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H-2 Gene Control of Resistance to P815-X2 Mastocytoma[1]

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ABSTRACT

Some, but not all, mutations at *H-2* loci can alter, in either direction, histocompatible tumor resistance in the model system of P815-X2 mastocytoma (DBA/2 origin) transplanted into DBA/2J or (C57BL/6 × DBA/2J)F_1 mice. This hybrid effect has been shown previously to depend on the immune system. We have examined eight mutants at *H-2K*^{b} (*bm1*, *bm4*, *bm6*, *bm7*, *bm8*, *bm10*, *bm11*, and *bm16*, two at *H-2D*^{b} (*bm13* and *bm14*), and one *I-A*^{b} mutant (*bm12*). When one parent is an *H-2*^{b} mutant (C57BL/6 mutant), the hybrid progeny (C57BL/6 mutant × DBA/2J)F_1 may have increased (*bm7* and *bm8*) or decreased (*bm10*, *bm11*, *bm12*, *and bm16*) survival times when compared to (C57BL/6 × DBA/2J)F_1 controls. Hybrids from *bm1*, *bm4*, *bm6* (all *H-2K*^{b}), and *bm13* and *bm14* (both *H-2D*^{b}) mutants showed no differences in survival. Several of the mutant molecules differ from those of the parental strain by only one or two amino acids. Apparently, these small changes in H-2 antigens are capable of altering resistance to a histocompatible tumor to produce an immune response gene-like effect.

The availability of *H-2* mutant strains and detailed data on the molecular nature of the gene products make the model presented in this report a particularly useful system to study.

INTRODUCTION

The major histocompatibility system of the mouse, *H-2*, originally of interest to immunologists primarily because of its role in tissue graft rejection, has grown to provide results of major importance for both general immunobiology and immunoregulation. A substantial number of traits appear to be controlled by the *H-2* system (6), several of which have potential relevance to tumor biology; *e.g.*, rejection of tumors in allogeneic systems. It is also widely accepted that genetic factors may be important in determining the ability of the host to mount a specific immune response to a neoplasm, but the particular genetic contributions remain unclear, primarily because of a dearth of good nonallogeneic experimental models.

The phenomenon of resistance to histocompatible tissue in F_1 hybrids has been studied for many years. The 2 basic systems are transplantation of nonmalignant parental-type bone marrow cells or the transplantation of malignant tumors to F_1 hybrids. The former system, referred to as hybrid resistance or the hematopoietic histocompatibility phenomenon, has been studied and described in detail by Cudkowicz *et al.* (2–4). It is most easily demonstrable in irradiated F_1 hybrid mice given injections of parental bone marrow and is thought to be associated with radioresistant natural killer cell activity. The latter system, the phenomenon under study in this paper, has been called allogeneic inhibition, syngeneic preference, or, more simply, the "hybrid effect" (5, 19), and has been of interest to tumor immunologists and immunogeneticists because of its potential for unraveling some mechanisms of tumor resistance. As has been described previously parental tumors often grow less well in their F_1 hybrids than in the strain of origin even though the F_1 hybrid recipients and parental strain tumors are theoretically histocompatible. This hybrid effect has been shown to be linked to the *H-2* complex (5) and, in our system, it can be transplanted into lethally irradiated parental-strain mice with anti-Thy-1 + complement-treated F_1 bone marrow, strongly suggesting that the mechanism of the hybrid effect in our model involves the immune system (21). The working hypothesis is that the superior histocompatible tumor resistance of F_1 hybrids is due to the contribution of immune response genes by the allogeneic parent which might heighten immune responsiveness against weak tumor-associated antigens.

Using *H-2* congenic lines and *H-2* recombinant strains available at the time, we initiated a series of experiments in 1975 to examine the role of the *H-2* complex genes in histocompatible tumor resistance (20). Results from these experiments suggested that more than one *H-2* gene was involved and led to the conclusion that tumor resistance in this model could not be attributed to simple I-region control via a single dominant immune response gene. However, the material available for the studies at that time did not permit the precise localization of the *H-2* genes involved.

