

## EXPRESSION OF Ly 1, Ly 2, Thy 1, AND TL DIFFERENTIATION ANTIGENS ON MOUSE T-CELL TUMORS

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The "differentiation antigens", Ly 1 and Ly 2, present on thymus cells and thymus-derived lymphocytes (T cells)<sup>1</sup> of mice (1) have been shown to mark differentiated functional subpopulations of normal peripheral T cells. By using cytotoxic elimination experiments, it was reported initially that in C57BL/6 (B6) mice, an Ly 1<sup>+</sup> Ly 2<sup>-</sup> (Ly 1 ↑, Ly 2 ↓)<sup>2</sup> subpopulation of peripheral T cells primed with sheep erythrocytes had helper activity for an in vitro sensitized trinitrophenol-specific antibody response; while among T cells of B6 mice immunized with cells allogeneic to B6, only those bearing the Ly 2 phenotype had killer activity in vitro against a fibroblast target of the same allotype as the cells used for immunization (2). In an expanded study, the Ly 1<sup>-</sup>2<sup>+</sup>3<sup>+</sup> (Ly 1 ↓, Ly 2,3 ↑) phenotype of cytotoxic effector cells was confirmed in B6 mice, which express the Ly 1.2, 2.2, 3.2 alleles. However, in B6/Ly 1.1 congenic mice which express the Ly 1.1, 2.2, 3.2 alleles (i.e., the congenic strain of mice in which the Ly 1.2 allele has been replaced by the Ly 1.1 allele) Ly 1<sup>+</sup>2<sup>+</sup>3<sup>+</sup> cells also were demonstrated to be cytotoxic effector cells (3). Moreover, T cells which are precursors of killer or helper cells apparently are differentiated for these Ly antigens before immunization (4), although the point at which this differentiation takes place has not been ascertained. Also under certain experimental conditions T cells, expressing the Ly 1<sup>-</sup>2<sup>+</sup> phenotype, are required for immune suppression (5). These initial observations of functional subsets with specific restricted Ly phenotypes have been confirmed, and extended to encompass a variety of T-cell functions (6-19).

Tumors with differentiated T-cell phenotypes would be potentially valuable for studies of T-cell function. Most T lymphocytic tumors in the mouse however, are spontaneous and induced tumors of thymic origin. The thymus is thought to be chiefly a lymphocytopoietic tissue containing relatively immature cell types. The original report in which Ly 1 and Ly 2 antigens were described (1) indicated that lymphomas varied in the expression of Ly antigens, although it was not reported whether the tumors expressed both Ly specificities when positive. That

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<sup>1</sup> Abbreviations used in this paper: ABS, absorption; B6, C57BL/6; C, complement; CT, cytotoxicity; ENU, 1-ethyl-1 nitrosourea; FI-GAM Ig or (heavy chain), fluorescent goat anti-mouse Ig or (Ig heavy chain); IF, immunofluorescence; T cells, thymus-derived lymphocytes; TL, thymus leukemia antigen.

<sup>2</sup> The +/- superscript nomenclature is a simplification of the conclusion from the original papers (2, 3) on functional subsets, one of which (3), suggested the Ly 1 ↓ : Ly 2,3 ↑ (i. e., arrows) nomenclature to indicate that the functional populations were relatively poor or rich in the respective antigens.

report also noted that the expression of TL, another T-cell antigen (20), was not correlated with the presence or absence of Ly antigen. Several questions about the phenotypic expression of Ly on neoplastic cells are not resolved: (a) Are both Ly 1 and Ly 2 antigens expressed equally on all T-cell tumors, or are the T-cell tumors, like peripheral T cells, differentiated for Ly phenotype?; (b) Are Ly antigens expressed only on T-cell lymphomas?, and (c) What is the relation of the antigenic phenotype to cell differentiation and leukemogenesis? To study these questions, we have examined early transplantation generations of a number of BALB/c lymphoid tumors induced by 1-ethyl-1-nitrosourea as well as early transplantation generations of spontaneous AKR lymphomas, for Ly 1, Ly 2, TL, and Thy 1 phenotypes.

### Materials and Methods

*Mice.* Mice used in these experiments for tumor passage were either BALB/c AnN, bred at the Animal Production Unit, National Institutes of Health, Bethesda, Md. or by Litton Bionetics, Kensington, Md., or AKR/J from The Jackson Laboratory, Bar Harbor, Maine.

Mice congenic with B6 were used for specificity controls with Ly and TL antisera, and were either obtained directly from Dr. E. A. Boyse, Memorial Sloan Kettering Cancer Center, New York, or bred in this Laboratory from stock obtained from Dr. Boyse. These congenic stocks are designated B6/Ly 1.1, B6/Ly 2.1, Ly 3.1, and B6/TL<sup>+</sup> following the simplified nomenclature of Shiku et al. (3). A/Thy 1.1 mice congenic with A strain mice, but expressing the Thy 1.1 rather than the Thy 1.2 antigen, were obtained from Dr. David Sachs, National Cancer Institute, for specificity controls with Thy 1 antisera.

