Factors Affecting Induction of Neurological Disorders in Mice by Paralysis-Inducing Friend-Related PVC Viruses

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ABSTRACT. Our previous studies showed that the passage of the Friend virus complex through rats generated variant MuLVs, designated PVC111, PVC211, PVC321 and PVC441, that induced neurological disorders associated with tremor and paralysis. In this study, we tested the pathogenicity of four different PVC viruses in mice. Although histopathological studies revealed spongiform degeneration in the spinal cords of NFS mice infected with each PVC virus, only PVC441 frequently induced tremor and paralysis. After a long latency, all of these viruses induced leukemia associated with severe anemia. Further studies with PVC441 revealed dose- and age-dependence for tremor induction. In contrast to NFS mice, BALB/c, DBA/2 and C57BL/6 mice infected with PVC441 virus showed no neurological symptoms, although the virus could be isolated from the tissues of central nervous system. Despite the absence of neurological symptoms, a high degree of neuronal degeneration in the lumbar spinal cord was found in PVC441-infected BALB/c mice. A low degree of neuronal degeneration was found in PVC441-infected DBA/2 or C57BL/6 mice. Genetic crosses of these resistant mice with susceptible NFS mice indicated that resistance to tremor induction by PVC441 was dominant in all mouse strains and suggested that various host genes may control the susceptibility of mice to tremor induction by PVC441 virus. — **KEY WORDS:** host genetic control, murine leukemia virus, neuropathogenicity, PVC virus, strain difference.

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There are several strains of murine leukemia viruses (MuLVs) inducing neurological disease in mice [1–3, 12, 13, 17, 20, 21]. We reported previously a unique group of MuLVs that cause a rapid neurodegenerative disease in rats [10]. These viruses, named PVC viruses, were generated by passing the Friend virus complex through rats. The individual PVC viruses differed in their neuropathogenicity in rats (in the order of PVC211, PVC321, PVC441, and PVC111), in the antigenicity of their SU envelope protein, and in the size of their TM envelope protein [19]. With the exception of PVC211 [7], the pathogenicity of PVC viruses in mice has not been reported.

Little is known about the host gene(s) that control the susceptibility of mice to MuLV-induced neurological diseases. Oldstone *et al.* reported that the *Fv-1* gene controlled susceptibility to disease induced by wild mouse ecotropic virus [14, 15]. Buller reported that two related genes restricted viral replication in mice and caused resistance to the development of disease caused by the neuropathogenic MCF variant of Friend MuLV [2].

In this report, we studied the pathogenicity of PVC viruses in mice and the host gene(s) affecting disease development.

MATERIALS AND METHODS

Viruses: NRK cells [4] chronically infected with PVC111, PVC211, PVC321, PVC441 virus or F-MuLV [9, 10, 16] were propagated in Eagle's minimal essential medium supplemented with 5% fetal bovine serum. The viruses were pelleted from the culture media by ultra centrifugation

with Beckmann type 19 rotor at 19,000 rpm for 3 hr. The pellets were resuspended with residual culture media to obtain high titer virus suspensions (about 100-fold concentration) and then used.

Infection of mice: NFS mice were provided by Dr. Ishimoto (Virus Research Institute, Kyoto University) and maintained in our laboratory by sister-brother mating. DBA/2 mice were purchased from Charles River Co. (Tokyo). BALB/c, and C57BL/6 mice were maintained in our laboratory by sister-brother mating.

Newborn mice from a litter or a few litters were inoculated intraperitoneally with concentrated preparations of viruses (50 μ l per mouse) within 24 hr of birth.

Virus titration: Spleen, brain and spinal cord collected from infected mice, were homogenized with culture medium, with glass homogenizers. The homogenates were aliquoted and stored at -70°C until used. The homogenates were thawed and centrifuged at 15,000 rpm (about 12,000 g) for 10 min at 4°C . Viruses in the supernatant fractions were assayed on NRK cells by the XC-test [10, 18].

Histopathological examination: Spinal cords from infected 30 day-old mice, and spleen, thymus and lymph node from leukemic mice were removed and fixed with Bouin's solution or with phosphate-buffered saline containing 10% formalin. Thin sections from these tissues were stained with hematoxylin and eosin for histopathological examination. A part of the spinal cord was fixed with 5% glutaraldehyde in phosphate-buffered saline (pH 7.2) and 1% osmium tetroxide for electron microscopic examination.

