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Host Genetic Determinants of Neurological Disease Induced by Cas-Br-M Murine Leukemia Virus

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Cas-Br-M is an ecotropic murine leukemia virus (MuLV) of wild-mouse origin that causes neurogenic hind-limb paralysis. By virtue of its N-tropism, the virus replicates well in tissues of mice bearing the *n* but not the *b* allele at the *Fv-1* locus. To determine if different *Fv-1*^{n} strains of mice were equally susceptible to virus-induced neurological disease, we inoculated NFS, C3H, DBA/2, CBA, AKR, C58, and NZB mice at birth with Cas-Br-M murine leukemia virus and observed them for the development of tremor and hind-limb paralysis. Three patterns of disease were observed: NFS and C3H mice developed disease within 3 months postinoculation; DBA/2 and CBA mice became affected between 8 and 15 months postinoculation; and no disease was observed in AKR, C58, or NZB mice up to 15 months after infection with Cas-Br-M murine leukemia virus. Studies of genetic crosses between intermediate-latency (DBA/2) or long-latency (AKR) strains with short-latency (NFS) strains showed that intermediate latency and long latency were semidominant traits determined by two or more interacting but independently assorting loci. These genes appear to determine the rate at which the virus replicates and at which viral gene products accumulate in the central nervous system.

Progressive hind-limb paralysis associated with the expression of murine leukemia virus (MuLV) and with lymphoma has been described in restricted populations of wild mice (2) and in laboratory mice inoculated with wild-mouse MuLV (8). The ecotropic MuLV isolates from wild mice have all been N-tropic in cultures. Breeding experiments between wild mice (*Fv-1*^{n}) and C57BL mice (*Fv-1*^{b}) (3) and between C57BR/cdJ mice (*Fv-1*^{n}) and C57BL mice (*Fv-1*^{b}) (10) showed that resistance to wild-mouse MuLV replication and induction of paralysis were dominant characteristics conferred by the *b* allele at the *Fv-1* locus. After neonatal inoculation of several *Fv-1*^{n} strains of mice with a wild-mouse ecotropic MuLV (Cas-Br-M), marked differences in the incidence and latency period of neurological disease were observed. To determine whether these differences were genetically determined, we examined parental, F1, and backcross mice with short latency × long latency and with short latency × resistant phenotypes for the frequency and latency of clinical neurological disease. To determine if the restriction affected viral replication, we assayed infectious MuLV and MuLV p30 levels in the spleens and brains of Cas-Br-M MuLV-infected short-latency (NFS) and intermediate-latency (CBA) mice.

MATERIALS AND METHODS

Mice. Pregnant C58/J, AKR/J, NZB/BlJ, C3H/HeJ, C3H.SW/SnJ, DBA/2J, and CBA/J mice were obtained from Jackson Laboratories, Bar Harbor, Maine. Pregnant NFS/N mice were obtained from the Small Animal Section, Veterinary Resources Branch, National Institutes of Health, Bethesda, Md., and pregnant C3H.OH/Sf mice were obtained from Donald Shreffler. (AKR/J × NFS/N)F1, (DBA/2J × NFS/N)F1, and backcross mice were bred in our colonies. All mice were inoculated intracerebrally with 0.03 ml of virus within the first 24 h of life. Beginning at 5 weeks of age, a clinical evaluation for evidence of tremor, hind-limb weakness, or paralysis was performed biweekly. Mice were sacrificed after neurological signs became clearly established or at 15 months if clinically normal. Brains and spleens from perfused mice were frozen at −70°C for infectious MuLV and MuLV p30 determinations or fixed in Formalin for histological evaluations.

Virus. Cas-Br-M MuLV was grown in SC-1 cells, and titers were determined by the XC assay as previously described (7). MuLV inocula contained $10^{4.5}$ to $10^{5.5}$ PFU/ml.

Viral protein determinations. MuLV p30 levels were determined in brain and spleen homogenates by a competition radioimmunoassay with ^{125}I-labeled Rauscher MuLV p30 and goat anti-wild-mouse MuLV antiserum as previously described (6).

