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The Genetic Control of Natural Killer Cell Activity and Its Association With In Vivo Resistance Against a Moloney Lymphoma Isograft

Győző, G., Petrányi[1,2], Rolf Kiessling[1], Susan Povey[3], George Klein[1], Leonard Herzenberg[4], and Hans Wigzell[5]

[1] *Department of Tumor Biology, Karolinska Institute, 104 01 Stockholm 60, Sweden*
[2] *Fellow, UICC, National Institute of Haematology and Blood Transfusion, Budapest, Hungary*
[3] *MRC Human Biochemical Genetic Unit, The Galton Laboratory, University College London, London, England*
[4] *Department of Genetics, Stanford University School of Medicine, Stanford, California*
[5] *Department of Immunology, Uppsala University, Uppsala, Sweden*

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Abstract. Spleens of normal young mice of certain strains contain lymphocytes that can kill strain A-derived YAC-1 lymphoma cells in a ^{51}Cr release cytotoxic assay in vitro. We have previously classified mouse genotypes as high or low reactors, according to their responses in this test. In vivo resistance to small numbers of YAC ascites lymphoma cells is correlated with in vitro cytolytic activity. In vitro and in vivo tests were carried out on the same individual $(A \times C57BL)F_1 \times A$ backcross mice. Natural in vitro killer cell activity appeared to be under polygenic control, including a strong H-2-linked factor. No linkage was found with five different isozyme loci, with the *Ig-1* locus or with C5 serum activity. Also in vivo resistance showed strong linkage with the *H-2* complex. In $(A \times CBA)F_1 \times A$ backcross mice, a weak linkage was found with the coat color locus *C*. There was a correlation between in vitro killer activity and in vivo resistance in the same backcross mice. In vivo resistance was particularly strong in mice that combined the $H\text{-}2^b$-linked resistance factor with a high cytolytic activity in vitro.

Introduction

There are several reports of tumor cell killing by lymphoid or monocytic cells from normal, nonsensitized individuals (Greenberg and Playfair 1974, Herberman *et al.* 1973, Kay and Sinkovic 1974, Petrányi *et al.* 1974a, Rosenberg *et al.* 1974, Skurzak *et al.* 1973, Takasugi *et al.* 1973, Gomard *et al.* 1974). We have reported previously that lymphocytes without B- or T-cell markers can kill the in vitro-growing Moloney lymphoma line YAC-1, and some other lines as well (Kiessling *et al.* 1975a, 1975b). Different mouse genotypes have shown considerable differences in killer activity. Spleen lymphocytes of A, A.CA, and A.SW mice exhibited low activity, whereas C57BL, C57L, CBA, and C3H were highly reactive. In F_1 hybrids between low and high reactive strains, high killing activity was dominant over low reactivity (Petrányi *et al.* 1975).

To assess whether the natural killer cell plays any role in vivo, we have compared the in vitro killer activity of spleen cells and in vivo resistance to

small numbers of YAC lymphoma cells in eight different semisyngeneic F_1 host genotypes. A clear positive correlation was found (Kiessling *et al.* 1975c). Our preliminary study on $(A \times C57BL)F_1 \times A$ backcross mice further suggested, that the activity of the natural killer cell is dependent on an *H-2*-linked genetic factor (Petrányi *et al.* 1975). A similar *H-2* association was demonstrated for the in vivo resistance in nonimmunized backcross mice (Kiessling *et al.* 1975c).

This is an extension of the study of the genetic control of in vitro killing and in vivo rejection in the same system. A large number of backcross mice were used and additional genetic markers were introduced for linkage analysis. Segregation of in vitro killing and in vivo resistance was compared in the same backcross mice.

Materials and Methods

Mice. The following inbred strains, maintained by continuous, single-line, brother-sister mating at our laboratory were used: A, C57BL/6, C57L, CBA. Backcross hybrids were produced by crossing $(A \times C57BL)$ F_1, $(A \times CBA)$ F_1 or $(A \times C57L)$ F_1 hybrids with strain A mice. All mice were from one to two months old.

Tumor. YAC is a Moloney virus-induced lymphoma of strain A origin, propagated in syngeneic mice in the ascites form. The in vitro YAC-1 subline was adapted to stationary suspension culture by Cikes *et al.* (1973). It was maintained in medium F13 (Grand Island Biological Company, Grand Island, New York) containing 10% heat inactivated fetal calf serum (Bio-Cult, Glasgow, Scotland) with antibiotics. The same medium was used as diluent and tissue culture medium throughout all experiments.