*Tumor Induction and Transplantation.* Specific tumor designations with further information for BALB/c tumors are shown in Table I. P1798, a long-term passage tumor line arose in a BALB/c mouse treated with estrogen (21). All of the other BALB/c tumors presented here are of recent origin and most were tested at less than 10 transplantation generations. These tumors were induced by 2 intraperitoneal injections of 1-ethyl-1-nitrosourea (ENU), 1 wk apart in 1- to 2-mo old animals. Each injection contained 1  $\mu$ mol ENU/g body weight. The ENU was prepared and injected by Dr. Jerry Rice, National Cancer Institute. Some of the tumors arose in ENU-treated mice that were subsequently given a single i.p. injection of pristane (2, 6, 10, 14 tetramethyl pentadecane) 1 mo later (22). Eight of the BALB/c tumors presented as primary thymic tumors with or without evidence of generalized spread of the leukemic process while two of them BALENTL 13 and 14 arose as generalized leukemias. The BALB/c tumors were transplanted by serial i.p. or s.c. passage. Solid tumor tissue was minced into suspension, or peritoneal tumor ascites cells were used when available for the tests performed.

The AKR transplantable tumors, AKRLS-12, AKRLS-13, and AKRLS-34, were initiated by s.c. or i.p. transplantation of primary spontaneous thymic tumors of AKR/J mice > 4 mo of age. Pathology of these tumors was judged only on a gross level and was comparable to the frequently described pattern of lymphoid tumors for this strain of mice: grossly enlarged thymus; enlarged lymph nodes; and enlarged spleen in some cases. The only selective basis for inclusion in this study was the availability of early transplantation generations (generations 5-11). All of the tumors have been frozen in liquid N<sub>2</sub>. Six recovered lines were tested including BALENTL 13, 14, P1798, and the 3 AKR tumors.

*Antisera.* Anti-Ly, -TL, and -Thy 1 antisera which were similar to those previously described (3), were a generous gift of Doctors E. A. Boyse and F-W. Shen, Memorial Sloan-Kettering Cancer Center, and are listed below (Table II). The antisera were selected for specificity of reaction only on thymocytes of B6 or the appropriate congenic strain. When necessary these antisera were absorbed with normal thymus and peripheral lymphoid tissue or tumors of the opposite allelic Ly specificity. The titers and dilutions of the antisera also are indicated in Table II.

*Cytotoxicity Test.* The cytotoxicity (CT) test of Gorer and O'Gorman (23) was used with the modifications described by Boyse et al. (24). Equal 0.05-ml vol of (a) antiserum serially diluted; (b) selected rabbit serum, diluted 1:15, in Ly tests (25, 26), or selected guinea pig serum, diluted 1:4, in TL and Thy 1 tests (both complement [C] sources were selected for low cytotoxicity against

TABLE I  
*BALB/c Tumors\**

Name	Induction	Days‡	Pathology		Transplantation generations tested¶
			Gross§	Microscopic	
BALENTL 3	ENU	132	T	S-LN, Thymus	3, 4
BALENTL 4	"	165	T	—	3¶
BALENTL 5	"	173	T	L-LN, Viscera	2, 3, 6, 15, 17
BALENTL 6	"	273	T	S-LN, Thymus	1, 2
BALENTL 7	ENU + Pristane	273	T	—	1¶
BALENTL 8	" "	311	T	S-LN, Thymus	1, 3
BALENTL 9	" "	344	G	S-LN, Thymus, early spleen	3¶
BALENTL 13	" "	145	G	S-LN, Lymph node; RCS-B, Liver	3, 4, ¶ 5, ¶ 12
BALENTL 14	ENU	443**	G	L-LN, Viscera	4, ¶ 12
P1798	Estrogen pellet	521**	T	S-LN, Thymus	189, 193, 194
BALENLM 11	ENU	260	G	S-LN, Lymph node, spleen (non-thymic)	2, 3, 4, 7, 10
BALENLM 15	ENU + Pristane	227	—	Giant cell sarcoma	1¶
BALENLM 16	" "	173	—	Microscopic plasma cell tumor	1¶
BALENLM 17	" "	235	G	S-LN (Mastocytoma)‡‡	1, ¶ 2

\*All of these tumors were induced in BALB/c AnN mice.

‡Days after treatment with ENU.

§T, thymic, G, generalized lymphoma.

||S-LN, small cell lymphocytic neoplasia; L-LN, large cell lymphocytic neoplasia; RCS-B, Dunn reticulum cell sarcoma type B.

¶A number of these tumors were tested from more than one animal at the same transplantation generation. The transplantation generation number indicates the number of times the tumor was transplanted prior to testing.

\*\*Actual age of mouse in days.

‡‡This tumor initially appeared to be mixed for cell type, but grew out as a mastocytoma on transplantation.