RESULTS

Neuropathogenicity of the PVC viruses: Newborn NFS mice were inoculated intraperitoneally with 1.8×10^3 – 5×10^3 plaque-forming unit (PFU) of each virus, and were observed. Thirty days after infection, two mice from each group were killed for measuring the virus titer. The other mice were observed further. Neurological signs became gradually evident in all weanling mice (from three weeks after infection) infected with PVC 441 virus, but not in those infected with PVC 111 or F-MuLV. The clinical signs induced by PVC441 virus were tremor and paralysis. In contrast, mice infected with PVC 211 or PVC 321 showed only a weak tremor and occasional hind limb paralysis. In each case, the observed tremors lasted until the mice died of leukemia. All PVC viruses, but not F-MuLV, induced spongiform degenerations in the lumbar spinal cords of infected NFS mice (data not shown) without inflammation.

To determine whether virus growth in the tissues of the central nervous system (CNS) correlated with the induction of neurological symptoms, virus titers of the spleen, brain and spinal cord of mice 30 days post infection were measured (Table 1). In general, the viruses grew higher titers in the spleen than in CNS tissues with the exception of PVC111 which showed generally poor growth, especially in CNS tissues. No correlation was observed between the virus titers at 30 days post infection and the development of neurological symptoms. F-MuLV showed comparable growth to those of PVC211, PVC321 or PVC441 in CNS tissues although it was non-neurovirulent.

Since the neurological disorder induced by PVC441 virus was the most evident, the factors influencing the induction of neurological disorder by this virus were further investigated. Mice inoculated with serial dilutions of PVC441 virus were observed for 30 days after infection (Table 2). All mice inoculated with 2,500 PFU of virus

Table 1. Virus recovery from tissues of NFS mice infected with various PVC viruses and F-MuLV^{a)}

Virus strain inoculated	Tremor induction ^{b)}	Recovery of virus [(PFU/0.1 g tissue) $\times 10^{-3}$] from					
		Mouse no.	Neurological symptoms	Spleen weight (g)	Spleen	Brain	Spinal cord
PVC111	0/8	1	–	0.258	3.9	0.05	0.06
		2	–	0.221	4.7	0.05	0.06
PVC211	10/10	1	+	0.160	30.6	3.0	3.2
		2	+	0.196	104.1	19.6	22.7
PVC321	6/6	1	+	0.187	60.4	16.1	16.6
		2	+	0.196	104.1	19.9	22.7
PVC441	6/6	1	+	0.135	28.9	1.9	1.6
		2	+	0.196	56.1	10.7	5.1
F-MuLV	0/7	1	–	0.278	230.2	5.9	8.0
		2	–	0.271	88.6	10.9	8.4

a) Newborn NFS mice were infected intraperitoneally with PVC111 (4.3×10^3 PFU/mouse), PVC211 (1.8×10^3 PFU), PVC321 (3.0×10^3 PFU), PVC441 (2.5×10^3 PFU), or F-MuLV (5×10^3 PFU). Thirty days after infection, two mice from each group were killed. Spleens, brains, and spinal cords were used for virus titration by XC assay.

b) No. of mice with neurological symptoms/no. of mice inoculated.

Table 2. Dose-dependent susceptibility of NFS mice to tremor induction by PVC441^{a)}

Inoculum dose (PFU/mouse)	Tremor induction	Recovery of virus [(PFU/0.1 g tissue) $\times 10^{-3}$] from					
		Mouse no.	Neurological symptoms	Spleen weight (g)	Spleen	Brain	Spinal cord
2500	5/5	1	+	0.160	68.6	3.2	4.0
		2	+	0.161	68.3	3.9	4.0
250	5/6	1	+	0.196	56.1	10.7	5.1
		2	+	0.135	28.9	1.9	1.6
25	3/5	1	+	0.226	185.8	10.0	5.5
		2	+	0.216	101.9	7.1	6.7
2.5	0/7	1	–	0.240	112.5	5.8	4.4
		2	–	0.193	19.7	0.03	0.1

a) Newborn NFS mice were infected with serially diluted PVC441. At 30 days post infection, organs were removed from diseased mice and viral titers were determined.

Table 3. Age-dependent susceptibility of NFS mice to tremor induction by PVC441^{a)}

Inoculated at (days of age)	Tremor induction	Recovery of virus [(PFU/0.1 g tissue) × 10 ⁻³] from					
		Mouse no.	Neurological symptoms	Spleen weight (g)	Spleen	Brain	Spinal cord
1	5/5	1	+	0.176	56.8	2.7	2.1
		2	+	0.162	31.5	1.8	61.3
3	7/7		+		ND ^{b)}	ND	ND
6	0/9		—		ND	ND	ND
9	0/7	1	—	0.213	7.1	0.3	0.07
		2	—	0.225	12.9	2.0	0.09

a) NFS mice of various age were infected with PVC441 (2.5×10^3 PFU/mouse). At 30 days post-infection, organs were used for virus titration.

b) Not determined.