RESULTS

Cas-Br-M MuLV-induced neurological disease in *Fv-1*^{n} mice. Newborn mice seven inbred *Fv-1*^{n} strains including three C3H *H-2* congenic strains were inoculated intracerebrally with Cas-Br-M MuLV and observed for the development of tremor and paralysis (Table 1). All NFS, C3H/He, C3H.OH, and C3H.SW mice developed paralysis after a latency period of 1 to 3 months. By comparison, all DBA/2 mice and three of seven CBA mice became symptomatic 8 to 15 months postinoculation. The brains of the clinically normal CBA mice showed mild spongiosis, gliosis, and some neuronal loss. These lesions were characteristic of Cas-Br-M MuLV-induced disease but were less severe and extensive than in mice showing signs of neurological disease. No clinical or histological evidence of disease was found in AKR, NZB, or C58 mice during a 15-month observation period.

Genetic studies of relative and complete disease resistance in DBA/2 and AKR mice. To evaluate the genetic components of the longer latency and relative resistance to Cas-Br-M MuLV-induced neurological disease demonstrated by DBA/2 mice, we inoculated Cas-Br-M MuLV into newborn (NFS × DBA/2)F1, (NFS × DBA/2)F1 × DBA/2, and (NFS × DBA/2)F1 × NFS mice and observed them for neurological

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TABLE 1. Incidence and latency of neurological disease in Cas-Br-M MuLV-inoculated *Fv-1*^{n} mice

Strain	*H-2* haplotype	Clinical signs of disease[a]	Latency (mo)
NFS	sq^4	22/26	1–3
C3H/He	*k*	6/6	1–3
C3H.OH	*o*	6/6	1–3
C3H.SW	*b*	12/13	1–4
DBA	*d*	18/18	8–15
CBA	*k*	3/7	8–10
AKR	*k*	0/14	>15
NZB	*d*	0/10	>15
C58	*k*	0/12	>15

[a] Clinical signs of disease included tremulousness, weakness, and paralysis. Expressed as number of mice with clinical signs of disease/total number of mice tested.

disease (Table 2). F1 mice developed neurological disease with a latency period longer than that in NFS mice but shorter than that in DBA/2 mice, suggesting that the long latency in DBA/2 mice was due to the effect of either a single semidominant gene or several interacting but independently assorting genes. The observations that (NFS × DBA/2)F1 × DBA/2 mice also developed disease with an intermediate latency period (5 to 8 months) and that disease in (NFS × DBA/2) F1 × NFS mice appeared after a short latency period (1 to 4 months) strongly suggest that the relative resistance of DBA/2 mice to Cas-Br-M MuLV-induced neurological disease reflects the interactions of more than two independently assorting loci.

Similar studies of disease-resistant AKR mice (Table 2) showed that (NFS × AKR)F1 mice developed disease after 4 to 6 months and that the latency period for disease in (NFS × AKR)F1 × NFS mice was similar to that in NFS mice. Of the (NFS × AKR)F1 × NFS mice, 76% developed disease by 3 months postinoculation, whereas all NFS mice had disease by this time. By analogy with the results from the DBA/2 studies, these findings indicate that the disease-resistant phenotype of AKR is due to the interactions of multiple loci.

Relationship of Cas-Br-M MuLV replication to disease latency. To determine whether the different latency periods for neurological disease observed in NFS and CBA mice were due to different levels of Cas-Br-M MuLV replication in these strains, we assayed extracts from spleens and brains of infected mice for infectious virus by the XC assay (Fig. 1A) and for MuLV p30 by a competition radioimmunoassay (Fig. 1B). Infectious MuLV and MuLV p30 levels in the spleens of CBA and NFS mice rose rapidly and were

TABLE 2. Incidence and latency of neurological disease in Cas-Br-M MuLV-inoculated F1 and backcross mice of long-, intermediate-, and short-latency strains

Strain	Clinical signs of disease[a]	Latency (mo)
NFS	22/26	1–3
DBA/2	18/18	8–15
AKR	0/14	>15
(NFS × DBA/2)F1	12/13	5–11
(NFS × DBA/2)F1 × DBA/2	12/13	5–8
(NFS × DBA/2)F1 × NFS	11/11	1–4
(NFS × AKR)F1	21/21	4–6
(NFS × AKR)F1 × NFS	21/21	1–5[b]

[a] See Table 1, footnote *a*.

[b] A total of 76% of mice developed neurological disease by 3 months after Cas-Br-M MuLV inoculation.

