LEUKEMIA-ASSOCIATED TRANSPLANTATION ANTIGENS RELATED TO MURINE LEUKEMIA VIRUS

THE X.1 SYSTEM: IMMUNE RESPONSE CONTROLLED BY A LOCUS LINKED TO *H-2**

BY H. SATO,‡ E. A. BOYSE, T. AOKI, C. IRITANI, AND L. J. OLD

(From the Division of Immunology, Memorial Sloan-Kettering Cancer Center, New York 10021, and the Viral Leukemia and Lymphoma Branch, National Cancer Institute, Bethesda, Maryland 20014)

(Received for publication 11 May 1973)

In the last few years we have encountered some remarkable instances in which transplanted mouse leukemias that elicit no discernible resistance in mice of the same inbred strain have been strongly rejected by F_1 hybrid recipients. As described below, a detailed study of one such system revealed that the leukemia cells carry a strong leukemia-related transplantation antigen (called X.1) to which the inbred host of origin appears genetically incapable of responding. Hybrids are evidently resistant to the same leukemia cells because they possess appropriate *Ir* (immune response)[1] genes, one of these being linked to *H-2*, introduced by the outcross parent. The results imply an immunological role for the *Rgv-1* (resistance to Gross virus) locus (ref. 1 for review) in linkage group IX (LG IX).

Materials and Methods

Mice.—With the exception of B10 and B10.D2 (new) (purchased from Jackson Laboratory, Bar Harbor, Maine), these were all obtained from our own colonies.

Leukemias.—The two BALB/c (BALB) leukemias that are most crucial to this study have the following history. Both were induced by X radiation in BALB mice of our own colonies and have been transplanted in these mice ever since: (*a*) BALB.RL♂1 (BALB radiation leukemia male 1) was induced by X radiation in 1962, soon converted to the ascites form, and has been passaged serially about 175 times, interrupted by a period of frozen storage (abbreviated RL♂1). (*b*) BALB.RL ♀ 1 was similarly induced in 1971, and is now in its 15th passage; it also has been stored frozen (abbreviated RL ♀ 1).

Cytotoxicity Test.—As described previously (2); the source of complement was rabbit serum selected for low toxicity and high complement activity, diluted 1/3 or 1/4, preabsorption with mouse cells proving unnecessary for the purposes of this study.

Immunoelectron Microscopy.—Details of the method used are given in ref. 3.

* This work supported in part by National Cancer Institute grant CA 08748.

‡ Fellow of the Damon Runyon Fund.

[1] *Abbreviations used in this paper*: B6, C57BL/6; BALB, BALB/c; *Ir*, *Immune response*; LG, linkage group; MuLV, murine leukemia virus; *Rgv-1*, *Resistance to gross virus-1*; RL, radiation leukemia.

RESULTS

Resistance of (BALB × B6) and Reciprocal Hybrid Mice to Challenge with BALB Leukemia Cells.—Table I shows that most (BALB × B6) and reciprocal hybrid mice, male and female, rejected up to 3×10^6 RL♂1 or 1×10^5 RL♀1 BALB leukemia cells (highest *initial* inoculum tested) given subcutaneously, although BALB mice have never survived inocula of 1×10^4 RL cells (the lowest number tested); we have observed no instance of a BALB mouse resist-

TABLE I

Resistance of (BALB × B6) and Reciprocal Hybrid Mice to the BALB Leukemias RL♂1 and RL♀1 Inoculated Subcutaneously (Summary of All Experiments)*

Recipient‡	Sex	Number of leukemia cells inoculated						
		1×10^4	5×10^4	1×10^5	2×10^5	5×10^5	1×10^6	3×10^6
		mice dead/total						
BALB leukemia RL♂1								
(BALB × B6)	♀	0/8	0/8		1/17	0/8	4/50	0/8
(BALB × B6)	♂				1/16	2/5		
(B6 × BALB)	♀						0/10	
(B6 × BALB)	♂				0/7	0/7		
BALB	♀	3/3	6/6		8/8	3/3	36/36	5/5
BALB	♂		3/3		10/10	5/5		
BALB leukemia RL♀1								
(BALB × B6)	♀			0/14				
(BALB × B6)	♂			0/4				
BALB	♀			11/11				
BALB	♂			4/4				

* The inoculum was made subcutaneously into shaved skin on the flank. In the hybrid the inoculum usually grew to palpable size (up to about 1 cm diameter) and then regressed. Most hybrids thereafter resisted sequential inocula of up to 200×10^6 leukemia cells (highest number tested) given intraperitoneally. These hyperimmunized mice were the source of X.1 antiserum.

‡ The female parent of each cross is the strain shown first.

ant to RL♂1 or RL♀1. By progressively increased inocula, the hybrids could be immunized to challenge with more than 200×10^6 leukemia cells.