Cytotoxic Assay. Spleen cell suspensions were prepared in F13 medium containing 10% fetal calf serum and HEPES buffer (pH 7.3). YAC-1 target cells were labeled with 200 μCi ^{51}Cr (sodium chromate) (in $5–10 \times 10^6$/0.5 ml concentration) for 30 minutes at 37°C. The cells were washed twice and adjusted to the desired cell density. Two or 4×10^4 isotope-labelled target cells were added to 10^6 effector cells in a total volume of 0.14 ml in glass tubes. Triplicate tubes with appropriate controls (for spontaneous and maximal release, using tissue culture medium and distilled water, respectively, along with target cells) were incubated at 37°C, in a 5% CO_2 incubator for 12 hours. After addition of 1 ml medium the samples were spun and the radioactivity of the supernatant and the pellet were measured in a gamma counter (Intertechnique, Paris, France). Results were expressed as percent lysis of the target cells, according to a formula described elsewhere (Kiessling *et al.* 1975a). Spontaneous release varied, from 15 to 25% of the total label. Maximum release varied from 70 to 75% of the total label. The sensitivity of the YAC-1 target cells for lysis varied considerably among different experiments. The following formula was used to permit a comparative evaluation of different experiments:

$$\text{corrected isotope release \%} = \frac{\text{percent lysis of the sample-percent lysis of the } \textit{lowest} \text{ value in the test}^1}{\text{percent lysis of the } \textit{highest} \text{ value in the test}^1\text{-percent lysis of the } \textit{lowest} \text{ value in the test}^1} \times 100$$

[1] Each backcross experiment contained 15 to 30 mice.

The corrected isotope release thus ranged between 0 and 100%, usually corresponding to 5 to 80% lysis. Only those experiments in which the lytic activity of the parental A strain and the reactive $(A \times C57BL)$ F_1, $(A \times C57Leaden)$ F_1, and $(A \times CBA)$ F_1 hybrid cells used as positive controls differed by at least 20% were evaluated.

In Vivo Resistance Assay. Ten days after the splenectomy for the in vitro cytotoxicity test was performed, the backcross mice were inoculated subcutaneously with 5×10^3 in vivo-maintained YAC ascites cells. Tumor growth was followed by inspection and palpation every second to third day. The take and the mean tumor diameter were registered.

H-2 Typing. *H-2* typing of $(A \times C57BL)F_1 \times A$ and $(A \times C57L)F_1 \times A$ backcross mice was carried out with an anti-$H\text{-}2^b$ serum, produced by hyperimmunizing A mice with C57L spleen cells. Complement-dependent cytotoxic tests were performed on spleen lymphocytes by the ordinary trypan blue exclusion technique.

Ig-1 Allotyping. The *Ig-1* allotype was determined by double immunodiffusion on agar-coated microscope slides, using antisera and methods previously described (Herzenberg and Herzenberg 1973).

C5 Determination. The C5 complement component was typed with a monospecific mouse anti-C5 reagent in a double diffusion assay (Ouchterlony 1958). The test serum was supplied by Leon T. Rosenberg.

Isozyme Typing. Enzymes were examined by horizontal starch-gel electrophoresis of aqueous extracts of kidney tissues, using the following methods with minor adaptations: autosomal glucose-6-phosphate dehydrogenase (*Gpd-1*) (Ruddle *et al.* 1968), malic enzyme (soluble NADP-dependent malate dehydrogenase, *Mod-1*) (Henderson 1966), glucose-phosphate isomerase (*Gpi-1*) (De Lorenzo and Ruddle 1969), Peptidase C (Dipeptidase, *Dip-1*) (Lewis and Truslove 1969), and Esterase 1 (Es-1) (Popp and Popp 1962, Ruddle and Roderick 1965).

Results

1. Distribution of In Vitro Natural Killer Cell Activity in a Backcross Population. One hundred sixty-six $(A \times C57BL)F_1 \times A$ backcross mice were tested in 14 separate experiments. Since the sensitivity of the YAC-1 target cells varied considerably in different experiments, a correction factor was introduced to allow joint evaluation (see *Materials and Methods*). All backcross mice tested were grouped according to their cytolytic activity against YAC-1.

Figure 1 shows the distribution of activity in the $(A \times C57BL)F_1 \times A$ backcross mice. The mean reactivity of A and $(A \times C57BL)F_1$ mice is shown for comparison.

2. Linkage Analysis of In Vitro Killer Cell Activity. Each $(A \times C57BL)F_1 \times A$ backcross mouse tested for in vitro spleen cell activity against YAC-1 was *H-2* and *Ig-1* allotyped and registered for coat color. Five different tissue isozymes in kidney and liver (listed in Table 1) and the C5 serum complement

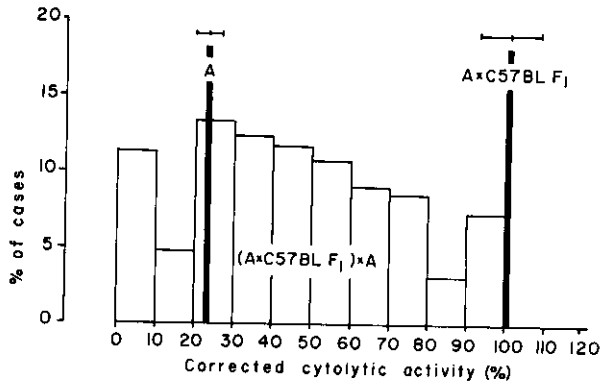

Fig. 1. The distribution of killer activity in 166 $(A \times C57BL)F_1 \times A$ backcrossed mice and the mean activity of 24 A and 25 $(A \times C57BL)F_1$ mice as representatives of the parent lines. ⊢O⊣mean corrected cytolytic activity ±S.E.

