[CANCER RESEARCH 30, 2073–2079, July 1970]

Antigenic Analysis of L Strain Cells: A New Murine Leukemia-associated Antigen, "L"

J. C. Leclerc, J. P. Levy, B. Varet, S. Oppenheim, and A. Senik

Laboratoire d'Immunologie des Tumeurs, Institut de Recherche sur les Leucémies et les Maladies du Sang, Hôpital Saint Louis, Paris 10ème, France

SUMMARY

Analysis of a normal tissue culture cell line (L strain) by serological methods and by induction of transplantation immunity revealed three antigens associated with murine leukemias present in these nonleukemic cells: (*a*) the group-specific antigen of murine leukemia viruses; (*b*) an antigen common to Friend, Moloney, Rauscher, and Graffi leukemias (FMRGi). The group-specific and FMRGi antigens are probably related to the nonleukemogenic type C virus present in L cells. The nature of this agent is briefly discussed; (*c*) a new antigen which is also present in various virus-induced leukemias of several strains and in the dimethylbenzanthracene-induced EL4 leukemia. It was called L antigen. L antigen was shown to be different from other already described murine leukemia antigens. No tumor rejection of L+ tumors was observed in hyperimmune mice despite the presence of cytotoxic antibodies in the blood. Enhancement of tumor growth was frequently observed in this situation. The origin of L antigen and its role in tumor immunity are briefly discussed.

INTRODUCTION

Various tissue culture lines originating from normal mice harbor type C particles identical with those of MuLV[1] (3, 4, 9). We report here the results of the antigenic analysis of L strain originating from normal C3H/An mice (5). Three types of antigens were detected in L cells: (*a*) the group-specific antigen of MuLV, as previously shown (4, 21); (*b*) the FMRGi antigen (14, 18); and (*c*) a new antigen, "L" antigen, which appears to be different from other known murine leukemia antigens. The new L antigen was shown also to be present on the leukemic cells in DMBA-induced EL4 leukemia and in many cases of Moloney, Rauscher, Gross, and Graffi virus-induced leukemia.

MATERIALS AND METHODS

L Strain Cells. L strain fibroblast tissue cultures were made in Eagle medium supplemented with 10% calf serum. For elimination of the possibility of viral contamination of our strain, in some experiments L strain, kindly provided by Dr. Maurin (Institut Pasteur) and Dr. Gresser (Institut de Recherches sur le Cancer, Villejuif), were used. Type C particles were regularly observed by electron microscopy as previously described by others (3, 4, 9). No oncogenic potency was observed when newborn BALB/c or C57BL/6 mice were inoculated with L strain agent extracted by a routine method (2).

Mice. Mice of the following inbred strains were obtained from our own colonies: C57BL/6, BALB/c, C3H/eb, C3H/He, AKR.

Leukemias. The nomenclature and origin of our leukemias are summarized in Table 1. Most of the leukemias arose in our laboratory after inoculation of newborn mice with MuLV. All were serially transplanted s.c. or i.p.

Antisera. Most anti-L cells antisera were prepared in 2-month-old C57BL/6 mice (Sera 32, 42, 52, 68, 72) and in some experiments in C3H/eb (Sera 200, 202) or C3H/He (Sera 205) mice. Four to 8 inoculations of 5×10^5 to 1×10^7 L cells were given at weekly intervals, the first with complete Freund's adjuvant s.c. and the following without adjuvant i.p. Anti-Gross (C57BL/6 anti-K36 AKR leukemia) and anti-FMRGi (anti-Graffi and anti-Moloney) were prepared as previously described (13, 14).

Cytotoxicity Tests. They were performed in a strictly isologous system with C57BL/6 antisera and C57BL/6 target cells, according to a previously described technique (14). Isologous C3H anti-L antisera have been also tested in some experiments against C57BL/6 or L strain cells.

Immunofluorescence Reactions. The indirect membrane immunofluorescence technique with living cells was performed according to the method of Möller (16).

Absorption Experiments. For absorption experiments, sera were inactivated by heating at 56° for 30 min, diluted up to 2 doubling dilutions beyond the 50% cytotoxic end point, and absorbed with various amounts of cells ranging from 1×10^6 to 5×10^7 for 0.1 ml serum. Absorbing cells were washed 3 times and packed; the serum was added to the cell pellet and the mixture was incubated with continuous shaking at 37° for 45 min. The cells were spun down at $2500 \times g$ for 10 min, and the supernatants were used for cytotoxic reactions. Cytotoxic indices of the absorbed sera were compared with those of unabsorbed sera or sera incubated with normal cells.

Virus Neutralization. The neutralization activity of anti-L antisera was tested against 3 different pseudotypes of MSV: (*a*) M-MSV (MLV), (*b*) M-MSV (RLV), (*c*) M-MSV (GiLV), with envelopes that are, respectively, those of Moloney, Rauscher, and Graffi leukemia viruses. The first two were kindly provided by Dr. J. Hartley (NIH, Bethesda, Md.), and the third was prepared in this laboratory (20). Tenfold dilu-

[1] The abbreviations used are: MuLV, murine leukemia virus; FMRGi, Friend, Moloney, Rauscher, and Graffi leukemias; DMBA, dimethylbenzanthracene; MSV, murine sarcoma virus; MLV, Moloney leukemia virus; RLV, Rauscher leukemia virus; GiLV, Graffi leukemia virus.