Fig. 1. Leukemogenicity of PVC viruses and F-MuLV. NFS mice were inoculated with the virus strains as described in the legend of Table 1. Symbols indicate the mice inoculated with virus strain of F-MuLV (○); PVC111 (▲); PVC211 (■); PVC321 (●); and PVC441 (□).

were tremulous, but the number of non-tremulous mice, or mice with weak tremor, increased as the virus dose was lowered, and all mice receiving 2.5 PFU of virus were non-tremulous during their life time (died of leukemia within 108 to 229 days after infection). As shown in Table 2, no apparent differences in the virus titers of spleen or CNS tissues were found among the mice inoculated with various doses of the virus except for the case of mouse no. 2 inoculated with 2.5 PFU of virus.

The age dependency of the susceptibility to neurological disease induction was also examined in NFS mice inoculated with 2,500 PFU of PVC441 virus (Table 3). All of the mice infected within 3 days of birth became tremulous, while mice infected 6 or 9 days after birth did not. Virus titers measured 30 days post infection in the spleens or CNS tissues of mice infected 9 days after birth were usually lower than those of mice infected 1 day after birth.

Leukemogenicity of PVC viruses: The virus-infected mice were observed for 300 days after infection to determine the leukemogenicity of each virus (Fig. 1). F-MuLV induced leukemia in all mice 64 to 148 days after infection, and

PVC111 virus induced leukemia gradually in all mice 137 to 296 days after infection. The other PVC viruses showed incubation periods for leukemia between those of F-MuLV and PVC111.

All of the leukemic mice showed hepatosplenomegaly and a part of them (approx. 10%) had enlarged thymus with or without enlarged mesenteric and/or axillary lymph nodes. When the hematocrits of leukemic mice that had been infected with each virus were measured, the values ranged from 12.3 to 16.1%, indicating severe anemia.

Leukemic spleen, thymus and axillary lymph node of a mouse infected with PVC321 were examined histopathologically. Cells proliferating in the spleen appeared to be erythroid precursor cells while those proliferating in the thymus or lymph node appeared to be of lymphoid origin (data not shown).

Strain difference of mice in the susceptibility to tremor induction: Since the neurological disorder induced by PVC441 was apparent in NFS mice (F_v-I^{nn}), the susceptibility of BALB/c (F_v-I^{bb}), DBA/2 (F_v-I^{nn}), C57BL/6 (F_v-I^{bb}) mice, or progeny of crosses between each mouse strain and NFS mice were tested with a high dose of this virus (4×10^4 PFU/mouse) (Table 4). Unlike the NFS strain, BALB/c, DBA/2, and C57BL/6 strains were all resistant to tremor induction.

When resistant BALB/c mice were crossed with susceptible NFS mice, only 1 out of 11 developed tremor after PVC441 infection and none of the DBA/2 × NFS F_1 mice were susceptible. Tremor was induced in 3 out of 15 C57BL/6 F_1 mice by PVC441 virus. F_2 and backcross mice suggested that resistance to tremor induction may be controlled by more than one gene.

Virus growth in these strains was measured in the spleen, brain and spinal cord. As shown in Table 5, virus titers from the spinal cords were quite variable from animal to animal, while those from spleens and brains seemed to be more stable. Higher recoveries in BALB/c and slightly lower recoveries in DBA/2 or C57BL/6 than those of NFS, were observed. The virus growth in spleens and brains of the various mouse strains differed by as much as 20-fold.

Histopathological changes in spinal cord of infected mice of various strains: Lumbar spinal cord specimens of four

Table 4. Susceptibility to induction of tremor by PVC441 infection in NFS, BALB/c, DBA/2, C57BL/6, and their crossed progeny

Mice	Incidence of tremor induction ^{a)}	p ^{b)}
Parent strain		
NFS	8/8	
BALB/c	0/6	
DBA/2	0/6	
C57BL/6	0/6	
F ₁		
BALB/c × NFS	1/11	
DBA/2 × NFS	0/11	
C57BL/6 × NFS	3/15	
F ₂		
BALB/c × NFS	1/27	0.012
DBA/2 × NFS	5/28	0.40
C57BL/6 × NFS	11/28	0.08
BC ₁		
NFS × (BALB/c × NFS)	18/28	0.11
NFS × (DBA/2 × NFS)	17/36	0.73
NFS × (C57BL/6 × NFS)	18/34	0.72

a) No. of mice with neurological symptoms/no. of mice inoculated.

b) Probability deduced from χ^2 -test when a single gene is postulated.

strains of mice infected with PVC441 were examined 30 days after infection. Spongiform vacuolations were found in the lumbar spinal cord of all of the four mouse strains (Fig. 2). In NFS, DBA/2, and C57BL/6 mice, the vacuolations were located in gray matter, although these pathological changes in DBA/2 and C57BL/6 were slighter than those of NFS mice and found in a limited area of the lumbar spinal cord, whereas the vacuolations were found in both gray and white matter of the spinal cord of BALB/c mice. The extent of degeneration was most severe in BALB/c mice. No inflammatory changes were found in any of the tested strains. Virions were found by electron-microscopic observation, budding from the cell membrane of the endothelial cells of small vessels into the subendothelial spaces in NFS mice, but not in glial cells or neurons (data not shown).