Table 1. Isozyme Marker Allotypes in the C57BL and A Strains

Marker	Symbol	Chromosome	Genotype	
			A	C57BL
Esterase-1	*Es-1*	8	*bb*	*aa*
Dipeptidase-1	*Dip-1*	1	*bb*	*aa*
Glucose-phosphate isomerase	*Gpi-1*	7	*aa*	*bb*
Glucose-6-phosphate dehydrogenase (autosomal)	*Gpd-1*	4	*bb*	*aa*
Malic enzyme (soluble NADP-dependent malate dehydrogenase)	*Mod-1*	9	*aa*	*bb*

component were determined as well. Mice of strain A are $H\text{-}2^a$,$Ig\text{-}1^c$ and they are C5-deficient; C57BL mice are $H\text{-}2^b$,$Ig\text{-}1^b$ and have normal serum C5-levels. In the $(A \times C57BL)F_1 \times A$ backcross, two coat color genes are segregating: albino (*c* vs. *C*) and brown-black (*b* vs. *B*). In addition, we studied five segregating isozyme markers (Table 1). The segregation thus involves six known chromosomes: *H-2*: 17, *C* and *Gpi-1*: 7, *B* and *Gpd-1*: 4, *Es-1*: 8, *Mod-1*: 9, *Dip-1*: 1. The chromosome carrying *Ig-1* and *C5* have not been identified.

The mean cytolytic activity was higher in the $H\text{-}2^a/H\text{-}2^b$ heterozygotes (52.8) than in the $H\text{-}2^a/H\text{-}2^a$ homozygotes (40.3) (Table 2). The significance of the difference was considerably higher in the larger animal groups included in the present study than in our last report on a smaller, preliminary series (Petrányi *et al.* 1975). As far as coat color genes are concerned, the difference between black (*B*) and brown (*b*) suggested by the small, preliminary series has now become insignificant (56.0 vs. 45.7; $0.1 > P > 0.05$). A previously unnoticed difference between colored and albino mice (49.6 for *Cc* vs. *42.5* for *cc*) that was more significant ($0{,}05 > P > 0{,}02$) appeared. There was no significant difference in relation to the *Ig-1* allotype or the C5 complement component. No sex difference was noted (Table 2).

Table 3 shows the genotype distribution of the $(A \times C57BL)F_1 \times A$ backcross mice with extremely low (0–10) and extremely high (90–100) corrected cytolytic activity. In spite of the small number of mice in each group, a striking relationship to the *H-2* genotype was displayed. It may be added, however, that there were a few "discordant" mice exhibiting extremely low reactivity in the *H-2* heterozygous group and extremely high reactivity in the homozygous group. This is consistent with our previous conclusion (Petrányi *et al.* 1975), that the reactivity is polygenically determined.

The killer activity in relation to isozyme type is represented in Table 2. There was no significant difference between heterozygotes and homozygotes with regard to the five markers studied. A slight difference, not statistically significant ($0.1 > P > 0.05$), in relation to *Es-1* and *Mod-1* was observed.

The $(A \times CBA)F_1 \times A$ backcross mice tested for spleen cell activity were registered only for coat color. Three coat color genes segregated in this backcross: color (*C* vs. *c*), agouti (*A* vs. *a*) and black-brown (*B* vs. *b*). No difference in cytolytic activity was displayed in relation to any of these markers (Table 4).

Table 2. Killer Activity, *H-2*, *Ig-1* and Isozyme Marker Types, Coat Color and C5 Deficiency in (A × C57BL)F_1 × A Backcross Mice