Received December 11, 1969; accepted March 6, 1970.

Table 1
Leukemias

Nomenclature	Origin	Strain
GiL1	Graffi-induced	C57BL/6
GiL4	Graffi-induced	C57BL/6
GiL10[a]	Graffi-induced	C57BL/6
GiC5	Graffi-induced	BALB/c
YL3	Moloney-induced	C57BL/6
YL5	Moloney-induced	C57BL/6
YL10	Moloney-induced	C57BL/6
YC8	Moloney-induced	BALB/c
YC10	Moloney-induced	BALB/c
RC18	Rauscher-induced	BALB/c
EδG2[b]	Gross-induced	C57BL/6
GC1	Gross-induced	BALB/c
GH1	Gross-induced	C3H/eb
K36[b]	Gross-induced	AKR
EL4[b]	DMBA-induced	C57BL/6
ERLD[b]	X-ray-induced	C57BL/6
C1498[c]	Spontaneous	C57BL/6

[a] Kindly provided by Dr. G. Pasternak (Berlin, Germany).
[b] Kindly provided by Dr. L. J. Old (New York, N. Y.).
[c] Kindly provided by Professor G. Mathé (Villejuif, France).

tions of the virus suspension were mixed volume for volume in half-diluted sera and incubated for 1 hr at room temperature. The mixture was then inoculated into newborn mice of the most sensitive strain, and the mice were examined every day for the appearance of tumor. Animals inoculated with virus preincubated in normal serum were used as controls.

Immunodiffusion Tests for Detection of MuLV Group-specific Antigen. The antigen was prepared from L strain cells and used in a modified Ouchterlony double diffusion test system [Geering *et al.* (6)]. Antiserum (anti-MuLV) was a W/Fu anti-Gross leukemia (C58NT)D kindly provided by Dr. E. A. Boyse (Sloan-Kettering Institute, New York, N. Y.).

Induction of Resistance against Transplantation of Isologous Leukemia. Four-month-old C57BL/6 mice were preimmunized with living L cells or disrupted EL4 cells (4 inoculations of 5×10^6 cells, the first with complete Freund's adjuvant) and challenged with EL4 cells (from 7×10^2 to 2.5×10^4). Mice given 1 injection of complete Freund's adjuvant were used as controls.

Four-month-old BALB/c mice were preimmunized in the same way with L strain cells, challenged with YC8 leukemias which contained both FMRGi and L antigen and compared with nonimmune mice.

Four-month-old BALB/c were preimmunized with EL4 cells, challenged with YC8 leukemia, and compared with nonimmune mice, or with mice given 1 injection of complete Freund's adjuvant.

RESULTS

Results of Cytotoxicity Tests and Immunofluorescence Reactions. The results of cytotoxicity reactions performed in strictly isologous systems can be summarized as follows:

All C57BL/6 anti-L cell antisera were cytotoxic for EL4 cells. The strongest of them were also slightly active against FRMGi+ leukemias but negative with EδG2, ERLD, and normal C57BL/6 cells. Demonstrative examples of these results, which have been repeatedly controlled, are given in Table 2. C3H/eb anti-L strain sera (Nos. 200, 202) were strongly cytotoxic for EL4 leukemias and L strain cells but inactive against C3H/eb, or C3H/He normal cells. C3H/He anti-L (No. 205) was completely inactive.

Among the 22 anti-FMRGi cytotoxic antisera tested, the 2 strongest were slightly active against EL4 cells. The other 20 were not cytotoxic for EL4 cells.

Anti-Gross antisera were cytotoxic for EδG2 (Gross-induced) and slightly active against FMRGi+ leukemias, as previously mentioned (14, 19), but were clearly negative with EL4 cells.

All sera were inefficient against ERLD and normal cells.

Immunofluorescence reactions gave results identical with those of cytotoxic tests as illustrated by an example (Table 3).

Results of Absorption Tests. The cytotoxicity test and the immunofluorescence reaction were positive when anti-L cell antisera were reacted with EL4 leukemia cells. Positive reactions, although weaker, were obtained when Moloney or Graffi leukemic cells which share FMRGi membrane antigen (14) were substituted for EL4 leukemia cells. However, EL4 is FMRGi negative (11, 14, 18). Absorption experiments were performed to determine whether different antigens were responsible for the reactivity of EL4 and FMRGi+ leukemias with anti-L antisera. Anti-L cells antisera were incubated with EL4, YL3, GiL10, and L cells (positive control) and with normal C57BL/6 spleen cells (negative control) and then tested either against EL4 or against GiL10 cells. Results were as follows:

The cytotoxic activity of the sera for EL4 cells (FMRGi–) was removed by L cells, EL4, GiL10, and YL3. Complete absorption was achieved in all cases; however, the quantity of the absorbing antigen on the 4 types of cells was different since complete absorption of the cytotoxic activity needed, respectively, about 2×10^6 L cells, 6×10^6 EL4 cells, 1.7×10^7 GiL10 cells, and 3×10^7 YL3 cells (Chart 1).

