THE EFFECT OF HISTOCOMPATIBILITY-2 TYPE ON RESPONSE TO THE FRIEND LEUKEMIA VIRUS IN MICE*

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That viruses can induce cancer in experimental animals requires no further proof. However, it is increasingly apparent that, even in the case of neoplasms known to be virus induced, the initial infection of a cell with a "tumorigenic" virus is only one event among a set of events and conditions which culminates in malignant tumors. Until the nature of these various factors can be elucidated, our understanding of viral tumorigenesis and the development of malignancy will remain incomplete.

The analysis of genetic differences between animal hosts of the same species which are, respectively, susceptible to and refractory to the tumorigenic effect of a given virus is an approach which may ultimately aid in defining the essential steps leading to malignancy. Thus, working with the Gross leukemia virus (1), which induces predominantly lymphoid neoplasms of thymic origin in susceptible mouse strains, we have previously shown (2, 3) that a major determinant of susceptibility to Gross virus leukemogenesis is a gene lying within or closely linked to *Histocompatibility-2* (*H-2*), a highly complex locus governing the strongest set of histocompatibility and blood group antigens of the mouse. However, little is known concerning the critical events which occur or fail to occur in Gross virus–susceptible mice during the prolonged period of latency preceding the appearance of leukemia. Therefore, it has not yet been possible to determine the basic mechanism of the *H-2*–associated susceptibility gene.

The Friend leukemia virus (4), in comparison with the Gross virus, induces an entirely different disease syndrome in susceptible mice, with a pronounced splenomegaly resulting from an almost explosive outgrowth of reticuloendothelial elements as the most obvious early symptom, followed eventually by the appearance of cells of a more frankly malignant histological appearance (5). Earlier experiments in our laboratories indicated an association between *H-2*

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type and susceptibility to the Friend disease syndrome (6). The experiments reported here confirm this finding with respect to the F-B strain (7) of Friend virus. The influence of *H-2* type is reflected in (*a*) the threshold virus dose required for the induction of the Friend disease, and (*b*) the incidence of recovery from the early splenomegalic phase of the disease syndrome. These *H-2*–associated effects are dependent upon the presence of a susceptible phenotype with respect to the Friend virus susceptibility gene demonstrated by Odaka (8) and by Axelrad (9), but this latter gene segregates independently with respect to the *H-2* locus.

Materials and Methods

Mice.—All the mice in these experiments were from our own colony of highly inbred mouse strains. The parental inbred strains, with their *H-2* genotypes, were: DBA/2 and BALB/-c-*T*($H\text{-}2^d$), and C57BL/6 ($H\text{-}2^b$). The BALB/c-*T* strain also carries the dominant mutation, *brachy* (*T*), which is linked to *H-2*. In addition, certain F_1 hybrids, as well as segregating backcross and F_2 generation mice, were studied. The mice were usually 6–10 wk old at the time of virus inoculation, and thereafter the degree of their splenomegaly, if any, was determined by palpation at frequent intervals for 2 or more months.

H-2 Typing.—Ascertainment of the *H-2* phenotypes of mice of segregating generations was performed on erythrocytes obtained by retro-orbital bleeding. The method was a modification of the hemagglutination technique of Gorer and Mikulska (10), using isoimmune sera obtained following three or more inoculations of normal lymphoid tissues or tumor cells into the appropriate allogeneic serum donors.

Friend Virus.—The variant F-B strain of Friend virus (7) was used exclusively in these experiments. It differs from the parental F-S virus strain most strikingly in its relative infectivity in mice of the DBA and BALB strains. Whereas the F-B virus strain is about equally infective in DBA and in BALB mice, the F-S parental virus strain is about one hundred times more infective in DBA mice than in BALB mice, according to the spleen focus assay (11). Susceptibility to F-B virus is dominant in crosses involving BALB or DBA mice (susceptible) and C57BL mice (resistant).

Virus preparations were obtained from the greatly enlarged spleens of BALB or DBA mice infected 2–3 wk previously with F-B virus from an isogeneic donor. The spleens were homogenized in nine times their weight of cold phosphate-buffered saline. Slow centrifugation at 4°C removed the large particulate matter from the homogenate, and the supernatant was recentrifuged for 4 min at 7000 *g*. This second supernatant, either as such or after filtration through Selas 02 filter candles, was the basic 1:1 dilution virus inoculum. When assayed in DBA or BALB mice, such preparations contain 3.5 ± 2.0 (SD) $\times 10^4$ focus-forming units (11) per 0.2 ml.

