GENETIC DIFFERENTIATION OF LYMPHOBLASTIC SARCOMA HISTOCOMPATIBILITY SITES IN DBA/1J AND C57BL/6 MICE[1]

RAYMOND M. GESINSKI, HOWARD W. LORSON, JR. AND THOMAS S. NAPOLETANO,
Department of Biological Sciences, Kent State University, Kent, OH 44242

Abstract. A chemically induced, 20-methylcholanthrene, lymphoblastic sarcoma, in DBA/1J mice was used to determine the histocompatibility of the tumor between the DBA/1J and C57BL/6J strains. Although 8 different histocompatibility sites exist between the 2 strains, this work demonstrated that these 8 sites were transmitted and expressed as a 5 unit discrete factor phenotypic variation. F_1 x F_1 hybrid animals were tested through the F_9 generation and showed that only 5 linkage groups are involved in the transmission of the various histocompatibility sites. Survivors of the implanted F_9 generation were used as progenitors for the F_{10} generation. Tumor take frequencies with the F_{10} generation demonstrated that the inheritance of tumor susceptibility behaved as a discrete function of a 5 unit inheritance factor.

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Numerous publications attest to the many independent units which constitute the mouse histocompatibility sites (Liebelt and Liebelt 1967; Lilly and Duran-Reynals 1972; Kearney *et al* 1975; Passmore and Hansen 1975). These histocompatibility sites have been assigned to linkage groups and have, in many instances, been mapped (Snell and Stimpfling 1966; Green 1971; Altman and Dittmer 1972). There exists a great deal of difference between C57BL/6 and DBA/1J histocompatibility sites (Snell and Stimpfling 1966; Green 1971; Altman and Dittmer 1972). The former has at least 8 histocompatibility sites not found in the latter. DBA/1J has 7 histocompatibility sites not found in the C57BL/6 strain. Thus a two-directional allelic difference of 15 histocompatibility factors, represented by at least 5 or more linkage groups, exists between these 2 strains of mice.

The C57BL/6 strain is resistant to methylcholanthrene chemical carcinogenesis, whereas the DBA/1J is highly susceptible (Scholes 1969). By using an established tumor (185 implant generations) from the DBA/1J strain, we determined to what extent implanted tumor susceptibility was transmitted to the C57BL/6 background. In addition, we hoped to be able to determine if any epigenetic mechanisms were readily detectable by utilizing crosses between the 2 strains of mice, and between the resulting hybrids.

MATERIALS AND METHODS

Experimental animals were sexually mature male and female mice of the DBA/1J and C57BL/6J inbred strain maintained in our laboratory. Mice were given food and water *ad libitum*, and had a 12 hour dark, 12 hour light regimen.

The tumor was maintained in the DBA/1J strain in which it was originally induced. Implants were done on the 10th or 11th day from the preceding tumor passage. Tumor implant passages 185 to 232 were used in this study. Histological sections stained with hematoxylin and eosin were done every fifth passage and microscopic examinations revealed no morphological change in the tumor. The tumor has maintained its same morphological appearance throughout its entire history and apparently is stable. The tumor has been coded RaVe, and according to the nomenclature of Bessis it is a lymphoblastic sarcoma type tumor (Bessis 1956).

Hybrids were developed according to the system of Green (1966) using the isogenic strains C57BL/6J and DBA/1J. It was hoped that hybrids which would be allogenic (genetically disparate) to the parental strains and congenic (differ at a single locus) to each other, with regard to tumor susceptibility, would ensue. Hybrid F_1 and F_2 were bred before they were used for tumor implants. Generations F_3

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through F_8 were not implanted. The F_9 generation was implanted with tumors prior to breeding and the survivors of the F_9 generation were then the progenitors for the F_{10} generation. The breeding scheme involved the use of cross-intercross and also cross-backcross (see fig. 1). Chi square proability values were used for statistical evaluation of the data.

