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Surface Antigenic Markers and Receptors for Human T and B Cells and Lymphoproliferative Disorders

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Over the past decade remarkable progress in understanding of the differentiation and surface markers of lymphocytes have been achieved. As a result it is well known that mammalian lymphocytes can be classed into two major groups, T cells and B cells[1]. These are distinguished by their function in immune responses. Of particular interest, there are intimate correlations between immunological function and surface markers, such as surface antigens and receptors of lymphocytes[2-4]. Therefore, it is convenient to classify and analyze lymphoid tumors by using these surface markers.

In the present work we have conducted tests on human cases of leukemias and lymphomas for the presence of surface antigens and receptors for T and B cells.

MATERIALS AND METHODS

Forty three male and female patients with lymphoproliferative disorders, including acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), lymphosarcoma cell leukemia (LSL) and malignant lymphoma, were investigated for their surface markers as shown in Table 1. Several cases of acute myeloid leukemia (AML), malignant reticulosis and multiple myeloma were also investigated.

Rosetting with sheep red cells (E-rosette) was used as the T cell marker. Heterologous antisera, which were rendered specific for human thymocytes[5], ATS-T, or for peripheral T cells[6], ALS-T, were also employed. In addition, we used several B cell markers such as surface bound Ig and a C3-receptor (EAC-rosette). Human B lymphocyte-specific antigens were defined by a heterologous anti-human B cell serum, ABS[7]. The methods of rosetting tests, cytotoxic test, immunofluorescence and preparation of antisera have been described elsewhere[4-8]. The specificities of these sera have been re-

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Table 1. *Surface Markers and Subpopulations*

markers	thymocyte	T cell	B cell
E 37°C	+	−	−
4°C	+	+	−
ALS-T	−*	+	−
ATS-T	+	−	−
C3-receptor	−	−	+
surface Ig	−	−	+
ABS	−	−	+

* medullary thymocytes are reactive with ALS-T.

ported previously[6,7,9]. It may be noted here that ATS-T reacts only with thymocytes. The percentage of cells which reacted to ALS-T was well correlated with that of E-bining cells in the peripheral lymphoid organs. In contrast, the number of ABS-sensitive cells was well correlated to that of EAC-binding cells. In the thymus, the majority of cells formed E-rosettes but only a minor population was lysed by ATS. ABS was entirely non-toxic for thymus cells. It should be noted that thymus-derived cells can be divided into two subsets according to their reactivity to ATS-T and ALS-T.

RESULTS AND DISCUSSION

Immunofluorescence studies of sections of normal and neoplastic lymphoid tissues.

It should be emphasized that an immunofluorescence study with ATS-T, ALS-T and ABS enabled us to demonstrate the histological localization of normal or neoplastic T and B cells in preserving the original structure of lymphoid organs or tumor tissues.

An indirect immunofluorescence study of a section of normal lymph node with ALS-T, i. e., anti-peripheral T cell serum, revealed that cells located in the lymphoid follicle were essentially devoid of fluorescence and lymphocytes located in the paracortical area, thymus-dependent area, were stained with ALS-T. In the human thymus, medullary thymocytes reacted intensely with ALS-T, whereas cortical thymocytes showed an almost negative fluorescence. ABS failed to stain thymocytes. ATS-T stained all thymocytes, but did not stain peripheral lymphocytes.

ABS stained cells in the lymphoid follicles intensely but did not stain cells in the paracortical area.