DISCUSSION

In this paper, we found that NFS mice infected with PVC441, PVC211 or PVC321 showed clinical signs of neurological disorders, although those infected with PVC211 or PVC321 showed weak tremor and rare paralysis, unlike the case in rats [10]. With the exception of PVC111, the failure of PVC211 or PVC321 virus to induce marked neurological symptoms was not due to the failure of these viruses to replicate well in the CNS (Table 1). All of the viruses caused pathological changes in the CNS of infected mice. Thus, the neurovirulence of PVC viruses altered in mice, when compared with those in rats with the exception of PVC111, and PVC viruses were virulent in the order of PVC441, PVC211, PVC321 and PVC111. The difference in neurovirulence was probably caused by viral genetic structure.

Our observations of the age-dependence and the viral dose-dependence of susceptible NFS mice indicate that the

Table 5. Virus recovery from tissues of NFS, BALB/c, DBA/2 and C57BL/6 mice infected with PVC441 virus^{a)}

Mouse strain	Recovery of virus [(PFU/0.1 g tissue) × 10 ⁻³] from				
	Mouse no.	Spleen weight (g)	Spleen	Brain	Spinal cord
NFS	1	0.176	56.8	2.9	2.1
	2	0.162	31.5	1.9	61.3
BALB/c	1	0.226	154.9	14.6	100.7
	2	0.199	175.9	25.0	7.8
DBA/2	1	0.093	26.2	2.8	0.3
	2	0.097	21.0	5.4	1.3
C57BL/6	1	0.084	10.1	0.3	0.3
	2	0.095	12.7	0.9	0.5

a) Newborn mice (7–9 mice for each infection) were inoculated with 4×10^4 PFU of PVC441 virus.

virus must replicate to a sufficiently high titer in the CNS before its post-natal change. Indeed, Hoffmann *et al.* [7] reported that the viral antigens appeared in CNS tissues at a fairly late stage and this indicates that virus growth sufficient to induce tremor is barely in time before the post-natal change. Lynch *et al.* [11] also indicated that post-natal change of the blood brain barrier might have an effect on disease expression.

We found three mouse strains that were resistant to tremor induction by PVC441, although they all showed spongiform degeneration in their spinal cords. Resistance to tremor induction does not appear to relate directly to the virus growth in these strains, because, with the exception of C57BL/6 mice, there were no significant differences in the virus titers in spleens and brains of the resistant and susceptible strains (Table 5). Curiously, the BALB/c strain was resistant to tremor induction by PVC441, though it showed a better growth of virus and a more severe extent of spongiform degeneration than the NFS strain, which was paralyzed after PVC441 infection. Thus, the resistance of BALB/c strain appears to control the clinical manifestation of neurodegeneration. A number of factors may be responsible for the resistance, since the resistance was not controlled by a single gene as shown in Table 4. In the case of the DBA/2 strain, its resistance might be controlled by a dominant gene since the probability obtained from the segregation rate was compatible with a single gene hypothesis, as shown in Table 4. This gene might relate to the neuronal degeneration, restricting the formation of vacuolation in the spinal cord, though it did not work completely as shown in Fig. 2. Although the pattern of resistance of the C57BL/6 strain resembled that of the BALB/c strain in the crossing experiments, C57BL/6 mice differed from BALB/c in the severity of vacuolar degeneration and in virus growth. Thus, the resistance gene of C57BL/6 mice seemed to restrict the formation of virus-induced vacuoles, or to be less susceptible to virus-induced neurodegeneration. Since some of the (C57BL/6 × NFS) F₁ mice showed susceptibility to tremor and the segregation ratio of susceptible mice in the backcross of these mice to NFS mice was compatible with a single gene hypothesis,

Fig. 2. Histopathological examination of lumbar spinal cord of various mouse strains infected with PVC441. NFS (A), BALB/c (B), DBA/2 (C), and C57BL/6 mice (D) were infected with 4×10^4 PFU of PVC441 virus. Spinal cords were removed 30 days after infection, fixed with 10% formalin in PBS, then thin sections were stained with hematoxylin-eosin, and observed (Magnification $\times 70$).

the resistance of the C57BL/6 strain to PVC441-induced neurological disease might be conferred by a recessive gene. Thus, the genetic basis of the resistance of the various mouse strains seemed to differ from each other, and various host genes might control the clinical expression of neurological disorders. These genes do not correlate with the *Fv-1* gene, or the *Fv-2* gene since all PVC viruses are NB-tropic [10] and C57BL/6 mice congenic for *Fv-2^{sa}* showed resistance to

tremor induction by PVC441.

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