Jpn. J. Cancer Res. 80, 257–264, March, 1989

Cell Surface Antigens of Radiation Leukemia Virus-induced BALB/c Leukemias Defined by Syngeneic Cytotoxic T Lymphocytes

Yukio Kaneko,[1] Herbert F. Oettgen,[2] Yuichi Obata[3] and Eiichi Nakayama[4]

[1]Clinical Center for Oncology, Mie University Hospital, 2-174 Edobashi, Tsu 514, [2]Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021, [3]Laboratory of Immunology, Aichi Cancer Center Research Institute, Chikusa-ku, Nagoya 464 and [4]Department of Oncology, Nagasaki University School of Medicine, 12-4 Sakamoto-cho, Nagasaki 852

Two cell surface antigens of mouse leukemias were defined by BALB/c cytotoxic T lymphocytes (CTL) generated against syngeneic radiation leukemia virus(RadLV)-induced leukemia, BALBRV1 or BALBRVD. Hyperimmunization of BALB/c mice with irradiated leukemias followed by *in vitro* sensitization of primed spleen cells resulted in the generation of CTL with high killing activity. The specificity of CTL was examined by direct cytotoxicity assays and competitive inhibition assays. A shared cell surface antigen, designated as BALBRV1 antigen, was detected by BALB/c anti-BALBRV1 CTL. BALBRV1 antigen was expressed not only on RadLV-induced BALB/c leukemias except for BALBRVD, but also on spontaneous or X-ray-induced BALB/c leukemias, chemically-induced leukemias with the H-2^d haplotype and some chemically-induced BALB/c sarcomas. In contrast, a unique cell surface antigen, designated as BALBRVD antigen, was detected by BALB/c anti-BALBRVD CTL. BALBRVD antigen was expressed only on BALBRVD, but not on thirty-nine normal lymphoid or tumor cells. These two antigens could be distinguished from those previously defined on Friend, Moloney, Rauscher or Gross murine leukemia virus (MuLV) leukemias, or MuLV-related antigens. Both cytotoxic responses were blocked by antisera against H-2K^d, but not H-2D^d. The relationship of BALBRV1 antigen and BALBRVD antigen to endogenous MuLV is discussed with regard to the antigenic distribution on tumor cell lines.

Key words: Radiation leukemia virus-induced leukemia — Cell surface antigens — Cytotoxic T lymphocytes

Murine leukemia virus(MuLV[5])-induced leukemias of the mouse have been classified into two groups on the basis of serologically detectable cell surface antigens: (a) leukemias induced by Friend, Moloney or Rauscher MuLV expressing the shared FMR antigens and (b) leukemias induced by Gross MuLV or occurring spontaneously in high leukemia incidence strains expressing Gross cell surface antigens (GCSA).[1,2] No crossreaction was observed between FMR antigens and GCSA.[3]

Consistent with these serologic findings, cytotoxic T lymphocytes (CTL) specifically reactive with FMR antigens or GCSA were obtained.[4,5] Thus, CTL generated against FMR antigen-positive leukemias killed only FMR antigen-positive leukemias, but did not kill FMR antigen-negative leukemias such as Gross MuLV-induced leukemias, spontaneous or X-ray-induced leukemias. Studies with Friend[6] or Moloney MuLV[7,8] immune CTL suggested that FMR antigens with which CTL reacted might be the type-specific antigenic determinant of gp70, coded by the *env* gene of MuLV. On the other hand, studies with Gross MuLV-immune CTL suggested that the cell surface antigens which were involved in T cell recognition might be GCSA, coded by the *gag* gene of GCSA.[9–11]

In contrast to these two cell surface antigens on MuLV-induced leukemias, little is known about the cell surface antigens on radiation leukemia virus (RadLV)-induced leukemia. Recently, we reported that RadLV-induced leukemia B6RV2, BALBRVB or BALBRVD expressed an individually distinct cell surface antigen that could be recognized by semisyngeneic CTL.[12,13] In the present study, we investigated the specificity of syngeneic CTL directed against two RadLV-induced BALB/c leukemias BALBRV1 and BALBRVD by direct cytotoxicity assays and competitive inhibition assays. The results demonstrated that a shared cell surface antigen and a unique cell surface antigen were detected by CTL sensitized against BALBRV1 or BLABRVD, respectively. The results also indicated the exclusive involvement of H-2K^d products in the interaction between CTL and leukemia BALBRV1 of BALBRVD.

[1] To whom correspondence should be addressed.

[5] Abbreviations used: MuLV, murine leukemia virus; CTL, cytotoxic T lymphocytes; RadLV, radiation leukemia virus; FMR, Friend, Moloney, Rauscher; GCSA, Gross cell surface antigens; MEM, minimum essential medium; MLTC, mixed lymphocyte tumor culture; MAb, monoclonal antibody; CAb, conventional antibody; MHC, major histocompatibility complex.