Resistance vs. Susceptibility of Different BALB Hybrids: Association with H-2 (LG IX).— Some strains when crossed to BALB confer resistance to BALB leukemia RL♂1 cells whereas others do not (Table II). We refer to the former as "responder" strains, and the latter strains, which confer little or not resistance, as "nonresponders." Strains B10 and AKR/*H-2*^{b} are responders, but their congenic partner strains B10.D2 and AKR, differing respectively only for the region of *H-2*, are nonresponders. Therefore the major locus conferring resistance to RL♂1 is linked to *H-2*.

TABLE II

*Different Mouse Strains Classified as X.1 Responders or X.1 Nonresponders (Including Low Responders) According to Their Capacity, when Crossed to BALB, to Confer Resistance to RL♂1 BALB Leukemia Cells**

X.1 Responders			X.1 Nonresponders		
Strain	Hybrid‡ recipient	Mice dead/ total	Strain	Hybrid recipient	Mice dead/ total
B6	(BALB × B6)♀	4/50	BALB♀	Control	36/36
B6	(BALB × B6)♂	1/16	BALB♂	Control	10/10
B6	(B6 × BALB)♀	0/10			
B6	(B6 × BALB)♂	0/7			
B10	(B10 × BALB)♀	1/20	B10.D2§	(B10.D2 × BALB)♀	9/10
			DBA/2 (=D2)	(BALB × DBA/2)♀	18/18
AKR/*H-2*^{b}§	(BALB × AKR/*H-2*^{b})♀	2/9	AKR	(BALB × AKR)♀	9/9
			AKR	(BALB × AKR)♂	5/5
HTI	(BALB × HTI)♀	2/13	HTG	(BALB × HTG)♀	11/11
HTI	(BALB × HTI)♂	0/5	HTG	(BALB × HTG)♂	4/4
C58	(BALB × C58)♀	2/10			
I	(BALB × I)♀	0/4			
			C3H/An	(BALB × C3H/An)♀	6/8
			A	(BALB × A)♀	6/7
			A	(BALB × A)♂	4/5

* 1 × 10^6 cells subcutaneously to female recipients, 2 × 10^5 to males.

‡ The female parent of each cross is of the strain shown first.

§ Congenic stocks differing from base strain only at the *H-2* region of linkage group IX. *Interpretation*: (*a*) Linkage group IX contains an *immune response* (*Ir*) locus conferring ability to respond to the leukemia-related transplantation antigen X.1 and so to reject X.1-positive leukemia cells. (*b*) Results with the *H-2* crossover stocks HTI vs. HTG (4) place this *Ir* locus in the K region of *H-2* [HTI shares its K region with B6 (a responder); HTG shares its K region with BALB (a nonresponder)].

Two entries in Table II suggest that the resistance locus is in the K rather than D region of *H-2*. HTG is an *H-2* crossover stock sharing its *H-2*(K) but not its *H-2*(D) region with BALB (4); it is a nonresponder. On the other hand, HTI, a different *H-2* crossover stock, possibly shares its *H-2*(D) region with BALB (nonresponder) and has the same K region as B6, which is a responder, and is itself a responder.

Table III summarizes experiments in which BALB backcross mice derived from crosses with B6 were typed for *H-2* and then challenged with one of the BALB leukemias RL♂1 or RL♀1. There was a close correlation between *H-2* type and capacity to resist challenge with the leukemia cells; therefore, again a substantial part of the resistance of the hybrid can be traced to LG IX. The presence of a second resistance locus in B6, not in LG IX, is suggested by the finding that a few *H-2*-compatible mice survived.

Serological Typing.—Pooled antisera (called "anti-X.1") were obtained by tail-bleeding (BALB × B6) mice that had received 5–15 inoculations of RL♂1 cells, in numbers rising from 1 × 10^5 to 2 × 10^8 intraperitoneally (see Table I). These antisera were positive in cytotoxicity tests with three BALB leukemias and one strain A leukemia (Fig. 1 and Table IV). [Similar results are given by (BALB × B6) anti-RL♀1 antisera.]

TABLE III

Further Identification of the Linkage Group IX Ir Locus That Confers Resistance to X.1-Positive ($X.1^+$) Leukemia Cells. Challenge of H-2-Typed BALB Backcross Mice with BALB Leukemia RL♂1 or RL♀1*

Exp. no.	Inoculum	Recipients*			
		$H\text{-}2^d/H\text{-}2^d$		$H\text{-}2^d/H\text{-}2^b$	
		♀	♂	♀	♂
		mice dead/total			
1	1×10^6 RL♂1 cells	18/20		4/18	
2	1×10^5 RL♀1 cells	9/12	12/12	4/13	7/13
3‡	1×10^5 RL♀1 cells	10/13	9/11	2/13	1/13

* The recipients were backcross mice of the type BALB × (BALB × B6) in various mating combinations (which did not significantly affect the outcome and are therefore not treated separately).

