

# Genetic Factors in Chronic Remittent Friend Disease<sup>1</sup>

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## SUMMARY

C57BL/6 and C57BL/Ks mice possess the alleles *H-2<sup>b</sup>* and *H-2<sup>d</sup>*, respectively. A study of Friend disease in C57BL/6 × DBA/2 F<sub>1</sub> and C57BL/Ks × DBA/2 F<sub>1</sub> mice showed that remittent disease occurred in the former hybrids and progressive disease in the latter. Since both hybrids have the same *Fv-1* and *Fv-2* genotypes the change in the character of the disease was ascribed to differences in the *Rgv-1* gene located near the *H-2* locus. The lymphatic leukemia-inducing virus that acts as helper for Friend virus was shown to replicate better in C57BL/Ks than in C57BL/6 mice, suggesting that *Rgv-1* gene may regulate the occurrence of remissions through its action on the helper virus.

## INTRODUCTION

Friend disease has been observed to run a chronic remittent course in C57BL/6 × DBA/2 F<sub>1</sub> (hereafter called B6D2F<sub>1</sub>) mice (2, 3, 10). Its course in these animals is characterized by 4 phases: (a) an early proliferative phase lasting 2 to 4 weeks; (b) a period of regression occurring from 2 to 8 weeks after inoculation; (c) a prolonged phase of remission when the disease is limited to a few tiny microscopic foci of dividing stem cells in the spleen; and (d) a brief terminal phase with rapidly progressive Friend disease. This is quite different from the course seen when susceptible inbred strains such as BALB/c are inoculated. In these mice splenomegaly is progressive and death occurs in 5 to 10 weeks with no detectable period of remission. Since the same virus preparation will induce progressive disease in BALB/c mice and remittent disease in B6D2F<sub>1</sub> hybrid mice, the change in the character of the disease in the hybrid must be due to host factors.

All preparations of FV<sup>2</sup> actually comprise a mixture of at least 2 viruses, namely SFFV and LLV. In addition, many virus preparations also contain a polycythemia-inducing agent and lactic dehydrogenase-elevating virus. While the former appears closely related to SFFV (17), the latter is

probably a contaminant; neither is considered separately in the present studies. LLV is present in all strains of FV (5, 6) and has been shown by classical *in vivo* and *in vitro* rescue techniques to function as a helper for SFFV, which is defective (9, 11).

The genetics of Friend disease have been well summarized by Lilly (15). Briefly, there are 2 major loci, *Fv-1* and *Fv-2*, while an additional gene, *Rgv-1*, associated with the *H-2* locus, plays a modifying role. *Fv-1* and *Fv-2* govern the response to SFFV, which induces erythroblastosis and stem cell proliferation in the spleen. Susceptibility is regulated primarily by *Fv-2*. Mice homozygous or heterozygous for the allele *Fv-2<sup>s</sup>* are highly susceptible to SFFV, whereas mice homozygous for *Fv-2<sup>r</sup>* are resistant. According to Lilly the *Fv-1* gene, with alleles *Fv-1<sup>n</sup>*, *Fv-1<sup>b</sup>*, governs susceptibility to LLV, the helper for SFFV. Naturally occurring leukemia viruses may be classified into 2 groups according to their ability to replicate in NIH Swiss and BALB/c mouse embryo cultures. The differences in response in the 2 cell lines are not absolute, but relative, being of the order of 100- to 1000-fold. Strains of virus replicating best in each cell line are termed N- and B-trophic, respectively. The ability of cell lines to support replication of the 2 types of virus is genetically determined. Susceptibility to both N- and B-trophic strains of helper virus (LLV) is recessive in *Fv-1<sup>n</sup>/Fv-1<sup>b</sup>* heterozygotes; they are, however, highly susceptible to NB strains of virus.