The recent introduction of *H-2* mutants provides a unique approach to investigate the effects of specific *H-2* genes on immune functions. Using mouse lines bearing mutations in the *H-2*^{b} haplotype, alterations in a single gene arising from various point mutations, single genes can be examined for effects on tumor resistance. Realizing this opportunity, we initiated studies in the model system of P815-X2, a lethal mastocytoma of DBA/2 origin, transplanted into DBA/2 and (DBA/2 × C57BL/6)F_1 or (DBA/2 × C57BL/6 mutant)F_1 hybrids. By comparing the survival of (DBA/2 × C57BL/6)F_1 and (DBA/2 × C57BL/6 mutant) F_1 mice after injection of P815-X2, where the C57BL/6 mutant differs from C57BL/6Kh only at *H-2K*^{b}, we found that differences in the *H-2K*^{b} gene alone could profoundly influence survival (22). The results we present here represent a broad sampling of the *H-2* complex using almost all of the available *H-2*^{b} mutants and extend those initial observations. Because biochemical studies reveal differences of only one or 2 amino acids between mutant and wild-type H-2K^b molecules (16), the data presented demonstrate that subtle amino acid changes in a single gene product of the major histocompatibility complex can influence histocompatible tumor resistance.

MATERIALS AND METHODS

Tumor Preparation. The P815-X2 tumor, a long-passaged mastocytoma of DBA/2 origin, was obtained from Dr. Eric Martz (University of

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Massachusetts, Amherst, Mass.) and was passaged in excess of 15 times in DBA/2J mice prior to cryopreservation. For each experiment, 5 × 10^6 viable tumor cells from this cryopreserved stock were thawed and injected i.p. for a single passage of 13 days duration in a DBA/2J animal.

Mice. Two inbred mouse strains, DBA/2J and C57BL/6Kh, were used. The former line was obtained from The Jackson Laboratory, and the latter was from our colony at Northwestern University. All of the known *H-2* mutations were selected by screening for unexpected graft rejection and frequently occurred without dramatic serologically detectable alterations in cell surface specificities (7). Most of the mutations have been derived from the *H-2K*^{b} gene, and many of these mutant antigens have been biochemically characterized (16). All mutant strains are congenic or coisogenic on the C57BL/6Kh background. Shown in Table 1, they consist of 8 mutations of *H-2K*^{b}, 2 of *H-2D*^{b}, and one of *I-A*^{b}. For simplicity, the mutants are referred to in the text by their superscripts. All F_1 hybrids were bred in our animal facilities; breeders from mutant strains *bm1*, *bm4*, and *bm14* were originally received by courtesy of D. W. Bailey and maintained thereafter in our facility.

Injection of Tumor. Hybrid progeny, about 10 weeks of age and age-matched to within approximately 21 days of each other, from matings of C57BL/6Kh or C57BL/6 mutant females with DBA/2J males were given injections of 10^6 viable P815-X2 tumor cells in a 0.05-ml volume s.c. through the left rear footpad using a separate 30-gauge needle for each mouse. Injections for each experiment were made from the same suspension of tumor cells, which had been passaged once in a DBA/2J mouse.

Analysis of Data. Mice were checked daily to determine the date of death. The statistical comparisons were done using the Mantel-Cox logrank test (17), and p values of <0.05 are considered significant regardless of absolute differences in the median survival times.

RESULTS

Growth of Tumor and Differential Resistance to Tumor. All animals developed swelling of the left rear footpad shortly after injection of the tumor and the progressively growing tumors enlarged in size until death of the host. Unequivocal differences in survival were found between (C57BL/6Kh × DBA/2J)F_1 (hereafter called B6D2F_1) and some (C57BL/6 mutant × DBA/2J)F_1 (hereafter called B6^{m}D2F_1) hybrid progeny. The earliest deaths, which were usually in the syngeneic control group (DBA/2J), occurred around Day 14, and all animals were dead by Day 60. There were no long-term survivors.

Results are based on the analysis of survival of female hybrids only, because surprisingly significant differences in survival were also found between F_1 hybrid females and males. This sex effect is possibly due to an X-linked histoincompatibility to the P815-X2 tumor (*H-X*^{d}) in male F_1 hybrids; within a single strain, it is always the male hybrids that survive longer than the female hybrids. As would be predicted, no such effect was observed for homozygous DBA/2J controls. This apparent response to H-X^d is described in detail in a separate report (9).

It is important to note that differences in the absolute magnitudes of the median survival times between identical strains in separate experiments may reflect numerous variables and, as such, cannot be legitimately compared.