Genotype	Phenotype	Number of Mice	Mean Corected cytolytic Activity ± S.E.	P
H-2^a/*H-2*^{a}		83	40.3 ± 2.9	0.002 > P > 0.0005
H-2^a/*H-2*^{b}		77	52.8 ± 3.1	
cc	white	104	42.4 ± 2.4	0.05 > P > 0.02
Cc	colored	82	49.6 ± 3.4	
BbCc	black	44	56.0 ± 3.9	0.1 > P > 0.05
bbCc	brown	31	45.7 ± 4.2	
Ig-1^a/*Ig-1*^{a}		42	43.0 ± 4.6	0.2 > P > 0.1
Ig-1^a/*Ig-1*^{b}		64	49.9 ± 3.3	
C5 negative		29	42.6 ± 5.6	0.4 > P > 0.3
C5 positive		28	39.8 ± 5.0	
Dip-1^b/*Dip-1*^{b}		37	51.8 ± 4.8	0.2 > P > 0.1
Dip-1^b/*Dip-1*^{a}		36	48.3 ± 4.3	
Gpi-1^a/*Gpi-1*^{a}		32	47.5 ± 4.3	0.4 > P > 0.3
Gpi-1^a/*Gpi-1*^{b}		44	49.8 ± 4.4	
Mod-1^a/*Mod-1*^{a}		45	42.7 ± 4.3	0.1 > P > 0.05
Mod-1^a/*Mod-1*^{b}		46	51.8 ± 4.2	
Gpd-1^b/*Gpd-1*^{b}		31	46.4 ± 4.8	0.3 > P > 0.2
Gpd-1^b/*Gpd-1*^{a}		25	51.6 ± 5.5	
Es-1^b/*Es-1*^{b}		49	43.3 ± 4.2	0.1 > P > 0.05
Es-1^b/*Es-1*^{a}		46	50.9 ± 3.9	
Female		42	48.3 ± 3.9	0.3 > P > 0.2
Male		45	52.8 ± 4.9	

Table 3. Genotype Segregation of (A × C57Bl)F_1 × A Backcross Mice with Extremely Low (0–10) or Extremely High (90–100) Cytolytic Activity (Corrected Value)

Genotype	Phenotype	Low	High	χ^2	P
H-2^a/*H-2*^{a}		16	4	9.41	0.005 > P > 0.001
H-2^a/*H-2*^{b}		3	9		
cc	white	11	4	2.33	0.2 > P > 0.1
Cc	colored	9	10		
BbCc	black	5	5	0.28	0.6 > P > 0.5
bbCc	brown	3	5		
Ig-1^a/*Ig-1*^{a}		6	4	0.06	0.9 > P > 0.8
Ig-1^a/*Ig-1*^{b}		6	5		

3. Linkage Analysis of In Vivo Resistance Against YAC Lymphoma Cells. Seventy-two splenectomized (A × C57BL)F_1 × A backcross mice were challenged subcutaneously with 5×10^3 live YAC ascites cells in three separate experiments. In vitro killer cell activity was determined for the spleen cell population of

Table 4. Killer Activity and Coat Color in an $(A \times CBA)F_1 \times A$ Backcross Population

Genotype	Phenotype	Number of Mice	Mean Corrected Cytolytic Activity, S.E. and P values	
cc	white	35	37.1 ± 4.8	$P = 0.4$
Cc	colored	31	37.1 ± 4.9	
bb	brown cinnamon	11	36.3 ± 8.5	
Bb	agouti black	20	38.2 ± 6.3	$P = 0.4$
aa	brown black	12	$34{,}6 \pm 8.4$	
Aa	agouti cinnamon	18	39.3 ± 6.6	$0.2 > P > 0{,}1$

Table 5. Tumor Growth and *H-2* type in $(A \times C57BL)F_1 \times A$ Backcross Mice Inoculated Subcutaneously with 5×10^3 YAC Ascites Cells

Days After Inoculation	Genotype				χ^2	P
	H-2^a/*H-2*^{a}		*H-2*^{a}/*H-2*^{b}			
	ratio[a]	%	ratio	%		
13	27/40	67	8/32	25	12.85	$P < 0.0005$
15	34/40	85	12/32	37	17.39	$P < 0.0005$
17	34/40	85	14/32	43	13.61	$P < 0.0005$
20	32/40	80	14/32	43	10.31	$0.005 > P > 0.001$
30	28/40	70	14/32	43	5.04	$0.025 > P > 0.010$

[a] Cumulative number of mice with progressively growing tumors over total number inoculated.

each individual mouse prior to the tumor cell challenge. In addition, *H-2* and *Ig-1* allotype, coat color, and five isozymes were typed for each backcross mouse. In another series, 43 $(A \times C57L)F_1 \times A$ backcross mice were tested for in vivo resistance in relation to *H-2* type and coat color. The resistance of 44 $(A \times CBA)F_1 \times A$ backcross mice was also determined, but only in relation to coat color.

In the $(A \times C57BL)F_1 \times A$ backcross mice a clear difference in resistance of *H-2* homozygotes and heterozygotes to primary YAC challenge was observed. Thirteen days after YAC inoculation, 67% of the homozygotes, but only 25% of the heterozygotes, had developed palpable tumors (Table 5). Beyond day 15, the tumor grew in 70 to 85% of the homozygotes and 43% of the heterozygotes. The difference was highly significant throughout the entire observation period.