‡ In this experiment the recipients had been preimmunized with the H-2-incompatible $X.1^+$ strain A leukemia RADA1 (see Table IV). *Interpretation:* (*a*) Linkage group IX contains an *immune response* (*Ir*) locus conferring ability to respond to the leukemia-related transplantation antigen X.1. (*b*) This *Ir* (IX) locus is a major but not the only determinant of X.1 resistance in hybrid recipients.

X.1-typing of leukemias and other tumors by absorption: Several leukemias and transplanted tumors of other kinds were typed for X.1 by absorption of X.1 antiserum diluted to near the end point, a sensitive serological method in vitro for detecting cell-surface antigens. The results are summarized in Table IV, where it can be seen that X.1 antigen does not originate from passage A Gross virus.

X.1 typing of normal mice by absorption in vivo: X.1 antigen was not demonstrable on thymocytes, lymph node cells, spleen, or bone marrow of young adult mice of strains with a high incidence of leukemia (AKR, C58), or of mice of other strains (including BALB), either by the direct cytotoxicity test or by absorption.

With AKR cells, however, the results of absorption in vitro were equivocal, suggesting that X.1 antigen might be present in small amounts in normal AKR mice. For this reason AKR and other mouse strains were typed for X.1 by absorption in vivo. Fig. 2 *A* illustrates how a selected standard amount of X.1 antiserum was found to be rapidly cleared by AKR mice but not by B6 mice. Table V shows mouse strains classified as $X.1^+$ or $X.1^-$ on the basis of absorption in vivo. Here it is seen that with the one exception of strain 129 the $X.1^+$ strains are all those that are life-long producers of murine leukemia virus (MuLV), which is highly suggestive that X.1 is associated with MuLV. This is further emphasized by the fact that C3Hf/Fg-Law alone of the three C3H sublines tested was $X.1^+$; this subline is noted for having acquired a high titer of MuLV and a high incidence of leukemia at some point in its history.

Tissue Representation of X.1 Antigen in AKR Mice.—Fig. 2 *B* illustrates that

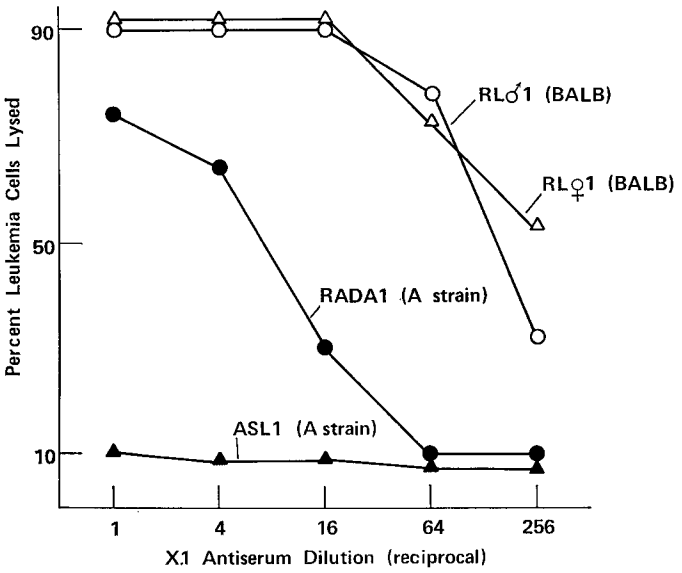

FIG. 1. Cytotoxicity tests with X.1 antiserum on four mouse leukemias.

treatment of AKR mice with cortisone greatly reduces their capacity to absorb X.1 antibody, the inference being that X.1 is represented primarily if not exclusively on lymphoid cells (see ref. 5 for the effect of cortisone on absorption of various alloantibodies in vivo).

Passive Immunization against BALB.RL Cells with X.1 Antiserum.—Passive protection against RL ♀ 1 BALB leukemia cells was tested for in (*a*) adult mice receiving X.1 antiserum by injection, and (*b*) the suckling progeny of (BALB × B6) mothers immunized with RL ♀ 1 and mated to BALB males. (The latter test has been applied previously with success in a similar context [ref. 6].) In both instances there was complete suppression (Table VI); a control serum prepared against MOPC-70A BALB myeloma cells (used because it was judged the most appropriate antiserum available against an X.1$^-$ BALB malignant cell) afforded no protection (Table VI).

Cytotoxic Antibodies in the Serum of Normal (BALB × B6) Mice.—The serum of many (BALB × B6) mice contains cytotoxic antibody against BALB.RL♂1 leukemia cells. This presumably is an anti-X.1 antibody. Normal (BALB × B6) serum also frequently contains antibody to leukemia EL4 (C57BL). But this latter antibody is not completely removed by absorption with RL♂1 cells, and therefore EL4 carries an antigen that does not belong to the X.1 system.

It is not surprising therefore that X.1 antisera are cytotoxic for leukemia EL4 although EL4 does not absorb activity from X.1 antiserum. The probability is that X.1 antiserum contains the naturally occurring cytotoxic antibody to EL4 that does not belong to the X.1 system.