The cytotoxic activity of the same sera for GiL10 cells (FMRGi+) was absorbed only by L cells, GiL10, and YL3 and was not completely removed by incubation with 5×10^7 EL4 cells after 2 successive absorptions (Chart 2).

It can be concluded that anti-L antisera revealed in these systems 2 different antigens: one which is common to L, EL4, YL3, and GiL10 and another which is not present on EL4 cells but is present on the others. Absorption experiments with anti-Moloney (Charts 3 and 4) revealed that L cells as well as YL3 and GiL10 cells absorb FMRGi antibodies and that EL4 cells do not.

From these results, it was concluded that antibody active against YL3, GiL10, and L cells was anti-FMRGi and that in addition there was present another antibody active against an antigen present on EL4 cells. We have called this the L antigen.

Presence of L antigen in Various Cells. Several absorption experiments were performed in order to detect L antigen in various leukemic and normal tissues. Table 4 summarizes the results of this investigation. L antigen was found to be present in L cells, in EL4 DMBA-induced leukemia, in several C57BL/6, BALB/c, C3H/eb, and AKR leukemias induced by Graffi, Moloney, Rauscher, and Gross viruses, in normal

C3H/He embryonic fibroblasts, and in the spleen of adults C3H/He mice.

On the contrary, no L antigen was found in normal organs and tissue cultures of BALB/c, C57BL/6, and C3H/eb L antigen was also not detected in several C57BL/6 leukemias including the Gross virus-induced EδG2 leukemia.

Induction of Transplantation Immunity. We failed to induce resistance against transplantation of Moloney (YL3) or Graffi (GiL10) leukemia in C57BL/6 mice by preimmunization with L cells; however, BALB/c mice preimmunized in the same way were able to reject up to 5×10^5 isologous YC8 Moloney-induced leukemia (Table 5). As YC8 cells contain both FMRGi and L antigens, it was not possible to assess the importance of L antigen in the rejection phenomenon. Therefore, the rejection of L+ leukemias was tested under conditions when the FMRGi antigen could not interfere. C57BL/6 mice were hyperimmunized with L cells; they failed to reject as low as 7×10^2 EL4 cells, even when the animals had cytotoxic anti-L antibodies in the blood. In another experiment, BALB/c mice hyperimmunized with EL4 cells (L+FMRGi−) were unable to reject YC8 isologous Moloney-induced leukemia (L+FMRGi+). Instead of rejection, the tumor growth was constantly enhanced, as shown in Table 6.

Detection of Group-specific Antigen of MuLV. The Ouchterlony double diffusion test with the use of a W/Fu anti-Gross serum revealed a common group-specific antigen in L strain cell agents, Gross and Rauscher viruses which have been previously disrupted by ether. Complete L strain agent did not react (Fig. 1).

Neutralization Tests. No neutralizing activity was found in

Table 2

Cytotoxic tests (cytotoxic indices)

All results are expressed in cytotoxic indices. A cytotoxic index lower than 0.20 is considered negative (14).

Antisera				Cells					
No.	Specificity	Dilution	C57BL/6 normal splenic cells	EL4	ERLD	EδG2	YL3	GiL4	GiL10
52	Anti-L cells	1/2	<0.05	1	<0.05	<0.05	0.36	0.48	0.52
		1/4	<0.05	1	<0.05	<0.05	0.32	0.39	0.47
		1/8	<0.05	1	<0.05	<0.05	0.26	0.23	0.40
		1/16		1	<0.05		0.14	0.15	0.36
		1/32		0.84	<0.05		<0.05	<0.05	<0.15
		1/64		0.83	<0.05		<0.05	<0.05	<0.05
		1/128		0.36			<0.05	<0.05	<0.05
		1/256		0.08			<0.05	<0.05	<0.05
72	Anti-L cells	1/2	<0.05	0.72	<0.05	<0.05	0.30	0.32	0.45
		1/4	<0.05	0.51	<0.05	<0.05	0.15	0.19	0.30
		1/8	<0.05	0.43		<0.05	<0.05	0.08	0.24
		1/16		0.30			<0.05	<0.05	0.08
		1/32		0.25			<0.05	<0.05	<0.05
		1/64		0.18				<0.05	<0.05
		1/128		0.05					<0.05
68	Anti-L cells	1/2	<0.05	0.48	<0.05	<0.05	<0.05	<0.05	<0.05
		1/4	<0.05	0.27	<0.05	<0.05	<0.05	<0.05	<0.05
		1/8		0.13					
		1/16		<0.05					
56	Anti-FMRGi (anti-Moloney)	1/2	<0.05	0.63	<0.05	<0.05	0.56	0.62	0.80
		1/4	<0.05	0.34	<0.05	<0.05	0.40	0.49	0.72
		1/8		0.18	<0.05		0.25	0.30	0.63
		1/16		<0.05			0.18	0.18	0.55
		1/32		<0.05			<0.05	0.08	0.30
		1/64		<0.05			<0.05	<0.05	0.19
		1/128		<0.05			<0.05	<0.05	<0.05
34	Anti-FMRGi (anti-Moloney)	1/2	<0.05	<0.05	<0.05	<0.05	0.32		0.44
		1/4		<0.05			0.22		0.31
		1/8		<0.05			0.11		0.20
		1/16		<0.05			<0.05		<0.05
48	Anti-Gross (anti-K36)	1/2	<0.05	<0.05	<0.05	0.92	0.21		0.36
		1/4		<0.05	<0.05	0.86	<0.05		0.18
		1/8		<0.05	<0.05	0.90	<0.05		<0.05
		1/32				0.80			<0.05
		1/128				0.42			
		1/256				0.17			
		1/512				<0.05			