RESULTS

H-2 and the Threshold Virus Dose for the Induction of Friend Disease.—

Intraperitoneal inoculation of 0.2 ml of full-strength virus preparations into susceptible DBA mice or (C57BL × DBA) F_1 hybrids results in a 100% incidence of splenomegaly which is first detectable by palpation at about 7–10 days and which attains its maximal degree at 14–18 days. Recovery from the disease is virtually nonexistent in these mice at this dosage level. C57BL mice, on the other hand, show no significant splenomegaly following this treatment.

Following inoculation of similar full-strength F-B virus preparations in a population of (C57BL × DBA) × C57BL backcross mice, significant splenomegaly appeared in 38 of 81 mice (47%), a proportion consistent with the interpretation that a single genetic locus determines susceptibility or resistance to the virus under these conditions. This result agrees, in general, with the finding of Odaka (8) and Axelrad (9) concerning single gene determination of susceptibility to their respective strains of Friend virus, using other mouse strain combinations and different methods of scoring susceptibility and resistance in individual mice.

At this high dosage level of the virus, no correlation was seen between virus susceptibility (splenomegaly) and *H-2* type: 17 of 41 heterozygous $H\text{-}2^b/H\text{-}2^d$ segregants (42%; P = 0.19) showed splenomegaly, and 21 of 40 $H\text{-}2^b$ homozygotes (52%) showed splenomegaly. From these data, it may be concluded that *H-2* and the gene determining this splenomegalic response to F-B virus segregate independently.

Another group of mice of this same (C57BL × DBA) × C57BL backcross generation was inoculated with 0.2 ml of a 1:10 dilution of the F-B virus preparation. In the population as a whole, 77 of 170 mice (45%) developed splenomegaly, a proportion not significantly different (P = 0.21) from the expected 50%. However, when the correlation between *H-2* type and development of splenomegaly was examined, a significant difference appeared: 42 of 77 $H\text{-}2^b/H\text{-}2^d$ heterozygotes (54%) were susceptible, while only 35 of 93 $H\text{-}2^b$ homozygotes (38%) were susceptible, this latter fraction being significantly different (P = 0.02) from the expected 50%.

A further group of (C57BL × DBA) × C57BL backcross mice were similarly inoculated with a 1:100 dilution of the basic virus inoculum. At this dosage level, only 12 of 69 mice (17%; P < 0.001) showed splenomegaly, and all of these were among the 36 $H\text{-}2^b/H\text{-}2^d$ segregants in the population; no $H\text{-}2^b$ homozygotes showed significant splenomegaly.

The results of these three experiments are illustrated in Fig. 1. Among heterozygous $H\text{-}2^b/H\text{-}2^d$ segregants, the incidence of splenomegaly did not differ significantly from the expected 50% at a 1:1 or a 1:10 dilution of the virus preparation, and this incidence decreased with only minimal statistical significance (P = 0.04) even at a 1:100 dilution of the virus inoculum. By contrast, the expected 50% incidence of splenomegaly among homozygous $H\text{-}2^b$ mice was seen only with full-strength virus preparations; at a 1:10 virus dilution, significantly fewer than 50% of the mice were susceptible, and at a 1:100 dilution, the incidence of splenomegaly decreased to zero.

H-2 and Recovery from Friend Virus–Induced Splenomegaly.—

In the previous section, an animal was considered susceptible to F-B virus if it had shown significant splenomegaly at palpation (representing an estimated minimum increase to four times its normal spleen weight) at any time during the 2 or more months of observation. In this section, our consideration is limited to those animals scored as susceptible by this criterion.

Among the 38 backcross mice which developed splenomegaly following inoculation of full-strength F-B virus preparations, eight mice (21%) recovered from their splenomegaly and regained a normal spleen size either transiently or permanently for the length of the observation period. All eight of these mice were among the 21 homozygous *H-2*^{b} susceptible segregants. This recovery phenomenon was never seen among DBA or (C57BL × DBA) F_1 mice at this level of virus dose.

