[CANCER RESEARCH 35, 1586-1590, June 1975]

H-2-linked Genetic Control of Resistance to Histocompatible Tumors[1]

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SUMMARY

The role of the major histocompatibility complex of the mouse (*H-2*) in resistance to two transplanted histocompatible tumors was evaluated by determining the differences in survival times between the syngeneic parent strain and various F_1 hybrids. C57BL/10nSn (B10) mice and their F_1 hybrids were given injections of a methylcholanthrene-induced fibrosarcoma of B10 origin. The B10 × B10.BR F_1, B10 × B10.M F_1, B10 × B10.WB F_1, and B10 × 5R F_1 hybrids survived significantly longer than the B10 parental strain or B10 × B10.D2 F_1 and B10 × 18R F_1 animals, while B10 × 2R F_1 mice succumbed significantly sooner than any of the above groups. Statistical comparisons of geometric mean survival times of the strain of tumor origin (B10) *versus* the F_1 hybrids showed the influence of genes coded for the *H-2* complex in the phenomenon, termed "hybrid resistance" or "allogeneic inhibition." However, tumor resistance did not occur in all hybrids and could not be attributed to a single dominant Ir gene localized in the *I* region as might be predicted if the phenomenon involved genetic control of immunological responsiveness to tumor-specific transplantation antigens. Similarly, in a second group of experiments, the mean survival times of DBA/2 × B10.D2 F_1 animals given injections of the P815 mastocytoma of DBA/2 (D2) origin was compared to the mean survival times of various hybrids with the D2 parent. Again, the results demonstrated the importance of the *H-2* gene complex in this phenomenon. However, the above results did not permit precise localization of the *H-2*-linked gene(s) responsible for differential resistance to the histocompatible tumor.

INTRODUCTION

The *H-2* gene complex, which codes for the major histocompatibility system of the mouse, has long been of interest to immunologists and tumor biologists because it was shown to be critically involved in the limitations to transplantation of cells and tumors in allogeneic systems (7). More recently, the complexity of the products and cellular reactivities controlled by the genetic system has been partially resolved to the extent that over a dozen polymorphic traits have been shown to be controlled by the *H-2* complex and closely associated genes (5, 17). Among such traits are several that have potential relevance to tumor biology; these include transplantation antigens (5, 11), mixed lymphocyte reactivity (1), the control of specific immune responsiveness (2), and susceptibility to viral-induced leukemogenesis (13). If one accepts the overwhelming body of information to support the conclusion that mammals have natural mechanisms that provide protection against spontaneous, induced, or transplanted neoplasms, then the study of the genetic controls over these phenomena should include an investigation of the possible role of the *H-2* region.

We began a series of experiments to examine histocompatible tumor resistance based on the simple hypothesis that histocompatibility-linked immune response genes control the ability to respond to tumor-specific antigens. The hypothesized existence of such histocompatibility-linked Ir genes for tumor-specific antigens could explain the well-documented phenomenon that syngeneic tumors grow less well in F_1 hybrids even though there are no histocompatibility barriers as defined by the classical laws of transplantation. The hypothesis was based on the concept that, regardless of the precise mechanism of oncogenesis, tumors with unique tumor-specific antigens would tend to escape immunological defenses more readily if the host lacked the appropriate histocompatibility-linked Ir gene(s) for the specific antigen(s) of the tumor. The F_1 hybrid would contribute a new repertoire of histocompatibility-linked Ir genes and thus would be more likely to have the appropriate dominant Ir gene for a response to an important tumor-specific antigen. Indeed, the superior histocompatible tumor resistance of F_1 hybrid mice, known as hybrid resistance or allogeneic inhibition, had already been shown to be controlled by the *H-2* complex (9). In addition, the observation by Lilly (12) that the induction of leukomogenesis by Gross virus is partially controlled by the *Rgv-1* gene, which mapped in the K end, *i.e.*, the *K* and *I* regions of the *H-2* complex, could also be explained by postulating that *Rgv-1* was an immune response gene for the tumor antigen(s) of the virally induced leukemia. Recent work by Sato *et al.* (16) supports such a conclusion. Since all carefully mapped *H-2*-linked Ir genes are located in the *I* region of the *H-2* complex, we sought to explore the possibility of *I*-region-determined control of tumor resistance based on the genetics of differences in MST's[3] when various F_1 hybrids

[1] Supported by National Cancer Institute Grant CA 14723.