MATERIALS AND METHODS

Mice BALB/c, C57BL/6, DBA/1 and A mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Other mouse strains were obtained from the breeding colony of Sloan-Kettering Institute.

Tumors The RadLV-induced leukemias were induced by injecting RadLV into newborn BALB/c and C57BL/6 mice.[14] The leukemias were maintained by passage in syngeneic mice. BALBRVA, BALBRVD and BALBRVE were passaged in the splenic form. The other leukemias were passaged in the form of ascites. BALBRVD cells were also maintained in *in vitro* cultures using Eagle's minimum essential medium (MEM) supplemented with 10% heat-inactivated fetal calf serum (HyClone, Sterile Systems, Inc., UT), 2 m*M* L-glutamine, 1% nonessential amino acids, 100 U/ml of penicillin and 100 μg/ml of streptomycin (complete MEM medium). Cultured BALBRVD cells were used *in vitro* as stimulator cells in mixed lymphocyte tumor cultures (MLTC) and as target cells in cell-mediated cytotoxicity assays.

BALB/c leukemia 18-4 (induced with Abelson virus) and DBA/2 leukemia P388D1 (induced with methylcholanthrene) were provided by Dr. N. Tada, Sloan-Kettering Institute. DBA/2 leukemia L1210 (induced with methylcholanthrene) were provided by Dr. F. Schmid, Sloan-Kettering Institute. The derivation of other tumors used in this study has been described in previous publications.[1, 15-19]

Antisera Goat anti-MuLV (AKR) gp70, anti-$G_{(ERLD)}$ ((B-G_{IX} × 129) F 1 monoclonal antibody (MAb) to $G_{(ERLD)}$), anti-NTD (rat (W/Fu×BN)F1 anti-W/Fu leukemia (C58NT)D), anti-TL (rat MAb to TL), anti-H-2^d (C57BL/6 anti-MethA(BALB/c)), anti-H-$2K^d$ (A anti-MethA) and anti-H-$2D^d$ (BALB.G anti-MethA) were described previously.[15-20] W/Fu rat anti-RadLV antiserum was provided by Dr. M. Lieberman, Stanford University School of Medicine, CA. Goat anti-MuLV (Rauscher) gp70, goat anti-MuLV(Rauscher) p30 and goat anti-MuLV(AKR) p15 were obtained from Dr. P. V. O'Donnell, Sloan-Kettering Institute. C57BL/6 anti-CI-4 (MAb to MuLV-related antigens) and (BALB/c × C57BL/6) F1 anti-CMS4 (MAb to transformation-related antigens) were obtained from Dr. A. B. DeLeo, Sloan-Kettering Cancer Center. Anti-Lyt-1.1 conventional antibody (CAb) ((BALB/c×C57BL/6)F1 anti-B6-Lyt-1.1), anti-Lyt-1.2 CAb (C3H/An anti-C3H.CH-Lyt-1.2:DS), anti-Lyt-2.1 MAb (B6-H-2^k anti-B6-H-2^k.CE-Lyt-2.1:DS), anti-Lyt-2.2 MAb (CBA/2 anti RL♂1) and anti-Lyt-3.2 MAb (C58 anti-CE/J) were obtained from Dr. N. Tada, Sloan-Kettering Institute.

Immunization of mice with RadLV-induced leukemias Male BALB/c mice were inoculated with 20×10^6 irradiated (10,000 rads) BALBRV1 or BALBRVD cells by 4–6 subcutaneous and intraperitoneal injections at intervals of 2 weeks unless otherwise stated. One to 4 weeks after the last immunization, spleen cells from immunized mice were used as responder cells in MLTC.

***In vitro* sensitization of spleen cells** Responder spleen cells (40×10^6) from immunized mice were mixed with 4×10^6 irradiated (10,000 rads) stimulator leukemia cells in 20 ml of complete MEM medium containing 5×10^{-5} *M* 2-mercaptoethanol. The cells were cultured for 5 days and used as effector cells in cell-mediated cytotoxicity assays.

Cell-mediated cytotoxicity assays The method was described previously.[17] In direct cytotoxicity assays, 1×10^4 ^{51}Cr-labeled target cells (100 μl) were incubated with serial dilutions of the effector cell suspension (100 μl). Assays were performed in duplicate in microtiter plates. The plates were incubated for 4 h at 37°C. The supernatant was then removed by a Titertek apparatus (Flow Laboratories, Rockville, MD) and assayed for radioactivity in a Packard scintillation counter. Percent specific lysis was calculated by using the following equation: $(a-b)/(c-b)\times100$, where a is cpm in the supernatant of target cells mixed with effector cells, b is cpm in the supernatant of target cells incubated alone and c is cpm after lysis of target cells with 2% Nonidet P-40.

