Brief Definitive Report

MALIGNANT LYMPHOMA WITH DUAL B AND T CELL MARKERS

Analysis of the Neoplastic Cells with Monoclonal Antibodies Directed against T Cell Subsets*

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The majority of human lymphoid neoplasms are readily assigned to either a B or T cell lineage (1). Many of the problems that arise in the attempt to categorize the remaining tumors reflect insufficient reactivity of the tumor cells with the available B and T cell reagents. However, several laboratories (2–10) have reported patients whose neoplastic lymphocytes simulataneously exhibit B and T cell surface characteristics. This unexpected finding was initially attributed to anti-sheep erythrocyte activity of the surface immunoglobulin (2) rather than to the presence of IgM and sheep cell receptor on the surface of the same cell. The availability of monoclonal anti-T cell antibodies (11, 12) permits the further analysis of these unusual tumors. We report five cases of lymphoid neoplasms in which cells with surface-associated IgM also reacted with a panel of monoclonal anti-T cell antibodies.

Materials and Methods

The patients reported herein were encountered in a systematic study of all patients with lymphoproliferative disease, whose neoplastic cells were examined in suspension by conventional surface marker techniques and with a panel of monoclonal T cell antibodies. The diagnosis of non-Hodgkin's lymphoma was made in the pathology department and subclassified by the Rappaport system (13). The diagnosis of chronic lymphosarcoma cell leukemia in case 2 was based on a preponderance of peripheral lymphocytes with clefted nuclei (14).

T cell surface antigens were identified with nonfluoresceinated mouse hybridoma antibodies kindly supplied by P. C. Kung and G. Goldstein of Ortho Pharmaceutical Corp., Raritan, N. J. (11, 12) and a fluoresceinated $F(ab')_2$ fraction of a goat anti-mouse gamma globulin antiserum (N. L. Cappel Laboratories Inc., Cochranville, Pa.), using a Zeiss ultraviolet light microscope (Carl Zeiss, Inc., New York) (15). Cell surface immunoglobulin was detected with fluorescein-conjugated antiserums specific for the human IgG and IgM heavy chains and for the κ and λ light chains (Meloy Laboratories Inc., Springfield, Va.) (16). The percentage of bright-staining cells was determined by interposing a 10% transmitting filter in the optical system (16). The fluorescence microscope was also used to evaluate thymus-related surface antigens detected by a previously described rabbit antiserum to human fetal thymus (16). Mononuclear cells that formed spontaneous rosettes with sheep erythrocytes (E-rosettes) (16), bore receptors for the Fc portion of immunoglobulin (IgGOx rosettes) (17), or bore receptors for complement (IgMEAC rosettes) (18) were enumerated as previously described.

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Results

Surface immunoglobulin of one heavy (IgM) and one light (κ) chain type was seen on the neoplastic lymphocytes from each patient (Table I). Furthermore, a major fraction of the cells stained brightly with the fluorescein-conjugated anti-immunoglobulin antisera. In case 1, lymph node cells were incubated with sheep erythrocytes and the rosettes isolated on a Ficoll-Hypaque gradient (16). Of the lymphoid cells recovered, 93 and 91%, respectively, were stained by anti-μ and anti-κ antiserums.

In case 1, 47% of the cells formed spontaneous rosettes with sheep erythrocytes, whereas in case 2, an average of 71% of cells were E-rosette positive. In cases 3, 4, and 5, <25% of cells formed E-rosettes. Spontaneous rosettes were not formed with sheep erythrocytes after incubation at 37°C without subsequent 4°C incubation. Complement receptors were detected on most of the neoplastic cells in cases 3 and 4, and on many cells in cases 2 and 5. The Fc receptor (IgGOx rosettes) was found on 10–29% of blood or lymph node mononuclear cells from the last four cases. Heterologous antithymocyte antiserum reacted with the lymphoma cells of all four patients tested.

With the exception of case 5, the majority of surface IgM-positive cells in each case reacted with OKT11, a monoclonal antibody directed against the sheep cell receptor (12). Furthermore, in all but case 2, surface Ig-positive cells reacted with OKT3, a monoclonal antibody that identified a marker on mature T cells (11–13). The surface IgM-positive cells in cases 1, 4, and 5 also reacted with OKT8, a monoclonal antibody that detects the cytotoxic/suppressor subset of T lymphocytes (11–13), whereas the lymphocytes from case 3 reacted with OKT4, which detects the inducer/helper T cell subset (11–13). Finally, the majority of cells in all five neoplasms were positive for the Ia-like antigen that is found on most B lymphocytes, monocytes, macrophages, and activated T cells (19).