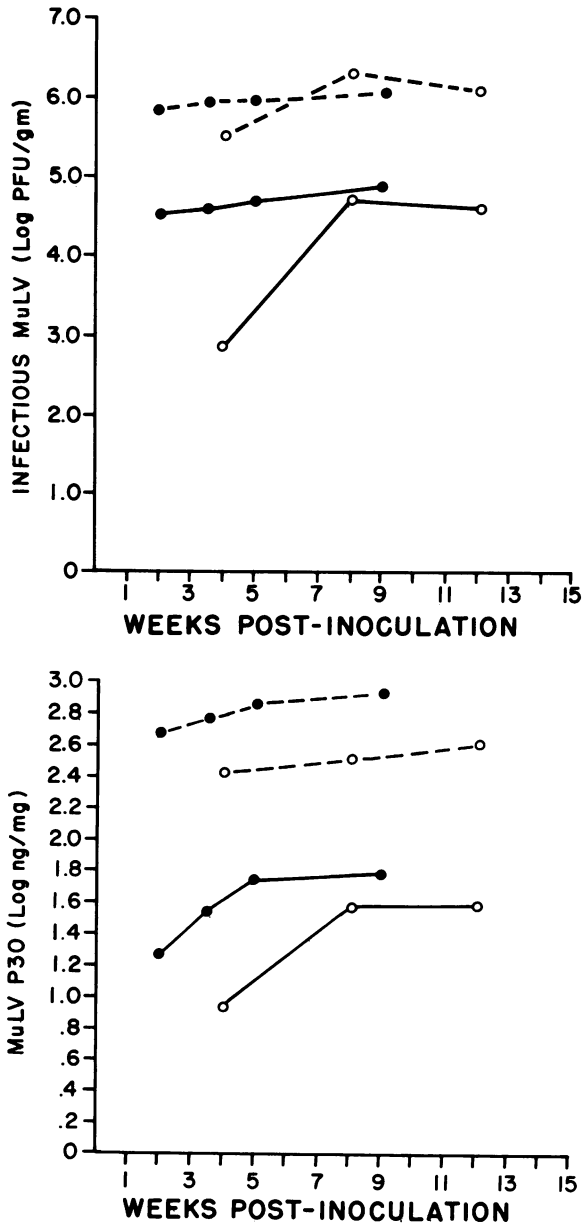

FIG. 1. (A) Infectious MuLV titers in spleens (----) and brains (——) of CBA (0) and NFS (●) mice inoculated at birth with Cas-Br-M MuLV. Each point represents the mean of four or more samples titrated by the XC assay (7) on duplicate plates. (B) MuLV p30 levels in spleens (----) and brains (——) of CBA (0) and NFS (●) mice inoculated at birth with Cas-Br-M MuLV. Each point represents the mean of four or more samples assayed in duplicate by a competition radioimmunoassay (6).

comparable by 4 weeks postinoculation. CBA mice had lower levels of infectious MuLV in their brains up to 8 weeks postinoculation than did NFS mice, whose levels were high 2 weeks postinoculation and rose slowly thereafter. CBA mice maintained lower levels of MuLV p30 in their brains than did NFS mice throughout their asymptomatic 12-week postinoculation period. However, MuLV p30 levels in the brains of CBA mice at the onset of neurological disease (>16 weeks postinoculation) were comparable to those in symptomatic NFS mice (>5 weeks postinoculation) (data not shown). Studies of neonatally infected DBA/2 mice showed that the

pattern of p30 accumulation in brains resembled that in CBA/J mice rather than that in NFS mice (data not shown).

DISCUSSION

The results of this study demonstrated that various strains of mice bearing the *n* allele at *Fv-1* differed markedly in their susceptibilities to induction of neurological disease by Cas-Br-M MuLV: NFS, C3H/He, C3H.OH, and C3H.SW mice developed disease in 1 to 3 months; DBA/2 and CBA mice developed disease between 8 and 15 months; and AKR, C58, and NZB mice were totally resistant to disease (Table 1).

For the *Fv-1*^{n} strains carrying the *k* allele at the *H-2* locus, short latency (C3H/He), intermediate latency (DBA/2), and long latency (AKR and C58) were observed. Congenic strains of C3H differing only at the *H-2* locus all demonstrated short latency (Table 1). These data suggest that the *H-2* locus plays no role in determining neurological disease expression in these strains.

