ^{125}I-rabbit anti-mouse immunoglobulin-binding assay.
‡ −, No significant binding over background counts of the monoclonal antibody to the cell line as measured in the ^{125}I-rabbit anti-mouse immunoglobulin-radiobinding assay.

line, the Hut 102 and Hut 78 Sezary T cell lines did not bind 3A1 antibody. In contrast, all cell lines tested bound the 4F2 antibody, and all except the IMR-32 neuroblastoma line bound 3F10 antibody (anti-HLA).

Determination of the presence of 3A1$^+$ T cells in the PB of MF or SS patients with high numbers of circulating Sezary cells. Since all three Sezary T cell lines did not bear the 3A1 antigen (Table II), we determined the cell-surface characteristics of PB Sezary cells from MF or SS patients. Normal subjects had a range of 65–80% 3A1$^+$ cells in their PB mononuclear cells (10), while our six CTCL patients had an average of 10.6±3.2% 3A1$^+$ PB mononuclear cells (Table IV). Conversely, the CTCL patients had an abnormally high percentage of 3A1$^-$ PB mononuclear cells (89.3±3.2%) compared to normal subjects (range 20–35%). Thus, most of the patients had very high percentages and absolute counts of circulating malignant Sezary cells (Table II) and most of these cells were 3A1$^-$ (Table IV). The flow cytofluorometry histogram of binding of directly fluoresceinated 3A1 antibody to normal unfractionated PB mononuclear cells (Fig. 1A) and to normal purified T cells (Fig. 1B) demonstrates that 75% of normal PB

TABLE IV

Percentage of 3A1$^+$ and 3A1$^-$ Cells in PB Mononuclear Cell Suspensions from MF or SS Patients

Patient	3A1$^+$	3A1$^-$
	%	%
155	1	99
185	1	99
167	18	82
163	10	90
110	18	82
152	16	84
Normal values*	64–80	20–35

* Range of values from studies of six normal PB mononuclear cell suspensions.

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 cytofluorometry, 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 toal 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,