TABLE II  
*Antisera\**

Specificity	Immunization	Test Thymocytes	Maximal CT	Titer
Anti-Ly 1.1	(BALB/c × B6)F <sub>1</sub> vs. B6/Ly 1.1 thymocytes	B6/Ly 1.1	>95%, 1/160	2,000
Anti-Ly 1.2	C3H/An vs. CE thymocytes	B6, BALB/c, AKR	>95%, 1/80	320
Anti-Ly 2.1	B6/H-2 <sup>k</sup> vs. CE thymocytes	B6/Ly 2.1, 3.1, AKR	>90%, 1/80	640
Anti-Ly 2.2	(C3H/An × B6/Ly 2.1)F <sub>1</sub> vs. ERLD‡	B6, BALB/c	>90%, 1/80	320
Anti-TL	(B6 × A/TL-)F <sub>1</sub> vs. ASL1	BALB/c	70%, 1/200	1,000
"	"	A, B6/TL+	>95%, 1/400	6,400
Anti-Thy 1.1	(B6 × A)F <sub>1</sub> vs. A/Thy 1.1 thymocytes	A/Thy 1.1, AKR	>95%, 1/80	2,000
Anti-Thy 1.2	(A/Thy 1.1 × AKR/H-2 <sup>k</sup> )F <sub>1</sub> vs. ASL1‡	A, BALB/c	>95%, 1/500	5,000

\*The maximal cytotoxicity is given as the percent dead cells at the serum dilution indicated. The titer is the reciprocal of the dilution which gives 50% lysis on the cells indicated.

‡ERLD and ASL1 are B6 and A strain tumors, respectively, which have long transplantation histories.

mouse thymocytes and high C activity); and (c) cells suspended in Medium 199 with Hanks' balanced salt solution ( $5 \times 10^6$  cells/ml) were incubated for 45 min at 37°C. The data are presented as percent dead (stained) cells after the addition of trypan blue in 0.15 M saline.

**Immunofluorescent Reagents.** Goat anti-mouse Ig heavy chain and anti-mouse polyvalent Ig antibodies were prepared by affinity chromatography and tested as described elsewhere (27).

Goat antibodies were coupled to fluorescein by reaction with fluorescein isothiocyanate to achieve a molar fluorescein: protein ratio of 2.5–5.7. Unconjugated fluorescein was removed by passage over Sephadex G-25 equilibrated with 0.01 M phosphate buffer in 0.15 M NaCl, pH 7.4. These fluorescent reagents are designated Fl-GAM (heavy chain) and Fl-GAMiG. All of these reagents react with <1% of normal thymocytes.

**Immunofluorescence Test.** The immunofluorescence (IF) test of Möller (28) was adapted as follows for use with mouse alloantisera. Equal vol (0.05 ml) of diluted alloantisera and cells prepared in Hanks' medium with 10% heat inactivated fetal calf serum and 0.1% sodium azide ( $20 \times 10^6$  cells/ml) were mixed on ice for 30 min. The suspensions were then diluted to 2 ml and washed twice with centrifugation at 220 g. Appropriate Fl-GAM (heavy chain) or Fl-GAMiG (0.05

ml), diluted to 0.5 mg/ml protein in phosphate-buffered saline was added to the pellet. The cells were suspended and incubated on ice again for 30 min and washed as before. The washed cell pellets were suspended in 1-drop vol and put on slides with cover slips and read under oil immersion with a Leitz fluorescent scope with Ploem illuminator (HBO 100W/2 mercury lamp with BG38 and BG12 excitation filters and an S546 barrier filter).

The use of the FI-GAM $\gamma_2$  reagent allowed us to type Ig<sup>+</sup>, H chain<sup>-</sup> tumors for Ly phenotype as discussed below. (See Results)

*Absorption Tests.* Tumors or normal lymphoid tissues for controls were prepared by suspension in Medium 199 with Hanks' balanced salt solution. The cells were washed twice, resuspended in 3-5-ml vol, and enough cells to yield a 0.05-ml cell pellet were dispersed into 50 × 6-mm tubes. After centrifugation at 800 g the supernate was aspirated and a 0.11-ml of appropriately diluted antiserum was added and mixed with the cells. The dilution of antiserum used in these tests was determined previously to be a dilution at which maximum positive reaction (either 90-95% CT or >90% IF) was observed on the appropriate thymus test cells. The absorptions (ABS) were carried out by mixing the cells on ice for 45 min, centrifuging the absorbing cells into a pellet, and transferring the absorbed serum supernate to a fresh tube for either a standard CT assay with serial twofold dilutions or IF on an antigen-positive thymus cell. This is the most critical test because antiserum specificity can be completely controlled by the use of B6 or congenic mouse thymocytes as the positive test cell after the absorption. Positive (+) ABS is indicated when no reactivity above complement controls or <1% IF was observed on the positive test cells. Negative (-) ABS indicates that no reduction was observed greater than that obtained with negative control, lymphoid tissue of mice with the opposite Ly (Thy 1 or TL) allele. Usually this does not exceed 5-10%. Exceptions are noted in the Tables.

## Results

*Preferential Expression of Either Ly 1 or Ly 2 on Lymphoma Cells.* Table III presents a summary of the CT, IF, and absorption data for the BALB/c and AKR tumors. 11 of these tumors were tested by all three tests. All of the tumors were tested at least twice and some of the tumors were tested a number of times (see Table I and below).