[2] Recipient of NIH Grant NS 11231 and a grant from the National Multiple Sclerosis Society.

Received December 16, 1974; accepted March 11, 1975.

[3] The abbreviation used is: MST, mean survival time.

are given a lethal inoculation of tumor that originated and was passaged in 1 parental strain. These experiments have led us to the conclusion that simple *I*-region control of tumor resistance via immune response genes, while presumably present and important in selected instances (16, 18), cannot adequately explain the results obtained with all tumors.

MATERIALS AND METHODS

The methylcholanthrene-induced fibrosarcoma used in these studies was kindly supplied by Dr. Richard Smith. University of Florida, who induced it in a female C57BL/10 mouse and supplied it to us in an early passage. The subline that we used, designated MB10.2, was the 2nd passage after a brief period of frozen storage. The P815 tumor, a long-passaged mastocytoma of DBA/2 origin, was obtained from Dr. Eric Martz (Department of Pathology, Harvard Medical School) and was passaged approximately 15 times in DBA/2J mice by us prior to use.

DBA/2J (D2), C57BL/10 nSn (B10), B10.BR, B10.D2, B10.A, A/J, C3H × D2 F_1, and C57BL/6 × DBA/2 F_1 (hereafter called B6D2F_1) mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. All other strains were maintained by brother-sister matings in our animal colony. With the exception of B6D2F_1 and C3H × D2 F_1 animals, all F_1 hybrids were bred in our animal facilities.

The MB10.2 tumor was dispersed by trituration in serum-free medium, and 5000 viable cells in a 0.1-ml volume were injected s.c. in the flank. One thousand P815 cells were injected i.p. in a 1.0-ml volume. Injections for each experiment were made from the same suspension of tumor cells, and the viability was determined not to have changed between the beginning and the end of the injection period. Within each experiment a few animals of different genotypes were randomly chosen for injection so that some animals of each genotype were given injections at virtually the same time. Animals were housed approximately 4 to 8 animals per cage and were checked daily for deaths. In most cases, normal, untreated animals were added to cages in which only 1 or 2 survivors remained. The geometric mean day of death (MST) for animals of each genotype was calculated and exact p values are given in the tables for a comparison to the defined low-resistance strain, which was identical to the tumor at the *H-2* locus. p values of <0.01 are considered significant regardless of the absolute differences in MST. The absolute magnitude of the MST for any particular recipient strain can be increased by changing the size or route of injection of the tumor inoculum, but the significance of differences remains similar (unpublished observations). In cases in which certain hybrid animals survived longer than 81 days, this value was arbitrarily used in the calculations for MST of that group.

RESULTS

All animals given injections of the MB10.2 fibrosarcoma developed nodules at the injection site after approximately 2 weeks. In most cases, progressively growing tumors enlarged in size until the death of the host. Most hybrids that survived past 81 days had no apparent tumors but, since early nodules were not biopsied, actual tumor regression among long-term survivors can only be inferred. There were also significant differences in MST between B10 and each otherwise syngeneic *H-2* hybrid when long-term survivors were excluded from the calculations.

Results of 3 separate experiments designed to compare homozygous $H\text{-}2^b$ B10 animals to *H-2* hybrids with 1 entire *H-2* haplotype distinct from $H\text{-}2^b$ are presented in Table 1. Syngeneic controls were included in each experiment to permit comparisons between groups. Differences in MST between control groups of separate experiments reflect such biological variables as the condition of the tumor. It is important to add that statistically significant differences ($p < 0.01$) could be reproduced in separate experiments. Of the hybrids tested, those carrying the $H\text{-}2^k$, $H\text{-}2^f$, or $H\text{-}2^{ja}$ haplotypes in heterozygous form with $H\text{-}2^b$ survived significantly longer than B10 animals when given injections of the $H\text{-}2^b$ tumor MB10.2. In contrast, $H\text{-}2^b/H\text{-}2^a$ heterozygous B10 × B10.D2 F_1 mice did not demonstrate a statistically significant increased survival time. In addition, 16 C57BL/10 × A F_1 animals that bear additional non-*H-2* genes contributed by the A/J parent were given injections of MB10.2. These mice had a MST of 47.2 days compared to 42.4 days for B10 animals ($p < 0.05$; Table 1).