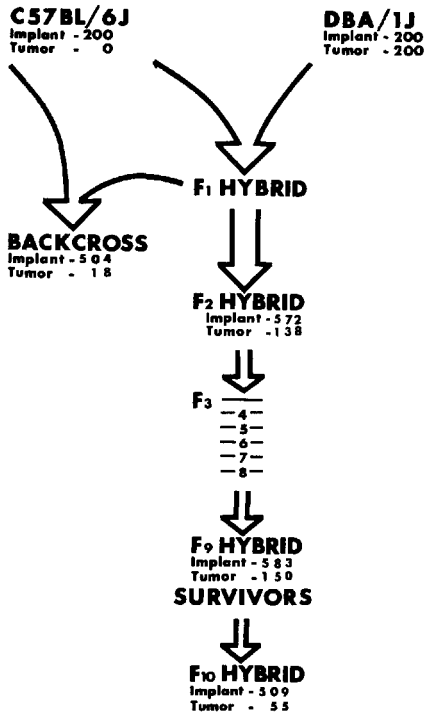

FIGURE 1. Flow diagram showing the breeding patterns followed to obtain mice for tumor implant. Also shown is the number of animals which received tumor implants, and the number which were susceptible to the tumor implant (tumor).

Tumors were harvested from previously implanted mice, cut into pieces approximately 8 mm^3 in size, placed in sterile saline and used for the propagation of the next tumor generation. For implanting, mice were anesthetized with sodium nembutal at a dose of 1.1 μl per gram body weight. A small incision approximately 0.5 cm long was made on the lateral aspect of the hypogastric region. Forceps were used to loosen the skin from the muscle wall toward the shoulder. A previously excised piece of tumor, kept in sterile isotonic saline, was inserted subcutaneously to approximately midway between the shoulder and hip and the skin smoothed back in place with forceps to facilitate healing of the incision.

RESULTS

The tumor maintained in the DBA/1J strain killed the animals in 8 to 12 days, with most of the animals succumbing on the 10th day. In contrast, the C57BL/6J animals were found to reject the tumor implant and survive. Hybrid (C57BL/6J X DBA/1J) mice either succumbed by the 12th day or survived. After 4 months, post implant, survivors were considered as having rejected the tumor implant, and were terminated.

No noticeable differences in the course of tumor development were observed between the DBA/1J strain and the hybrid (C57BL/6J X DBA/1J). Nor was there any difference between male and female in the development or rejection of the tumor by hybrid mice.

Two hundred pure strain C57BL/6J mice were implanted with the tumor and none of the mice developed a tumor (fig. 1). With the F_1 hybrid (C57BL/6J X DBA/1J) generation all 200 implanted mice developed the tumor and died within 12 days. All F_1 mice had a black coat coloring. The backcross of F_1 hybrid to the C57BL/6J strain was done with 504 mice, and 18 of these mice developed the tumor.

Four coat colors appeared in the F_2 hybrid generation: black, dark brown, gray and light brown in a ratio of 10:3:2:1. No differences in tumor susceptibility could be correlated with coat color. Hence coat color could not be used as a marker in determining tumor susceptibility, nor was any other utilizable marker observed in the hybrid. The F_2 hybrid generation had 572 mice implanted and the tumor developed in 138 of the mice.

Generations F_3 through F_8, were not implanted and were used only as progenitors for the next generation, up to the F_9 generation. The F_9 generation had 583 mice implanted and the tumor developed in 150 of the mice. Survivors from the F_9 generation were then bred to form the F_{10} generation. In the F_{10} generation 509 mice were implanted and the tumor developed in 55 of the mice.

DISCUSSION

Considering the fact that all the F_1 generation were susceptible to the tumor, it was assumed that the histocompatibility for tumor development was controlled by dominant genes. The prog-

TABLE 1

Statistical treatment of sarcoma histocompatability sites in DBA/J and C57BL/6 mice.