At the dosage level of a 1:10 dilution of the virus inoculum, 33 of 77 mice (43%) recovered from their splenomegaly. Again, a strong correlation with *H-2* type was apparent: 26 of 35 *H-2*^{b} homozygotes (74%) recovered, while only 7 of 42 *H-2*^{b}/*H-2*^{d}

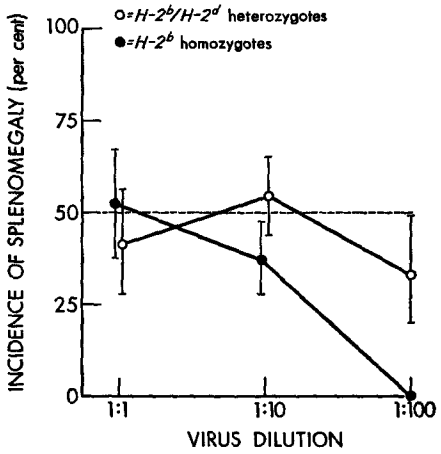

FIG. 1. The incidence of splenomegaly in mice of the (C57BL/6 × DBA/2) × C57BL/6 backcross generation, according to *H-2* type, following inoculation of a 1:1, 1:10, or 1:100 dilution of standard F-B virus preparations.

heterozygotes (17%) did so. When the virus inoculum was reduced to a 1:100 dilution—a dilution at which none of 33 *H-2*^{b} homozygotes showed detectable splenomegaly—7 of the 12 susceptible *H-2*^{b}/*H-2*^{d} heterozygotes (58%) recovered at least transiently.

These data are compiled in Fig. 2, showing the pronounced differences at each dosage level in the recovery rate from F-B virus-induced splenomegaly among the two classes with respect to *H-2* type.

This correlation between *H-2* type and recovery from Friend disease was also seen in another series of experiments, in which segregating generations of the cross BALB/c-*T* (susceptible) × C57BL (resistant) were observed following challenge with full-strength preparations of F-B virus. An additional genetic marker was present and segregating in these crosses: the dominant gene *brachy* (*T*), which causes abnormalities in the tails ("short-tail") of heterozygotes (*T*/+), with a penetrance of about 85–95% in the crosses studied here. *Brachy* is lethal at an early embryonic stage for homozygotes (*T*/*T*). The *brachy*

locus is in the ninth linkage group of the mouse, being linked to *H-2* with a recombination frequency of about 15% in females and 5% in males.

BALB males heterozygous for *brachy* ($H\text{-}2^dT/H\text{-}2^d+$) were mated with normal C57BL females. Short-tail F_1 females ($H\text{-}2^dT/H\text{-}2^b+$) were then crossed with C57BL males to provide the backcross generation, and short-tail F_1 males were mated with normal-tail F_1 females to produce the F_2 generation ($H\text{-}2^d+/H\text{-}2^b+ \times H\text{-}2^dT/H\text{-}2^b+$). 222 backcross and 252 F_2 generation mice were observed for their response to inoculation of full-strength F-B virus, and the results are summarized in Table I.

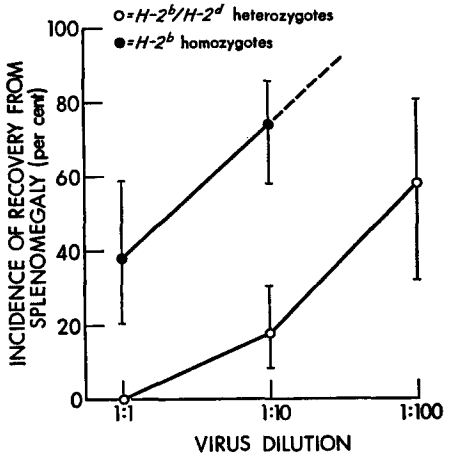

FIG. 2. The incidence of recovery from splenomegaly induced by inoculation of a 1:1, 1:10, or 1:100 dilution of standard F-B virus preparations in susceptible mice of the (C57BL/6 × DBA/2) × C57BL/6 backcross generation, according to *H-2* type. No mice of the homozygous $H\text{-}2^b$ type developed splenomegaly following inoculation of a 1:100 dilution of the virus preparation, and the broken line in the chart indicates that the incidence of recovery among these animals might be expected to approach 100% at about the same virus dilution at which the incidence of splenomegaly among them (Fig. 1) approaches zero.

