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Genetic Aspects of Resistance to Friend Leukemia Virus.* (30974)

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Resistance of inbred C57BL mice to Friend's leukemic agent has been documented in a number of studies(1,2,3). Analysis of F_2 and backcross progeny from RF $\times$ C57BL crosses suggested that resistance is a simple recessive character(4). In an attempt to differentiate susceptibility in the CFW and DBA2 strains from C57BL-resistance by standard mendelian methods, an alternate form of gene expression was suggested by our findings. These observations are described here.

Materials and methods. Mice. C57BL/Crgl and DBA2/Crgl mice were obtained from the Cancer Research Genetics Laboratory, University of California, Berkeley. Inbred CFW mice were obtained from the Lobund Laboratory, University of Notre Dame, Notre Dame, Ind. *Virus.* BALB/c-adapted Friend virus was obtained from Dr. W. Bostick, California College of Medicine, Los Angeles. It was specifically adapted to DBA and CFW mice by serial passage. After 7 passes in each respective strain, 2 pools of virus were prepared from spleen homogenates in the manner described by Fieldsteel *et al* (2). Virus titers of DBA-derived preparations were determined in DBA and CFW mice respectively as described by Friend(1). A similar reciprocal titration was made with CFW-derived virus. The results summarized in Table I indicate negligible differences. Passage history appeared to have little or none of the effects previously described(1,2). Stock virus for subsequent experiments consisted exclusively of DBA-derived preparations. *Inoculation and subsequent animal observations.* Cell-free preparations containing approximately 20 ID_{50} of virus were inoculated intraperitoneally in 35-40 day old test

TABLE I. Titration of Friend Virus* in CFW and DBA Mice.

Passage history	Assay strain	Titer per ml (log_{10})
DBA	DBA	3.48
DBA	CFW	3.56
CFW	CFW	3.43
CFW	DBA	3.35

* Virus prepared from spleen homogenates 21 days after infection: 0.5 ml was inoculated intraperitoneally into each of a group of 10 mice per decimal dilution.

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mice. Simultaneous inoculation of indicator DBA mice with 5 ID_{50} of virus provided a check on the efficacy of this procedure. Twenty-one days after inoculation, mice were killed by cervical dislocation, and their spleens removed and weighed. Spleen weight frequency distributions were plotted on a 0.05 g scale in an attempt to detect genetic variance. Similar methodology has been employed in other tumor studies(5,6).

Results. CFW × C57BL cross. Spleen weight distributions of parental CFW, parental C57BL, and subsequent generations inoculated with 20 ID_{50} of Friend virus are shown in Fig. 1. In CFW × C57BL and reciprocal C57BL × CFW F_1 progeny, average spleen weights were 0.59 ± 0.02 g and 0.57 ± 0.02 g respectively. A *t* test did not show a significant difference ($P = >0.05$) between spleen weight means of the F_1 progeny, indicating absence of maternal effects and sex linkage. Distribution of spleen weight in both groups of F_1 hybrids closely approximates that of parental CFW mice, suggesting the dominance of susceptibility in this cross. The bimodality of spleen weight distribution in C57BL × (C57BL × CFW) backcross progeny is evidence that a single major gene differentiates susceptibility in the 2 parent strains.

DBA × C57BL cross. Spleen weight distributions of parental DBA, parental C57BL, and subsequent generations inoculated with 20 ID_{50} of Friend virus are shown in Fig. 2. No significant difference was found when the average spleen weight (0.11 ± 0.01 g) of DBA × C57BL F_1 progeny and that (0.11 ± 0.02 g) of reciprocal C57BL × DBA × DBA F_1 hybrids were compared by the *t* test. This was interpreted as evidence of no maternal effects and no sex linkage. The similarity of both F_1 frequency histograms to the parental C57BL distribution suggested dominance of resistance in this cross. The bimodality of spleen weight distribution in progeny from DBA × (C57BL × DBA) backcross matings is indicative of a major single gene difference between the parental strains.

CFW × DBA cross. In an attempt to determine whether separate loci were responsible for susceptibility in CFW and DBA mice, reciprocal F_1 hybrids and progeny from (CFW × DBA) F_1 × (DBA × CFW) F_1 matings were inoculated with 20 ID_{50} of Friend virus. The susceptibility of the parent strains was not remeasured. Average spleen weight in CFW × DBA F_1 progeny was 0.55 ± 0.02 g. The mean for reciprocal DBA × CFW F_1 hybrids was 0.58 ± 0.02 g. A *t* test for significance between F_1 means suggested neither maternal effects nor sex linkage ($P = >0.05$). Spleen weight in F_2 progeny averaged 0.63 ± 0.01 g. The frequency distributions about these means are shown in Fig. 3. All 3 histograms are essentially unimodal. Only 4 of 105 progeny had spleen weighing less than 0.50 g, and these were 0.44, 0.46, 0.47, and 0.47 g respectively.