The same technique was applied to lymphoid malignancies. The tech-

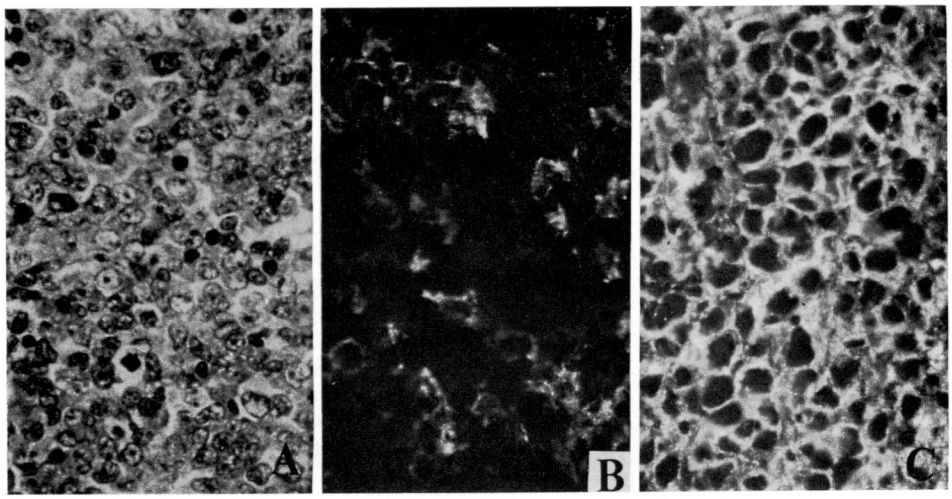

Fig. 1. A: Malignant lymphoma diagnosed as reticulum cell sarcoma after Hematoxyln-Eosin staining.
B: Immunofluorescent staining of the same preparation as in (A) with ALS-T. No tumor cells are stained.
C: Immunofluorescent staining of the same section with ABS. Note that the tumor cells are ABS-positive. Therefore this tumor was diagnosed as a B cell lymphoma.

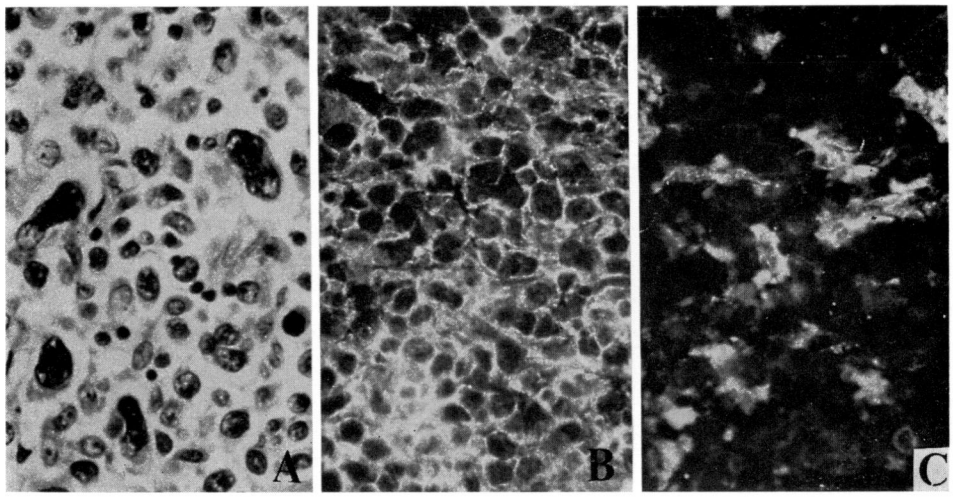

Fig. 2. A: Histology of a nasopharyngeal tumor. Hematoxylin-Eosin staining.
B: Immunofluorescent staining of the same section with ALS-T. The tumor surface is intensely stained.
C: Immunofluorescent staining of the adjacent section with ABS. Necrosis is non-specifically fluorescent, but tumor cells are ABS-negative. Therefore this tumor was diagnosed as a T cell lymphoma.

nique was highly satisfactory in the diagnosis of lymphomas with special regards to their origin by using immunofluorescence technique with ALS-T and ABS. When we investigated cell markers with a single cell suspension, we often encountered difficulty in evaluating the results of the rosette tests, cytotoxic tests and membrane fluorescence in suspended cells. This is because of the contamination of non-neoplastic cells in the cell suspension. In such cases, immunofluorescence studies with ATS-T, ALS-T and ABS on tissue sections, using hematoxylin-eosin staining of the same section as referrence were very useful in discerning whether the fluorescent cells were neoplastic or normal.