TABLE IV

Leukemias and Other Tumors Classified as X.1$^+$ or X.1$^-$*

Strain of origin	Description	X.1 type	
		By direct cytotoxicity test	By absorption in vitro
BALB	RL♂1, RL♀1, RL♀2 (radiation-induced leukemias)	+	+
	Meth A (ascites sarcoma; chemically induced); MOPC-70A, S19 (myelomas)	−	−
A	RADA1 (radiation-induced leukemia)	+	+
	ASL1, ASL8 (spontaneous leukemias)	−	−
AKR	15 primary spontaneous leukemias	±/+	+
	K36 (long-transplanted ascites leukemia)‡	−	−
B6	E♂G2 (leukemia induced by passage A Gross virus)	−	−
	ERLD (radiation-induced leukemia)	−	−
C3H (sub-line?)	BP8 (ascites sarcoma)	−	−
I	I-29 (spontaneous leukemia)	−	−

* With the exception of MOPC-70A, S19, and BP8, all these arose in mice of our colonies; details of their origins appear in various previous papers from this laboratory (MS-KCC).

‡ This leukemia is carried in (B6 × AKR)F_1 hybrids, which may account for its being X.1$^-$ (see text).

Partial Resistance of RL♂1 BALB Leukemia Cells after Passage in (BALB × B6) Hybrids.—A line of RL♂1 cells that had been passed eight times intraperitoneally in untreated (BALB × B6) mice was found to exhibit greatly reduced sensitivity to cytotoxic X.1 antiserum and to be more resistant to rejection by (BALB × B6) recipients. In the latter test, 4/5 (BALB × B6) recipients survived the standard challenge inoculum of 1×10^6 RL♂1 BALB leukemia cells as opposed to 1/5 (BALB × B6) recipients challenged with the 1×10^6 cells of the F_1-passaged line. Quantitative absorption of X.1 antiserum (for method see ref. 5) shows the F_1-passaged line to contain only 15–20% of the X.1 antigen carried by the original BALB-passaged line. A count of C-type particles in electron micrographs, carried out by Dr. C. Stackpole (of Sloan-Kettering Institute), suggests a corresponding decrease in the number of virions produced by the relatively resistant F_1-passaged line.

Immunoelectron Microscopy with X.1 Antisera.—Fig. 3 is an electron micro-

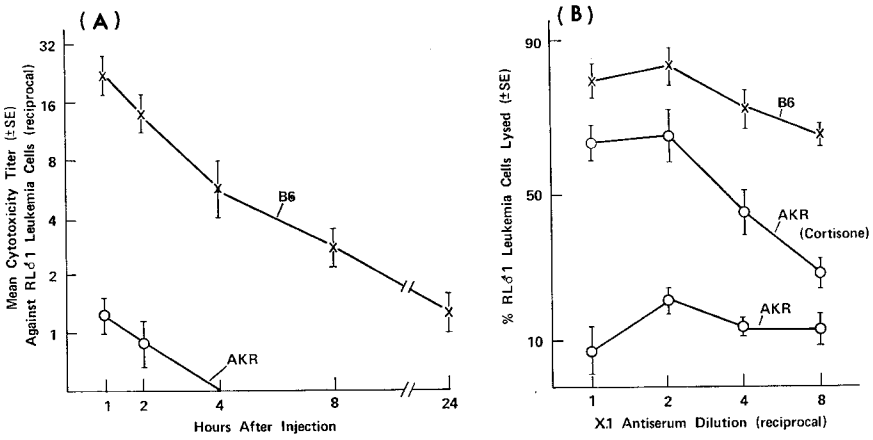

FIG. 2. Clearance of X.1 antibody injected into B6 or AKR mice (*A*) or into AKR mice treated with cortisone (*B*). Each group of eight mice, 6–7 wk old, of the same sex and similar weights, received X.1 antiserum (same pool) intravenously, 0.6 ml for *A* and 0.3 ml for *B*. Serum samples were taken from each mouse at intervals as indicated (*A*) or after 1 h (*B*) and titrated for residual cytotoxic antibody against RL♂1 cells (after heating at 56°C for 30 min to reduce the anticomplementary action of mouse serum). The initial titer of the X.1 antiserum pool [(BALB × B6)F_1 anti-BALB RL♂1 leukemia cells] was 1/128 (end point 50% RL♂1 cells lysed). The cortisone-treated mice (*B*) received 25 mg of cortisone acetate intramuscularly 3 days before injection of antiserum. *Interpretation:* (*a*) Normal AKR mice express X.1 antigen. (*b*) X.1 antigen is expressed mainly on lymphoid tissues, hence the greatly reduced clearance after cortisone. (*c*) B6 mice do not express X.1 antigen.

graph illustrating an RL♂1 cell with budding virions. The sites of X.1 antigen are shown by the marker southern bean mosaic virus according to the hybrid antibody method (8). There is labeling of sectors of the cell surface and also of virions.