	Backcross	F2	F9	F10**
OBSERVED	18	138	150	55
EXPECTED	$15.75(\frac{1}{2})^5$	$136(\frac{3}{4})^5$	$138(\frac{3}{4})^5$	54.5
OB−EX*	2.25	2	12	.5
$\frac{(OB-EX)^2}{EX}$	0.3214	0.0294	1.043	0.0046
p	$0.99>p<.95$	$1.00>p<.99$	$.95>p<.90$	$.99>p<.95$

*OB=OBSERVED EX=EXPECTED

**The F10 expected value is derived from the possible combination available for histocompatibility to be achieved. Graphic representation of the possible combinations is shown in table 2.

eny from the backcross and intercross indicated that the histocompatibility for tumor development behaved as a discrete hybrid variation. Furthermore, the tumor take ratios developed with the backcross and the F_2 generation pointed to a 5 unit inheritance factor. Chi-square p value for the tumor take in the backcross was $0.99>p<0.95$ and for the F_2 generation it was $1.00>p<0.99$ (table 1). These values are excellent for substantiating the tumor take susceptibility as a five unit inheritance factor.

Eight generations were bred in keeping with the standard procedures to establish a stable gene pool in a population (Green 1966). The F_9 generation received tumor implants and again the inheritance of tumor susceptibility behaved as a discrete function of a 5 unit inheritance factor. Once again the $0.95>p<0.90$ value for the F_9 generation substantiated the 5 unit inheritance pattern.

Additional proof was obtained by using F_9 tumor implant survivors, animals which rejected tumor implant, as progenitors for the F_{10} generation. If the original hypothesis of a discrete 5 unit inheritance factor was valid, then there should exist in the surviving F_9 individuals units of inheritance which would represent: 4/5; 3/5; 2/5; 1/5 and 0/5 of the total susceptibility factor. The possibilities presented here are in reality a binomial expansion problem. A diagramatic representation of this binomial with numerals representing the possible and available chromosome(s) and the letter X the probable distribution of the involved histocompatibility chromosome(s) for tumor susceptibility is shown in table 2. The F_{10} tumor take $1.00>p<$ 0.99 (table 1) verified that the probable distribution of the histocompatibility sites in F_9 survivors did match and indeed coincide with the graphic representation given in table 2.

TABLE 2

Matrix illustrating possible combinations for a 5 factor distribution.

1										
2										
3	1/32									
4										
5										
1	1	1	1	X*						
2	2	2	X	2						
3	3	X	3	3	2/32					
4	X	4	4	4						
X	5	5	5	5						
1	1	1	X	1	1	X	X	X	1	
2	2	X	2	2	X	2	X	2	X	
3	X	3	3	X	3	3	3	X	X	4/32
X	4	4	4	X	X	X	4	4	4	
X	X	X	X	5	5	5	5	5	5	
1	1	X	1	X	X	1	X	X	X	
2	X	2	X	2	X	X	2	X	X	
X	3	3	X	X	3	X	X	3	X	8/32
X	X	X	4	4	4	X	X	X	4	
X	X	X	X	X	X	5	5	5	5	
X	X	X	X	1						
X	X	X	2	X						
X	X	3	X	X	16/32					
X	4	X	X	X						
5	X	X	X	X						
X										
X										
X	1/32									
X										
X										

*X marks the linkage group(s) which could possibly be carrying the factors for tumor histocompatibility. Numbers represent non-histocompatible sites. The fraction indicates the possible number of combinations for each group in that set which would result in tumor histocompatability being achieved.

As mentioned earlier, there exist 15 different two-directional allelic histocompatibility sites between the 2 strains of mice employed in this study. At the maximum, however, only 8 incompatibilities would be involved in the take frequency of this tumor. Although most of the histocompatibility groups have been assigned linkage groups (Snell and Stimpfling 1966; Green 1971; Altman and Dittmer 1972), several histocompatibility sites have not. The results obtained in our work indicate that all the histocompatibility sites involved belong to no more than 5 linkage groups.

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