The gene, *Rgv-1*, localized to the "K end" of the *H-2* locus, also influences Friend disease (13, 14). This gene exerts its effect relatively late in the course of the disease. Characteristically, mice homozygous at the *H-2<sup>b</sup>* locus require a significantly larger inoculum of virus to produce Friend disease and are more likely to undergo remission than mice homozygous for *H-2<sup>d</sup>*. This gene also governs susceptibility to Gross leukemia virus (12). Thus, C57BL/6 mice with the *H-2<sup>b</sup>* allele are resistant to infection with Gross virus, whereas the congenic strain with the *H-2<sup>k</sup>* allele is highly susceptible (13).

The genotype of the B6D2F<sub>1</sub> hybrid is *Fv-1<sup>n</sup>/Fv-1<sup>b</sup>*, *Fv-2<sup>s</sup>/Fv-2<sup>r</sup>*, *H-2<sup>b</sup>/H-2<sup>d</sup>*. If the genetics of FV infections are those outlined above, then B6D2F<sub>1</sub> mice should be susceptible to FV and the disease, under the influence of the *H-2<sup>b</sup>* allele, should remit. C57BL/Ks mice, which originated from C57BL/6J, now differ at 4 histocompatibility loci, namely *H-1*, *H-2*, *H-8*, and *H-12* (18). Only the *H-2* locus is known to be involved in the genetic control of susceptibility to FV and is relevant to the present experiments. At this locus the C57BL/Ks mice have the allele *H-2<sup>d</sup>*. The genotype of C57BL/Ks × DBA/2 F<sub>1</sub> (hereafter

<sup>1</sup> This work was supported in part by Grant DRF-1123 from the Damon Runyon Memorial Fund for Cancer Research, a grant from the American Cancer Society, Oregon Division, Inc., and by USPHS Grant CA-07868 from the National Cancer Institute.

<sup>2</sup> The abbreviations used are: FV, Friend virus; SFFV, spleen focus-forming virus; LLV, lymphatic leukemia-inducing virus; FV(LLV-H), Hartley pseudotype of FV; ID<sub>50</sub>, 50% of mice developed the infection.

Received May 10, 1973; accepted June 28, 1973.

called BkD2F<sub>1</sub>) mice will differ from B6D2F<sub>1</sub> mice at the *H-2* locus where they will be homozygous for *H-2<sup>d</sup>*.

Under the postulated influence of the *Rgv-1* gene, located near the *H-2* locus, Friend disease in BkD2F<sub>1</sub> mice would be expected to be progressive. This change in the character of the disease might, as Lilly (15) has suggested, be on immunological grounds. It might also be dependent on the relative susceptibility of the 2 strains to the helper virus (LLV), although this might in fact have an immunological basis. In order to confirm the latter hypothesis it would be necessary to show that (a) C57BL/6 and C57BL/Ks mice bear identical alleles at *Fv-1* and *Fv-2* loci, (b) Friend disease does not have a remittent course in BkD2F<sub>1</sub> hybrids, and (c) LLV replicates better in BkD2F<sub>1</sub> than in B6D2F<sub>1</sub> hybrids.

## MATERIALS AND METHODS

**Mice.** C57BL/6 and C57BL/Ks mice were obtained from The Jackson Laboratories, Bar Harbor, Maine, and C57BL/6 and DBA/2 mice were from Simonsen Laboratories, Gilroy, Calif. C57BL/6 mice from the 2 colonies retained skin grafts from one another indefinitely. B6D2F<sub>1</sub> and BkD2F<sub>1</sub> mice were bred in our laboratory.

**Virus.** The origin of our strain of FV and the isolation of LLV from it, together with the methods for virus titration, have been described (5, 7). FV was in the 10th passage in BALB/c mice and LLV in the 6th passage in rats at the time of these experiments. Our original strain of FV is known to be NB trophic (8). Using a lymphatic leukemia virus isolated from a strain of FV grown *in vitro* by Hartley, an N-trophic FV pseudotype [FV(LLV-H)] was prepared by our *in vitro* method (9). A Moloney pseudotype of FV was prepared by the same method. Spleen focus assays were performed by the method of Axelrad and Steeves (1). The presence or absence of Friend disease or lymphatic leukemia was confirmed histologically in all experiments.