Preliminary Results with I-29, a Second Leukemia That Is Rejected by Hybrid Recipients.—This leukemia arose spontaneously in a mouse of our I colony in 1961. It has undergone about 130 passages and has been stored frozen. It is rejected strongly by some I hybrids, but never by I mice, and a cytotoxic antibody is produced that is not identical with anti-X.1; as shown in Table IV, leukemia I-29 is X.1$^-$.

DISCUSSION

The most remarkable feature of the X.1 system in the two BALB leukemias RL♂1 and RL♀1 is the considerable strength of the rejection response of resistant hybrids such as (BALB × B6) in the face of an apparently total lack of any immune response in syngeneic BALB hosts. Resistance to inoculation of 2×10^8 intraperitoneal RL♂1 cells is the rule in (BALB × B6) recipients whose resistance has been built up by progressive immunization, and no doubt

TABLE V

Mouse Strains Classified as X.1$^+$ or X.1$^-$(*)

X.1$^+$	X.1$^-$
AKR (21‡)	A (4)
AKR/*H-2^b*§ (6)	A.BY§ (2)
AKR/*H-2^a*§ (4)	A/TL$^-$§ (3)
C58 (6)	BALB (8)
NZB (2, old)	DBA/2 (3)
129 (8)	B6 (8)
129/G$_{1X}^-$§ (2)	B6/G$_{1X}^3$§ (2)
	B6/Ly-1.1§ (3)
	B6/Ly-2.1§ (8)
	B6/*H-2^k*§ (5)
C3Hf/Figge-Law (4)	C3H/An (3)
	C3Hf/Bi (3, young)
	GR/A (2)
	I (2)
	SJL (2)
	SWR (2)
	CBA.T6 (2)

* According to clearance of X.1 antibody in vivo as illustrated in Fig. 2. Each mouse received 0.25 ml of an X.1 antiserum pool with a titer of 1/64 against RL♂1 cells; serum recovered 30 min later was titrated against RL♂1 cells starting at a dilution of 1/2. There is a clear distinction between X.1$^+$ and X.1$^-$ strains: with X.1$^+$ mice the serum recovered shows very little cytotoxic activity (always <40% lysis at 1/2); serum from X.1$^-$ mice shows 70–80% cells lysed at 1/2 and demonstrable lysis up to 1/8–1/16.

‡ Number of mice tested.

§ Congenic strains differing from the background strain only in the region of the locus indicated.

even larger inocula would have been rejected. So X.1 is a highly effective transplantation antigen, but one to which the BALB host is evidently unresponsive.

We shall not undertake here to comment generally on the subject of resistance of hybrid mice to cells of parental origin. Useful reviews and extensive references are provided by Huemer (9) and Bennett (10).

Genetics of the Immune Response to X.1$^+$ Leukemia Cells.—A large part of the resistance in (BALB × B6) hybrids is conferred by a locus in the K region of *H-2*. Very probably this is *Rgv-1*, which is known to lie in this region and has a dominant allele conferring resistance to leukemogenesis by inoculated Gross MuLV (1). In that event the mechanism by which *Rgv-1* controls susceptibility to MuLV is immunological and it may provisionally be classed with the *Ir* (*Immune response*) genes that are linked to the K end of *H-2* and are known to determine high vs. low response to a number of antigens (11).

As more than one *Ir* locus may be involved in the immune response to X.1,

TABLE VI

Passive Immunization with X.1 Antibody: Protection against Challenge with X.1$^+$ Leukemia Cells

Recipients	Challenged with RL ♀1 BALB leukemia cells	Antiserum	Mice dead/ total	Time to death
				days
Experiment 1				
Adult BALB ♀ ♀	1×10^5	Anti-X.1	0/3	All survived
		Anti-PC.1 (control)	3/3	33, 33, 36
		None	3/3	36, 41, 47
Experiment 2				
Newborn progeny of immune ♀ ♀ *	1×10^4	Anti-X.1 (by maternal transmission)	0/11	All survived
	5×10^4		0/12	All survived
BALB (aged 1 day)	1×10^4	None	6/6	41, 41, 42, 42, 48, 49
	5×10^4		6/6	28, 34, 34, 36, 36, 36

Anti-X.1 serum = (BALB × B6)F_1 anti-RL ♂1 BALB leukemia cells, administered intraperitoneally (0.3 ml) 3–4 h before challenge with RL ♀1 BALB leukemia cells subcutaneously. Anti-PC.1 control serum = (B6 × DBA/2)F_1 anti-MOPC-70A BALB myeloma cells, administered as above. This cytotoxic antiserum recognizes PC.1 alloantigen that is present on myeloma cells but not on leukemia cells (7).