*CT Tests on BALB/c Tumors.* Typical CT data for two of the tumors tested with anti-Ly 1.2 and anti-Ly 2.2 are present in Fig. 1. A high level of CT >90% at the 1/80 dilution of anti-Ly 1.2 was observed for BALENTL 13, while CT for BALENTL 5 was 28% under the same conditions. CT with anti-Ly 2.2 indicated that BALENTL 5 cells were >90% positive (BALENTL 13 CT under the same conditions was <10%). The initial dilutions of both antisera were chosen to be on a maximum plateau of kill for normal BALB/c thymocytes. In Table III the CT data for these tumors and other similar tumors are presented in summary at this plateau dilution. It is evident that some of these tumors had high levels of expression of either Ly 1 or Ly 2 antigen, indicated as Ly 1<sup>+</sup> or Ly 2<sup>+</sup>, comparable to the Ly phenotypic expression of normal thymus cells, but clearly both antigens were not expressed to the same degree. With these criteria BALENTL 3-8 and 14 are Ly 1<sup>-2+</sup> and BALENTL 13 is Ly 1<sup>+2-</sup>. It also is evident that two of the BALB/c lines, P 1798 and BALENTL 9 were positive for both Ly antigens. The level of CT, however, was lower for both of these tumors compared to normal thymocytes.

CT results on tumor cells prepared from lymphoid or solid tumor tissues suggested that some of the tumors which were scored as negative for one of the Ly antigens had a subpopulation of about 15% positive cells. This low degree of positive reactivity was absent in ascites tumors.

Occasionally these tumors were tested with antisera to Ly 1.1 or Ly 2.1, the

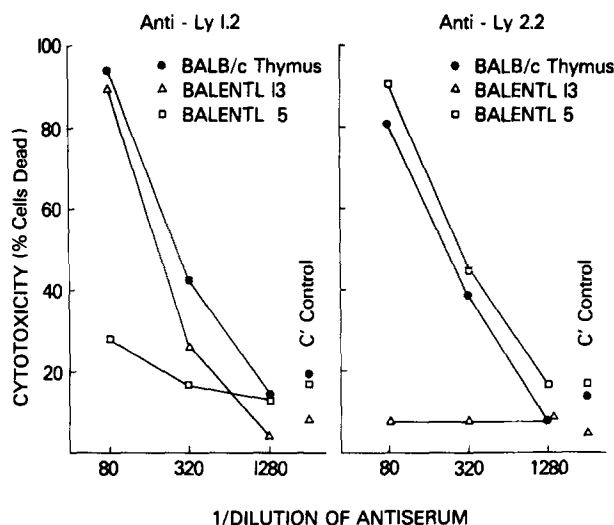


FIG. 1. Cytotoxicity of anti-Ly 1.2 and anti-Ly 2.2 on BALENTL 13 and BALENTL 5. Cytotoxicity = percent dead cells (stained with trypan blue) on ordinate; 1/dilution of antiserum on abscissa. BALENTL 13 was tested at generation 5 in ascites form (See Table VI) and BALENTL 5 was tested at generation 2 as a suspension of a solid subcutaneous tumor. Control normal BALB/c thymus cells were obtained from a 2-mo old animal. Serum controls (serum 1:80, without C added, not pictured) were <10% for both antisera with either tumor.

opposite alleles to those detected on BALB/c cells, and no positive tumors were observed with either antiserum.

BALENLM 11, 15, and 17 tumor cell suspensions had reduced phenotypic expression of both Ly 1 and Ly 2 antigens and were also Thy 1.2<sup>-</sup> and TL<sup>-</sup>.

*IF Assay of Ly Antigen Expression.* Because CT tests as performed here with anti-Ly sera cannot distinguish with satisfaction reactions on subpopulations of cells, we developed immunofluorescence tests for use with mouse alloantisera. This approach had the potential of asking whether tumors with low antigen expression by CT, e.g., 20% had (a) reduced antigen expression on all the tumor cells thus reducing the sensitivity of the cells to lysis with antibody and C, or (b) a minor subpopulation of positive cells with normal thymus levels of Ly antigen expression.

With these high CT anti-Ly sera, either Fl-GAM<sub>1</sub> or Fl-GAM<sub>2</sub> reagent was satisfactory for IF tests on normal thymus cells, because the maximum number of Ly<sup>+</sup> cells were labeled with either reagent. However, the fluorescence intensity of the Fl-GAM<sub>2</sub> was lower and therefore the Fl-GAM<sub>1</sub> reagent was employed whenever possible. When tested, Fl-GAM<sub>1</sub>, Fl-GAM<sub>1</sub>A, and Fl-GAM<sub>1</sub>M did not give satisfactory stains with these anti-Ly sera. The specificity of the sera used in this report was confirmed by tests on the appropriate congenic thymocytes. Under the conditions described, fluorescent dots on >90% of the cells could be seen when the sera had been selected and/or absorbed to react specifically with thymocytes from B6 or the proper Ly congenic strain. Since the Fl-GAM<sub>2</sub> reagent reacts with <1% of spleen cells and <5% of Ig<sup>+</sup> tumor cells, this reagent, when used with anti-Ly sera, allowed us to test Ig<sup>+</sup>