Competitive inhibition assays In competitive inhibition assays, 1×10^4 ^{51}Cr-labeled target cells (100 μl) were incubated for 4 h at 37°C with different numbers of unlabeled inhibitor cells (50 μl) and effector cells (50 μl) at a fixed effector cell to target cell ratio. The data are given as percent specific lysis or percent inhibition, based on the following equation: (1 − percent specific lysis with inhibitor cells/percent specific lysis without inhibitor cells) × 100.

Antibody blocking assays In antibody blocking assays, serially diluted antiserum (50 μl), effector cell suspension (50 μl) and 1×10^4 ^{51}Cr-labeled target cells (50 μl) at a fixed effector cell to target cell ratio were incubated together for 4 h at 37°C. The data are given as percent inhibitor cells/percent specific lysis without inhibitor specific lysis with antibody/percent specific lysis without antibody) × 100.

Pretreatment of effector cells with Lyt antibody and complement The method was described previously.[21]

RESULTS

***In vitro* generation of cytotoxic effector cells reactive with BALBRV1 and BALBRVD leukemias** Conditions for generating *in vitro* syngeneic cytotoxic effector cells reactive with RadLV-induced leukemias BALBRV1 and BALBRVD were examined. As shown in Table I, no cytotoxicity was detected in spleen cells from non-immunized mice even after *in vitro* sensitization. Spleen

Table I. Generation of Cytotoxic Cells Reactive with BALBRV1 and BALBRVD

Leukemia	*In vivo* immunization	*In vitro* sensitization[a]	Specific lysis (%)			
			80:1[b]	40:1	20:1	10:1
BALBRV1	−	−	0	0	0	0
	−	+	5	4	4	0
BALBRV1	2×	−	0	2	0	4
	2×	+	32	24	13	9
BALBRV1	5×	−	2	0	0	0
	5×	+	55	40	39	29
BALBRVD	−	−	9	2	0	0
	−	+	10	1	0	0
BALBRVD	4×	−	0	0	0	0
	4×	+	60	42	31	12

a) Spleen cells from normal or immunized mice were cultured for 5 days with or without irradiated leukemia cells.
b) Effector cell to target cell ratio.

Table II. Cytotoxicity of BALB/c Effector Cells Generated against BALBRV1: Direct Cytotoxicity Assays

Exp. No.	Target cells	Specific lysis (%)			
		20:1[a]	10:1	5:1	2.5:1
1	BALBRV1	57	32	26	15
	BALBRVA	79	36	24	10
	BALBRVC	55	30	14	10
	BALBRVE	59	37	22	15
	B6RV1	6	1	3	5
	B6RV2	7	1	3	2
	RL♂1	72	52	35	15
	Pu5	73	60	40	20
	L1210	76	47	30	16
2	BALBRV1	42	33	31	21
	BALBRV4	42	29	26	21
	BALBRVD	0	2	1	0
	LSTRA	0	0	0	2
	EL-4	1	0	1	4

a) Effector cell to target cell ratio.

cells from immunized mice were also nonreactive when cultured alone, but cytotoxicity could be induced in the cells by *in vitro* sensitization. The mice that had been immunized 4 to 5 times *in vivo* consistently gave rise to effector cells with high killing activity and were used in subsequent analyses.

T-cell characteristics of cytotoxic effector cells reactive with BALBRV1 and BALBRVD leukemias Pretreatment of BALB/c anti-BALBRV1 effector cells with Lyt-1.2, Lyt-2.2 or Lyt-3.2 antibody and complement abolished their cytotoxic activity. Effector cell activity was not affected by pretreatment with control Lyt-1.1 or Lyt-2.1 antibody and complement. These results indicated that BALB/c anti-BALBRV1 effector cells were Lyt-$1^+2^+3^+$ T cells. Similarly, BALB/c anti-BALBRVD effector cells were also shown to be Lyt-$1^+2^+3^+$ (data not shown).

Specificity of BALB/c effector cells generated against BALBRV1 leukemia Table II shows two individual experiments on direct cytotoxicity and Fig. 1A illustrates a representative competitive inhibition assay with BALB/c anti-BALBRV1 effector cells. Table III shows the results of direct cytotoxicity and competitive inhibition assays with a series of leukemia cells and other tumor cells of BALB/c, DBA/2 or C57BL/6 origin. No discrepancy was observed between the results of these two assays. These results indicated that a shared cell surface antigen on BALBRV1 was recognized by BALB/c anti-BALBRV1 effector cells.