The dual marker cases were restricted to non-Hodgkin's lymphomas of follicle center cell lineage. Thus, dual marker cases were not detected among 17 cases of chronic lymphocytic leukemia or three cases of diffuse, well-differentiated lymphocytic lymphoma, both of which represent neoplastic proliferation of medullary cord B lymphocytes (20). Among the lymphomas of follicle center cell lineage (20), a single case of chronic lymphosarcoma cell leukemia was doubly marked; there were 2 dual marker cases among 12 nodular poorly differentiated lymphocytic lymphomas, one case among four diffuse, poorly differentiated lymphocytic lymphomas, and 1 case among 18 diffuse histiocytic lymphomas.

Discussion

Since 1974, when Brouet and Prieur (2) investigated two patients with lymphocytic leukemia, whose neoplastic cells bore surface immunoglobulin and formed rosettes with sheep erythrocytes, there have been numerous reports (3–10) of malignant lymphocytes bearing both B and T cell surface markers. These dual cases can be placed in several categories. One group consists of patients with lymphoblastic lymphoma, childhood acute lymphocytic leukemia, and T cell chronic lymphocytic leukemia, whose malignant cells were both E-rosette positive and positive for complement receptors (5, 6). Such cases appear to represent proliferations of T lymphocytes bearing complement receptors; these cells closely resemble immature thymocytes with similar markers that are present during the 10th to the 15th wk of gestation (21). A second group consists of patients whose neoplastic cells bear clonal surface immuno-

TABLE I

*Neoplastic Human Lymphocytes with Both B and T Cell Surface Markers**

Case number/tissue examined	Diagnosis‡	Surface immunoglobulin					Rosettes			Heterologous anti-thymocyte antiserum	Monoclonal antibody§									
		G	M	κ	λ	Bright staining cells	E	IgMEAC	IgGOx		T1	T3	T4	T6	T8	T9	T10	T11	M1	I1
1. Node	NPDL	2	59	53	0	0.6	47	9	0	36	60	77	22	10	59	11	12	86	6	60
E-rosette$^+$ LNC			93	91	0	0.9														
2. Blood MNC (6/16/80)	LSCL	2	63‖	53	0	0.6	77	39	29	86	71	14				3	0			65
Blood MNC (11/7/80)		2	43	42	1	0.1	61	3	25	86	81	54	9	0	36	5	12	69	3	52
Blood MNC (1/13/81)		6	56	50	0	0.1	74	30	27	91	89	39	12	0	19	5	11	63	6	61
3. Orbit	NPDL	46	75‖	80‖	11	0.7	24	83	10	82‖	28	52	60	45	28	26	35	91	42	80
4. Conjunctiva	DPDL	58	71‖	81‖	3	0.4	17	60	21	59	61	64	11	42	75	17	44	82	26	80
5. Abdominal node	DHL	15	85	96	2	0.8	1	30	13		21	61	30	11	56	96	14	41	9	71

* All results are expressed in percent except for the bright staining cells, which are expressed as decimal fractions.

‡ Abbreviations: LNC, lymph node cells; MNC, mononuclear cells; NPDL, nodular poorly differentiated lymphocyte lymphoma; LSCL, chronic lymphosarcoma cell leukemia; DPDL, diffuse poorly differentiated lymphocytic lymphoma; DHL, diffuse histiocytic lymphoma.

§ The following monoclonal antibodies were used: OKT1; OKT3, PAN T cells; OKT4, inducer/helper T cells; OKT6, common thymocyte; OKT8, cytotoxic/suppressor T cells; OKT9, early thymocyte; OKT10, early thymocyte; OKT11, sheep cell receptor; OKM1, monocyte, granulocyte; OKI1, Ia-like antigen.

‖ Spontaneous polar cap formation observed.

globulin and are reactive with anti-T cell antisera (8–10). These cases are more difficult to explain, to some extent because of the differing and incompletely defined specificities of anti-T cell antisera.

The final category of dual marker case are patients whose neoplastic cells bear clonal surface immunoglobulin and are E-rosette positive. One of the early patients of Brouet and Prieur (2) falls into this category; subsequently, additional patients have been described with similar cell surface markers (3, 4, 7). Several investigators ascribed these cases to anti-sheep erythrocyte activity of the membrane-bound IgM antibody of otherwise unexceptional B cells. The evidence for this hypothesis appeared convincing; it included the finding that protease treatment of the neoplastic cells abolished rosette formation, that treatment with appropriate anti-immunoglobulin heavy and light chain antisera interfered with rosette formation, and that these neoplastic cells formed rosettes at elevated temperatures, whereas, normally, reduced temperatures favor rosette formation (2, 7). However, it should be noted that E-rosette formation of normal T cells is also abrogated by protease treatment (22), and that similar abnormal temperature dependence of rosette formation was observed in otherwise typical malignant and nonmalignant T cells (23), and also that one group of investigators was unable to interfere with rosette formation of doubly marked cells by treatment with either anti-immunoglobulin antiserum or by induced polar cap migration of the surface IgM (4). Thus, the anti-sheep erythrocyte activity of surface IgM of neoplastic cells might not explain all such dual marker cases.