TABLE III  
Antigen Distribution on BALB/c and AKR Tumors\*

Tumor	Ly 1.2			Ly 2‡			TL			Thy 1‡	
	CT	IF	ABS	CT	IF	ABS	CT	IF	ABS	CT	ABS
BALENTL 3		<5			95			>90			95
BALENTL 4	20			90			95				
BALENTL 5§	21	11	-	>90	>90	+	>95	72	+	>95	+
BALENTL 6	14	<5	-	52	>95	+	>95	95	+		+
BALENTL 7	15		-	95		+			+		(60%)
BALENTL 8	<10		-	36	>95	+	>90		+	51	+
BALENTL 9	31	>95	+	50	>95	+	76	>95	+	76	+
BALENTL 13§	90	90	+	10	<5	-	<10	<10	-	34	+
BALENTL 14		<1-20	-		58-90	+	>90	>90	+	>95	+
P 1798	50	50	+	27	90	+	>95	>95	+	43	+
BALENLM 11¶	(-)	27		(-)	22		(-)	1		16	+
BALENLM 15	28	<5		28	<5		<5			<10	
BALENLM 16¶		<5			13			<5			
BALENLM 17	24	11	-	4	<5	-	<10	<1	-	<5	
AKR LS 12	>90			<10			<5				
AKR LS 13	<5	<5	-	51	90	+	<5	<1	-	23	+
AKR LS 34	21	<1	-**	42	95	+	21	<5	-	17	+

\*CT, percent dead (trypan blue stained) cells. CT data are presented at the 1/80 dilution for anti-Ly 1.2, 1/40 or 1/80 for anti-Ly 2.2, 1/200 for anti-TL, 1/500 for anti-Thy 1.2, 1/40 for anti-Ly 2.1 and 1/80 for anti-Thy 1.1. IF, percent fluorescent cells. Antisera to Ly 1, Ly 2, and TL were routinely used one twofold dilution less than indicated for CT tests. Control cell preparations incubated with FI-GAMlg were routinely <5% except for BALENLM 11, 15, 16, and 17, which were 37->95% Ig<sup>+</sup> but which were <1% positive with FI-GAM<sub>1,1</sub>. ABS (absorption data) are summarized as: positive ABS (+), <1% IF (or <5% CT) on positive indicator cells. Negative ABS (-), 90-95% IF or CT on positive indicator cells. B6 or B6/Ly congenic thymocytes were used for testing absorbed anti-Ly sera, BALB/c thymus cells were used for testing absorbed anti-TL, and A/J or A/Thy 1.1 thymus cells were used for testing absorbed anti-Thy 1. C controls on tumors were about 10% (5-10) in most cases.

‡The Ly 2.2 and Thy 1.2 alleles are expressed on normal BALB/c thymocytes and BALENTL lymphomas. The Ly 2.1 and Thy 1.1 alleles are expressed on AKRLS lymphomas and normal AKR thymocytes.

§More detailed data are presented for BALENTL 5 and BALENTL 13 in Tables IV and V.

||This tumor absorbed 60% of the anti-Thy 1.2 CT compared to complete absorption with an equal volume of normal packed BALB/c thymus cells.

¶CT results were not greater than controls 15-20% dead cells, however, cell loss during the test incubation was a major problem with BALENLM 11 as well as for BALENLM 16.

\*\*Under the conditions described, partial absorption of 30% was observed. This low degree of absorption was assumed to be from contaminating host cells (see text).

tumors for Ly antigen. For tests of Ig<sup>+</sup> tumors, the Ly sera were centrifuged at 100,000 g for 20 min in a Beckman Airfuge (Beckman Instruments, Fullerton, Calif.) to remove immunoglobulin complexes and aggregates.

When lymphoid tumors (BALENTL 3, 5, 6, 8, 13, and 14) were tested by IF for Ly expression and when only large or intermediate sized cells (i.e., presumptive tumor cells) were scored for fluorescence, the tumor cells consistently appeared to be totally negative for one of the Ly antigens and strongly positive for the other. The pattern of surface dots with anti-Ly sera and FI-GAMlg was quite distinct and was similar on normal thymus and tumor cells. The question of low positive reactions was clarified by IF tests since a subpopulation of smaller cells was observed with brightly fluorescent dots, probably indicating a contaminating host cell population rather than a low level of antigen expression on the total population of tumor cells.

*Lack of Ly on Ig<sup>+</sup> Tumors.* Initial attempts to type several of the BALB/c tumors by cytotoxicity tests were unsatisfactory because of a depletion of test cells during the 37°C incubation with C. When BALENLM 11, 15, 16, 17, were tested for surface Ig by IF with polyvalent FI-GAMlg, a clear pattern of fluorescent membrane dots and rims was observed. This suggested that these tumors either had bound Ig passively in vitro or had intrinsic surface Ig. These

surface Ig<sup>+</sup> tumors were subsequently tested for Ly and TL antigens by IF with Fl-GAM $\gamma_2$ . In these tests, where loss of tumor cell was not a problem, the Fl-GAM $\gamma_2$ -negative tumors were also negative with either anti-Ly 1.2 or anti-Ly 2.2 sera. Preliminary tests of ABL5, PL 1, and PL 2, Abelson virus-induced tumors, also indicate a lack of Ly antigens on these tumors (M. Potter and B. J. Mathieson, unpublished data).