Specificity of BALB/c effector cells generated against BALBRVD leukemia BALBRVD leukemia was not lysed by BALB/c anti-BALBRV1 effector cells. In competitive inhibition assays, BALBRVD showed no inhibitory activity on BALBRV1 target cells (Fig. 1A). BALBRVD target cells, however, were effectively lysed by BALB/c anti-BALBRVD effector cells (Table I). Table IV shows the results of individual direct cytotoxicity assays of BALB/c anti-BALBRVD effector cells. Specificity analyses with a wide variety of tumors showed that BALB/c anti-BALBRVD effector cells lysed only BALBRVD target cells, and that only BALBRVD cells were inhibitory to the lysis of BALBRVD target cells in competitive inhibition assays (Fig. 1B and Table III).

Table III. Specificity of BALB/c Effector Cells Generated against BALBRV1 or BALBRVD: Results of Direct Cytotoxicity Assays and Competitive Inhibition Assays[a]

Cells	Effector cells							
	BALB/c anti-BALBRV1				BALB/c anti-BALBRVD			
	Direct C[b]		Competitive I[c]		Direct C[b]		Competitive I[c]	
	40:1[d]	20:1	20:1[e]	10:1	40:1[d]	20:1	60:1[e]	30:1
RadLV-induced leukemias								
BALBRV1(BALB/c)	52	42	58	47				
BALBRVD(BALB/c)					64	56	61	58
BALBRVE(BALB/c)					0	0	8	2
BALBRV4(BALB/c)					0	0	10	0
B6RV1(C57BL/6)							5	2
B6RVTC1(C57BL/6)	0	0						
B6RV2(C57BL/6)							0	7
X-ray-induced leukemias								
RL♂1(BALB/c)			53	36				
RL♂4(BALB/c)	73	67	53	27			8	5
RL♀8(BALB/c)	51	47	59	44			6	0
ERLD(C57BL/6)			0	0			0	2
Chemically-induced leukemias								
L1210(DBA/2)			73	48			0	0
P388D1(DBA/2)	59	37			0	2		
EL-4(C57BL/6)			11	0			0	0
Spontaneous leukemia								
Pu5(BALB/c)			74	66			7	0
Methylcholanthrene-induced sarcomas								
MethA(s)[f] (BALB/c)	41	26			7	10		
MethA(a)[g] (BALB/c)	2	0			6	3		
CMS1 (BALB/c)	30	20			14	9		
CMS2 (BALB/c)	26	15			7	4		
CMS3 (BALB/c)	0	0			3	4		
CMS4 (BALB/c)	0	0			8	8		
CMS5(BALB/c)	3	0			8	3		
CMS8(BALB/c)	10	4			3	0		
CMS11(BALB/c)	1	1			6	0		
CI-4(BALB/c)	0	0			0	0		
Gross virus-induced leukemia								
E♂G2(C57BL/6)			0	0			0	3
Moloney virus-induced leukemia								
LSTRA(BALB/c)			0	0	5	4	29	8
Rauscher virus-induced leukemia								
RBL5(C57BL/6)	9	5	8	0	0	0		
Abelson virus-induced leukemia								
18-4(BALB/c)	1	0	0	0			12	0
Mastocytoma								
P815(DBA/2)	8	4	3	3	8	6	0	11
Myeloma								
MOPC70A(BALB/c)	0	1			6	3		
Normal thymocytes								
BALB/c			0	0			3	5
ConA blast (thymocytes)								
BALB/c	5	5	0	10	0	0	0	3
ConA blast (spleen cells)								
BALB/c	3	0	7	7	0	0	0	0
C57BL/6	0	0	7	10	0	0	0	15
DBA/1	0	0	9	5				
C3H	1	0	0	0	10	7	0	8
B10.S	0	0	5	0	4	2	0	0
BALB-H-2[j]	0	2	0	2	4	2	21	8

a) Methylcholanthrene-induced BALB/c sarcomas, P388D1 and MOPC70A were used only as target cells in direct cytotoxicity assay. ERLD, E♂G2 and normal thymocytes were used only as inhibitor cells in competitive inhibition assays. *b*) C, cytotoxicity assays. The data are given as percent specific lysis. *c*) I, inhibition assays. The data are given as percent inhibition. *d*) Effector cell to target cell ratio. *e*) Inhibitor cell to target cell ratio. *f*) MethA solid, maintained *in vitro*. *g*) MethA ascitic, maintained *in vitro*.