Table 3

Immunofluorescence reactions

All results are expressed in fluorescent indices. An index lower than 0.20 must be considered negative.

Antisera				Cells			
No.	Specificity	Dilution	Normal splenic cells	EL4	ERLD	EδG2	GiL10
52	Anti-L cells	1/4	<0.05	0.62	<0.05	<0.05	0.52
		1/8	<0.05	0.50	<0.05	<0.05	0.40
		1/16		0.45	<0.05	<0.05	0.30
		1/32		0.38		<0.05	0.18
		1/64		0.30			<0.05
		1/128		0.25			<0.05
		1/256		0.16			<0.05
		1/512		<0.05			<0.05
56	anti-FMRGi (anti-Moloney)	1/4	<0.05	0.35	<0.05	<0.05	0.65
		1/8	<0.05	0.27	<0.05	<0.05	0.55
		1/16		0.20	<0.05	<0.05	0.40
		1/32		<0.05			0.32
		1/64		<0.05			0.27
		1/128		<0.05			0.08
		1/256		<0.05			<0.05

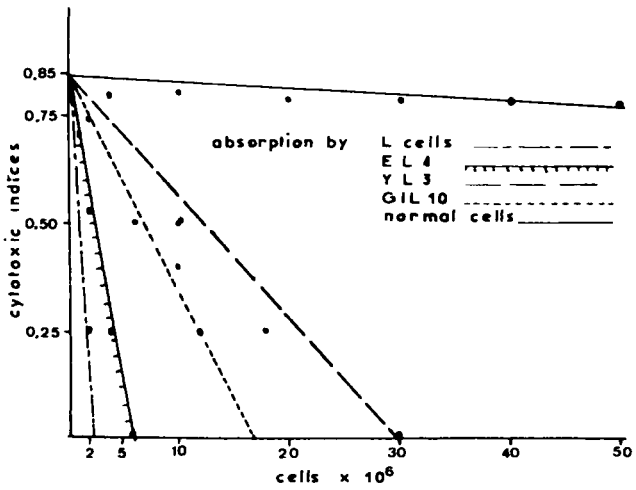

Chart 1. Absorption curves of a C57BL/6 anti-L antiserum (No. 52). The serum was incubated with one of the following cells: L cells, EL4, GiL10, YL3, normal C57BL/6 splenic cells. The absorbed serum was tested for its cytotoxic activity against EL4 cells (nonviral).

anti-L antisera against M-MSV (MLV), M-MSV (RLV), and M-MSV (GiLV).

DISCUSSION

These experiments demonstrate that L strain, which is a tissue culture line arising from normal mice and which bears a type C virus apparently unable to induce leukemia, contains at least 3 different types of antigens, all associated with murine leukemias or MuLV.

The 1st antigen is the group-specific antigen of MuLV already detected in several tissue culture strains by Hall *et al.* (9) by complement-fixation and recently shown by Schäfer *et al.* (21) in L strain cells with a rabbit antiserum. Preliminary experiments have shown that the group-specific antigen extracted from L cells does not differ from the MuLV-gs 1 of Geering *et al.* (6). This result indicates that the group-specific antigen is not related to the leukemogenic potency of these leukemic viruses.

The 2nd antigen detected in L strain cells is FMRGi common to Friend, Moloney, Rauscher (18), and Graffi leukemias (13, 14). At least 2 explanations can account for the presence of FMRGi in the 3 batches of L strain which were tested. (*a*) There is an external *in vitro* contamination by a known virus of the "FMRGi-inducing subgroup." This seems rather