In Chart 1, survival of B10 animals is compared to that

Table 1

Survival of B10 and various F_1 hybrid animals after s.c. injection of 5000 viable MB10.2 fibrosarcoma cells of B10 origin

Experiment	Strain	n	*H-2*	MST (days)	p[a]	Survivors at 81 days
1	B10	36	*b/b*	36.5		0
	B10 × B10.BR F_1	26	*b/k*	50.8	0.0003	7
	B10 × B10.M F_1	33	*b/f*	59.1	<0.000001	15
2	B10	22	*b/b*	42.4		0
	B10 × B10.WB F_1	18	*b/ja*	64.1	0.000002	10
	B10 × A F_1	16	*b/a*	47.3	0.043	0
3	B10	25	*b/b*	31.8		0
	B10 × B10.D2 F_1	27	*b/d*	36.44	0.053	0

[a] Student's t test.

for hybrids produced between B10 females and males with various recombinant *H-2* alleles. Haplotype differences between the hybrids and B10 animals are depicted diagramatically. B10 × 5R F_1 animals survived significantly longer than B10's, and there were 5 animals in which the tumor presumably regressed. Survival of B10 × 18R F_1 animals was indistinguishable from that of B10 mice, while B10 × 2R F_1 animals succumbed significantly sooner than B10 or any of the other injected groups.

Data from 2 experiments designed to determine the role of various *H-2* alleles in resistance of animals to death after injection of the P815 (*H-2*^{d}) mastocytoma are summarized in Table 2. In both experiments, D2 × B10.BR F_1 or C3H × D2 F_1 (*H-2*^{d}/*H-2*^{k}) animals failed to survive longer than D2 × B10.D2 F_1 (*H-2*^{d} homozygous) mice. In contrast, B6D2F_1 or D2 × B10 F_1 (*H-2*^{d}/*H-2*^{b}) and D2 × B10.S F_1 (*H-2*^{d}/*H-2*^{s}) animals survived significantly longer than either *H-2*^{d}/*H-2*^{d} or *H-2*^{d}/*H-2*^{k} animals. D2 × B10.D2 F_1 hybrids were used as controls for these experiments to rule out any contributions of non-*H-2* genes derived from the B10 background. There were no statistically significant differences in MST of the parental DBA/2 strain and the D2 × B10.D2 F_1 hybrids (p = 0.062).

An attempt to analyze the role of various regions of the *H-2* locus in determining relative resistance to the P815 mastocytoma is reflected by the data in Chart 2. MST's of all hybrid mice are compared to those for D2 × B10.D2 F_1 mice. Only D2 × 5R F_1 and D2 × B10.BR F_1 mice failed to survive significantly longer than D2 × B10.D2 F_1 mice. The significance of the increased MST of D2 × B10.A F_1 mice was only marginal (p = 0.282), particularly in view of the fact that large numbers (n = 38) of such animals were available for comparison to D2 × B10.D2 F_1 mice (n = 24). B6D2F_1 and D2 × 18R F_1 hybrids all survived significantly ($p < 0.01$) longer than the *H-2*^{d} homozygous controls. Only 6 D2 × 4R F_1 animals were available for study, and the significance of the difference in their MST compared to D2 × B10.D2 F_1 was less than 2% (p = 0.0162) despite the small value of n.

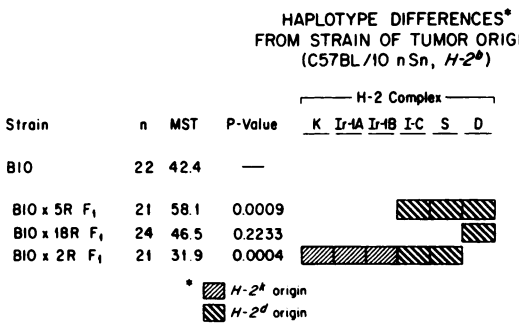

Chart 1. Survival of C57BL/10nSn (B10) mice and various F_1 hybrids with *H-2* recombinant fathers after injection with 5000 viable MB10.2 fibrosarcoma cells (B10 origin). The haplotype differences of the hybrids compared to the strain of tumor origin (B10) are depicted diagramatically.

