

UNUSUAL HUMAN LYMPHOMA PHENOTYPE  
DEFINED BY MONOCLONAL ANTIBODY

BY ALAN C. AISENBERG AND BARBARA M. WILKES

*From the Huntington Memorial Laboratories, Massachusetts General Hospital, Boston, Massachusetts  
02114*

The development of monoclonal antisera by the hybridoma technique permits the expansion of knowledge about human lymphoma. We report a remarkable lineage among the large cell lymphomas that is devoid of Ia-like antigen, exhibits clonal surface (or cytoplasmic) IgG, and reacts with monoclonal antisera previously demonstrated to react only with early thymocytes (1). The present findings: (a) extend our knowledge of the specificity of a group of monoclonal antisera widely employed and (b) provide an additional discriminant for the subdivision of human lymphoma. If, as some have proposed (2), lymphomas represent expansions of specific lymphocyte subsets, this phenotype may represent a significant stage in lymphocyte development.

**Materials and Methods**

*Conventional Lymphocyte Surface Markers.* The three cases that were the subject of this investigation were all designated by the Pathology Department (Massachusetts General Hospital, Boston, Mass.) as non-Hodgkin's lymphomas of the large cell or histiocytic type: the first and third were of diffuse histology; the second was nodular. Cell suspensions of lymphoma tissue were prepared in medium 199 (Microbiological Associates, Walkersville, Md.) by sieving tissue, obtained fresh from the operating room, through a stainless steel mesh. All surface membrane studies were performed on the day of biopsy after viability had been assessed by trypan blue dye exclusion.

Cell surface immunoglobulin (SIg) was determined with fluorescein-conjugated goat antisera specific for the IgG, IgM, and IgD heavy chains of human immunoglobulin and for the kappa and lambda light chains (Meloy Laboratories, Inc., Springfield, Va.). Lymphoid cells ( $10^6$  in 0.05 ml of medium 199) were incubated with 0.05 ml of undiluted fluorescein-conjugated antisera for 1 h at 37°C, washed three times at room temperature with phosphate-buffered saline (pH 7.4), and suspended in phosphate-buffered glycerine. The cells were placed on a slide, overlaid with a cover slip, and examined with a Zeiss ultraviolet microscope equipped with an Osram HBD 200 mercury arc lamp and a fluorescein isothiocyanate-495 nm interference primary filter. A minimum of 200 lymphocytes were examined; thereafter, a 10% transmitting filter was interposed in the optical system; 200 cells were again examined. Bright cells were defined as those visible with the reduced light. The technical details have been described in earlier publications (3-5). Cytoplasmic immunoglobulin (CIg) was determined with 1:10 dilutions of the same fluorescein-conjugated antisera and air-dried methanol-fixed lymphocyte preparations.

Cell suspensions that formed spontaneous rosettes with sheep erythrocytes were assessed by adding lymphoid cells ( $0.5 \times 10^6$  in 0.4 ml of medium 199 that contained 0.1% gelatin) to sheep erythrocytes (0.4 ml of an 0.5% suspension in medium 199 of washed pooled sheep erythrocytes obtained from BBL Microbiology Systems, Becton, Dickinson & Co., Cockeysville, Md.) in the presence of 9% absorbed and inactivated AB serum. The mixture was incubated for 10 min at 37°C, centrifuged at room temperature for 5 min at 150 g, and incubated for at least 2 h at 4°C (6). Receptor for the Fc portion of the immunoglobulin molecule was assayed

with sheep erythrocytes coated with IgG (IgGEA rosettes) (7). The IgG fraction of rabbit anti-sheep cell stroma was obtained from N. L. Cappel Laboratories, Cochranville, Pa. Complement receptor was detected with sheep erythrocytes sensitized by the addition of hemolysinamboceptor and mouse complement by the method of Pincus et al. (8).

Thymus-related surface antigens were evaluated with the globulin fraction of a rabbit antiserum to human fetal thymus absorbed with A-positive erythrocytes and chronic lymphocytic leukemia cells, as described previously (9). The nonfluorescent anti-thymus globulin reacted with lymphoma cells that were then washed and reincubated with a fluorescein-conjugated goat anti-rabbit globulin antiserum (Meloy Laboratories, Inc.). The percentage of lymphoma cells that fixed the rabbit globulin was determined with the fluorescence microscope. Terminal deoxynucleotidyl transferase (TdT) was also determined by an indirect immunofluorescence assay; the reagents employed were obtained from the Bethesda Research Laboratories, Rockville, Md. (10).