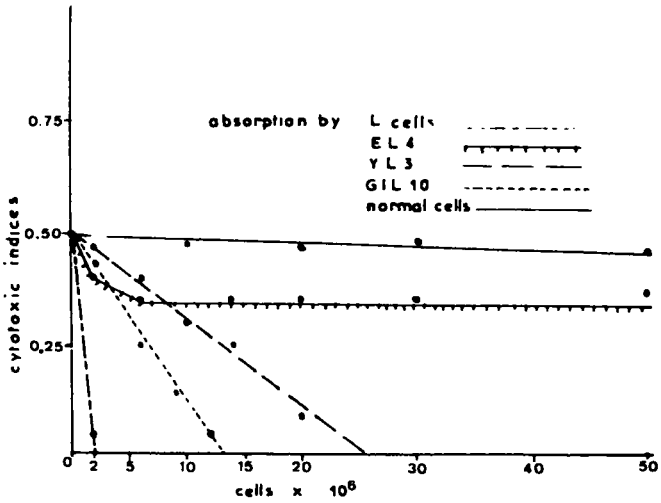

Chart 2. Absorption curves of a C57BL/6 anti-L antiserum (No. 52). The serum was incubated with one of the following cells: L cells, EL4, GiL10, YL3, normal C57BL/6 splenic cells. The absorbed serum was tested for its cytotoxic activity against GiL10 (Graffi virus induced).

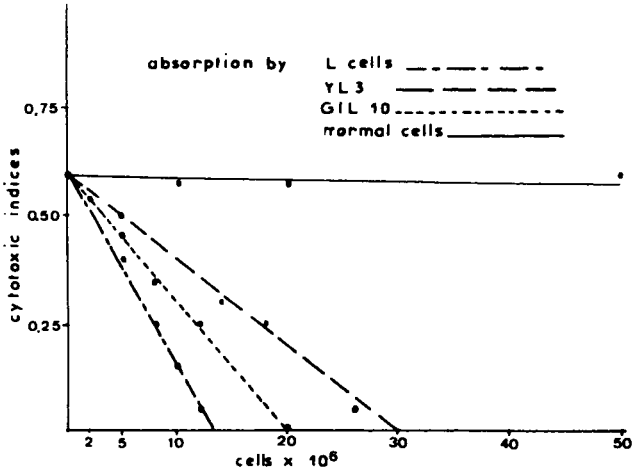

Chart 3. Absorption curves of a C57BL/6 anti-FMRGi serum (No. 34, anti-Moloney). The serum was incubated with one of the following cells: L cells, YL3, GiL10, normal C57BL/6 splenic cells. The absorbed serum was tested for its cytotoxic activity against GiL10 cells (FMRGi+).

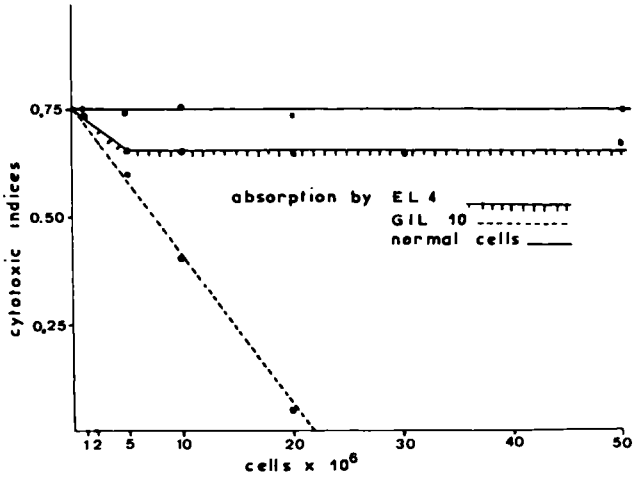

Chart 4. Absorption curves of a C57BL/6 anti-FMRGi serum (No. 34 anti-Moloney). The serum was incubated with one of the following cells: EL4, GiL10, normal splenic cells. The absorbed serum was tested for its cytotoxic activity against GiL10 (FMRGi+).

Table 4

Search for L antigen in various leukemias and normal cells

Absorbing cells			Results of absorption experiments[a]
Nomenclature	Origin	Strain	
EL4	DMBA leukemia	C57BL/6	+++
ERLD	X-ray leukemia	C57BL/6	−
C1498	Spontaneous leukemia	C57BL/6	−
EdG2	Gross virus	C57BL/6	−
GC1	Gross virus	BALB/c	+
GH1	Gross virus	C3H/eb	+
K36	Gross virus	AKR	+
GiL1	Graffi virus	C57BL/6	++
GiL4	Graffi virus	C57BL/6	++
GiL10	Graffi virus	C57BL/6	++
GiC5	Graffi virus	BALB/c	++
YL3	Moloney virus	C57BL/6	++
YL5	Moloney virus	C57BL/6	++
YL10	Moloney virus	C57BL/6	++
YC8	Moloney virus	BALB/c	++
YC10	Moloney virus	BALB/c	++
RC18	Raucher virus	BALB/c	++
Spleen	Normal adult	BALB/c	−
Spleen	Normal adult	C57BL/6	−
Spleen	Normal adult	C3H/He	++
Spleen	Normal adult	C3H/eb	−
Liver	Normal adult	C57BL/6	−
Thymus	Normal adult	C57BL/6	−
Brain	Normal adult	C57BL/6	−
Kidneys	Normal adult	C57BL/6	−
L strain	Normal mice	C3H/An	++
Fibroblast	Normal embryo	BALB/c	−
Fibroblast	Normal embryo	C57BL/6	−
Fibroblast	Normal embryo	C3H/eb	−
Fibroblast	Normal embryo	C3H/He	++

[a] +++, cytotoxic indices negative after absorption of 0.1 ml serum by less than 5 × 10^6 cells; ++, cytotoxic indices negative after absorption of 0.1 ml serum by more than 5 × 10^6 cells and less than 25 × 10^6 cells. +, cytotoxic indices decreased but not negative after absorption of 0.1 ml serum by 25 × 10^6 cells. −, cytotoxic indices unchanged (<0.10) after absorption by 25 × 10^6 cells.