DISCUSSION

The phenomenon of increased resistance of F_1 hybrids to the growth of injected histocompatible tumor has been known for many years, and some early work with *H-2* congenic strains demonstrated that *H-2* complex genes played an important role in this phenomenon (8). It was not generally appreciated, however, that only specific hybrid combinations manifest such so-called allogeneic inhibition or hybrid resistance with respect to a single tumor and that not all hybrids were equally as resistant to the same tumor. This report demonstrates that differences at certain *H-2* complex genes specifically confer resistance to a histocompatible tumor, while other *H-2* alleles lack this characteristic. Thus, B10 × B10.BR F_1, B10 × B10.M F_1, and B10 × B10.WB F_1 hybrids survived longer than the parental B10 animals or the B10 × B10.D2 F_1, B10 × 18R F_1, and B10 × 2R F_1 hybrids when given injections of MB10.2 tumor. Likewise, D2 × B10 F_1, B6D2F_1, and D2 × B10.S F_1 mice survived longer than the D2 × B10.D2 F_1 control animals when given injections of the P815 tumor. Thus, *H-2* heterozygosity or the presence of "foreign" *H-2* gene products is an inadequate explanation for the presumably suppressed tumor growth in more resistant hybrids.

The data presented in this report were obtained with 2 distinct tumors in different genetic hybrid combinations. Congenic mice were used in most of these experiments to

Table 2

Survival of various DBA/2 (D2) hybrids given injections of 1000 viable P815 mastocytoma (D2 origin) cells

Experiment	Strain	n	*H-2*	MST (days)	p[a]
1	D2 × B10.D2 F_1	29	*d/d*	30.1 ± 1.3[b]	
	D2	42	*d/d*	26.8 ± 1.3	0.062
	D2 × B10.BR F_1	28	*d/k*	28.8 ± 1.0	0.305
	C3H × D2 F_1	24	*d/k*	28.3 ± 1.7	0.085
	D2 × B10 F_1	20	*d/b*	35.7 ± 2.4	<0.0001
	B6D2 F_1	26	*d/b*	49.7 ± 2.5	<0.0001
	D2 × B10.S F_1	22	*d/s*	37.6 ± 2.3	<0.0001
2	D2 × B10.D2 F_1	24	*d/d*	22.7	
	D2 × B10.BR F_1	12	*d/k*	21.8	0.537

[a] Student's t test. The D2 × B10.D2 F_1 hybrid was used as the standard for statistical comparison to control for any influences attributable to non-*H-2* genes of the B10 congenic partner.

[b] Mean ± S.D.

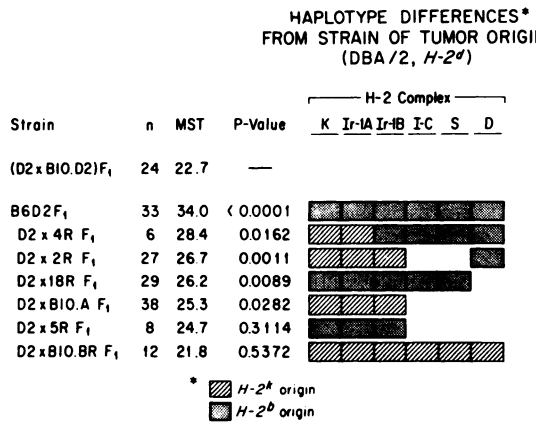

Chart 2. Survival of various DBA/2 (D2) hybrids after injection with 1000 viable P815 mastocytoma cells. The haplotype differences of the hybrids, which are heterozygous at portions of the *H-2* complex compared to the $H\text{-}2^d$ homozygous D2 × B10.D2 F_1 animals, are depicted diagramatically.

minimize the possible influences of non-*H-2* genes. However, similar patterns of tumor resistance could be obtained using F_1 hybrids of standard strains (Table 2). The data further indicate that in these systems simple *I* region genetic control is inadequate to explain the observed hybrid resistance. It is apparent, for example, that B10 × 5R F_1 animals were significantly ($p < 0.001$) more resistant to the MB10.2 tumor than were homozygous $H\text{-}2^b$ B10 animals. Since 5R and B10 share the *K*, *Ir-1A*, and *Ir-1B* subregions of the *H-2* complex, this difference cannot be attributed to a single dominant gene (immune response or otherwise) localized in the same region of the chromosome that codes for all currently mapped immune response genes (2). The increased resistance of the B10 × 5R F_1 hybrids is surprising since the recombinant $H\text{-}2^{i5}$ haplotype carried by the 5R strain consists of segments of the $H\text{-}2^b$ and $H\text{-}2^d$ haplotypes, neither of which is associated with significantly increased tumor resistance (Table 1). These results suggest localization of the responsible genes outside the *H-2* complex or the occurrence of genetic interaction between the *K* and *D* ends of the $H\text{-}2^{i5}$ haplotype. Interestingly, we have recently demonstrated genetic interaction in the $H\text{-}2^{i5}$ haplotype between 2 genes that control immune responsiveness to certain synthetic polypeptide antigens (6). In addition, the lack of hybrid resistance in the B10 × 18R F_1 mice to the MB10.2 tumor suggests that the *D* region gene(s) difference is not significant to confer tumor resistance.