Genetics of Resistance to P815-X2. Hybrid progeny of 5 of the 8 tested mutants at *H-2K*^{b} and the *I-A*^{b} mutant, *bm12*, differed significantly ($p < 0.05$) in resistance to the tumor when compared to B6D2F_1 controls. Furthermore, the differences in survival relative to B6D2F_1 determined by these mutations were in both directions; *i.e.*, either longer or shorter survival, depending on the mutation.

Hybrids from *bm7* and *bm8* demonstrated significantly increased survival times compared to controls (Table 2).

In contrast, hybrids from *bm10* (Table 2) [*bm11*, *bm12*, and *bm16* (Table 3)] demonstrated shorter survival times compared to controls. The results from *bm11* and *bm12* hybrids confirm the significantly shorter survival times associated with these 2 mutations observed in our initial studies (22).

Of special interest are the results with *bm10* hybrids. The single amino acid change of Val → Met at Residue 165 (and possibly one additional amino acid change) in the H-2K molecule of *bm10* (Table 1) was associated with disappearance of the hybrid effect altogether; *i.e.*, *bm10* hybrid progeny showed no advantage in survival over homozygous DBA/2J controls after injection of the tumor ($p = 0.852$) (Table 2).

Hybrids from *bm1*, *bm4*, *bm6*, and both *H-2D*^{b} mutants (*bm13* and *bm14*) failed to exhibit significant differences in survival when compared to B6D2F_1 controls (Tables 2 and 3).

Table 1

Genetic mapping and biochemical analysis of the H-2^b *mutants*

Mutant	Locus and allele	CNBr fragment	Amino acid position	Amino acid interchange	
bm1	K^b	Ia	155	Arg → Tyr	(16)
			156	Leu → Tyr	
bm3[a]	K^b	Ib	77	Asp → X	(16)
			89	Lys → X	
bm4	K^b	Ia			(16)
bm5[a]	K^b	Ib	116	Tyr → Phe	(16)
bm6	K^b	Ib	116	Tyr → Phe	(16)
			121	Cys → Arg	
bm7[b]	K^b	Ib	116	Tyr → Phe	(16)
			121	Cys → Arg	
bm8[b]	K^b	IIIn	23	Met → X	(16)
bm10[a]	K^b	Ia	165	Val → Met	(16)
		Ia			
bm11[a]	K^b	Ib	77	Asp → X	(16)
bm12[a]	$I\text{-}A^b$				(10)
bm13	D^b				(15)
bm14	D^b				(15)
bm16[a]	K^b	Ib	116	Tyr → Phe	(16)

[a] Hybrids from *bm3* and *bm5* (described previously in Ref. 22) and *bm10*, *bm11*, *bm12*, and *bm16* (Tables 2 and 3) showed shorter survival times compared to those of B6D2F_1 controls.

[b] Hybrids from *bm7* and *bm8* showed longer survival times compared to those of B6D2F_1 controls (Table 2).

DISCUSSION

Our studies in the P815-X2 hybrid effect model support 2 overall conclusions. (*a*) Some, but not all, modifications in *H-2*^{b} gene products are capable of modifying the resistance to histocompatible tumor (for summary of results, see Table 1, footnotes). These experiments implicate the independent roles of more than one *H-2* gene in the hybrid effect. (*b*) The results from these studies offer unexpected evidence for the role of Class I molecules in controlling immune responses, since immune response genes are generally thought to be localized to the I-region of the *H-2* complex. There are at least 4 other reports of *H-2*-linked immune response genes which map outside of the I-region (8, 18, 23, 24). Experiments with hybrid progeny from *bm12*, the only existing I-region mutant, nonetheless suggest that I-region immune-response control of histocompatible resistance to P-815-X2 is also present and important (Table 3) (22).