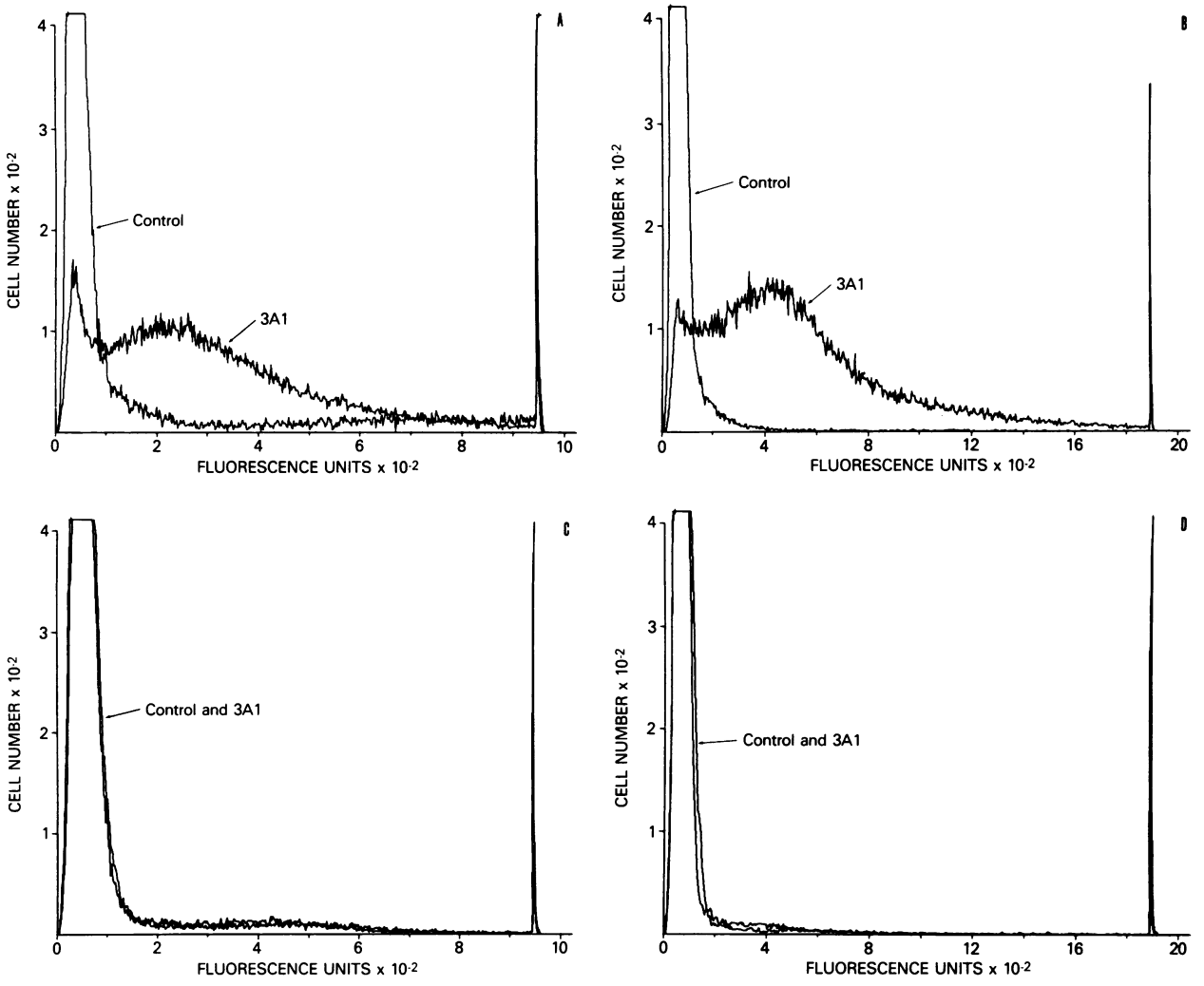

FIGURE 1 Figure illustrates the flow cytofluorometry histograms of binding of directly labeled 3A1 antibody to normal unfractionated PB mononuclear cells (A), normal purified T cells (B), Sezary PB mononuclear cells from patient 185 (C), and PB Sezary T cells (patient 185) (D). Whereas 75% of normal PB mononuclear cells and 85% of normal PB T cells are $3A1^+$, none of the PB cells of patient 185 are $3A1^+$.

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

Patient	E-rosette+	HTLA+‡	Ia+‡	3A1+§	4F2+§	FcR* IgG	FcR* IgM	Sezary cell morphology
				%				
110	86	100	0	0	100	0	30	95
155	95	100	0	0	100	1	10	97
163	60	100	0	0	100	21	0	98
167	65	100	0	0	100	ND‖	ND	90
185	99	100	0	0	100	7	8	97

* 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

	Patient 155	Patient 110
Total PB lymphocytes, *cells/mm³*	27,750	123,500
3A1⁺ Diploid lymphocytes, %	1	3
3A1⁺ Hyperdiploid lymphocytes, %	0	15
3A1⁻ Diploid lymphocytes, %	0	0
3A1⁻ Hyperdiploid lymphocytes, %	99	82

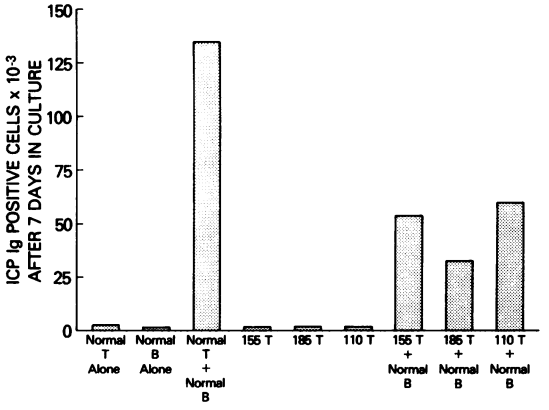

FIGURE 2 Ability of normal T cells and Sezary T cells to induce PWM-driven intracytoplasmic immunoglobulin in B cells in vitro. Normal or Sezary T cells were cultured alone (10^6 cells/culture) or were added to purified normal allogeneic B cells (containing 40% sIg⁺ B cells, 45% monocytes and 15% null cells) in a 1:1 ratio (0.5×10^6 cell to 0.5×10^6 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.

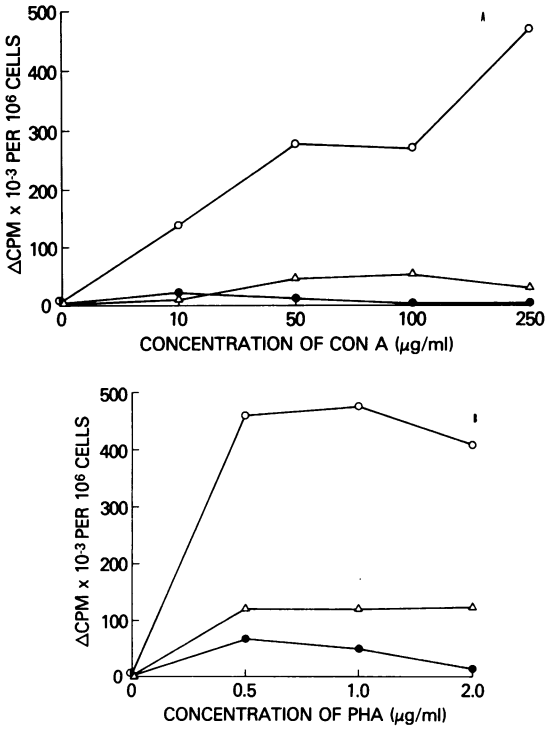

FIGURE 3 Blast transformation to Con A (A) and PHA (B), as measured by tritiated thymidine incorporation of normal PB T cells (○), and Sezary T cells from patient 155 (Δ) and 185 (●). Sezary T cells responded submaximally to both PHA and Con A.

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 maligant 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 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

Cell type	E-rosette	sIg	FcR* IgM	FcR* IgG	Ia‡	HTLA‡	3A1§	4F2§
			% cells positive					
Normal PB T cells‖	100	0	55–70	5–15	0–5	100	75–90	0¶
ALL T cell lines**	40–70	0	0	0	100	100	100	100
PB Sezary cells	50–90	0	0–30	0–21	0	100	0–8	100
Hut 78 and Hut 102 Sezary cell lines**	40–80	0	0	0	100	100	0	100

* 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.

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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