*Expression of TL and Thy 1.2 Antigens on BALB/c Tumors.* Two of the tumors, P 1798 and BALENTL 9, undifferentiated for the T-cell markers Ly 1 and Ly 2, similar to normal thymus cells, are TL<sup>+</sup>. In addition most of the BALB/c tumors with a single Ly phenotype also express TL. On at least two of the Ly 2<sup>+</sup> TL<sup>+</sup> tumors, BALENTL 5 and 6, the TL specificities observed are at least TL.2 and TL.1, 4 or 5 (20, 29); and TL.3 is not expressed in addition to the normal TL.2 specificity. This conclusion is drawn from the following observations: anti-TL serum absorbed with either BALENTL 5 or 6 remains cytotoxic on either A strain or B6/TL<sup>+</sup> thymocytes but not on BALB/c thymocytes, and anti-TL serum absorbed with ERLD, a TL.1, 2, 4 positive tumor, removes cytotoxic reactivity to the BALENTL tumors.

Most of the tumors were tested for Thy 1. CT with anti-Thy 1 sera is often greatly reduced on tumors but maximal CT of 95% could be obtained with increased serum concentrations. Only the T-cell tumors tested, i.e., tumors that were Thy 1<sup>+</sup> and/or TL<sup>+</sup>, express Ly antigens, while several non-T-cell tumors do not.

*Absorption Tests for T-Cell Antigens.* Since CT and IF tests are direct tests on tumors, it was necessary to confirm the tumor cell phenotypes by absorbing known positive sera with tumor cells under experimental conditions which would allow maximum sensitivity, and then testing the absorbed sera on positive indicator normal thymocytes to check for specific ABS. The summary table (Table III) indicates those tumors which were tested by ABS and the results completely agree with the phenotypes determined by CT and IF. On some of the tumors, the phenotype was confirmed for all of the T-cell antigens by ABS tests.

*Stability of Surface Phenotype in BALB/c T-Cell Lymphomas.* The surface phenotype is stable over a number of generations, as demonstrated by BALENTL 13, an Ly 1.2<sup>+</sup>, 2.2<sup>-</sup> tumor (Table IV) and BALENTL 5, an Ly 1.2<sup>-</sup>, 2.2<sup>+</sup> tumor (Table V). Repeated tests on BALENTL 13 indicated an Ly 1<sup>+</sup>2<sup>-</sup> phenotype in two sublines and on different tumor cell sources within a single transplant host. The isolated observation of a substantial Ly 2<sup>+</sup> population in a solid peritoneal tumor mass was not repeated when tested several generations later and remains unexplained.

*AKR Tumors.* Transplanted AKR Thy 1.1<sup>+</sup> lymphomas (AKRLS 12, 13, 34) also appear to have restricted expression of Ly antigens (Table III). Preliminary data (not shown) from an additional group of AKR thymic lymphomas have indicated that the one notable difference between these tumors and BALB/c tumors is the low incidence of TL<sup>+</sup> AKR tumors, which is consistent with previously reported data for lymphomas of this strain (20). Nearly all (9 of 10) of the BALB/c T cell tumors and 0 of 3 of the AKR tumors presented here are TL<sup>+</sup>.

TABLE IV  
Ly Antigen Phenotype of BALENTL 13 – Repeated Testing\*

Transplan- tation generation and subline†	Tumor cell source	Ly 1.2			Ly 2.2			TL		Thy 1.2	
		CT	IF	ABS	CT	IF	ABS	CT	ABS	CT	ABS
3-S	Ascites	>95 (C 16)			15 (C 12)						
	Mesenteric lymph node	>95 (C <10)			25 (C <10)						
4-A	Ascites	85 (C <10)			<5 (C <5)			<10 (C <10)			
	Solid peritoneal tumor mass	78 (C <15)			51 (C <15)			31 (C <17)			
4-S	Ascites	>95 (>1)	+		4§ (<1)	-					
5-S	Ascites	90 (C <5)			10 (C <5)			12 (C <10)		34 (C <5)	
12-S	Solid peritoneal tumor mass	>90	+		<5	-		-		+/<10	
17-A	Ascites	>95			<1					18 (C <5)	+/57

\*Sera were used as described in Table III and tests were performed as detailed in the text. C controls are indicated in parentheses below the test results. This tumor was consistently Ig<sup>-</sup> (<5%) when tested by IF at generations 4, 12, and 17.

†The number indicates the passage generation; the letters S and A indicate whether the line was initially passed from the primary solid tumor in the chest cavity (S), or from the peritoneal ascites cells of the primary tumor (A).

‡No large tumor cells positive.

||AKR tumors (Thy 1.1\*) used for control absorptions did not decrease the CT on A/J thymus cells in either case.