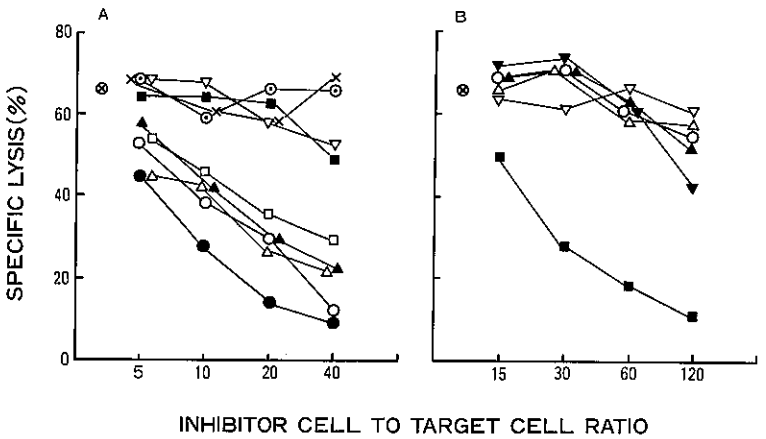

Fig. 1. Competitive inhibition assays with BALB/c effector cells generated against BALBRV1 (A) and BALBRVD (B). Unlabeled inhibitor cells were added at different ratios to 1×10^4 ^{51}Cr-labeled target cells. A: BALBRV1 cells were used as target cells. The effector cell to target cell ratio was 10:1 B: BALBRVD cells were used as target cells. The effector cell to target cell ratio was 60:1. Inhibitor cells used were BALBRV1 (○), BALBRV4 (●), BALBRVA (△), BALBRVC (▲), BALBRVE (□), BALBRVD (■), B6RV1 (⊙), B6RVTC1 (▽), B6RV2 (×), RL♂1 (▼). The symbol ⊗ indicates % specific lysis without inhibitor cells.

Table IV. Cytotoxicity of BALB/c Effector Cells Generated against BALBRVD: Direct Cytotoxicity Assays

Exp. No.	Target cells	Specific lysis (%)			
		80:1[a]	40:1	20:1	10:1
1	BALBRVD	48	30	16	9
	BALBRV1	14	6	4	8
	BALBRVC	7	1	0	0
	B6RVTC1	1	0	0	0
	RL♂1	11	1	1	0
2	BALBRVD	63	44	30	18
	BALBRVA	0	0	0	0
	B6RV1	4	0	0	0
	B6RV2	9	0	1	8
	RL♀4	6	0	0	0
	RL♀8	0	0	0	0
	Pu5	13	9	0	2
	18-4	5	2	0	0
	L1210	10	1	2	3
	EL-4	0	0	0	0

a) Effector cell to target cell ratio.

Blocking assays with alloantisera and heteroantisera to cell surface antigens of mouse lymphoid cells To examine the determinants recognized by BALB/c anti-BALBRV1 effector cells and BALB/c anti-BALBRVD effector cells, antisera were added without the addition of exogenous complement. Tables V and VI show the results of these antibody blocking assays. Antisera against H-2^d or H-2K^d blocked both cytotoxic responses. In contrast, antisera detecting H-2D^d, TL, RadLV, MuLV, MuLV-related antigens and transformation-related antigens had no blocking effect.

DISCUSSION

In the present study, the cell surface antigens recognized by syngeneic CTL reactive with two RadLV-induced BALB/c leukemias BALBRV1 and BALBRVD were investigated. To induce CTL with high killing activity, it was necessary to immunize mice repeatedly *in vivo* with irradiated leukemias before *in vitro* sensitization of primed spleen cells. The specificity of CTL thus generated was examined by direct cytotoxicity assays, competitive inhibition assays and blocking assays with conventional or monoclonal antibody to H-2, RadLV, MuLV, TL or MuLV-related antigens or transformation-related antigens. The results indicated that a shared cell surface antigen (designated as BALBRV1 antigen) was recognized by BALB/c anti-BALBRV1 CTL. On the other hand, a unique cell surface antigen (designated as BALBRVD antigen) was recognized by BALB/c anti-BALBRVD CTL. There are several MuLV-related cell surface antigens that have been serologically defined. These are GCSA,[1,2] G_{IX},[20] $G_{(RADA1)}$,[16] $G_{(ERLD)}$,[18] $G_{(AKSL2)}$,[14] and FMR antigens.[3] BALBRV1 does not express any of these antigens on its surface (Table VII). GCSA and FMR antigens have been shown to be recognized by CTL.[4-11] However, GCSA-positive P815[20] and MOPC70A[1] or FMR antigen-positive LSTRA[22] and 18-4[23] were not lysed by BALB/c anti-BALBRV1 CTL. The distribution pattern of BALBRV1 antigen on some tumors is distinct from those of the antigens described above. Declève *et al.* reported that RadLV possesses the envelope glycoprotein gp71, unlike other murine type C viruses.[24] In this regard, it is possible that BALBRV1 antigen is related to RadLV. Alternatively, BALBRV1 antigen may be an epitope related to endogenous MuLV. MethA(a), CMS4 and CMS5 that lack the expression of MuLV structural antigens and MuLV-

Table V. Antibody Blocking Assays on the Cytotoxic Responses of BALB/c anti-BALBRV1 Effector Cells[a]

