Brief Definitive Report

AN ANTIGEN SHARED BY A HUMAN T CELL SUBSET AND B CELL CHRONIC LYMPHOCYTIC LEUKEMIC CELLS

Distribution on Normal and Malignant Lymphoid Cells*

By LAURENCE BOUMSELL, HELENE COPPIN, DANIELE PHAM, BRIGITTE RAYNAL, JEAN LEMERLE, JEAN DAUSSET, AND ALAIN BERNARD

From the Institut National de la Santé et de la Recherche Médicale, Hôpital Saint-Louis, 75010 Paris; and the Laboratoire des Tumeurs de l'Enfant and the Service d'Experimentation Animale, Institut Gustave Roussy, 94800 Villejuif, France

We have previously defined a set of antigenic determinants shared by human T cells and the surface Ig-bearing cells from a large number of patients with B cell chronic lymphocytic leukemia (B-CLL) (1, 2). These antigenic determinants were recognized by rabbit antisera raised against human T cells. Absorption experiments showed that T cells from human peripheral blood (PBL), from the thymus, and from T cell chronic lymphocytic leukemia (T-CLL) displayed all these antigenic determinants; cells from patients with B-CLL may express all, none, or an incomplete set of these determinants. Finally, these antigens are confined to the E-rosetting population of PBL and are not expressed on B cell lymphoid cell lines (B-LCL) in culture, B cell Burkitt-like lymphomas, and plasmocytomas. Absorptions also revealed that investigations of the exact distribution pattern of these antigens on T cells were not possible using these rabbit antisera, because they primarily contained antibodies specific for human T cells. These investigations are reported here for one of these antigenic determinants shared by human T cell and B-CLL cells that is defined by a monoclonal antibody.

Materials and Methods

Preparation of the Monoclonal Antibody. Biozzi's high responder strain of mice were immunized by one intravenous injection of 50×10^6 T-CLL cells as briefly described elsewhere (3, 4). After 4 d, mice were sacrificed, and 10^8 spleen cells were mixed with the nonsecretor myeloma cell line NS1 (5) at a ratio 10:1 and pelleted by centrifugation. The cells were resuspended in 1 ml polyethylene glycol (PEG 6000 [Sigma Chemical Co., St. Louis, Mo.] 33% in RPMI-1640 [Grand Island Biological Co., Grand Island, N. Y.] Hepes). After 3 min at room temperature, the mixture was centrifuged for another 3 min. PEG was then removed, and the cells were gently resuspended in 4 ml fetal calf serum (FCS) for 10 min. The cells were then diluted by adding 20 ml of RPMI-1640, containing pyruvate, L-glutamine, and antibiotics, and plated in 0.1-ml portions into a 96-well microculture plate (Falcon 3040; Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.). Progressive selection was carried out for 2 wk with hypoxanthine-aminopterin-thymidine (HAT) medium. HAT 0.1 ml was added after 24 h, and half the medium (0.1 ml) was replaced with fresh HAT medium on days 2, 3, 6, 9, and 13. Hypoxanthine, thymidine medium was then added every 2–3 d for another 2 wk. The cell cultures were then maintained in RPMI-1640, containing 15% FCS with pyruvate, L-glutamine, and antibiotics. Clones of living hybrids were observed by day 7 or 8 under the microscope, and the supernates

* Supported by Délègation Genèrale à la Recherche Scientifique et Technique 79.7.0503; Institut National de la Santé et de la Recherche Médicale 79.5073-1c and 79.5073-1d; Institut Gustave Roussy 80A 10; and Association de la Recherche sur le Cancer.

of hybrids were screened repeatedly for antibody production from the 3rd wk onward. Whenever a positive well was detected, the culture was expanded in a 24-well Costar plate (Costar 3524; Costar Data Packaging, Cambridge, Mass.), and the hybrid was cloned, while the screening was repeated. Cloning of the cells, achieved by serial dilution of cell suspensions, was performed twice on relevant hybrid. The cloned hybrid cells were then injected intraperitoneally into nude mice resulting in tumors that were serially transplanted. Ascites fluid from the tumor-bearing nude mice were individually checked for antibody activity.

Antibody Assays. Antibodies to the immunizing T cells were detected in a complement-dependent microcytotoxicity test as described in (6). A panel of normal T and B cells, thymus cells, B cell lines, and cells from patients with various B and T cell malignancies was also used for the screening. Cells were used fresh or after freezing, with identical results.

Rabbit Antisera. The preparation and specificity of these antibodies has been described in detail elsewhere (2, 7).