TABLE V  
Ly Antigen Phenotype BALENTL 5 – Repeated Testing\*

Trans- plantation generation	Tumor cell source	Anti-Ly 1.2			Anti-Ly 2.2			Anti-TL			Anti-Thy 1.2		
		CT	IF	ABS IF on B6	CT	IF	ABS IF on B6	CT	IF	ABS CT on BALB	ABS CT on A	CT	ABS CT on A
2	SC	28			>90			>95					
3	SC	21			>90	>95		>95	80				
6	SC		15	-/>90		80	+/<5		64	+/<15	-/>90	>90	+/<5
15	ASC	15	(+)	§	39	>95		>95	>95			76	
15	SC		(+)	§		>95		>95	>95			>95	
17	ASC	22	<10	-/>90	50	>95	+/<5	>95	>95	+/<15	-/82	54	+/<5
17	IP Solid	20	<10		37	>95		>95	>95			85	

\*Sera were similar to those described in Table II and tests were performed as described in the text. This tumor was consistently Ig<sup>-</sup> (<5%) each time it was tested by IF.

†Most of these positive cells were smaller than the typical tumor cell.

‡Although these cells appeared slightly positive the typical dot pattern of IF with anti-Ly was not observed with these cells. Since this tumor appears to have some viral-related specificities, this positive result may be an artifact. A subsequent generation tested by absorption was negative for Ly 1.2.

## Discussion

We have used three methods for testing the Ly and TL phenotype of T-cell tumors of BALB/c and AKR mice and two methods for detecting Thy 1 antigen. In 11 of the 13 tumors either Ly 1 or Ly 2 antigen is expressed at a level comparable to Ly antigen expression in the thymus and the tumors are "negative" for the other Ly antigen. Absorption and C-dependent cytotoxicity have indicated no more than a minor background (probably normal host cell contamination) of positive cells for one of the Ly antigens: IF has indicated that large tumor cells are negative for the corresponding antigen. However, we



cannot eliminate the possibility that the phenotypic expression is below the level of sensitivity of our tests, for the "negative" Ly antigen. Nor can we eliminate the possibility that the minor population of small Ly 1<sup>+</sup>2<sup>+</sup> cells present in the in vivo passaged tumors are stem cells for large tumor cells with restricted Ly phenotype. This is unlikely in view of preliminary observations which have indicated a lack of small positive cells among cells of established in vitro lines of these tumors.<sup>3</sup> Therefore we conclude that there is preferential but not necessarily exclusive expression of either Ly 1 or Ly 2 on most tumors of these strains.

It is remarkable that so many of the thymic tumors described here are "differentiated" for Ly 1 or Ly 2 since nearly all thymus cells express both antigens. Partial lysis and sequential lysis experiments (2, 4) have distinguished four subclasses of normal peripheral T cells with phenotypes indicated as follows: Ly 1<sup>+</sup>2<sup>+</sup>; Ly 1<sup>+</sup>2<sup>-</sup>; Ly 1<sup>-</sup>2<sup>+</sup>; Ly 1<sup>-</sup>2<sup>-</sup>. Thus these tumors may reflect (a) neoplastic induction of a partially differentiated thymus cell or alternatively, (b) neoplastic conversion of a peripheral T cell which returns to the thymus to produce a tumor. The first possibility is more likely since the majority of the BALB tumors are TL<sup>+</sup>.

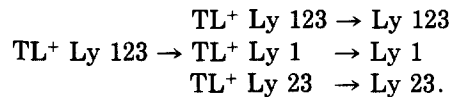
TL antigen is expressed on thymus cells and on tumors of presumed T-cell origin but has not been found on peripheral T cells (20). Since some tumors are Ly 1<sup>+</sup>2<sup>-</sup> or Ly 1<sup>-</sup>2<sup>+</sup> and also TL<sup>+</sup>, the presence of TL on these tumors might represent the derepression of a tumor-specific TL antigen after differentiation has restricted the surface phenotype to either Ly 1<sup>+</sup> or Ly 2<sup>+</sup>. Although the BALB/c tumors have not been tested for all of the TL specificities, it appears that most of these tumors express more than the normal BALB/c thymus TL.2 specificity. However, if the TL<sup>+</sup> phenotype is simply the result of derepression of tumor-specific TL, it is peculiar that the majority of these tumors (9 of 10) derived from a TL.2<sup>+</sup> strain (BALB/c) are TL<sup>+</sup> tumors while the majority of tumors in AKR, a TL<sup>-</sup> strain are TL<sup>-</sup> (20, 30). Thus the proportion of TL<sup>+</sup> tumors reflects the TL genotype of the target for leukemogenesis.

The normal differentiation process may involve the restriction of Ly before the differentiative loss of TL. The tumor phenotype might indicate that the target cell for leukemogenesis is a partially differentiated thymocyte. In spontaneous leukemogenesis of AKR mice (31, 32), radiation leukemogenesis and RadLV virus-induced leukemogenesis in B6 mice (31) the preleukemic thymus becomes populated with cells expressing a low Thy 1, high H-2 antigen phenotype. Such a phenotype is characteristic of peripheral T cells rather than thymocytes which normally are high Thy 1, low H-2 cells. The tumors presented here were also characteristically less susceptible to lysis with anti-Thy 1 and C. Thus the target cell population for thymic leukemogenesis may represent a normally minor thymus cell population which has reduced Thy 1 expression quantitatively and at least in part is differentiated for Ly antigen expression. Since a subpopulation of thymocytes, capable of synergy in graft-versus-host

<sup>3</sup> Kim et al. Manuscript in preparation.