(CFW × C57BL) F_1 × (DBA × C57BL) F_1 cross. To obtain additional information on the interrelationship of CFW- and DBA-mediated susceptibility, progeny from (CFW × C57BL) F_1 × (DBA × C57BL) F_1 matings were inoculated with 20 ID_{50} of Friend virus. The distribution of spleen weights of 169 such progeny is shown in Fig. 4. The bimodality of this distribution was similar to the results obtained with (CFW × C57BL) × C57BL backcross progeny, suggesting that the presence of the DBA genome had a minor effect on the determination of susceptibility.

(CFW × DBA) F_1 × (DBA × C57BL) F_1 cross. The inheritance of DBA-mediated susceptibility in an alternate triparental combination was tested with progeny from (CFW × DBA) F_1 × (DBA × C57BL) F_1 crosses. The spleen weight distribution of 133 such progeny inoculated with 20 ID_{50} of Friend virus is shown in Fig. 5. This histogram is also bimodal with clear-cut separation between two groups. If division of these groups is at approximately 0.37 g, then a group of low susceptibility would include 35 animals and a group for high susceptibility would include 97 animals. This 3:1 ratio of susceptible to resistant mice ($X^2 = 0.3$) is additional evidence of a negligible effect by DBA genome on genetic determination of susceptibility in this cross. It would appear that the single gene difference between inbred CFW

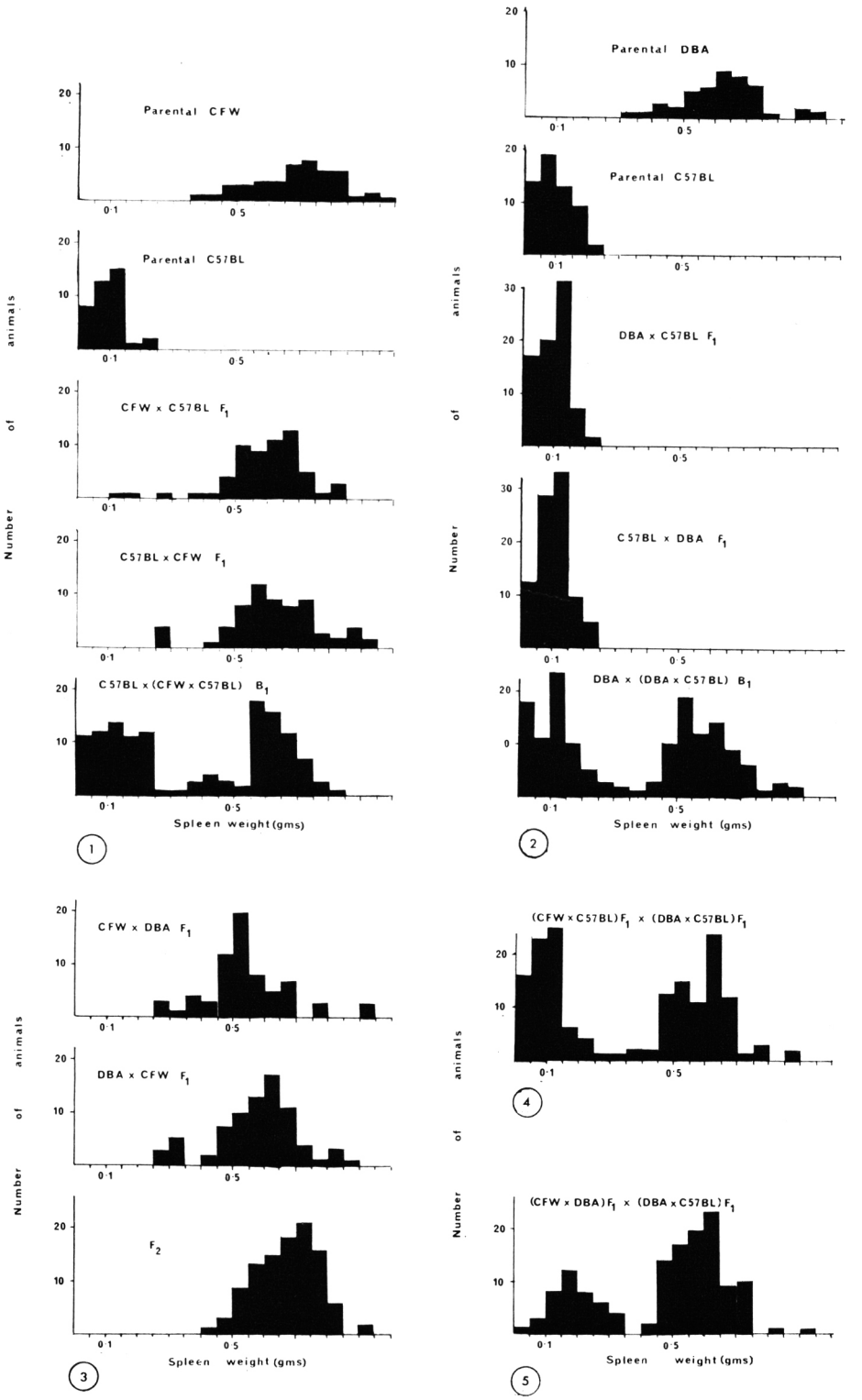
Parental CFW
Parental C57BL
CFW x C57BL F1
C57BL x CFW F1
C57BL x (CFW x C57BL) B1
Number of animals
Spleen weight (gms)
1
Parental DBA
Parental C57BL
DBA x C57BL F1
C57BL x DBA F1
DBA x (DBA x C57BL) B1
Number of animals
Spleen weight (gms)
2
CFW x DBA F1
DBA x CFW F1
F2
Number of animals
Spleen weight (gms)
3
(CFW x C57BL)F1 x (DBA x C57BL)F1
Number of animals
4
(CFW x DBA)F1 x (DBA x C57BL)F1
Number of animals
Spleen weight (gms)
5