## RESULTS AND DISCUSSION

**Susceptibility of C57BL/6 and C57BL/Ks Mice to FV.** Groups of 26 mice of each strain were inoculated i.p. with  $10^{3.6}$  ID<sub>50</sub> of FV; one-half were killed 35 and the other half 180 days postinoculation. The mean spleen weights of the C57BL/6 mice were  $220 \pm 50$  and  $140 \pm 30$  mg, respectively, and those of the C57BL/Ks mice were  $200 \pm 55$  and  $140 \pm 30$  mg, respectively. None of these mice showed histological evidence of Friend disease.

**Susceptibility of B6D2F<sub>1</sub> and BkD2F<sub>1</sub> Hybrids to FV.** A simultaneous titration of FV was carried out in 9-week-old mice of both strains. One-half of the animals were killed 5 weeks after inoculation, and the remainder were killed 12 weeks after inoculation. The results (Table 1) show that the titer of the virus in B6D2F<sub>1</sub> mice was significantly lower in mice killed 12 weeks after inoculation than in those killed 5 weeks after inoculation. The mice killed 12 weeks after inoculation had both smaller spleens and a lower incidence of Friend disease than those killed earlier, indicating that

remission had occurred. On the other hand, the titer of FV and microscopic incidence of Friend disease in the BkD2F<sub>1</sub> hybrids was the same at 5 and 12 weeks after inoculation, while the mean spleen weight had increased rather than decreased. Thus, Friend disease in BkD2F<sub>1</sub> mice did not remit as it did in B6D2F<sub>1</sub> mice. Nevertheless, it did not run the rapidly progressive course seen in BALB/c and DBA/2 mice, which are homozygous *Fv-2<sup>s</sup>/Fv-2<sup>s</sup>*. Since all reports indicate total dominance of *Fv-2<sup>s</sup>* over *Fv-2<sup>r</sup>*, it is possible that the differences observed are due to another gene.

**Fv-1 Genotype of C57BL/Ks Mice.** If the difference in response to the 2 hybrids is to be ascribed to a difference in the *H-2*-related *Rgv-1* locus, then it is necessary to show that they both have the same alleles at *Fv-1*, although for present purposes the genotype need not be determined directly. Since the DBA/2 parent is known to be *Fv-1<sup>n</sup>/Fv-1<sup>n</sup>*, only 2 possibilities exist in the C57BL/Ks hybrids, viz. *Fv-1<sup>n</sup>/Fv-1<sup>n</sup>* or *Fv-1<sup>n</sup>/Fv-1<sup>b</sup>*. As susceptibility to both N- and B-trophic strains of Friend virus is recessive in the *Fv-1<sup>n</sup>/Fv-1<sup>b</sup>* genotype, it follows that there will be a difference in the response between the 2 genotypes to N-trophic and NB-trophic FV, as the heterozygote is highly susceptible to the NB strain of FV (15).

Using the spleen focus assay, the response of B6D2F<sub>1</sub> and BkD2F<sub>1</sub> hybrid mice to our NB-trophic strain of FV and the N-trophic pseudotype FV(LLV-H) was determined. The results (Table 2) indicate that both hybrids have the same *Fv-1<sup>n</sup>/Fv-1<sup>b</sup>* genotype. By exclusion, these data suggest that unless some unknown gene is operating, the difference in response between the 2 hybrids is due to the *Rgv-1* gene.

**Mode of Action of Rgv-1 Gene.** The most likely modes of action appear to be either immunological, as Lilly (15) has suggested, or related to the action of the *Rgv-1* gene on susceptibility to the lymphatic leukemia viruses which can act as helpers for FV. The *Ir-1* gene, which plays an important role in the antigenic response to certain synthetic polypeptides, has been localized at the "K end" of the *H-2* locus (16). This is very close to the position of the *Rgv-1* gene (14), so that the 2 genes may be related or even identical. Previous experiments by us (2, 3) have shown that only very low levels of neutralizing antibody to FV can be detected in the sera of B6D2F<sub>1</sub> mice with regressed Friend disease. This led us to speculate that LLV, the naturally occurring helper for FV, might be replicating at different rates in the 2 hybrid strains of mice. A simultaneous comparative titration of LLV showed a significantly higher titer of  $> 10^{-5.5}$  ID<sub>50</sub>/ml in newborn BkD2F<sub>1</sub> compared to  $10^{-3.6}$  ID<sub>50</sub>/ml in newborn B6D2F<sub>1</sub> mice.