There was no significant difference in resistance against YAC ascites cells in relation to *Ig-1* allotype (Table 6), to albino (*c*) or brown-black (*b*) coat

Table 6. *Ig-1* Allotype and Tumor Growth in (A × C57BL)F_1 × A Backcross Mice Inoculated Subcutaneously with 5×10^3 YAC Ascites Cells

Days After Inoculation	Genotype				χ^2	P
	Ig-1^c/*Ig-1*^{c}		*Ig-1*^{c}/*Ig-1*^{b}			
	ratio[a]	%	ratio	%		
13	13/30	43	22/41	53	0.74	0.4 > P > 0.3
15	19/30	63	27/41	65	0.09	0.9 > P > 0.8
17	20/30	66	27/41	65	0.01	P > 0.9
20	21/30	69	27/41	65	0.14	0.8 > P > 0.7
30	21/30	69	23/41	56	1.42	0.3 > P > 0.2

[a] Cumulative number of mice with progressively growing tumors over total number inoculated.

Table 7. Coat Color and Tumor Growth in (A × C57BL)F_1 × A Backcross Mice Inoculated Subcutaneously With 5×10^3 YAC Ascites Cells

Days after Inoculation	Genotype				χ^2	P
	cc		*Cc*			
	Ratio[a]	%	Ratio	%		
13	20/37	54	15/35	42	0.90	0.4 > P > 0.3
15	26/37	70	21/35	60	0.83	0.4 > P > 0.3
17	25/37	67	21/35	60	0.44	P = 0.5
20	25/37	67	20/35	57	0.83	0.4 > P > 0.3
30	23/37	62	19/35	54	0.45	P = 0.5
Days After Tumor Cell Inoculation	Genotype				χ^2	P
	bbCc		*BbCc*			
	Ratio[a]	%	Ratio	%		
13	10/23	43	5/12	41	0.01	P > 0.9
15	15/23	65	6/12	50	0.76	0.4 > P > 0.3
17	14/23	60	7/12	58	0.02	0.9 > P > 0.8
20	13/23	56	7/12	58	0.01	P > 0.9
30	13/23	56	7/12	58	0.01	P > 0.9

[a] Cumulative number of mice with progressively growing tumors over total number inoculated.

color locus (Table 7) or to three isozyme markers studied (Table 8). There was no sex difference with regard to in vivo resistance to YAC-cells in this backcross population (tumor takes on day 12: in females 20/40, 50%: in males 16/32, 50%). The resistance of (A × C57L)F_1 × A backcross mice to a primary challenge of YAC ascites cells and their *H-2* types are shown in Table 9. On days 13 to 17

Table 8. Isozyme Marker Allotypes and Tumor Growth in $(A \times C57BL)F_1 \times A$ Backcross Mice Inoculated Subcutaneously With 5×10^3 YAC Ascites Cells

Days After Inoculation	Genotype				χ^2	P
	Gpi-1^a/*Gpi-1*^{b}		*Gpi-1*^{a}/*Gpi-1*^{a}			
	Ratio[a]	%	Ratio	%		
13	7/26	27	9/21	43	1.31	0.3 > P > 0.2
15	15/26	58	13/21	62	0.09	0.8 > P > 0.7
17	13/26	50	11/21	52	0.03	0.8 > P > 0.7
20	13/26	50	11/21	52	0.03	0.8 > P > 0.7
30	13/26	50	10/21	48	0.03	0.8 > P > 0.7
Days After Inoculation	Genotype				χ^2	P
	Mod-1^a/*Mod-1*^{b}		*Mod-1*^{a}/*Mod-1*^{a}			
	Ratio[a]	%	Ratio	%		
13	13/27	48	14/30	46	0.01	P > 0.9
15	15/27	55	19/30	63	0.35	0.6 > P > 0.5
17	14/27	52	18/30	60	0.38	0.6 > P > 0.5
20	13/27	48	19/30	63	1.33	0.3 > P > 0.2
30	9/27	33	17/30	56	3.11	0.10 > P > 0.05
Days After Inoculation	Genotype				χ^2	P
	Es-1^a/*Es-1*^{b}		*Es-1*^{b}/*Es-1*^{b}			
	Ratio[a]	%	Ratio	%		
13	13/27	48	12/29	41	0.26	0.7 > P > 0.6
15	19/27	70	16/29	55	1.37	0.3 > P > 0.2
17	19/27	70	14/29	48	2.82	0.10 > P > 0.05
20	19/27	70	14/29	48	2.82	0.10 > P > 0.05
30	16/27	59	11/29	37	2.45	0.2 > P > 0.1

[a] Cumulative number of mice with progressively growing tumors over total number inoculated.

a highly significant, *H-2*-linked difference, comparable to the $(A \times C57BL)F_1 \times A$ backcross was observed. On days 20 to 30, the difference disappeared, suggesting that an initial resistance eventually broke down. The final take incidence was similar in *H-2* homozygotes and heterozygotes.