* Aged 2–5 days; progeny of (BALB × B6)F_1 females immunized against RL ♀1 leukemia cells and producing high titers of X.1 antibody (mating was with BALB ♂ ♂).

it is possible that the resistance of some other hybrids might be due to a locus other than *Rgv-1*; this would have to be tested in each case by the criterion of association with *H-2* type. As X.1 antigen is present on both virion and cell surface, the resistance to viral leukemogenesis conferred by the relevant *Rgv-1* allele (1) could involve neutralization of MuLV as well as elimination of MuLV-infected cells.

The Relation of X.1 to MuLV.—This is established by: (*a*) the reaction of X.1 antibody with the viral envelope, seen in immunoelectron microscopy, and (*b*) the finding that mouse strains with a high incidence of leukemia are X.1$^+$ and other strains X.1$^-$ (with the exception of strain 129 mice). But X.1 antigen distinguishes a sub-type of MuLV distinct from passage A Gross virus, and the serological evidence suggests that it is not the predominant MuLV of AKR and other strains with a high incidence of leukemia, hence the poor representation of X.1 antigen in these strains despite their high output of MuLV.

Naturally Occurring Antibodies to Leukemia Cells in the Serum of (BALB × B6) and Reciprocal Hybrid Mice.—Cytotoxic antibodies against leukemia cells,

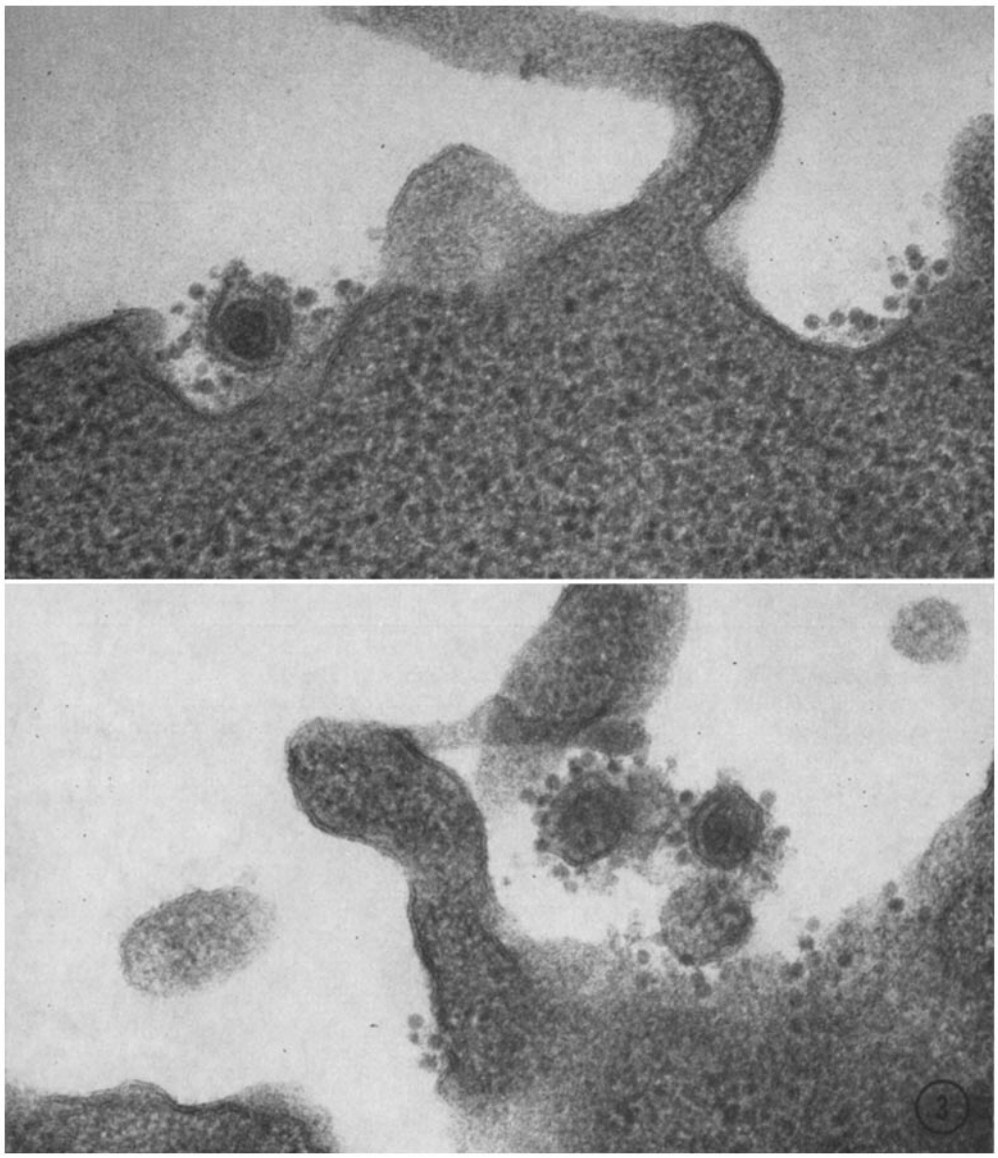

FIG. 3. BALB♂RL1 leukemia cells were reacted with X.1 antiserum and the antigen sites marked with SBMV (southern bean mosaic virus) according to the hybrid antibody method (8). Small areas on the cell surface, and the envelope of the virus, are labeled with SBMV. × 90,000.

directed to X.1 and probably at least one other leukemia-related cell-surface antigen, are common in the serum of (BALB × B6) and reciprocal hybrids. It seems likely that these, and similar natural antibodies in other mouse strains, originate from exposure of mice bearing the appropriate *Rgv/Ir* alleles to the respective sub-types of MuLV, endogenous or exogenous.