Exp. No.	Antiserum	Percent inhibition		
1		1:12[b]	1:24	1:48
	anti-H-2^d	78	72	60
	anti-H-$2K^d$	46	50	36
	anti-H-$2D^d$	0	0	0
	NMS[c]	4	0	0
2		1:12	1:24	1:48
	anti-H-2^d	100	100	100
	anti-MuLV(AKR)gp70	0	0	0
	anti-MuLV(Rauscher)gp70	0	0	0
	anti-MuLV(Rauscher)p30	0	0	0
	anti-MuLV(AKR)p15	0	0	0
	anti-$G_{(ERLD)}$	4	0	0
3		1:30	1:60	1:120
	anti-H-2^d	78	78	76
	anti-RadLV	0	0	0
	anti-NTD	0	0	0
	anti-MuLV-related antigens	0	0	0
	anti-transformation-related antigens	0	0	0
	anti-TL	0	13	11

a) Cytotoxic activity of BALB/c anti-BALBRV1 effector cells was assayed on ^{51}Cr-labeled BALBRV1 target cells. The effector cell to target cell ratio was 25:1 in Experiment 1, 60:1 in Experiment 2 and 30:1 in Experiment 3. Percent specific lysis without the addition of antibody was 50% in Experiment 1, 27% in Experiment 2 and 46% in Experiment 3.
b) Antiserum dilution.
c) NMS, normal mouse serum.

Table VI. Antibody Blocking Assays on the Cytotoxic Responses of BALB/c anti-BALBRVD Effector Cells[a]

Antiserum	Percent inhibition		
	1:30[b]	1:60	1:120
anti-H-2^d	91	88	78
anti-H-K^d	45	24	24
anti-H-$2D^d$	3	5	9
NMS	0	0	12
anti-RadLV	0	0	5
anti-MuLV(AKR)gp70	0	0	5
anti-MuLV(Rauscher)gp70	0	0	3
anti-MuLV(Ranuscher)p30	0	12	8
anti-MuLV(AKR)p15	0	0	3
anti-NTD	0	0	0
anti-$G_{(ERLD)}$	0	5	6
anti-MuLV-related antigens	0	0	9
anti-transformation-related antigens	5	20	11
anti-TL	0	6	1

a) Cytotoxic activity of BALB/c anti-BALBRVD effector cells was assayed on ^{51}Cr-labeled BALBRVD target cells. The effector cell to target cell ratio was 30:1. Percent specific lysis without the addition of antibody was 65%.
b) Antiserum dilution.

related cell surface antigens do not express BALBRV1 antigen, while MethA(s) that expresses MuLV-related antigens and MuLV gp70 expresses BALBRV1 antigen. It was shown that leukemogenesis by RadLV increased the expression of H-2 antigen on thymocytes.[25] RadLV may cause an alteration of endogenous MuLV antigen, resulting in its recognition by CTL as BALBRV1 antigen.

BALBRVD antigen, on the other hand, is expressed only on BALBRVD leukemia. The restricted expression of BALBRVD antigen suggests that BALBRVD antigen may belong to a class of individually distinct antigens that were originally found on chemically induced murine sarcoma MethA as tumor-specific transplantation antigens.[15] Morishita *et al.* recently reported that two RadLV-induced BALB/c leukemias BALBRVB and BALBRVD expressed individually distinct cell surface antigens that could be recognized by semisyngeneic CTL.[13] In this study, it was not determined whether syngeneic and semisyngeneic CTL recognized the identical antigens on BALBRVD. This should be further investigated.

Blocking experiments with anti-H-2 sera indicated that the products of H-$2K^d$ region of MHC are involved in the

Table VII. Expression of BALBRV1 Antigen, BALBRVD Antigen and Serologically Defined MuLV-related Cell Surface Antigens on Tumor Cells

Tumor cells	BALBRV1 antigen[a]	BALBRVD antigen[b]	GCSA	G_{IX}	$G_{(RADA1)}$	$G_{(ERLD)}$	$G_{(AKSL2)}$	MuLV		
								gp70	p30	p15
BALBRV1	+	−	−	−	−	−	−			
BALBRVD	−	+								
RL♂1	+	−	−	+	−	+	−	+	−	+
MOPC70A	−	−	+	−	−	−	−	−	−	+
MethA(a)	−	−	−	−	−	−	−	−	−	
MethA(s)	+	−	+	+	+	+	−	−	−	
CMS1	+	−	+	+			−	+	+	
CMS2	+	−						+		
CMS3	−	−	+	+	+			+	+	
CMS4	−	−	−	−	−	−	−	−	−	
CMS5	−	−	−	−	−			−	−	
CMS8	−	−						+		
CMS11	−	−	−	−	−	−	−			

a) Defined by BALB/c anti-BALBRV1 effector cells.
b) Defined by BALB/c anti-BALBRVD effector cells.