Characterization of the Monoclonal Antibody. Immunoglobulins from the ascites fluid of tumor-bearing nude mice were concentrated by ammonium sulfate precipitation (35% saturation) and examined by immunodiffusion with antisera prepared in rabbits against purified murine myeloma proteins. These antisera and the reference myeloma proteins were purchased from (Bionetics Laboratory Products, Litton Bionetics Inc., Kensington, Md.). The protein concentration was determined by the Lowry method (8).

Results

Obtention of a Monoclonal Antibody Recognizing an Antigen Shared by Human T Cells and B-CLL Cells. We isolated hybrids that produced antibodies to E^+ PBL and not E^- PBL. A few of these antibodies also recognized cells from patients with B-CLL. We were able to clone an hybrid of this type twice and named it A50. In nude mice, A50 gave rise to a transplantable tumor and ascites fluid, containing antibody in high titer (reacting at 10^{-4}–10^{-3} dilution with human E^+ PBL in a complement [C]-dependent cytotoxic test). The immunoglobulin produced by A50 is an IgG_{2a} K chain, as revealed by immunodiffusion assays (results not shown).

A50 recognized the E^+ PBL from every normal donor tested. On a panel of 39 B-CLL, A50 recognized cells from 17 patients. Table I compares the cytotoxicity of A50 on this panel of B-CLL with that of four rabbit antisera obtained by immunization with various T cells and absorption on B-LCL. By absorption experiments, we had previously shown that these antisera recognized human T cell-specific antigens and a set of antigens shared by T cells and B-CLL cells, but lacking on B cell peripheral

TABLE I

Comparison of the Cytotoxic Patterns of a Monoclonal Antibody and Four Rabbit Anti-T Cell Antisera on B-CLL

B-CLL tested	A50	Rabbit antisera raised against			
		Sézary	Thymus	XLA PBL*	T cell lymphoma
3, 33, 39, 41, 42, 43, 44, 50, 62, 63, 66, 67, 68, 69	+‡	+	+	+	+
18, 32, 70	+	+	−	+	+
1	−§	−	+	−	−
2, 4, 5, 6, 7, 10, 13, 19, 23, 25, 34, 49,‖ 52, 54, 59, 60, 61, 65, 72, 73, 76	−	−	−	−	−

* PBL obtained from a patient with X-linked agammaglobulinemia (Bruton's type) (XLA).

‡ A B-CLL was considered positive if ≥70% of the cells were lysed by the antiserum.

§ A B-CLL was considered negative if ≤20% of the cells were lysed by the antiserum.

‖ B-CLL 49 had 40% E^+ cells, and 35–45% of the cells were recognized by the antisera: it was therefore considered negative.

blood lymphocytes (B-PBL) (1, 2). With the exception of an antiserum raised against thymus cells, A50 recognized the same B-CLL cells as the antisera raised against Sézary cells, T cell peripheral blood lymphocytes (T-PBL), and T cell lymphoma cells (Table I).

Distribution of the Antigen Shared by Human T Cells and B-CLL Cells on Normal Lymphocytes. Table II shows the populations of normal lymphocytes recognized by A50. Cells forming sheep erythrocyte rosettes (E^+) from various organs are recognized by A50. However, in the thymus, only a proportion of cells — (varying from 33 to 59%) — is recognized. This proportion of cells does not seem to vary with the age of the donor, and cells carrying this antigen could readily be detected in the thymus of a 14-wk-old fetus. We could not find, in any fraction containing B cells and monocytes, any cell recognized by A50. Nor did we find any other positive non-T cell malignancy: we have now investigated the cells from 14 patients with common acute lymphoblastic leukemia (ALL), 1 patient with pre-B cell ALL, 11 patients with B cell lymphoma, 1 patient with plasmocytoma, 4 patients with acute myeloblastic leukemia, and the cells from 3 B-LCL.

T Cell Malignancies Displaying the Antigen Shared by Human T Cells and B-CLL Cells. As A50 did not recognize all thymocytes, and presumably these were the immature ones, we have investigated various T cell malignancies. We have previously shown, using rabbit antisera, that T cell malignancies can be subdivided on the basis of the surface

TABLE II

Distribution of the Antigen Shared by Human T Cell and B-CLL Cells on Normal Lymphoid Cells

Origin of the cells		Number of individuals	Antisera		
			A50	α-Thymus*	α-B-CLL*
Thymuses		7			
Fetal	14 wk old		59‡	95	≤5
	16 or 17 wk old		33	51	12
	22 wk old		63	95	8
	28 wk old		45	95	9
Child	8 mo old		39	95	≤5
	4 yr old		37	94	≤5
	Unknown		51	95	≤5
Lymph nodes					
E^-§		1	≤5	≤5	95
E^+§			89	89	≤5
E^-		1	26	27	74
E^+			93	90	11
Spleen					
E^-		1	≤5	≤5	75
E^+			88	88	≤5
E^-		1	≤5	≤5	60
E^+			79	85	≤5
Tonsils					
E^-		1	≤5	≤5	95
E^+			46	65	≤5
PBL					
E^-		16	≤5–20‖	≤5–20	60–90
E^+			70–90‖	70–90	≤5–10

* Preparation of the antisera against thymus cells and against B-CLL cells has been described elsewhere (2, 7).