FIG. 1. Distribution of spleen weights in Friend virus-infected CFW and C57BL inbred mice, their reciprocal F_1 hybrids, and progeny from (CFW × C57BL) × C57BL matings.

FIG. 2. Distribution of spleen weights in Friend virus-infected DBA and C57BL inbred mice, their reciprocal F_1 hybrids, and progeny from (DBA × C57BL) × DBA matings.

FIG. 3. Distribution of spleen weights in Friend virus-infected (CFW × DBA) F_1 hybrids, reciprocal (DBA × CFW) F_1, and their F_2 progeny.

FIG. 4. Distribution of spleen weights in Friend virus-infected progeny from (CFW × C57BL) F_1 × (DBA × C57BL) F_1 matings.

FIG. 5. Distribution of spleen weights in Friend virus-infected progeny from (CFW × DBA) F_1 × (DBA × C57BL) F_1 matings.

and C57BL mice solely accounts for the bimodality of the frequency distribution.

Discussion. As indicated by respective matings to resistant C57BL mice, susceptibility of the CFW and DBA strains to Friend virus appears to be dependent on discrete determinative factors. Unequivocal evidence of separate loci for susceptibility in CFW and DBA animals was not obtained by the infection of F_2 progeny from appropriate crosses. The incidence of virus-induced splenomegaly in (CFW × DBA) F_1, reciprocal (DBA × CFW) F_1, and F_2 progeny closely matched that observed in the parental strains. Suggestion of isoallelism in the parent strains is negated by the results obtained with (CFW × C57BL) F_1 and (DBA × C57BL) F_1 hybrids.

Evidence that the allele for susceptibility in CFW mice, the allele for resistance in C57-BL, and their counterpart in the DBA strain are at the same locus is suggested by the results obtained with progeny of triparental lineage. The distinct bimodality of spleen weight distribution in infected (CFW × C57BL) F_1 × (DBA × C57BL) F_1 progeny does not support a separate locus hypothesis for susceptibility in CFW and DBA mice. The question of assigning a putative genotype to the DBA strain is enigmatic. If susceptibility in CFW animals and resistance in C57BL mice are symbolized SS and ss respectively, segregants from the (CFW × C57BL) F_1 × (DBA × C57BL) F_1 cross would be S/S°, S/s, S°/s and s/s; where S°S° is the putative genotype of inbred DBA animals. Since the 4 classes of segregants mendelize in equal proportions, it seems likely that the resistant progeny observed were either s/s or S°/s (the latter being the genotypic equivalent of DBA × C57BL F_1 hybrids). Similarly in progeny from (CFW × DBA) F_1 × (DBA × C57BL) F_1 matings, genotypic categories would be S/S°, S/s, S°/S°, and S°/s. Heterogyzotes for S°/s (¼ of the progeny) would be the only resistant animals. Such an explanation makes the observed 3:1 ratio appear meaningful. Inherent in such a scheme is the assumption that the locus in DBA mice is 'inactive' and that the various phenotypes do not reflect a conventional multiallelic series.

Somewhat similar findings have been reported for resistance to Rous sarcoma virus (RSV). Resistance to RSV in chick embryos appears to be recessive in certain crosses(7, 8), but dominant in others(9). The significance of the viral capsid in genetically determined host response to RSV has been suggested by recent experiments(10). The relevance of these data in Friend virus infection has not been established.

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