Several points concerning the results deserve further comment. Two *H-2*^{b} mutant strains, *bm7* and *bm8*, when compared

Table 2
P815-X2 resistance in (C57BL/6Kh or C57BL/6 mutant) × DBA/2J hybrids (females): Experiment 1

Mother	No. tested	Survival (days) Median	Survival (days) Mean ± SE	Difference from C57BL/6Kh (*p*)	Difference from DBA/2J (*p*)
DBA/2J	19[a]	22	22.4 ± 1.50	0.0002	
C57BL/6Kh	19	28	28.6 ± 2.00		0.0002
B6.C-*H-2*^{bm1}[b]	15	31	32.3 ± 2.00	0.0585	<0.0001
B6-*H-2*^{bm6}	12	29	30.3 ± 1.90	0.2955	0.0004
B6.C-*H-2*^{bm7}	14	33	33.6 ± 2.70	0.0200	0.0001
B6-*H-2*^{bm8}[b]	12	33	35.8 ± 3.20	0.0059	<0.0001
B6.C-*H-2*^{bm10}[b]	23	23	23.5 ± 1.20	0.0002	0.8520
B6.C-*H-2*^{bm14}	20	29	31.7 ± 2.80	0.0774	<0.0001

[a] Includes both female and male (homozygous) DBA/2J since they are identical for *H-X* and in their survival times. For all other strains, male hybrids are not included in the analysis because of an apparent X-linked histoincompatibility (9).
[b] Comparable results were obtained in 2 to 3 separate experiments with similar numbers of mice tested for hybrids from *bm1* (22) and *bm8* and *bm10*.[3]

Table 3
P815-X2 resistance in (C57BL/6Kh or C57BL/6 mutant) × DBA/2J hybrids (females): Experiment 2

Mother	No. tested	Survival (days) Median	Survival (days) Mean ± SE	Difference from C57BL/6Kh (*p*)	Difference from DBA/2J (*p*)
DBA/2J	26[a]	22	21.1 ± 1.50	0.0001	
C57BL/6Kh	15	31	32.7 ± 1.90		<0.0001
B6.C-*H-2*^{bm4}	10	30	31.4 ± 3.00	0.8542	<0.0001
B6.C-*H-2*^{bm11}[b]	19	27	27.8 ± 1.40	0.0072	<0.0001
B6.C-*H-2*^{bm12}[b]	24	27	27.7 ± 1.10	0.0044	<0.0001
B6.C-*H-2*^{bm13}	14	30	31.1 ± 3.10	0.6646	<0.0001
B6-*H-2*^{bm16}	8	27	27.4 ± 1.50	0.0139	0.0117

[a] Includes both female and male (homozygous) DBA/2J since they are identical for *H-X* and in their survival times. For all other strains, male hybrids are not included in the analysis because of an apparent X-linked histoincompatibility (9).
[b] Comparable results were obtained in 1 to 2 separate experiments with similar numbers of mice tested for hybrids from *bm11* and *bm12* (22).

to *H-2*^{b} are associated with longer survival times after injection of histocompatible tumor (Table 2). In the case of *bm7*, this mutant shares identical amino acid changes with *bm6* at positions 116 and 121 of the H-2K molecule (Table 1) and appears to be otherwise indistinguishable from *bm6* by skin grafting and cell-mediated cytotoxicity (12); yet, only *bm7* hybrid progeny were associated with significantly longer survival when compared to B6D2F$_1$ controls. Since *bm6* is coisogenic (recovered in a homozygous C57BL/6Kh) on the C57BL/6Kh strain background while *bm7* is congenic [originally recovered in a (C57BL/6Kh × BALB/c)F$_1$ hybrid], the prolongation of survival seen in *bm7* hybrids may be due to residual non-*H-2* genes derived from the BALB/c strain. We have begun to test this possibility. However, it is unlikely that residual non-*H-2* BALB/c genes contribute significantly to increased resistance to P815-X2, based on the observation that (BALB/cKh × DBA/2J)F$_1$ hybrids do not demonstrate significantly increased (or decreased) survival when compared to homozygous DBA/2J controls after injection with the same preparation of P815-X2 ($p > 0.05$).[3] On the other hand, the *bm8* mutation is coisogenic and, thus, there can be no contamination by genes from other strains. Therefore, we can tentatively conclude that at least one mutation in *H-2K*^{b} results in a strain which is superior (in terms of survival) to the wild type in this F$_1$ hybrid model. The H-2K molecule of this strain has a Met → X change at Residue 23 (Table 1). This apparently unique situation is seen in the only mutant presently known to have a change in the IIIn CNBr fragment of the molecule (Table 1). The relevance of this unique position in the molecule awaits the identification and testing of more IIIn mutants.