Analysis of these data reveals that the incidences of splenomegaly corresponded closely to those expected on the assumption that a single genetic locus, independent of *H-2*, determines susceptibility and resistance to F-B virus in these mice.

With respect to recovery from splenomegaly in susceptible segregants from these crosses, this phenomenon was again, as in the C57BL × DBA crosses described above, strongly associated with the homozygous $H\text{-}2^b$ type. In addition, the association of this recovery phenomenon with the absence of the *brachy* trait was almost as pronounced as its association with the homozygous $H\text{-}2^b$ type. Thus, the genetic factor responsible for this ability to recover from splenomegaly was again clearly situated within the region of the ninth linkage group containing *H-2* and *brachy*. Both the incomplete penetrance of *brachy*

and the quantitative nature of the effects of the gene governing recovery from splenomegaly make it impossible to obtain from these data a definite linear ordering of the genes.

Three coat color traits were also segregating in these BALB × C57BL crosses, controlled by the genes *agouti* (linkage group V), *brown* (VIII), and *albino* (I). There was no association between the occurrence of any of these traits and either susceptibility to splenomegaly or recovery from splenomegaly.

TABLE I

Incidence of Splenomegaly and of Recovery from Splenomegaly among Mice of the (C57BL × BALB/c-T) × C57BL Backcross and the (C57BL × BALB/c-T) F_2 Generations, According to H-2 (A) and Brachy (B) Phenotypes

Phenotype	Incidence of splenomegaly		Incidence of recovery among splenomegalic mice	
	No.	%	No.	%
Backcross generation:				
A. $H\text{-}2^b/H\text{-}2^d$	56/112	50	4/56	7
$H\text{-}2^b/H\text{-}2^b$	48/110	44	39/48	81
B. Short-tail	46/93	49	5/46	11
Normal-tail	58/129	45	38/58	66
F_2 generation:				
A. $H\text{-}2^d/H\text{-}2^d$	44/56	79	1/44	2
$H\text{-}2^b/H\text{-}2^d$	92/132	70	2/92	2
$H\text{-}2^b/H\text{-}2^b$	43/64	67	19/43	44
B. Short-tail	86/109	79	3/86	3
Normal-tail	93/143	65	19/93	20

DISCUSSION

These studies of response to the F-B strain of Friend virus indicate that, in the crosses studied, *essential* susceptibility to the induction of splenomegaly at high virus doses is determined by a single gene, with a dominant allele for susceptibility and a recessive allele for resistance, which segregates independently with respect to *H-2*. However, this essential susceptibility is markedly influenced by the host's genotype with respect to *H-2* or a gene closely associated with it. In mice with an essentially susceptible genotype, *H-2* type influences the threshold virus dose required for the induction of splenomegaly and also the prognosis for recovery from splenomegaly at a given dosage level of virus.

It is important to emphasize that the influence of *H-2* in these experiments is expressed as a quantitative character, and that an essentially susceptible

genotype is required for its expression. Thus, with respect to the (C57BL × DBA) × C57BL backcross generation, for example, a high dose of F-B virus can be selected at which the expected 50% of mice of both *H-2* types become splenomegalic, and a low dose of virus can be selected at which no mice of either *H-2* type respond with splenomegaly; however, intermediate doses of virus can be selected at which the incidence of splenomegaly is markedly reduced among $H\text{-}2^b$ homozygotes but not among heterozygous $H\text{-}2^b/H\text{-}2^d$ segregants. The *H-2*–associated gene apparently has no effect in the absence of an essentially susceptible genotype.

It seems likely that the greater rate of recovery from splenomegaly, at a given virus dosage level, among the $H\text{-}2^b$ homozygotes as compared with the $H\text{-}2^b/H\text{-}2^d$ heterozygotes in the population might be a further reflection of the same basic mechanism by which *H-2* influences the virus dose threshold for the splenomegalic response. Whatever this underlying mechanism is, it appears to take effect only *after* cellular infection and virus proliferation have begun, so that it seems unlikely to depend upon the presence or absence of hypothetical virus receptor sites on the cell surfaces.