*Surface Marker Analysis with Hybridoma Antisera.* Nonfluoresceinated monoclonal mouse hybridoma antisera were kindly supplied by P. C. Kung and G. Goldstein of Ortho Pharmaceutical Corp., Raritan, N. J. as lyophilized ascites proteins. These antisera have been extensively studied in other laboratories (1, 11-14).  $1 \times 10^6$  lymphocytes were incubated with 50  $\mu$ l (10  $\mu$ g/ml) of each of the following mouse hybridoma antisera: OKT1 (all T cells [11]), OKT3 (peripheral T cells [1]), OKT4 (inducer/helper T cells [12]), OKT5 and OKT8 (suppressor/cytotoxic T cells [1]), OKT6 (common thymocytes [1]), OKT9 (early thymocytes [1]), OKT10 (early thymocytes [1]), OKM1 (monocytes [13]), and OKI1 (Ia antigen [14]). After a 30-min incubation at 37°C, the lymphoma cells were washed twice with phosphate-buffered saline and reincubated for 60 min at the same temperature with 50  $\mu$ l of a 1:5 dilution of fluorescein-conjugated goat anti-mouse gamma globulin (F[ab']<sub>2</sub> fraction obtained from N. L. Cappel Laboratories, Inc.). The cells were again washed twice and then examined under the fluorescence microscope as described above for surface immunoglobulin.

## Results

The results from three cases of large cell (histiocytic) lymphoma are presented in Table I and Fig. 1. With conventional surface marker techniques, the first two were identified as clonal proliferations of lymphocytes that bore brightly staining IgG heavy chain and lambda light chain on their surface. The third specimen was devoid of surface immunoglobulin, but the lambda light-chain was detected in the cytoplasm of the fixed lymphoma smear, and a monoclonal immunoglobulin (3,250 mg/100 ml) of IgG heavy chain and lambda light chain was demonstrated in the serum by immunoelectrophoresis and agarose gel electrophoresis. In all three neoplasms, only a minor population of cells reacted with the conventional T cell reagents, i.e., rabbit anti-thymus antiserum and sheep erythrocytes.

The findings with the monoclonal antisera were unexpected and constitute the basis of this report. The great majority of lymphoma cells from all three tumors reacted with OKT9 and OKT10, considered from earlier studies to detect early thymocyte antigens (1), whereas very few cells reacted with the monoclonal antibody with Ia specificity (OKI1). (The reactivity of OKI1 has been confirmed in our laboratory by positive studies [48-80% of cells staining brightly] in four out of four cases of common acute lymphocytic leukemia, two out of two cases of chronic lymphocytic leukemia, and three other large cell lymphomas of B lineage.) A low level of positivity with the monoclonals that identify mature T cells (OKT1, OKT3, OKT4, OKT5, and OKT8) was consistent with our findings with conventional T cell reagents.

TABLE I  
Surface Markers in Malignant Lymphoma

Case	Primary site	B lymphocyte markers						EAC*	Fc‡	T cell markers		TdT	Ortho monoclonals¶										
		Sig			Clg					ATS§	E		T1	T3	T4	T5	T6	T8	T9	T10	M1	II	
		G	M	D	κ	λ	κ																λ
1	Abdominal lymph node	82**	0	0	3	88**	0	0	38	13	17	13	—	9	8	3	6	1	5	85	91**	2	2
2	Tonsil	79**	24	0	1	50	—	22	4	13	17	0	0	17	16	3	18	0	18	37	58**	0	18
3¶¶	Mediastinal mass	0	0	0	0	0	0	0	0	168§	248§	0	0	118§	—	48§	—	—	8	90**	76	—	98§
	Control lymph nodes	17 ± 14	10 ± 8	6 ± 6	13 ± 7	8 ± 6		21 ± 10	2 ± 2	49 ± 17	32 ± 17	0	0	54 ± 12	52 ± 12	43 ± 17	9 ± 5	0	12 ± 6	4 ± 3	1 ± 1	1 ± 1	14 ± 8

\* EAC, complement receptor (EAC rosettes).

‡ Fc, Fc receptor (IgGEA rosettes).

§ ATS, anti-thymus heteroantiserum.

|| E, E rosettes.

¶ Ortho monoclonals: T1, all T cells; T3, peripheral T cells; T4, inducer/helper T cells; T5 and T8, suppressor/cytotoxic T cells; T6, common thymocytes; T9 and T10, early thymocytes; M1, monocytes; II, Ia antigen. The figures for control lymph nodes are the mean for nine hyperplastic nodes ± 1 SD. All results are expressed as percentage of cells reacting.

\*\* Bright staining; >50% of cell visible by fluorescence microscopy when incidence light was reduced to 10%.

‡‡ A monoclonal IgG of λ type was demonstrated in the patient's serum by immunoelectrophoresis and agarose gel electrophoresis.

§§ Small lymphocytes distinguishable from large tumor cells.