In the $(A \times CBA)F_1 \times A$ backcross group, there was a significant linkage between resistance and the color (*C*) locus. On day 27 the tumor was growing in 75% of the albino (*cc*) and 37% of the colored (*Cc*) mice. The number of colored mice was small; within the colored group there was no significant relationship between resistance and the agouti (*A*) or black (*B*) locus (Table 10).

4. Correlation among In Vivo Resistance, Natural Killer Cell Activity, and H-2 Genotype. Our previcus studies on A mice and eight different F_1 hybrids

Table 9. Tumor Growth and *H-2* Type in $(A \times C57L)F_1 \times A$ Backcross Mice Inoculated subcutaneously With 5×10^3 Ascites Cells

Days After Inoculation	Genotype				χ^2	P
	H-2^a/*H-2*^{a}		*H-2*^{a}/*H-2*^{b}			
	Ratio[a]	%	Ratio	%		
13	20/25	80	5/18	28	11.73	0.001 > P > 0.0005
15	22/25	88	7/18	38	11.50	0.001 > P > 0.0005
17	21/25	84	9/18	50	7.61	0.01 > P > 0.005
20	21/25	84	12/18	66	1.76	0.2 > P > 0.1
30	21/25	84	14/18	77	0.26	0.7 > P > 0.6

[a] Cumulative number of mice with progressively growing tumors over total number inoculated.

Table 10. Tumor Growth and Coat Color in $(A \times CBA)F_1 \times A$ Backcross Mice Inoculated Subcutaneously With 5×10 YAC Ascites Cells

Genotype	Phenotype	Ratio[a]	%	χ^2	P
cc	white	12/16	75	5.79	0.025 > P > 0.010
Cc	colored	10/27	37		
bb	brown cinnamon	4/9	44	0.02	0.9 > P > 0.8
Bb	agouti black	6/18	33		
aa	brown black	5/14	35	0.02	0.9 > P > 0.8
Aa	agouti cinnamon	4/12	33		

[a] Cumulative incidence of tumor bearers 27 days after challenge over total number inoculated.

involving the A strain showed a clear correlation between the natural killer cell activity against YAC-1 tumor cells in vitro and resistance against small YAC ascites tumor inocula in vivo. Backcross experiments showed that both in vitro cytolysis and in vivo resistance were influenced by an *H-2*-linked factor (Petrányi *et al.* 1975, Kiessling *et al.* 1975c). In the present study the same individual backcross mice were tested with regard to both properties. Figures 2 and 3 show the corrected spleen killer cell activity, *H-2* type, and mean tumor diameter of 89 backcross mice, 62 $(A \times C57BL)F_1 \times A$ and 27 $(A \times C57L)\ F_1 \times A$ – on days 12 and 20, respectively. Generally, it was found that the tumor grew better in backcross mice with low cytotoxic activity than in mice with high cytotoxic activity, but there were individual exceptions in both directions. In the group with low spleen cell activity (0–50% corrected lysis), the mean tumor diameter was 2.45 mm on day 12, and 5.40 mm on day 20. In the group with high activity (50–100% corrected lysis), it was 1.10 mm on day 12, and 3.56 mm on day 20 (Table 11). Table 12 shows a similar analysis based on tumor take, rather than tumor size.

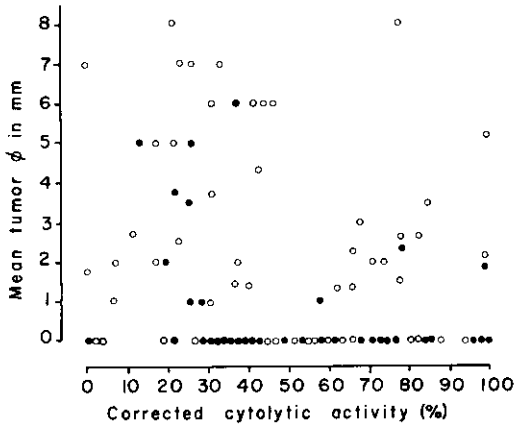

Fig. 2. The corrected spleen cell activity, *H-2* type, and mean tumor diameter of 62 $(A \times C57BL)F_1 \times A$ and 27$(A \times C57L)F_1 \times A$ backcross mice on day 12 after challenge. Each point represents one individual mouse. Challenge subcutaneously with 5×10^3 YAC ascites cells. ○ *H-2*^{a}/*H-2*^{a}, ● *H-2*^{a}/*H-2*^{b}