A more likely hypothesis is that the *H-2*–associated gene influences the ability of the host to stage an effective immunological response to the virus or to virus-induced antigens of infected cells (12). McDevitt (personal communication) has found that the genes governing the level of antibody response in mice to two synthetic polypeptide antigens are closely associated with the *H-2* locus. Similarly, Aoki et al. (13) found natural antibody to the G (Gross) leukemia antigen in homozygous $H\text{-}2^b$ and in heterozygous $H\text{-}2^k/H\text{-}2^b$ segregants but not in homozygous $H\text{-}2^k$ segregants from crosses of AKR ($H\text{-}2^k$, G+) and C57BL ($H\text{-}2^b$, G−). It remains problematic whether such determinants of immunological responsiveness act by direct genetic control of the antibody–combining sites or by some other mechanism. One may speculate, for example, that some isoantigen determined by the $H\text{-}2^d$ allele but not by $H\text{-}2^b$ cross-reacts to some extent with an important Friend virus–induced antigen; thus mice possessing an $H\text{-}2^d$ allele would, by reason of their immunological tolerance for the particular $H\text{-}2^d$ component, be impaired in their ability to respond to the virus-specific antigen, as well.

On the other hand, nonimmunological mechanisms may also be postulated to explain the effect of *H-2* on response to F-B virus. Perhaps the cellular milieu in homozygous $H\text{-}2^b$ mice does not permit replication of the virus as rapidly or efficiently as that in mice of other *H-2* types, so that the host's immunological response is more efficient in eliminating the infection. Alternatively, some difference in interferon production according to *H-2* type could conceivably explain the association.

Our unpublished studies of susceptibility to the F-S strain of Friend virus in the (C57BL × DBA) × DBA backcross and the (BALB × C3H/Bi) F_2 gen-

eration have so far revealed no significant association between *H-2* type and response to this virus strain. However, the homozygous $H\text{-}2^b$ type does not occur in these crosses, and experiments in the (C57BL × DBA) F_2 generation, where it does occur, are now underway to determine the response of $H\text{-}2^b$ homozygotes to F-S virus.

The observations of Fefer et al. (14) concerning the spontaneous regression rates of tumors induced in newborn mice with the Moloney sarcoma virus, which is antigenically related to the Friend virus, may be of a similar nature to the findings reported here. Newborn BALB/c, C57BL/6, and (BALB × C57BL) F_1 mice were all equally susceptible to tumor induction by the virus, but the regression rates of the tumors in the three groups of mice differed greatly: 3% in BALB mice, 24% in the F_1 hybrids, and 47% in C57BL mice.

Studies of the mechanism underlying the association of *H-2* type and response to Friend virus should be greatly facilitated by the use of mouse strains congenic with each other but differing with respect to the *H-2* chromosomal segment. We are currently undertaking a breeding program designed to create a DBA/2-$H\text{-}2^b$ and a BALB/c-$H\text{-}2^b$ strain. The existing congenic pair, C3H/DiSn ($H\text{-}2^k$) and C3H.SW ($H\text{-}2^b$) may also prove useful in this regard.

SUMMARY

Two types of quantitative response to the F-B strain of Friend virus in segregating generations of a cross involving a susceptible (DBA/2 or BALB/c; $H\text{-}2^d$) and a resistant (C57BL/6; $H\text{-}2^b$) mouse strain show a marked correlation with the *H-2* type of the mice. *Essential* susceptibility, as determined by the splenomegalic response to high virus doses, is controlled by a single pair of alleles which segregates independently with respect to the *H-2* locus. However, *relative* susceptibility, as determined by the incidence of the splenomegalic response at moderate or low levels of virus dosage, is significantly greater among mice homozygous or heterozygous for the $H\text{-}2^d$ allele than among $H\text{-}2^b$ homozygotes in these populations. In addition, the incidence of recovery from splenomegaly induced by a given level of virus dosage is significantly greater in $H\text{-}2^b$ homozygotes than in segregants of other *H-2* types among their littermates. Possible mechanisms responsible for these effects are discussed.

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