Table 5

Study of leukemia transplantation immunity in anti-L hyperimmune mice

				Results		
	Immunization	Challenge				
Mice	by	Cells	No.	No. of inoculated mice	No. of positive mice	% positive
BALB/c	L cells	YC8	10,000	8	0	0
	0	YC8	10,000	11	4	36
	L cells	YC8	20,000	15	4	26
	0	YC8	20,000	15	8	53
	L cells	YC8	50,000	21	2	9
	0	YC8	50,000	27	16	55
	L cells	YC8	500,000	11	1	9
	0	YC8	500,000	12	12	100
C57BL/6	L cells	YL3	30,000	11	6	54
	0	YL3	30,000	18	7	38
	L cells	YL3	60,000	11	8	72
	0	YL3	60,000	21	11	52
	L cells	YL3	100,000	8	6	75
	0	YL3	100,000	11	11	100
C57BL/6	L cells	GiL10	10,000	9	8	88
	0	GiL10	10,000	9	4	44
	L cells	GiL10	20,000	9	9	100
	0	GiL10	20,000	9	8	88
	L cells	GiL10	50,000	9	9	100
	0	GiL10	50,000	7	7	100
C57BL/6	L cells	EL4	700	12	12	100
	0	EL4	700	14	13	93
	L cells	EL4	3,000	11	11	100
	0	EL4	3,000	14	14	100
	L cells	EL4	6,000	23	23	100
	0	EL4	6,000	24	24	100
	L cells	EL4	13,000	12	12	100
	0	EL4	13,000	10	10	100
	L cells	EL4	25,000	10	10	100
	0	EL4	25,000	12	12	100

Table 6

Study of transplantation immunity in BALB/c mice hyperimmunized against EL4 (L + FMRGi−)

	Challenge		Results		
Immunization	Cells	No.	No. inoculated	No. positive	% positive
EL4 cells	YC8	10^3	10	7	70
EL4 cells	YC8	10^4	11	11	100
EL4 cells	YC8	3 × 10^4	22	22	100
EL4 cells	YC8	6 × 10^4	12	12	100
Control	YC8	10^3	11	5	45
Control	YC8	10^4	11	4	36
Control	YC8	3 × 10^4	24	7	29
Control	YC8	6 × 10^4	12	11	91

unlikely since the injection of the L strain agent failed to induce leukemia. (*b*) Another virus of the same subgroup may be present in C3H/An normal mice, but well expressed only in tissue culture, as occurs with the EB virus. This "FMRGi-inducing virus" differs from other viruses in the subgroup in that it is not able to induce leukemias and the results of neutralization tests indicate that it does not share the type-specific

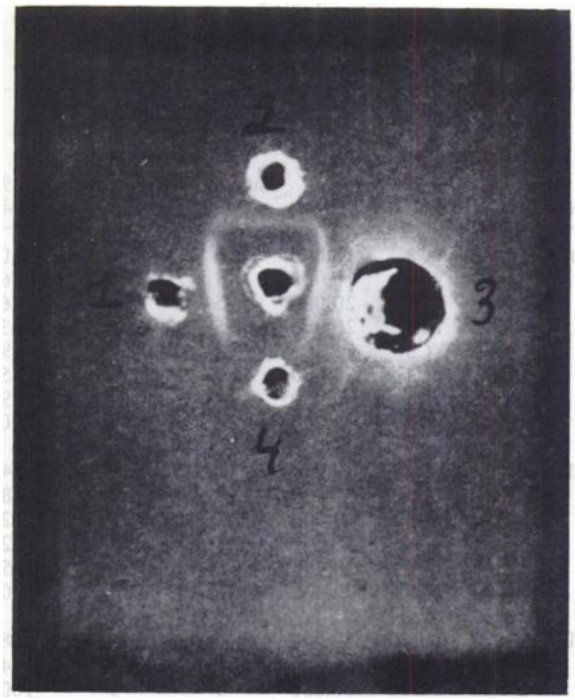

Fig. 1. Ouchterlony double diffusion test. *Center well,* W/Fu rat anti-Gross serum; *peripheral wells: 1,* ether-treated Rauscher virus; *2,* ether-treated Gross virus; *3,* ether-treated L strain agent; *4,* complete L strain agent.