Nomenclature.— Our choice of "X" in designating this antigenic system revealed by the immune response of hybrid mice was based on preference for a simple and noncommittal provisional notation, and because of certain similarities to earlier work on leukemia antigens called X by Gorer and Amos (12). These antigens, like X.1, were demonstrable by using antibody to protect mice against challenge with a syngeneic leukemia, the antiserum being prepared in this case by immunizing a second inbred strain with the leukemia cells. No in vitro serological test for antibody was successful; this may be attributable to the primitive state of the cytotoxicity test at that time, and to the fact that such antisera contain various alloantibodies that must be absorbed out before a test for anti-X is feasible. The original X antigens were seemingly highly diverse, each leukemia having a different one, even in a single inbred strain; cross-reactivity was either absent or minimal (13, 14). This led Gorer to conclude that X antigens could not be associated with leukemia virus (13). That view is less compelling today when we have so much evidence of the diversity of MuLV by such criteria as neutralization of virus in vitro (see ref. 15), range of host susceptibility (reviewed in ref. 1), as well as immunoelectron microscopy (3, 16); indeed no fewer than four distinguishable isolates of MuLV can be obtained from a single inbred (BALB) mouse (17), and there is no reason to think that this is an upper limit. Gorer did not observe rejection of his leukemias by hybrids, but he noted that "X tests" (i.e. giving antiserum to protect mice against challenge with leukemia cells) were usually more effective in hemisyngeneic hybrids than in the syngeneic strain of origin, suggesting augmentation of resistance by an active immune response in the hybrid. All Gorer's leukemias had been passed for long periods in hybrids and may thus have acquired a relative resistance comparable to that of our RL♂1 line after passage in hybrids, which would also account for the weakness of protection by antibody in most of his experiments. Gorer noted in a single experiment that ability to produce an X antibody was linked with *H-2* (13), but the relation of this finding to our observation of *H-2*-linked ability to reject leukemia cells is uncertain because the former effect was genetically recessive (13) whereas the latter is dominant, as expected for an *Ir* gene in LG IX.

Are X Systems likely to Be Found in All Leukemias?—This is an important question, e.g., in its relation to the prospect of using X antisera to arrive at a comprehensive classification of MuLV that could be used for identification purposes and thus help in elucidating the biology of these viruses. The second case of strong hybrid resistance that we are studying, alluded to briefly in Results (last section), involves the I strain leukemia I-29, and it is already

apparent that the I-29 antigen is not identical with X.1. The fact that most leukemias are not strongly rejected by hybrids might seem to speak against X antigens as a general feature of leukemias. But this may be misleading, because evidently resistance develops with relative ease simply on passage in hybrid recipient. It may be that X antigens are characteristic of leukemias generally but only uncommonly act as strong histocompatibility antigens. Future search for other X systems will therefore not be limited to the criterion of strong histoincompatibility responses on the part of hybrids, but will include tests for X antibodies in hybrids immunized with material other than viable leukemia cells. It remains to be seen whether the presence of X antigens is invariably associated with production of virions.

By an "X system" we imply an antigen or set of antigens, associated with leukemia cells, to which some strains of mice can respond, but not the strain of origin of the leukemia. How the features of the X.1 system—(*a*) presence of antigen on virions and cell surface, (*b*) transplantation resistance and antibody production by hybrid recipients, and (*c*) transfer of resistance to leukemia cells by means of antiserum—could or should be used as criteria for defining a class of (X) leukemia antigens may be clearer when more of these systems have been studied.

SUMMARY

Two BALB radiation leukemias are strongly rejected by hybrids of BALB with certain other mouse strains, although BALB mice themselves exhibit no detectable resistance whatever. Hybrids immunized with progressively increased inocula are resistant to 200×10^6 or more leukemia cells; their serum is cytotoxic for the leukemia cells in vitro and protects BALB mice against challenge with these BALB leukemias. The antigenic system thus identified has been named X.1.

In (BALB × B6) hybrids the major determinant of resistance was shown to be a B6 gene in the K region of *H-2*. This is likely to be the *Rgv-1* (*Resistance to gross virus*) locus of Lilly, which may thus be identified in this case as an *Ir* (*Immune response*) allele conferring ability to respond to X.1 antigen on MuLV and leukemia cells, and so responsible for production of X.1 antibody and the rejection of $X.1^+$ leukemia cells by hybrid mice.