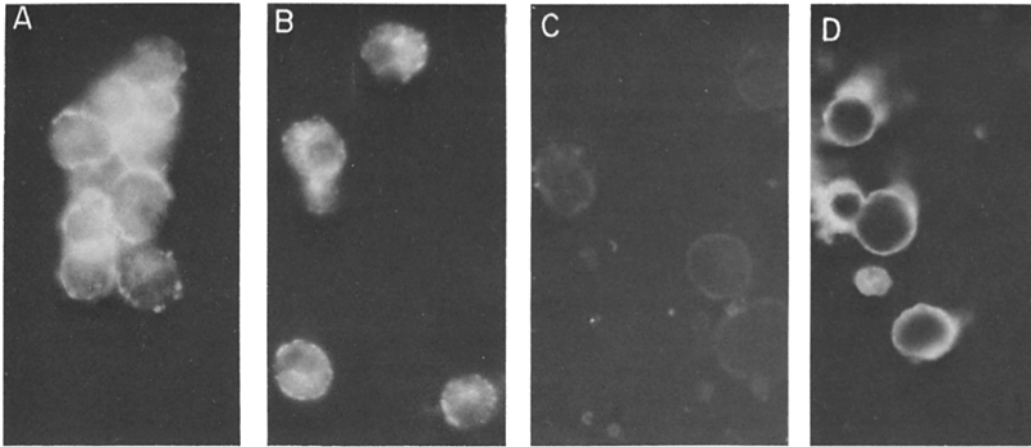


FIG. 1. Staining of case 3 with fluorescein-conjugated antisera. (A) surface staining with Ortho monoclonal OKT9, (B) surface staining with Ortho monoclonal OKT10, (C) cytoplasmic staining with goat anti-kappa light-chain antiserum, (D) cytoplasmic staining with goat anti-lambda light-chain antiserum. See Materials and Methods for details. Initial magnification  $\times 380$ .

### Discussion

Three human malignant lymphomas have been identified with monoclonal IgG either on the surface membrane or within the cytoplasm. These neoplasms react with the Ortho monoclonal antisera of hybridoma derivation OKT9 and OKT10, heretofore employed to identify early thymocytes (1), and fail to react with a monoclonal antiserum that identifies the Ia antigen. One might suggest that these malignant lymphocytes possess both B and T cell markers, an unusual finding in man, but one that has been described in the mouse (2, 15). Although this possibility is not excluded, it appears more likely that OKT9 and OKT10 are not specific for the T lineage and react with an antigen present on some B cells. Thus, the specificity of the OKT9 and OKT10 antigens could be considered analogous to the Ia antigen first observed widely on B lymphocytes (16-18), and only more recently shown to be regularly present on activated T cells (14, 19).

The absence of the Ia antigen on three lymphoid tumors purported to be of B lineage also requires comment. It was not surprising to find the third neoplasm in Table I devoid of Ia because, despite its lymphoid morphology, this tumor was clearly differentiated along the plasma cell pathway with the loss of SIg and the presence of immunoglobulin in the cytoplasm: plasma cells lack the Ia antigen (17, 20). At the present time it seems plausible that the first two tumors (Table I), which still have SIg, are also proceeding along the plasma cell differentiation pathway and have lost the Ia antigen as a consequence.

The present findings raise other considerations that will require investigation of additional human lymphomas for resolution. First, is the reciprocal relationship between the Ia antigen and the antigens responsible for OKT9 and OKT10 reactivity fortuitous, or does it have biological meaning in lymphocyte differentiation? Second, will the observation that all three tumors bear IgG, an immunoglobulin found less frequently than IgM on the surface of non-Hodgkin's lymphoma cells (21), be born out by subsequent observations? (Indeed, the three lymphomas are all of lambda

light-chain type, whereas kappa is the common light chain on the surface of lymphoid tumor cells as it is in normal circulating human immunoglobulin [21].)

Finally, how frequently will this subset be observed in human lymphoma? Approximately 50–60% of the large cell or histiocytic lymphomas have been identified as B cell neoplasms on the basis of clonal surface immunoglobulin (21, 22), but there is no reason to suggest that the major fraction of these is of the phenotype described here (SIgG+ or CIgG+, OKT9+, OKT10+, Ia–). Indeed, the available evidence suggests that most large cell lymphomas are Ia-positive (22). Nonetheless, the recognition of three such tumors in a period of a few months suggests that they are not uncommon. Further investigation is needed to establish the frequency of this phenotype and its significance in lymphocyte differentiation.

### Summary

The phenotype of three large cell (histiocytic) lymphomas of man has been defined with conventional lymphocyte surface marker techniques, a panel of monoclonal antisera of hybridoma derivation (Ortho Pharmaceutical Corp.), and the fluorescence microscope. Two tumors exhibited surface IgG of lambda light-chain type, whereas the third was negative for surface Ig but contained the same Ig in the cytoplasm. With the monoclonal antisera, all three were found to bear two surface antigens (OKT9 and OKT10) previously described on early thymocytes, and were devoid of the Ia antigen. The available knowledge suggests that these neoplasms represent the expansion of a clone of B lymphocytes along the pathway that leads to plasma cell differentiation rather than a clone with both B and T surface membrane markers.

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