If one is to ascribe the differences in the responses to FV of the 2 strains of mice to differences in susceptibility to the LLV helper, then it must be recognized that, unlike defective Rous sarcoma virus, susceptibility to helper virus does not strictly parallel the ability to develop Friend disease (4). In an experiment in which groups of 13 eight-week-old C57BL/6 and C57BL/Ks mice were inoculated with  $10^{2.9}$  ID<sub>50</sub> of the Moloney pseudotype of FV, none of the mice in either group developed Friend disease, although

Table 1  
Comparative titration of FV in 9-week-old B6D2F<sub>1</sub> and BkD2F<sub>1</sub> mice

FV was titrated in groups of 9-week-old B6D2F<sub>1</sub> and BkD2F<sub>1</sub> mice, one-half of which were killed 5 weeks p.i.<sup>a</sup> and the remainder 12 weeks p.i.

Dilution of virus (-log <sub>10</sub> )	B6D2F <sub>1</sub>				BkD2F <sub>1</sub>			
	Killed 5 wk p.i.		Killed 12 wk p.i.		Killed 5 wk p.i.		Killed 12 wk p.i.	
	Mean spleen weight (mg)	FD/total <sup>b</sup>	Mean spleen weight (mg)	FD/total	Mean spleen weight (mg)	FD/total	Mean spleen weight (mg)	FD/total
1	300 ± 170 <sup>c</sup>	8/8	190 ± 80	6/8	490 ± 250	7/8	530 ± 180	8/8
2	280 ± 90	5/8	180 ± 50	5/8	350 ± 140	7/8	430 ± 60	8/8
3	260 ± 100	7/8	150 ± 50	3/8	300 ± 180	6/8	420 ± 260	7/8
4	220 ± 140	5/8	130 ± 20	0/8	270 ± 100	5/7	270 ± 50	3/7
5	150 ± 20	0/8	150 ± 10	0/8	170 ± 20	0/7	170 ± 120	1/7
Virus titer (-log <sub>10</sub> /ml)	4.5		3.0		4.7		4.7	

<sup>a</sup> p.i., postinoculation. FD, number with Friend disease confirmed microscopically.

<sup>b</sup> Total number of mice inoculated with FV.

<sup>c</sup> Mean ± S.D.

Table 2

Comparison of susceptibilities of B6D2F<sub>1</sub> and BkD2F<sub>1</sub> hybrid mice to N- and NB-trophic FV

Comparative titrations of an N-trophic FV pseudotype and an NB strain of FV were performed by the focus-forming assay.

Virus	Titer (focus-forming units/ml) <sup>a</sup>	
	B6D2F <sub>1</sub>	BkD2F <sub>1</sub>
N-trophic FV (LLV-H)	3.18	3.18
NB-trophic FV	5.06	5.11

<sup>a</sup> -log<sub>10</sub> ID<sub>50</sub>.

4 of the BkD2F<sub>1</sub> mice died with lymphatic leukemia. This result, which supports earlier work (4), indicates that the ability of the helper virus to replicate is not the only requirement for a mouse strain to be susceptible to FV.

The foregoing data strongly support the hypothesis originally proposed by Lilly (13) that 3 separate genes are involved in FV infection. The *Rgv-1* gene not only controls replication of helper virus (LLV) but also in some way regulates the occurrence of remissions, possibly through its close relationship to the *Ir-1* gene. However, this remains to be determined.

## ACKNOWLEDGMENTS

We wish to thank Judy Maylie for her skillful technical help.

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