Surface markers of acute lymphoblastic leukemia

Using these antisera and other markers, lymphoid malignancies were investigated for their surface characteristics. Table 2 shows a summary of ALL. Null cell ALL was most common. A few cases were T cell ALL. These were ATS-T reactive but ALS-T negative, which fact suggests their thymic origin.

In contrast to other previous reports, B cell ALL were rather frequent.

Table 2. *Acute Lymphoblastic Leukemia (ALL)*

Case No.	T cell			B cell		
	E	ALS-T	ATS-T	EAC	IgS	ABs
1 (1, F)	2		0*	1		0
2 (3, M)	1		3*	3		0
3 (1, F)	4		1*	2		1
4 (6, M)	2		0*	1		
5 (8, M)	1		0*	4		
6 (14, M)	10	2	0*	16		21
7 (6, M)	7		70*	5		8
8 (14, F)	2			3		0
9 (16, M)	72			14		
10 (18, M)	42	0	98	0		0
11 (4 m, M)	0	0	2*	11		83
12 (2, M)	2	3		57	1	88
13 (10, M)	1	1		34	3	82
14 (3, M)	14	5		4	0	86
15 (4, F)	3	4		8	0	73
16 (5, M)	9	0		5	45	60

M: male F: female

* anti-T developed from anti-thymus serum.

The reason why B cell ALL were frequent in our series, was that ABS detected B cell antigens of the lymphocytes which were lacking in other B cell markers. Otherwise, they would be overlooked and diagnosed as null cell ALL.

Surface markers of chronic lymphocytic leukemia and lymphosarcoma cell leukemia

The majority of chronic lymphocytic leukemia, CLL, showed B cell markers (Table 3). It was shown that, in some cases, there is a disparity between the expression of receptors and surface antigens on tumor cells, which might reflect the difference of their differentiation. T cell CLL was very rare in our series.

We experienced three ceses of lymphosarcoma cell leukemia, two of

Table 3. *Chronic Lymphocytic Leukemia (CLL)*

Case No.	T cell			B cell		
	E	ALS-T	ATS-T	EAC	sIg	ABS
1 (53, M)	3		0*	83	70	86
2 (67, M)	1			93		
3 (49, M)	16		11*	82		
4 (63, M)	1			65		88
5 (63, M)	6	3	0	47		96
6 (67, M)	5			3	50	82
7 (38, M)	7			83		81
8 (56, M)	11	4		24		86
9 (80, F)	2	2		56	0	85
10 (56, M)	2	2		80	0	85
11 (54, M)	70	86		7		15

M: male F: female
* anti-T developed from anti-thymus serum.

Table 4. *Lymphosarcoma Cell Leukemia (LSL)*

Case No.	T cell			B cell		
	E	ALS-T	ATS-T	EAC	sIg	ABS
1 (26, M)	2		11*	21		88
2 (19, M)	1		4	66	97	99
3 (58, F)	48	80	0	90	0	0
	80	98		43		0

M: male F: female
* anti-T developed from anti-thymus serum.

which had B cell markers (Table 4). One case was T cell leukemia with double markers.

Surface markers of malignant lymphoma and related disorders

Non-Hodgkin lymphomas were investigated (Table 5). The majority of them were B cell lymphomas. T cell lymphomas were not common. As mentioned above, immunofluorescence studies with ALS-T and ABS on tissue sections were helpful to distinguish T and B cell lymphomas.

Most of the lymphomas previously classified as reticulum cell sarcoma according to their morphological features were diagnosed as lymphosarcomas on the basis of lymphocyte-surface markers. They presented morphological features of transformed lymphocytes.