In the cases reported here, the B cell membrane characteristic was established by the demonstration of surface-bound immunoglobulin that was clonal both with respect to the IgM heavy chain and the κ light chain. Furthermore, the presence of Ia-like antigen and complement receptors on the neoplastic cells is consistent with a B cell lineage, although the latter characteristics are not restricted to B lymphocytes (19). The present investigation extends the characterization of these doubly marked cases through the use of monoclonal anti-T cell antibodies. In each of the five cases, the neoplastic lymphocytes reacted with a monoclonal antibody that detects the sheep erythrocyte receptor; all but one reacted with a monoclonal antibody directed against a determinant present on normal peripheral blood T cells, and all but one reacted with a monoclonal antibody specific for either the inducer/helper or the cytotoxic/suppressor T cell subsets. In addition, lymphocytes from two of the five were E-rosette positive. The combined evidence strongly suggests that the neoplastic lymphocytes of our patients exhibit both B and T surface membrane characteristics.

A number of mechanisms have been proposed for the presence of B and T determinants on the same cell: derepression of genetic material as a result of neoplastic transformation (3), transformation of a stem cell before divergence of the B and T lymphocyte pathways (4), and neoplastic expansion of a population of normal cells that express both markers (8). The evidence does not permit a confident choice between these alternatives, nor are these mechanisms mutually exclusive.

Our findings suggest that cases with double marker cells share two characteristics: (*a*) variability in expression of T cell surface antigens on the neoplastic cells and (*b*) the tendency for the neoplastic cells to be of follicle center B cell lineage. One example of the variability of T cell surface properties is the varying avidity for sheep erythrocytes; the avidity ranges from high (binding of erythrocytes occurring at 37°C) (2) to normal (binding occurring at 4°C but not at 37°C), as in our first two cases, to

nonexistent (no binding at 4°C or 37°C despite evidence for the presence of the erythrocyte receptor antigen on the neoplastic cells), as in our last three cases. Similarly, we observed cases whose neoplastic cells bore surface determinants of the cytotoxic/suppressor T cell subset or of the inducer/helper subset or of neither subset.

A second shared characteristics of the dual marker cases relates to their follicle center lineage (20). This B lymphocyte line is characterized by clefted nuclei and abundant surface-associated immunoglobulin (16, 20). A disproportionate number of the previously reported cases (2–4, 7) with double marker cells (four of six) were from nodular, poorly diffentiated lymphocytic lymphoma or its leukemic counterpart, chronic lymphosarcoma cell leukemia. Both of these diseases involve neoplastic proliferation of B lymphocytes of the follicle center (20). All five of our cases were of follicle center B cell lineage.

Thus, the dual marker cases might represent neoplastic transformations of a follicle center B lymphocyte; such transformation might be accompanied by the expression of genes that regulate T cell antigens. The restriction of this expression to cells of follicle center lineage suggests that derepression is not random but is directed or limited by the degree of differentiation of the transformed cell. Finally, it might be considered that double-marker cases represent neoplastic proliferation of normal lymphocytes bearing dual markers; such cells have been detected among peripheral blood mononuclear cells (24). Perhaps these cells normally reside in lymphoid follicles; the cells reported by Poppema et al. (25) located at the periphery of the germinal center in secondary follicles might be candidates for examination for the presence of dual markers.

Summary

In the course of analyzing human lymphoma tissue with conventional surface marker techniques and with monoclonal antibodies directed against T cell subsets, five tumors were encountered with dual B and T cell determinants. All bore on their surface membrane IgM of κ light chain type, complement receptors, and the Ia-like antigen. In each of the five cases, the neoplastic lymphocytes reacted with a monoclonal antibody that detects the sheep erythrocyte receptor (OKT11); all but one reacted with a monoclonal antibody for peripheral T cells (OKT3); and all but one reacted with a monoclonal antibody specific for either the inducer-helper (OKT4) or the cytotoxic-suppressor (OKT8) T cell subsets. In addition, lymphocytes from two of the five cases formed spontaneous rosettes with sheep erythrocytes (E-rosettes). These tumors with dual B and T surface characteristics were confined to human malignant lymphomas that originate from B lymphocytes of the follicle center.

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