The B10 × 2R F_1 group represents an example of less tumor resistance by F_1 hybrids when compared to the homozygous strain of tumor origin. This result is difficult to explain and also suggests the interaction of several genes in this phenomenon since the *H-2* complex of 2R contains the *K*, *Ir-1A*, and *Ir-1B* subregions of $H\text{-}2^k$, a known resistant *H-2* allele (see Table 1), and the *I-C* and *S* subregions of another known resistant hybrid B10 × 5R F_1. In light of the increasingly apparent number of *H-2*-complex-associated genes that are related to immunological functions (17), it is likely that so-called "immune" parameters of tumor resistance may be influenced by more than 1 gene in this region. While the mechanism of action of such genes remains to be determined, the possibility of enhanced tumor growth through suppression of anti-tumor-immune responsiveness must also be considered.

An analysis of resistance patterns of various D2 hybrids given injections of the P815 tumor likewise does not allow assignment of this trait to a single dominant *I*-region gene. For this tumor, the D2 × B10.BR F_1 and D2 × 5R F_1 animals failed to survive longer than the $H\text{-}2^d$ homozygotes (Chart 2). Marginally significant differences were noted with the D2 × B10.A F_1 ($p = 0.028$) and D2 × 4R F_1 hybrids ($p = 0.016$) with the D2 × B10.D2 F_1 controls. In contrast, the D2 × 2R F_1 and D2 × 18R F_1 hybrids demonstrated highly significant tumor resistance (Chart 2). The data do not permit identification of a single genetic region that confers tumor resistance.

The fact that B6D2F_1 animals were much more resistant than animals that share many portions of the $H\text{-}2^b$ complex may conceivably be due to other factors. These could include the lack of a DBA/2 maternal influence and perhaps the presence of C57BL/6 as opposed to C57BL/10 non-*H-2* loci. Indeed, D2 × B10 F_1 animals were significantly more resistant than D2 × B10.D2 F_1 mice, but they did not manifest the dramatic differences noted with the B6D2F_1 animals. In a separate experiment (Table 2), we observed an MST of 49.83 days in the B6D2F_1 hybrids compared to 35.71 days in D2 × B10 F_1 mice ($p < 0.0001$). Further studies concerning the role of non-*H-2* genes in histocompatible tumor resistance will be presented in a separate report (R. M. Williams, in preparation).

Taken together the results of these studies concerning the role of *H-2* complex genes in the phenomenon of enhanced histocompatible tumor resistance by F_1 hybrids support 2 general conclusions: (*a*) there are several cases in which heterozygosity at part or all of the *H-2* complex does not confer relative resistance; (*b*) relative resistance to the fibrosarcoma and mastocytoma used here cannot be attributed to single *I*-subregion genes. Detailed analysis of our results with these tumors do not rule out the participation of more than 1 *H-2* or closely associated genes. Although precise localization of the genes involved is not possible at this time, D end genes or other genes outside of *H-2* but still in the same linkage group, may be important for control of histocompatible tumor resistance. In this regard, an analogy can be drawn to several other *H-2*-influenced phenomena that may be controlled by several genes. These include the mixed-lymphocyte reaction, graft-*versus*-host reaction, and the phenomenon of irradiated hybrid resistance to parental bone-marrow grafts (1, 3–5, 10, 17). In view of the facts that individual tumors may have numerous antigens (14, 15) and that there are numerous ways in which the growth of histocompatible cells may be retarded, it is not unreasonable to assume that several *H-2* complex genes may participate in tumor resistance through more than 1 mechanism. Our results with the particular tumors used in no way preclude the possibility of *I*-region immune-response control of specific antitumor responses in other systems, particularly those in which oncogenic viruses may be involved, such as the *Rgv-1* gene (16).

ACKNOWLEDGMENTS

We thank Barbara Gricus for her excellent assistance in the preparation of the manuscript.

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