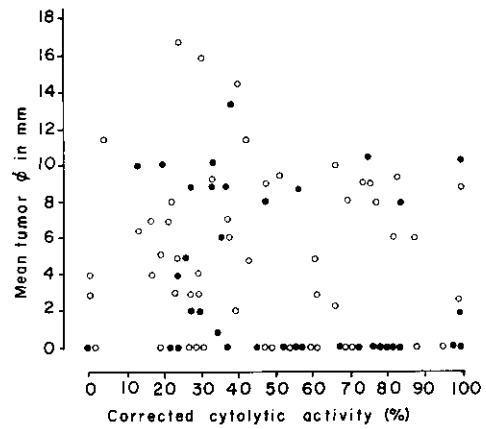

Fig. 3. The corrected spleen cell activity, *H-2* type, and mean tumor diameter of 62 $(A \times C57BL)F_1 \times A$ and 27 $(A \times C57L)F_1 \times A$ backcross mice on day 20 after challenge. Each point represents one individual mouse. Challenge subcutaneously with 5×10^3 YAC ascites cells. ○ *H-2*^{a}/*H-2*^{a}, ● *H-2*^{a}/*H-2*^{b}

To evaluate the influence of *H-2* type on in vitro activity and in vivo resistance, we assembled the take incidence and mean tumor diameter values in the low and high in vitro cytolytic groups, respectively, according to their *H-2* genotype (Tables 11 and 12). In the *H-2*^{a}/*H-2*^{b} heterozygotes the tumor grew less than in the *H-2*^{a}/*H-2*^{a} homozygotes; this was true in both the low and high in vitro cytolytic groups. This *H-2*-dependent difference was less pronounced on day 20 than on day 12 (see also Fig. 3). A similar pattern emerges when comparing in vivo growth in relation to in vitro activity. As shown in Table 11, both the *H-2*^{a}/*H-2*^{a} homozygotes and the *H-2*^{a}/*H-2*^{b} heterozygotes were less resistant to the tumor when their in vitro spleen cell activity was low. This suggests that both an *H-2*-linked factor and non-*H-2*-linked

Table 11. In Vitro Killer Cell Activity, *H-2* Type and Tumor Size in $(A \times C57BL)F_1 \times A$ and $(A \times C57L)F_1 \times A$ Backcross Mice Inoculated subcutaneously with 5×10^3 YAC Ascites Cells. Values of the Mean Tumor Diameter in the Low (0–50 Corrected Lysis) and High (50–100 Corrected Lysis) Activity Groups Are shown

Genotype	Number of Mice	Low	High	
		Mean[a] S.E. and P[b]	Mean S.E. and P	
12 Days After Inoculation				
$H\text{-}2^a/H\text{-}2^a + H\text{-}2^a/H\text{-}2^b$	89	2.45 ± 0.36	1.10 ± 0.27	$0.005 > P > 0.002$
$H\text{-}2^a/H\text{-}2^a$	50	3.30 ± 0.51	1.91 ± 0.15	$0.02 > P > 0.01$
$H\text{-}2^a/H\text{-}2^b$	39	1.29 ± 0.44	0.28 ± 0.49	$0.05 > P > 0.02$
		$0.005 > P > 0.002$	$0.002 > P > 0.005$	
20 Days After Inoculation				
$H\text{-}2^a/H\text{-}2^a + H\text{-}2^a/H\text{-}2^b$	89	5.40 ± 0.65	3.56 ± 0.66	$0.05 > P > 0.02$
$H\text{-}2^a/H\text{-}2^a$	50	6.34 ± 0.91	4.85 ± 0.86	$0.1 > P > 0.05$
$H\text{-}2^a/H\text{-}2^b$	39	4.62 ± 0.98	2.16 ± 0.91	$0.05 > P > 0.02$
		$0.2 > P > 0.1$	$0.02 > P > 0.01$	

[a] Mean tumor diameter in millimeters: mice without tumors calculated as zero.
[b] P values of Student's t-test. Mice without tumors calculated as zero.

Table 12. In Vitro Killer Cell Activity, *H-2* Type and Tumor Take Incidence in $(A \times C57BL)F_1 \times A$ and $(A \times C57L)F_1 \times A$ Backcross Mice Inoculated Subcutaneously With 5×10^3 YAC Ascites Cells. The cumulative Number of Tumor Bearers Over Total Number Inoculated in the Low (0–50 Corrected Lysis) and High (50–100 Corrected Lysis) Activity Groups is shown

Genotype	Low		High		P Value of χ^2 test
	Ratio[a]	%	Ratio	%	
12 Days After Inoculation					
$H\text{-}2^a/H\text{-}2^a + H\text{-}2^a/H\text{-}2^b$	31/50	62	16/39	41	$0.05 > P > 0.02$
$H\text{-}2^a/H\text{-}2^a$	23/29	79	13/21	62	$0.2 > P > 0.1$
$H\text{-}2^a/H\text{-}2^b$	8/21	38	3/18	16	$0.2 > P > 0.1$
	$0.005 > P > 0.001$		$0.005 > P > 0.001$		
20 Days After Inoculation					
$H\text{-}2^a/H\text{-}2^a + H\text{-}2^a/H\text{-}2^b$	38/50	76	19/39	48	$0.010 > P > 0.005$
$H\text{-}2^a/H\text{-}2^a$	24/29	82	14/21	66	$0.2 > P > 0.1$
$H\text{-}2^a/H\text{-}2^b$	14/21	66	5/18	27	$0.025 > P > 0.010$
	$0.2 > P > 0.1$		$0.025 > P > 0.010$		

[a] Cumulative number of mice with progressively growing tumors over total number inoculated.

factor(s) influence in vivo resistance and the partially related in vitro cytolytic activity.