antigen of Rauscher, Moloney, or Graffi viruses. It has been shown previously that M-MSV (MLV) and M-MSV (RLV) are strongly neutralized by anti-Moloney or anti-Rauscher antisera but not by anti-Graffi antisera, the contrary being observed with M-MSV (GiLV) (15, 20). Thus, several different type-specific antigens are present on the viruses of the "FMRGi-inducing subgroup" and one of these antigens could be specific of the L strain agent. A new pseudotype of MSV, for which envelope is that of L strain agent, has been recently obtained in our laboratory. This virus may help to resolve the question of the nature of the L antigen since it induces tumors in BALB/c and C3H/eb mice. The reason for the nonleukemogenicity of a virus which: (*a*) bears the group-specific antigen of MuLV; (*b*) is able to induce the FMRGi subgroup antigens; and (*c*) can be the "helper virus" of a defective MSV, seems especially worthy of study. The absence of cross-neutralization between Moloney, Rauscher, Graffi, and L strain viruses which all are able to induce cellular FMRGi antigen confirms the probable nonidentity of viral and cellular antigen in murine leukemias (15).

The use of anti-L cells antisera and EL4 leukemia as target cells provides an experimental system which allows the definition of a 3rd antigen, the L antigen. This antigen is revealed on EL4 cells by homologous (C57BL/6) or isologous (C3H/eb) anti-L cytotoxic antisera. The L antigen appears to be distinct from all the antigens previously described in murine leukemias. The strictly isologous system eliminates the responsibility of a histocompatibility antigen in the reaction. θ antigens are different from L antigens for the same reasons. TL antigens cannot be concerned since EL4 is TL– (17); furthermore, anti-L sera were inactive against TL+ ERLD leukemia. No confusion is possible with G antigen (Gross), EL4 being unable to absorb anti-G as already established (19). Moreover, anti-L sera did not react with E♂G2 Gross-induced leukemia. Absorption experiments showed that FMRGi and L are different. ML antigen can be ruled out since EL4 is ML– (E. A. Boyse, personal communication). The group-specific antigen of MuLV cannot be confused on the basis of our experiments; in addition, the MuLV group-specific antigen is not detected by mice antisera (6). The possibility of identity between E and L was first considered [for this reason, L was initially called E′ (12)], but Aoki *et al.* (1) have recently demonstrated that E and L are different: E is present in only 4 leukemias; 1 of them is also L+ (EL4), the 3 other E+ leukemias are devoid of L antigen. In addition, several virus-induced leukemias of C57BL/6 and BALB/c, which seem to be devoid of E, bear L antigen on their membrane. Another leukemia antigen called "X" was described by Gorer and Amos (8) in EL4 leukemia, but it was detected in an allogenic system and was never revealed by serological methods. It must be concluded that L antigen is different from the other murine leukemia antigens previously described.

As mentioned above, the nature of L antigen has not been determined in this study. It seems that it is not an organ-specific antigen or an antigen naturally present in all tissue cultures. The existence of this antigen in several virus-induced leukemias and in L strain which is infected by a virus of the MuLV group suggests that the L antigen can be virus induced. In this hypothesis, L could be an expression of the viral genome on cell membranes different from G or FMRGi. However, EL4 is a DMBA-induced nonviral leukemia (7). In fact, type A particles of the intracytoplasmic as well of the intracisternal type are present in EL4 (22). Thus an incomplete MuLV, either etiological or passenger, could be present in EL4 and responsible for L antigen. It must be emphasized that, even if it is a viral-induced antigen, L probably does not require the presence of type C particles to be expressed since EL4 is devoid of such particles (22). Another problem arises from the presence of L in C3H/He normal tissues. It can be suggested that this substrain carries the same viral agent as L strain cells since C3H/He originates from C3H/An (23). The incapacity of C3H/He mice to develop anti-L antibodies is in favor of this hypothesis. C3H/eb originates also from C3H/He by intrauterine transplantation of C3H/He ova into C57BL/6 mice carried out with the object of eliminating mammary tumor virus (23); it is possible that L strain virus was eliminated simultaneously. Therefore C3H/eb would not be tolerant to this agent, while C3H/He would be tolerant. A cellular origin of L cannot be definitively ruled out by these experiments, and further studies are necessary.

The role of L antigen in tumor rejection is not clear. We did not obtain tumor rejection with anti-L hyperimmune mice, in spite of a high titer of circulating anti-L antibodies. Surprisingly, immunization of BALB/c with EL4 cells which share the L antigen but not FMRGi antigen (11, 14, 19) induces an enhancement of tumor growth of YC8 leukemia (L antigen+). On the contrary, we saw that BALB/c immunized simultaneously against L and FMRGi antigens (by inoculations of L strain cells) rejected the same YC8 leukemia. These results

suggest that immunization against the L antigen may lead to immunological enhancement rather than rejection. Our findings support the recent work of Hellström *et al.* (10), who have demonstrated the enhancement phenomenon in the MSV system. Further experiments now in progress indicate that immunological enhancement can be transmitted with the serum of mice immunized against EL4 leukemia cells.