Immunoelectron microscopy with X.1 antiserum (from immunized hybrids) shows labeling both on the cell surface and on virions produced by the leukemia cells. It is not known whether X.1 comprises only one or more than one antigen.

Three radiation-induced BALB leukemias, one A strain radiation-induced leukemia, and 15/15 AKR primary spontaneous leukemias were typed $X.1^+$ by the cytotoxicity test. Several other leukemias, including one induced by passage A Gross virus and one long-transplanted AKR ascites leukemia carried in (B6 × AKR)F_1 hybrids, were $X.1^-$. Normal mice of strains with a

high incidence of leukemia and one other strain (129) express X.1 antigen, but evidently in amounts too small for certain detection in vitro; by the method of absorption in vivo, however, these strains could be typed $X.1^+$ and other strains $X.1^-$.

We ascribe the X.1 antigen system tentatively to a sub-type of MuLV that is not passage A Gross virus and is probably not the dominant sub-type in strains with a high incidence of leukemia.

After repeated passage in hybrids, one of the BALB leukemias became relatively resistant to rejection by the hybrid, partially lost its sensitivity to X.1 antiserum in vitro, and in electron micrographs was seen to produce fewer virions.

The serum of untreated (BALB × B6) hybrids often contains cytotoxic antibody against leukemia cells, some of it probably anti-X.1. But another commonly occurring antibody, which is cytotoxic for C57BL leukemia EL4, appears to belong to another (undefined) system.

REFERENCES

1. Lilly, F., and T. Pincus. 1973. Genetic control of murine viral leukemogenesis. *Adv. Cancer Res.* **17**:231.
2. Boyse, E. A., L. Hubbard, E. Stockert, and M. F. Lamm. 1970. Improved complementation in the cytotoxic test. *Transplantation.* **10**:446.
3. Aoki, T., and T. Takahashi. 1972. Viral and cellular surface antigens of murine leukemias and myelomas. *J. Exp. Med.* **135**:443.
4. Gorer, P. A., and Z. B. Mikulska. 1959. Some further data on the H-2 system of antigens. *Proc. R. Soc. Lond. B Biol. Sci.* **151**:57.
5. Boyse, E. A., M. Miyazawa, T. Aoki, and L. J. Old. 1968. Ly-A and Ly-B: two systems of lymphocyte isoantigens in the mouse. *Proc. R. Soc. Lond. B Biol. Sci.* **170**:175.
6. Boyse, E. A., L. J. Old, and E. Stockert. 1962. Some further data on cytotoxic isoantibodies in the mouse. *Ann. N. Y. Acad. Sci.* **99**:574.
7. Takahashi, T., L. J. Old, and E. A. Boyse. 1970. Surface alloantigens of plasma cells. *J. Exp. Med.* **131**:1325.
8. Hämmerling, U., T. Aoki, E. A. Boyse, and L. J. Old. 1968. Use of hybrid antibody with anti-γG and anti-ferritin specificities in locating cell surface antigens by electron microscopy. *J. Exp. Med.* **128**:1461.
9. Huemer, R. P. 1969. Further studies on inhibition and adaptation of a parental tumor in F_1 hybrid mice. *Cancer Res.* **29**:610.
10. Bennett, M. 1972. Rejection of marrow allografts. *Transplantation.* **14**:289.
11. Benacerraf, B., and H. O. McDevitt. 1972. Histocompatibility-linked immune response genes. *Science (Wash. D. C.).* **175**:273.
12. Gorer, P. A., and D. B. Amos. 1956. Passive immunity in mice against C57BL leukosis EL4 by means of iso-immune serum. *Cancer Res.* **16**:338.
13. Gorer, P. A. 1961. The antigenic structure of tumors. *In* Advances in Immunology. W. H. Taliafferro and J. H. Humphrey, editors. Academic Press, Inc., New York.

14. Gorer, P. A., M. A. Tuffrey, and J. R. Batchelor. 1962. Serological studies on the X antigens. *Ann. N. Y. Acad. Sci.* **101:**5.
15. Eckner, R. J., and R. A. Steeves. 1972. A classification of the murine leukemia viruses. Neutralization of pseudotypes of Friend spleen focus-forming virus by type-specific murine antisera. *J. Exp. Med.* **136:**832.
16. Aoki, T., and G. Todaro. 1973. Antigenic properties of endogenous type C viruses from spontaneously transformed clones of BALB/3T3. *Proc. Natl. Acad. Sci. U.S.A.* **70:**1598.
17. Rowe, W. P., D. R. Lowy, N. Teich, and J. W. Hartley. 1972. Some implication of the activation of murine leukemia virus by halogenated pyrimidines. *Proc. Natl. Acad. Sci. U.S.A.* **69:**1033.