Also informative in correlating our hybrid effect studies with the biochemical data is the comparison of *bm5* and *bm16*, which share the single amino acid change Tyr → Phe at Residue 116 of the parental H-2K molecule (Table 1). Hybrid progeny from both *bm5* (described previously in Ref. 22) and *bm16* (Table 3) exhibited shorter survival times compared to wild-type hybrid controls. Similarly, the H-2K molecules of *bm3* and *bm11* share an Asp → X interchange at position 77 (Table 1), and, although "X" is unidentified at present, it is thought to be the same for both mutants based on peptide analysis.[4] The *bm3* mutant contains an additional mutation, Lys → X, at position 89 (Table 1). P815-X2-injected hybrid progeny from both strains demonstrated shorter survival times compared to wild-type hybrid controls based on the present experiments (*bm11*) and those described previously (22) which included both *bm3* and *bm11*.

Finally, although hybrids from both of the available *H-2D*^{b} mutants (*bm13* and *bm14*) failed to show significant differences in survival when compared to B6D2F$_1$ controls, these limited results do not rule out the possible importance of D-region genes (*H-2D*, *H-2L*, *H-2M*, and *H-2R*) in the control of histocompatible tumor resistance.

The novel feature of these experiments is the introduction of increased precision into the analysis of the roles of single *H-2* genes in tumor resistance. Investigations into the mechanisms that account for survival differences among the F$_1$ hybrids after injection with the same histocompatible tumor should be informative. In other words, how do changes as subtle as a single amino acid difference in *H-2* molecules produce such dramatic changes in survival? Reference may be made to other well-known *H-2*-linked phenomena that are controlled by more than one gene. These include, for example, the mixed lymphocyte reaction and the graft-*versus*-host reaction. In consideration of the fact that tumors may have numerous antigens, it is not unlikely that responsiveness to such different antigens may be controlled by different major histocompatibility complex genes having immune responses or immune response-like properties. Whether these immune response-like genes function through independent mechanisms or by some type of genetic interaction remains to be seen.

There are indications in other systems that genes with immune response-like functions might be located outside the I-region of the mouse. Schmitt-Verhulst and Shearer (18) demonstrated that the generation of cytotoxic effector cells to trinitrophenyl-modified self components was under the control of genes mapping in the K- as well as I-regions of *H-2*. Zaleski and Klein (23), using the *H-2* mutant, CBA-*H-2*^{kml}, have shown that one of the genes controlling the plaque-forming cell response to the Thy-1.1 antigen apparently resides in the K-region and might even be identical with the *H-2K* locus. Further studies using *H-2* mutants have revealed genes controlling the primary antibody immune response to Thy-1 antigens in both the K- and D-regions (24). Recently, studies of the regulation of the autoimmune response to mouse thyroglobulin have demonstrated genetic interaction

[3] L. W. Kwak, O. Kucuk, R. Melvold, and R. M. Williams, unpublished data.

[4] S. G. Nathenson, personal communication.

between I-region and D-end gene products (8). Using *H-2* mutants, the model system of hybrid resistance to P815-X2 has potential for producing new insights into the relationship between genes and immune functions in a model pertinent to the regulation of autochthonous tumor growth.

Since the discovery of the first *H-2* mutation 16 years ago (1), *H-2* mutations have made increasing contributions to immunology, (7, 10; for a review, see Ref. 6). For example, *H-2* mutations have been invaluable in studies demonstrating that single loci control responses such as the mixed-lymphocyte reaction and the graft-*versus*-host reaction (6) and particularly as models for the study of T-cell activation (11). The available evidence suggests that most of the *H-2* variants are true mutations which can be attributed to nucleotide substitutions (6, 7, 16).

We are currently investigating the potential roles of C57BL/6 background genes in histocompatible resistance to P815-X2. Construction of F_1 hybrids between recombinant-inbred strains and DBA/2 parents should provide evidence for or against the possible resistant effects of non-*H-2* genes which could interact with the various $H\text{-}2^b$ genes already shown to control the response to histocompatible tumor. The precedent for this type of interaction has already been established in minor histocompatibility antigens (13, 14).

ADDENDUM

After this paper went to press, further evidence became available indicating that the *H-2* variants are germ-line mutations attributable to changes in DNA sequences (25, 26).

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