‡ Values represent the cytotoxic index calculated as follows: percent dead in experimental − percent dead in C control/100 − percent dead in C control × 100.

§ Cells were separated over a Ficoll gradient after rosetting with 2-aminoethylisothiouronium bromide hydrobromide-treated sheep erythrocytes as previously described (2), into E^+ and E^- cells.

‖ Ranges of values of the cytotoxic index.

T cell antigens they display (7, 9). Table III shows that A50 did not recognize all T cell malignancies; all T-CLL cells and Sézary cells we tested were recognized but no T cell-ALL cells and only the cells from some T lymphomas were recognized. This panel of T cells was also investigated with several rabbit anti-human T cell antisera (7). Whereas the antiserum raised against fetal thymus cells contained only T cell-specific antibodies, the other rabbit sera contained both T cell-specific antibodies and antibodies to the antigenic determinants shared by human T cells and B-CLL cells. The same panel of T cell lymphoblasts recognized by A50 was also recognized by one rabbit antiserum raised against Sézary cells (Table III).

Discussion

The monoclonal antibody A50, obtained after immunization against T-CLL cells, recognizes an antigen present on the surface of T cells and B-CLL cells. We had previously shown, using rabbit antisera, that T cells and B-CLL cells share a set of

TABLE III

Distribution of the Antigen Shared by Human T Cell and B-CLL Cells on T Cell Malignancies

T cell malignancies	A50	Rabbit antisera raised against*					
		Sézary	Sézary	Fetal thymus	Child thymus	T cell lymphoma	XLA PBL‡
T-ALL§	−‖	−	−	−	+	+	−
	−	−	−	−	+	+	−
	−	−	−	−	+	+	−
	−	−	−	−	+	+	+
	−	−	−	+‖	+	+	−
	−	−	−	+	+	+	+
T cell lymphoma	−	−	−	−	+	+	−
	−	−	−	−	+	+	−
	−	−	−	−	+	+	−
	−	−	−	−	+	+	−
	−	−	−	−	+	+	−
	−	−	−	−	+	+	−
	−	−	−	−	+	+	+
	−	−	−	−	+	+	+
	−	−	−	+	+	+	+
	−	−	−	+	+	+	+
	+	+	+	+	+	+	+
	+	+	+	+	+	+	+
	+	+	−	+	+	+	+
	+	+	+	−	+	+	+
	−	−	+	−	+	+	+
T-CLL	+	+	−	−	+	+	+
	+	+	−	−	+	+	+
	+	+	+	−	+	+	+
Sézary	+	+	+	−	+	+	+
	+	+	+	−	+	+	+
	+	+	−	−	+	+	+
	+	+	−	−	+	+	+
	+	−	−	−	+	+	+
	+	−	−	−	+	−	+

* The rabbit antisera are used at a dilution where they recognized both T cell-specific antigens and the antigen(s) shared by human T cell and B-CLL cells.

‡ Same as Table I.

§ Results of individual patients with T cell acute lymphoblastic leukemia (T-ALL) (6 patients), T cell lymphoma (15 patients) T-CLL (3 patients), and Sézary disease (6 patients).

‖ A sample was considered positive if ≥50% of the cells were lysed in a cell suspension that contained 80% or more malignant cells. A sample was considered as negative if 10% or less of the cells were lysed in a cell suspension that contained 80% or more malignant cells.

antigens, and by absorption experiments, we could enumerate at least three different determinants within this set (2). The determinant recognized by A50 is not present on all B-CLL cells displaying antigens belonging to this set, although it is present on most of them. It is present on the T-PBL from every normal subject tested, as are the determinants defined by rabbit antisera. The restricted distribution of these determinants on B-CLL cells suggests that this set of antigens is more complex than initially suspected. Further investigation of the cellular distribution, together with biochemical investigations, will permit us to study whether these determinants belong to different molecular species or to related molecular structures.

The fact that we could not find any positive B cells, normal or malignant, other than B-CLL cells, does not exclude the possibility that these antigens might be present on the surface of a limited population of normal B cells. Alternatively it should be determined why B-CLL cells, contrary to all other malignant B cells, display surface antigens normally present on T cells only.