REFERENCES

1. Aoki, T., Stuck, B., Old, L. J., Hämmerling, U., and de Harven, E. E Antigen: A Cell Surface Antigen of C57BL Leukemias. Cancer Res., *30:* 244–251, 1970.
2. Chenaille, P., Levy, J. P., Tavitian, A., and Boiron, M. A Routine Method for Concentration and Partial Purification of a Murine Leukemia Virus (Rauscher). Nature, *213:* 107–108, 1967.
3. Cromack, A. S. An Electron Microscopy Study of a Virus-like Particle in Chick Embryo and L Cell Cultures. J. Gen. Virol., *2:* 195–198, 1968.
4. Dales, S., and Howatson, A. F. Virus Like Particles in Association with L Strain Cells. Cancer Res., *21:* 193–197, 1961.
5. Earle, W. R., Shilling, E. L., Sturck, T. H., Strans, N. P., Brown, M. E., and Shelton, E. Production of Malignancy *in Vitro.* V. The Mouse Fibroblasts Cultures and Changes Seen in the Living Cells. J. Natl. Cancer Inst., *4:* 165–212, 1943.
6. Geering, G., Old, L. J., and Boyse, E. A. Antigens of Leukemias Induced by Naturally Occurring Leukemic Virus: Their Relationship to the Antigen of Gross Virus and Other Murine Leukemia Virus. J. Exptl. Med., *124:* 753–772, 1966.
7. Gorer, P. A. Antibody Response to Tumor Inoculation in Mice with Special Reference to Partial Antibodies. Cancer Res., *7:* 634–641, 1947.
8. Gorer, P. A., and Amos, D. B. Passive Immunity in Mice against C57BL/6 Leukosis EL4 by Means of Isoimmune Serum. Cancer Res., *16:* 338–343, 1956.
9. Hall, W. T., Hartley, J. W., Sanford, K. K., Evans, V. J., and Andersen, W. F. Virus Production and Murine Leukemia Virus Complement Fixing Antigen in Neoplastic and Non Neoplastic Cell Lines. Science, *156:* 85–88, 1967.
10. Hellström, I., and Hellström, K. E. Studies on Cellular Immunity and Its Serum Mediated Inhibition in Moloney-Virus Induced Mouse Sarcomas. Intern. J. Cancer, *4:* 587–600, 1969.
11. Klein, E., and Klein, G. Antigenic Properties of Lymphomas Induced by the Moloney Agent. J. Natl. Cancer Inst., *32:* 547–568, 1964.
12. Leclerc, J. C., Levy, J. P., Varet, B., and Oppenheim, S. Communauté Antigénique entre la Souche L et Certaines Leucémies Murines. Compt. Rend., *266:* 2206–2209, 1968.
13. Levy, J. P., Leclerc, J. C. Oppenheim, S., Varet, B., and Silvestre, D. Étude Sérologique sur les Leucémies Expérimentales de Moloney et de Graffi. Nouvelle Rev. Franc. Hematol., *7:* 705–710, 1967.
14. Levy, J. P., Leclerc, J. C., Varet, B., and Oppenheim, S. Study of the Antigenic Specificity of Graffi Leukemia Cells. J. Natl. Cancer Inst., *41:* 743–750, 1968.
15. Levy, J. P., Varet, B., Oppenheim, S., and Leclerc, J. C. Neutralization of Graffi Leukemia Virus. Nature, *224:* 606–608, 1969.
16. Möller, G. Demonstration of Mouse Isoantigens at the Cellular Level by the Fluorescent Antibody Technique. J. Exptl. Med., *114:* 415–434, 1961.
17. Old, L. J., Boyse, E. A., and Stockert, E. Antigenic Properties of Experimental Leukemias. I. Serological Studies *in Vitro* with Spontaneous and Radiation Induced Leukemias. J. Natl. Cancer Inst., *31:* 977–986, 1963.
18. Old, L. J., Boyse, E. A., and Stockert, E. Typing of Mouse Leukemias by Serological Methods. Nature, *201:* 777–779, 1964.
19. Old, L. J., Boyse, E. A., and Stockert, E. The G (Gross) Leukemia Antigen. Cancer Res., *25:* 813–819, 1965.
20. Oppenheim, S., Levy, J. P., and Leclerc, J. C. Propriétés Antigeniques d'un Pseudotype Graffi du Virus du Rhabdomyosarcome Murin (MSV). Compt. Rend., *268:* 620–623, 1969.
21. Schäfer, W., Anderer, F. A., Bauer, H., and Pister, L. Studies on Mouse Leukemia Viruses. I. Isolation and Characterization of a Group-specific Antigen. Virology, *38:* 387–394, 1969.
22. Silvestre, D., and Leclerc, J. C. Étude des Particules Virales de la Leucémies EL4. Pathol. Biol. Semaine Hop., *16:* 305–307, 1968.
23. Staats, J. Standardized Nomenclature for Inbred Strains of Mice: Third Listing. Cancer Res., *24:* 147–168, 1964.