Genetic analysis of the relatively resistant strain DBA/2 and the fully resistant strain AKR in crosses with susceptible NFS mice (Table 2) demonstrated that in both cases, the resistant phenotypes resulted from the interactions of multiple but independently assorting genes (Table 2).

The total resistance of AKR and C58 mice to the induction of neurological disease by Cas-BR-M MuLV cannot be attributed to interference with Cas-Br-M MuLV replication as a result of high-level early expression of infectious ecotropic MuLV in these strains. This view is based upon independent observations (J. W. Hartley and W. P. Rowe, personal communication) that the amount of infectious ecotropic MuLV detected in tissues of (AKR × NFS)F1 mice was equivalent to that detected in tissues of age-matched AKR mice. However, in this study, (AKR × NFS)F1 mice developed neurological disease, whereas AKR mice did not (Table 2).

The observation that NZB mice were resistant to the induction of neurological disease by Cas-Br-M MuLV may be related to the fact that these mice have a variant of the *n* allele, termed *nr*, at the *Fv-1* locus, and N-tropic ecotropic MuLVs replicate with lower efficiency in tissues of *Fv-1*^{nr} mice than in tissues of mice bearing the *n* allele at this locus (J. W. Hartley and W. P. Rowe, unpublished data). It is likely that the replication of Cas-Br-M MuLV is also restricted by *Fv-1*^{nr}.

The possibility that mink cell focus-forming (MCF) MuLV might contribute to the neurological disease induced by Cas-Br-M MuLV was suggested by the early appearance of MCF viruses in the spleens of Cas-Br-M MuLV-infected mice (5). However, the absence of MCF virus-related gp70 in the brains of paralyzed animals (5) and the failure of MCF MuLV recovered from wild-mouse ecotropic MuLV-infected mice to induce disease in susceptible newborn mice (9) indicated that recombinant MCF MuLV did not contribute in a major way to the pathogenesis of this disease. Further support for this view comes from studies of genetic crosses with the intermediate-latency strain DBA/2. The replication of MCF MuLV in tissues of DBA/2 mice is restricted by the dominant *r* allele at the *Rmcf* locus (4); NFS mice bear the *s* allele at this locus, and their tissues support the growth of MCF MuLV. If *Rmcf* were an important determinant of neurological disease in DBA/2 mice infected with Cas-Br-M MuLV, it would be expected that *Rmcf*^{r} mice in the (DBA/2 × NFS)F1 × NFS cross would have a longer latency period than *Rmcf*^{s} mice. The observation that all these backcross mice developed disease with a short latency period suggests that *Rmcf* and thus MCF MuLV do not play a significant role in this disease. Thus, the relative resistance or susceptibility to Cas-Br-M MuLV-induced neurological disease among *Fv-1*^{n} mice is influenced by several genes other than those known to affect the course of non-neurological MuLV-induced diseases.

The means by which these genes influence the latency period for Cas-Br-M MuLV-induced neurological disease appears to be related to the control of viral replication in the target tissue. Cas-Br-M MuLV replicates early in the spleen and reticuloendothelial system, produces viremia, and disseminates to the central nervous system (CNS), where it replicates primarily in endothelial cells (1). Cas-Br-M MuLV replicated rapidly in the spleens of both CBA and NFS mice; however, Cas-Br-M MuLV replication was slower in the CNS of CBA mice than in the CNS of NFS mice. Ultimately, the CNS of CBA mice had infectious MuLV and MuLV p30 levels equivalent to those in NFS mice. The Cas-Br-M MuLV titers and MuLV p30 levels in the brains of symptomatic NFS, CBA, and DBA/2 mice did not differ. This was also true for infectious MuLV and MuLV p30 levels in the spinal cord, which were comparable to those in the brain. These data support previous observations for several other inbred strains that MuLV replication and MuLV p30 accumulation in the CNS must reach a critical level before neurological disease is clinically expressed (7, 9, 10). The mechanism by which Cas-Br-M MuLV replication and MuLV p30 accumulation result in spongiform encephalomyelopathy and clinical neurological disease is unclear. However, this study demonstrates that host genetic factors in addition to *Fv-1* can influence the rate of viral replication and MuLV p30 accumulation in the CNS and the expression of neurological disease.

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