The determinant recognized by A50 is not present on all immature T cells. Surface antigens defined by monoclonal antibodies and restricted to a subpopulation of human T cells have already been described (10–12). These monoclonal antibodies provide us with new insights into the stages of intrathymic differentiation and will permit a better analysis of malignant T cell proliferations. We have previously defined three subsets of malignant T lymphoblasts that are correlated with other markers of T cell maturation using rabbit anti-human T cell antisera (9). A similar subclassification of T cell lymphoblasts has also been observed using monoclonal antibodies (10). The demonstration of the heterogeneity of malignant lymphoid proliferations has many implications including those of direct clinical importance.

Summary

We obtained a monoclonal antibody, A50, after immunizing Biozzi's high responder strain of mice with T cell chronic lymphocytic leukemia (T-CLL) cells. A50 recognized an antigen present on the surface of B cell chronic lymphocytic leukemia cells from many patients and from cells of T lineage from any subject we tested. We could not find this antigen either on the surface of normal B cell or on other non-T cell malignancies. On T cells, this antigen was present on a subpopulation of thymus cells, and on most peripheral T cells. The antigen was present on the surface of cells from T-CLL, Sézary's disease, and a subset of T cell lymphoma. The antigen seemed to belong to a complex set of antigenic determinants that we had defined with rabbit antisera.

The authors wish to thank Dr. G. Biozzi for providing the mice and for his valuable comments; Dr. C. Gosse for maintaining the hybridomas in germfree nude mice; Doctors L. Degos, G. Flandrin, and F. Valensi for providing specimens from patients; Ms. Martine Beauval and Mr. Laurent Jeannière for skillful technical assistance; and Dr. L. Vogler for kindly reviewing the manuscript.

Received for publication 21 February 1980 and in revised form 8 April 1980.

References

1. Boumsell, L., A. Bernard, V. Lepage, J. Lemerle, and J. Dausset. 1978. Analysis of shared antigenic determinants between chronic lymphocytic leukemia and T cell. *Fed. Proc.* **37:** 1289.

2. Boumsell, L., A. Bernard, V. Lepage, L. Degos, J. Lemerle, and J. Dausset. 1978. Some chronic lymphocytic leukemia cells bearing surface immunoglobulins share determinants with T cells. *Eur. J. Immunol.* **8**:900.
3. Biozzi, G., D. Mouton, O. A. Sant'Anna, H. C. Passos, M. Gennari, M. H. Reis, V. C. A. Ferreira, A. M. Heumann, Y. Bouthillier, O. M. Ibanez, C. Stiffel, and M. Siqueira. 1979. Genetics of immunoresponsiveness to natural antigens in the mouse. *Curr. Top. Microbiol. Immunol.* **85**:31.
4. Boumsell, L., and A. Bernard. 1979. Intérêt des races de souris bonnes productrices d'anticorps de Biozzi pour la production d'anticorps monoclonaux. *C. R. Acad. Sci. Paris.* **289**:723.
5. Milstein, C., K. Adetugbo, N. J. Cowan, G. Köhler, D. S. Secher, and C. D. Wilde. 1976. Origins of lymphocyte diversity. Somatic cell genetics of antibody-secretory cells: studies of clonal diversification and analysis by cell fusion. *Cold Spring Harbor Symp. Quant. Biol.* **41**: 793.
6. Mittal, K. K., M. R. Mickey, D. P. Singal, and P. I. Terasaki. 1968. Refinement of microdroplet lymphocyte cytotoxicity test. *Transplantation (Baltimore).* **6**:913.
7. Boumsell, L., A. Bernard, H. Coppin, Y. Richard, C. Penit, P. Rouget, J. Lemerle, and J. Dausset. 1979. Human T cell differentiation antigens and correlation of their expression with various markers of T cell maturation. *J. Immunol.* **123**:2063.
8. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265.
9. Bernard, A., L. Boumsell, C. Bayle, Y. Richard, H. Coppin, C. Penit, P. Rouget, C. Micheau, B. Clausse, R. Gérard-Marchant, J. Dausset, and J. Lemerle. 1979. Subsets of malignant lymphoma in children related to the cell phenotype. *Blood.* **54**:1058.
10. Reinherz, E. L., P. C. Kung, G. Goldstein, R. H. Levey, and S. F. Schlossman. 1980. Discrete stages of human intrathymic differentiation: analysis of normal thymocytes and leukemic lymphoblasts of T cell lineage. *Proc. Natl. Acad. Sci. U. S. A.* **77**:1588.
11. McMichael, A. J., J. R. Pilch, G. Galfré, D. Y. Mason, J. W. Fabre, and C. Milstein. 1979. A human thymocyte antigen defined by a hybrid myeloma monoclonal antibody. *Eur. J. Immunol.* **9**:205.
12. Levy, R., J. Dilley, and L. A. Lampson. 1978. Human normal and leukaemia cell surface antigens. Mouse monoclonal antibodies as probes. *Curr. Top. Microbiol. Immunol.* **81**:164.