Tablle 5. *Malignant Lymphoma and Related Disorders*

Case No.	T. cell			B cell			Section	
	E	ALS-T	ATS-T	EAC	sIg	ABS	T	B
1 (79, M)	2		2*	10				
2 (66, F)	1			0	95	93		
3 (58, M)	4	3	0	86		96		
4 (25, F)	23	25	0	62		73	−	+
5 (29, M)	0	2		85		96		
6 (56, F)	5	4	0	75		85		
7 (44, F)	47	42		49		40	−	+
8 (71, M)	36	10		51		65		
9 (58, F)	51			49			−	+
10 (38, M)	26	17		85		81		
11 (74, F)	13	15		35		80		
12 (44, F)[a]	56	98	0	2		0	+	−
13 (56, M)[b]	76			2		3	+	−

M: male F: female

* anti-T developed from anti-thymus serum.

a mycosis fungoides.

b nasal gangrene.

Surface markers of other hematological malignancies

Acute myeloid leukemias, malignant reticulosis and multiple myeloma showed no surface markers of lymphocytes (Table 6).

It is generally accepted that cell surface markers regulate the individuality of the cells and have an intimate correlation with the biological characteristics of the cell. Therefore, it seems reasonable that the diagnosis and

Table 6. *Acute Myeloid Leukemia (AML), Malignant Reticulosis (MR) and Multiple Myeloma (MM)*

Case No.	T cell			B cell		
	E	ALS-T	ATS-T	EAC	sIg	ABS
AML 1 (12, M)	9		2*	6		0
2 (6, M)	2			8		0
3 (20, F)	10	2	0	8		6
4 (26, F)	2	2	0	16		3
MR 1 (14, F)	0		1*	2		2
2 (26, F)	3	1	0	2		2**
	6	0		1		5***
MM (42, F)	8			6		5

M: male F: female

* anti-T serum developed by absorbing antithymus serum with human red cells and CLL cells of B cell origin.

** materials from skin lesion.

*** materials from cerebrospinal fluid.

classification of lymphoid tumors should be based on cell surface markers. Several investigators have been diagnosing and classifying lymphoid malignancies using cell surface markers[10,11]. However, they did not employ anti-T nor anti-B cell sera, because specific antisera was not available.

In this study, the usefulness of the specific antisera for thymocytes, peripheral T or B cells was clearly demonstrated in the detection of surface antigens of lymphocytes which were lacking in other markers and in the distinguishing of the origin of the tumors using immunofluorescence in tissue section studies.

We are accumulating cases of lymphoproliferative disorders and investigating the relationship between cell surface markers and morphological features of tumor cells including the clinical course, and sensitivity to therapy or prognosis of patients.

SUMMARY

Forty-three cases of leukemias and lymphomas were tested for the presence of surface antigens and receptors for human T and B cells. Rosetting with sheep erythrocytes was used as a T cell marker. Heterologous antisera, which were rendered specific for human thymocytes (ATS-T) or for peripheral T cells (ALS-T), were also employed. In addition, we used several B cell markers, such as surface bound Ig and a C3-receptor. Human B lymphocyte specific antigens were defined by a heterologous anti-human B cell

serum (ABS). ALS-T reacted only with thymocytes, while ALS-T recognized antigens that existed selectively on peripheral T cells and medullary thymocytes. ABS, on the other hand, reacted with B cells but not with thymic or peripheral T cells. By using these markers, lymphatic leukemias and lymphomas were classified into either T, B or null cell type. It should be noted that T cell tumors were divided into two subclasses according to their reactivity with ATS-T and ALS-T. It was also shown that, in some cases, there was a disparity between the expression of receptors and surface antigens on tumor cells, which might reflect the difference of their differentiation. Another point to which special attention should be paid was that immunofluorescence study with these sera enabled us to demonstrate the histological localization of normal or neoplastic T and B cells in preserving the original structure of lymphoid organs or tumor tissues.

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