<sup>4</sup> Preliminary examination of 3 (BALB/c × DBA/2)F<sub>1</sub> tumor transplantation lines suggests that one of these lines is Ly 1<sup>+</sup>2<sup>-</sup> and TL<sup>+</sup>.

reactions, are eliminated with anti-TL serum (33), there may indeed be a normal phenotypic counterpart to the type of cell observed in these tumors. Thus, we may have to re-examine the model of normal T-cell differentiation (16, 34) which proposes that normal thymocytes lose the potential for TL expression before the differentiation of subpopulations restricted for Ly 1 or Ly 2, and before the acquisition of the capacity to react specifically with antigens. In addition the following model of T-cell differentiation should be considered:



An alternative explanation for the high proportion of TL<sup>+</sup> tumors exists: the anti-TL serum used in these tests may detect Qa-2 or T-cell differentiation antigens related to TL by genetic proximity on chromosome 17 (35). This question is still under investigation.

The stability of the phenotype over several generations and the finding of tumors with either the Ly 1<sup>+</sup> or Ly 2<sup>+</sup> phenotype supports the concept that these tumors represent states of T-cell differentiation. The stability further indicates that these antigens are not subject to modulation during transplantation of these tumor lines, but rather that the restricted Ly surface phenotype is an intrinsic property of these T-cell tumors.

Although there appears to be a predominance of BALB/c lymphomas with the Ly 1<sup>-</sup>2<sup>+</sup> phenotype, this is not true of AKR lymphomas. The Ly phenotype may depend on the method of tumor induction, the strain of mice used, or it may simply reflect a sampling error due to the relatively small number of tumors tested from these strains. Eight of the BALB/c primary T-cell tumors presented as thymic tumors with or without evidence of generalized spread of the leukemic process, while three tumors, each with a different Ly phenotype, BALENTL 9, 13, and 14, arose as generalized T-cell leukemias possibly originating from peripheralized T lymphocytes.

Tumors have been described which subserve functions such as suppression (36), or autoaggression (37, 38), the Ly phenotype of those tumors may correspond with previously described phenotypes of normal functional immune cells. Since there is no exclusion of either of the restricted Ly phenotypes in these tumors, we find no support for the concept that all T-cell lymphomas will express the Ly phenotypes described for normal killer and suppressor or normal helper cells. However, individual tumor lines with such phenotypes may express appropriate functions under particular experimental conditions.

Beside the advantage of obtaining homogeneous populations with functional activity, these tumors have other obvious uses. In general, tumors with restricted Ly phenotypes may be useful as immunogens. BALENTL 13 has been used to produce anti-Ly 1.2 serum (F-W. Shen, personal communication). Since tumor tissue can be obtained in large amounts, and with the apparent restriction of the Ly antigen these tumors will be invaluable as a source for biochemical preparation of the antigen. Finally, the satisfactory description and availability of tumors such as these as models for T cells may enable us to expand our knowledge of the cellular aspects of the immune response as much

as plasmacytomas have enabled investigators to expand our knowledge of B cells and antibody in immune responses.

### Summary

Transplanted lymphomas, most of thymic origin, induced in BALB/c mice with 1-ethyl-1-nitrosourea (ENU) and transplanted spontaneously occurring lymphomas of AKR mice were examined for the expression of the T-cell antigens Ly, TL, and Thy 1 by using three serological methods. Most (11 of 13) of the Thy 1<sup>+</sup> and/or TL<sup>+</sup> tumors, i.e., T-cell tumors, expressed high levels of either Ly 1 or Ly 2 antigen, but not both. Thus most thymic lymphocytic tumors expressed restricted Ly phenotypes comparable to phenotypes previously described for functional peripheral T cells. Because tumor phenotypes were stable over a number of transplant generations, they therefore appeared to be an intrinsic property of the specific tumors. The majority of the BALB/c lymphomas were Ly 1<sup>-</sup> 2<sup>+</sup> and also positive with anti-TL antiserum. This predominant phenotype on the BALB/c tumors may be related to either the mode of tumor induction or to the mouse strain, but since the restricted Ly pattern was observed both in BALB/c and AKR tumors, the phenotypic restriction itself is not a consequence of either of these factors. Tumor induction by ENU per se is not responsible for Ly or TL antigen expression since several non-T-cell BALB/c tumors, also induced by ENU, did not express either Ly or TL antigens.

Data presented here suggest that the target cell for leukemogenesis may be a partially differentiated thymus cell. The restricted expression of Ly antigens on differentiating thymus cells to either the Ly 1<sup>+</sup> (Ly 1 ↑) or Ly 2<sup>+</sup> (Ly 2 ↑) phenotype may occur before the loss of TL antigen.

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