Discussion

The following conclusions appear to be justified: (a) Natural killer cell activity against in vitro-growing YAC-1 lymphoma cells is under polygenic control. An *H-2*-linked factor plays an important, but not exclusive role; non-*H-2*-linked gene(s) appear to be involved as well. There is a possible linkage to the *C* locus, a definite correlation requires further study. (b) In vivo resistance against small numbers of YAC ascites cells is largely controlled by an *H-2*-linked factor. However, non-*H-2*-linked gene(s) appear to have some influence as well. (c) There is a correlation between in vitro cytolytic activity and in vivo resistance in individually tested backcross mice. This is more apparent in $(A \times C57BL)F_1 \times A$ than in $(A \times C57L)F_1 \times A$ backcross hybrids, because the in vivo resistance of the latter tends to break down gradually after inoculation; this effect is much less pronounced in the former group. The correlation is not absolute, since some mice with low in vitro cytolytic activity exhibit strong in vivo resistance, and vice versa. (d) In vivo resistance was particularly strong in mice that combined the $H\text{-}2^b$-linked resistance factor with a high cytolytic activity in vitro. This suggest a possible interaction between the two functions, but we cannot exclude the possibility that the *H-2*-linked genetic factor contributes to in vivo resistance, at least in part, via the natural killer cell.

Although *Ir* genes influencing immune responsiveness against tumor-associated antigens have not yet been clearly identified, such genes undoubtedly exist and probably play a major role in determining the ability of the host to respond to tumor-associated antigens. For virus-induced tumors, the evidence for immune surveillance is very strong (Allison 1975). It is likely that the natural hosts of ubiquitous, potentially oncogenic viruses have been selected for a high degree of immune responsiveness against the corresponding, potentially neoplastic cells. There is evidence, although not entirely conclusive (Kiessling *et al.* 1975a), that the killing activity of nonimmune spleen cells against the YAC-1 Moloney lymphoma is actually directed against Moloney virus-determined surface antigen(s). It is therefore possible that we are actually dealing with a surveillance mechanism against the neoplastic products of horizontally transmitted oncogenic mouse C-type viruses. The polygenic control of responsiveness would be consistent with this possibility, since it can be predicted that strong selective pressures tend to fix multiple controls (Klein 1974).

The possible role of *H-2*-linked factors in the genetic control of tumor surveillance has recieved much attention recently. In addition to earlier reports concerned with the effect of the *Rgv-1* gene on Gross-virus leukemogenesis and the role of another *H-2*-linked factor in mammary tumor genesis (Lilly 1971, Mühlbock and Duc 1971), Sato *et al.* recently described an *H-2*-linked factor, influencing resistance against X-1, a radiation-induced leukemia (Sato *et al.* 1973). Oth *et al.* found an *H-2*-linked factor in relation to the rejection of polyoma virus-induced transplanted tumor (Oth *et al.* 1975). The function of these *H-2*-linked factors is unknown. There is only one report, by Aoki *et al.*, suggesting a parallelism between antibody production against the X-1 tumor cell and the in vivo resistance against the same tumor (Aoki *et al.* 1968). Our observation may also be relevant in showing that the natural killer cell

may play an important role in resistance against the Moloney virus-induced YAC lymphoma cell in vivo, and that both in vivo resistance and in vitro killer activity are under the influence of an *H-2*-linked factor.

In addition to our previous fractionation studies, the non-T nature of the killer cell has been further strengthened by our recent finding that nude mice and T-lymphocyte-depleted hosts of genetically responsive types resist small YAC inocula as well as do intact mice (Kiessling *et al.* 1975d). Interestingly enough, linkage has been found between the spontaneous cytotoxicity of human peripheral, non-T lymphocytes against mouse fibroblasts and the *HL-A 2,12,* and possibly *HL-A 3,7,* haplotypes in a human system (Petrányi *et al.* 1974a, 1974b, Varga *et al.* 1975).

Dealing with a similar, if not identical phenomenon, Herberman *et al.* (1974, 1975a) suggested that the natural killer cell activity may be directed against naturally spread C-type viruses or their neoplastic cell products. The *H-2*-linked factor might represent an *Ir*-gene function controlling a possible "natural" immunization by horizontal virus transmission or by other antigen crossreacting with MLV antigens.

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