interaction between syngeneic CTL and BALBRV1 or BALBRVD target cells. Exclusive involvement of H-2K^d region products was also reported in T cell recognition of FMR antigen-positive leukemias.[4,26] In contrast, the products of the H-2D^d region of MHC were shown to be involved in the cytotoxic responses of (C57BL/6× BALB/c)F1 CTL directed to a unique cell surface antigen of X-ray-induced BALB/c leukemia RL♂1.[27] Blocking experiments with antibody to RadLV, MuLV, TL and MuLV-related antigens or transformation-related antigens indicated that none of these antibodies had a blocking effect on BALBRV1 and BALBRVD target cells. However, it is generally known that antibodies to a wide range of MuLV components have no blocking effect on the cytotoxic responses of FMR antigen- or GCSA-specific CTL.[22,28] These findings, therefore, suggest that our observations in the antibody blocking assays do not necessarily exclude the possibility that BALBRV1 antigen and BALBRVD antigen may be related to RadLV or endogenous MuLV.

ACKNOWLEDGMENTS

The authors thank Drs. N. Tada, F. Schmid, M. Lieberman, P. V. O'Donnell and A. B. DeLeo for their gifts of tumor cells and antibodies.

(Received October 24, 1988/Accepted January 5, 1989)

REFERENCES

1) Old, L. J. and Stockert, E. Immunogenetics of cell surface antigens of mouse leukemia. *Ann. Rev. Genet.*, **17**, 127–160 (1977).
2) Old, L. J., Boyse, E. A. and Stockert, E. The G(Gross) leukemia antigen. *Cancer Res.*, **25**, 813–819 (1965).
3) Old, L. J., Boyse, E. A. and Stockert, E. Typing of mouse leukemias by serological methods. *Nature*, **201**, 777–779 (1965).
4) Gomard, E., Duprez, V., Reme, T., Colombani, M. J. and Levy, J. P. Exclusive involvement of H-2D^b or H-2K^d product in the interaction between T-killer lymphocytes and syngeneic H-2^b or H-2^d viral lymphomas. *J. Exp. Med.*, **146**, 909–922 (1977).
5) Green, W. R., Nowinski, R. C. and Henney, C. S. The generation and specificity of cytotoxic T cells raised against syngeneic tumor cells bearing AKR/Gross murine leukemia virus antigens. *J. Exp. Med.*, **150**, 51–66 (1979).
6) Collins, J. K., Britt, W. J. and Chesebro, B. Cytotoxic T lymphocyte recognition of gp70 on Friend virus-induced erythroleukemia cell clones. *J. Immunol.*, **125**, 1318–1324 (1980).
7) Taniyama, T. and Holden, H. T. *In vitro* induction of T-lymphocyte-mediated cytotoxicity by infectious murine type C oncornaviruses. *J. Exp. Med.*, **150**, 1367–1382 (1979).
8) Enjuanes, L., Lee, J. C. and Ihle, J. N. Antigenic specificities of the cellular immune response of C57BL/6 mice to the Moloney leukemia/sarcoma virus complex. *J.*

Immunol., **122**, 665–674 (1979).
9) Plata, F. and Lilly, F. Viral specificity of H-2-restricted T killer cells directed against syngeneic tumors induced by Gross, Friend or Rauscher leukemia virus. *J. Exp. Med.*, **150**, 1174–1186 (1979).
10) Green, W. R. H-2-restricted cytolytic T lymphocytes specific for a subclass of AKR endogenous leukemia virus-induced tumors: correlation of tumor susceptibility with expression of the Gross cell surface antigen. *J. Immunol.*, **125**, 2584–2590 (1980).
11) Abastado, J-P., Plata, P., Merello, D., Daniel-Vedele, F. and Kourilsky, P. H-2 restricted cytolysis of L cells doubly transformed with a cloned H-2K^b gene and cloned retroviral DNA. *J. Immunol.*, **135**, 3512–3519 (1985).
12) Nakayama, E., Uenaka, A., Stockert, E. and Obata, Y. Detection of a unique antigen on radiation leukemia virus-induced leukemia B6RV2. *Cancer Res.*, **44**, 5138–5144 (1984).
13) Morishita, H. Shiku, H., Horibe, K., Obata, Y., Stockert, E., Oettgen, F. H., Old, L. J. and Yamada, K. Cell surface antigens of murine leukemias induced by radiation leukemia virus. Recognition of individually distinct cell surface antigens by cytotoxic T cells on leukemias expressing crossreactive transplantation antigens. *J. Exp. Med.*, **163**, 452–457 (1986).
14) Stockert, E., DeLeo, A. B., O'Donnell, P. V., Obata, Y. and Old, L. J. $G_{(AKSL2)}$: a new cell surface antigen of the mouse related to the dualtropic mink cell focus-inducing class of murine leukemia virus detected by naturally occurring antibody. *J. Exp. Med.*, **149**, 200–215 (1979).
15) DeLeo, A. B., Shiku, H., Takahashi, T., John, M. and Old, L. J. Cell surface antigens of chemically induced sarcomas of the mouse. I. Murine leukemia virus-related antigens and alloantigens on cultured fibroblasts and sarcoma cells: description of a unique antigen on BALB/c MethA sarcoma. *J. Exp. Med.*, **146**, 720–734 (1977).
16) Obata, Y., Stockert, E., O'Donnell, P. V., Okubo, S., Snyder, H. W., Jr. and Old, L. J. $G_{(RADA1)}$: a new cell surface antigen of mouse leukemia defined by naturally occurring antibody and its relationship to murine leukemia virus. *J. Exp. Med.*, **147**, 1089–1105 (1978).
17) Nakayama, E., Shiku, H., Takahashi, T., Oettgen, H. F. and Old, L. J. Definition of a unique cell surface antigen of mouse leukemia RL♂1 by cell-mediated cytotoxicity. *Proc. Natl. Acad. Sci. USA*, **76**, 3486–3490 (1979).
18) Obata, Y., Stockert, E., DeLeo, A. B., O'Donnell, P. V., Snyder, H. W., Jr. and Old, L. J. $G_{(ERLD)}$: a cell surface antigen of the mouse related to xenotropic MuLV defined by naturally occurring antibody and monoclonal antibody. Relation to G_{IX}, $G_{(RADA1)}$, $G_{(AKSL2)}$ systems of MuLV-related antigens. *J. Exp. Med.*, **154**, 659–675 (1981).
19) Palladino, M. A., Obata, Y., Stockert, E. and Oettgen, H. F. Characterization of interleukin 2-dependent cytotoxic T-cell clones: specificity, cell surface phenotype, and susceptibility to blocking by Lyt antisera. *Cancer Res.*, **43**, 573–576 (1973).
20) Stockert, E., Old, L. J. and Boyse, E. A. The G_{IX} system. A cell surface allo-antigen associated with murine leukemia virus; implications regarding chromosomal integration of the viral genome. *J. Exp. Med.*, **133**, 1334–1355 (1971).
21) Nakayama, E., Shiku, H., Stockert, E., Oettgen, H. F. and Old, L. J. Cytotoxic T cells: Lyt phenotype and blocking of killing activity by Lyt antisera. *Proc. Natl. Acad. Sci. USA*, **76**, 1977–1981 (1979).
22) Gomard, E., Levy, J. P., Plata, F., Henin, Y., Duprez, V., Bismuth, A. and Reme, T. Studies on the nature of the cell surface antigen reacting with cytolytic T lymphocytes in murine oncorna virus-induced tumors. *Eur. J. Immunol.*, **8**, 228–236 (1978).
23) Risser, R., Stockert, E. and Old, L. J. Abelson antigen: a viral tumor antigen that is also a differentiation antigen of BALB/c mice. *Proc. Natl. Acad. Sci. USA*, **75**, 3918–3922 (1978).
24) Declève, A., Lieberman, M., Ihle, J. N., Rosenthal, P. N., Lung, M. L. and Kaplan, H. S. Physicochemical, biological and serological properties of a leukemogenic virus isolated from cultured RadLV-induced lymphomas of C57BL/Ka mice. *Virology*, **90**, 23–35 (1978).
25) Meruelo, D., Nimelstein, S. H., Jones, P. P. Lieberman, M. and McDevitt, H. O. Increased synthesis and expression of H-2 antigens on thymocytes as a result of radiation leukemia virus infection: a possible mechanism for H-2 linked control of virus-induced neoplasia. *J. Exp. Med.*, **147**, 470–487 (1978).
26) Weiss, A., Brunner, K. T., MacDonald, M. R. and Cerottini, J-C. Antigenic specificity of the cytolytic T lymphocyte response to murine sarcoma virus-induced tumors. III. Characterization of cytolytic T lymphocyte clones specific for Moloney leukemia virus-associated cell surface antigens. *J. Exp. Med.*, **152**, 1210–1225 (1980).
27) Duprez, V., Gomard, E. and Levy, J. P. Cell-mediated antitumor response in the RL♂1 system. I. T nature, H-2D^d involvement and antigenic specificity of the effector cells. *Eur. J. Immunol.*, **8**, 650–655 (1978).
28) Green, W. R., Nowinski, R. C. and Henney, C. S. Specificity of cytotoxic T cells directed against AKR/Gross virus-induced syngeneic leukemias: antibodies directed against H-2K, but not against viral proteins, inhibit lysis. *J. Immunol.*, **125**, 647–655 (1980).