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Isolation of Paralysis-Inducing Murine Leukemia Viruses from Friend Virus Passaged in Rats

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Four clones of murine leukemia viruses (PVC-111, PVC-211, PVC-321, and PVC-441) were isolated from a paralyzed Fischer rat which had been infected with rat-passaged Friend leukemia virus. PVC-211 and PVC-321 viruses induced hind leg paralysis in rats and killed them within 1 month, and PVC-441 did so within 2 months after infection, whereas PVC-111 did not within 4 months. PVC-321 and PVC-441 but not PVC-111 virus grew well in brain and spinal cord media. The viral antigens were found often in glia cells and rarely in neurons of the rats infected with each of these PVC viruses. All of the PVC viruses induced neuronal degeneration but neither inflammation nor leukemic infiltration in the spinal cord. The isolated viruses were all ecotropic and NB-tropic. Age dependency of the susceptibility of rats to paralysis induction was observed.

Dawson et al. have observed that rats infected with Friend leukemia virus (FLV) often develop hind leg paralysis due to the infiltration of leukemic cells in vertebral column and meninges after slow development of leukemia (over 3 months) (1). During the course of study on the susceptibility of rats to FLV infection (5), we found that all rats infected with rat-passaged FLV developed hind leg paralysis and most rats died within 7 weeks after infection. Necropsy revealed no histopathological changes, such as the enlargement of thymus, spleen, or lymph node and leukemic infiltration in various tissues. We suspected that this disease might have been induced by a specific virus like murine leukemia virus (MuLV) derived from wild mice (3, 4). We tried here to isolate the virus which induces paralysis in rats.

Paralysis-inducing viruses were obtained by serial passages of BALB/c-adapted NB-tropic FLV (5, 7) in rats (Fig. 1). Unless otherwise stated, 0- to 2-day-old Fischer rats were used and observed for at least 4 months. The inocula were prepared from the spleens and given to rats intraperitoneally. By the second passage, we did not observe paralysis, although some rats developed splenomegaly. However, at the third passage (step. 3), all infected rats showed a slight-to-moderate tremor of both hind limbs which subsequently became paralyzed. The rats showed weight loss, ruffling of hair, and bowel and bladder incontinence. These clinical observations were quite similar to those on spontaneously paralyzed wild mice (4). The illness lasted for 7 to 14 days until death. Of 12 rats, 10 died within 47 days after infection.

A homogenate was prepared from a survivor and used in two ways, in vivo and in vitro (step. 4). Seven rats injected with this homogenate developed paralysis. In inoculated NRK cells (2), neither cytopathic effect nor microorganism growth was observed. The infected NRK culture was passaged three times, and the culture medium was tested for paralysis induction (step. 5). Seven inoculated rats were paralyzed.

To determine what kind of agent(s) was involved in the development of paralysis, we tested whether the endpoint of XC-plaque formation coincided with that of paralysis induc-

tion. Since XC-positive culture media but not XC-negative media induced paralysis, we began to clone XC-positive viruses by endpoint dilution and obtained four virus clones (PVC-1, PVC-2, PVC-3, and PVC-4). We further purified these clones by two cycles of endpoint dilution, testing the inducibility of paralysis (steps 6, 7, and 8). Four clones, PVC-111, PVC-211, PVC-321, and PVC-441, were obtained from PVC-1, PVC-2, PVC-3, and PVC-4, respectively.

All rats infected with PVC-2 or PVC-3 virus were paralyzed and died within 1 month, and all rats infected with PVC-4 virus did so within 2 months after infection. However, all rats infected with PVC-1 virus developed neither paralysis nor leukemia. Twice purified subclones showed the same pathogenicity as the parent clones (Fig. 2). Thus, the property of each virus clone was stable and distinguishable from each other.

We investigated the propagation of cloned viruses in central nervous system (CNS) tissue as well as in spleens of infected rats (Table 1). NB-tropic F-MuLV which had been cloned from myeloproliferative virus stock (5, 10) was used as a control. All viruses grew well in the spleen. However, in the CNS tissue, PVC-321 and PVC-441 but not PVC-111 virus grew well. Even at the nonparalytic stage, considerable amounts of virus were recovered from the CNS of PVC-441-infected rats no. 1 and 2. Surprisingly, the cloned F-MuLV grew considerably well in CNS tissue. The virus titers in the sera of PVC-441-infected rat no. 3 and 4 were 380 and 300 PFU/0.1 ml, and those in the sera of PVC-111-infected rats no. 3 and 4 were 40 and 30 PFU/0.1 ml, respectively. These results indicate that the blood contamination contributes negligibly to the virus titers of CNS tissue.

By the indirect fluorescent antibody (IFA) method with rabbit antiserum to Rauscher MuLV, viral antigens were detected in the spinal cord of rats infected with each of the cloned viruses. In the rats infected with PVC-211, PVC-321, or PVC-441 virus, many glia cells were stained strongly. Neurons were rarely and very weakly stained (Fig. 3). In the rats infected 130 days previously with PVC-111 virus, a few glia cells were stained. In the rats infected 128 days previously with cloned F-MuLV, as many glia cells were stained as in rats infected with PVC-211, PVC-321, or PVC-441 virus. These findings also indicate that the virus recovered
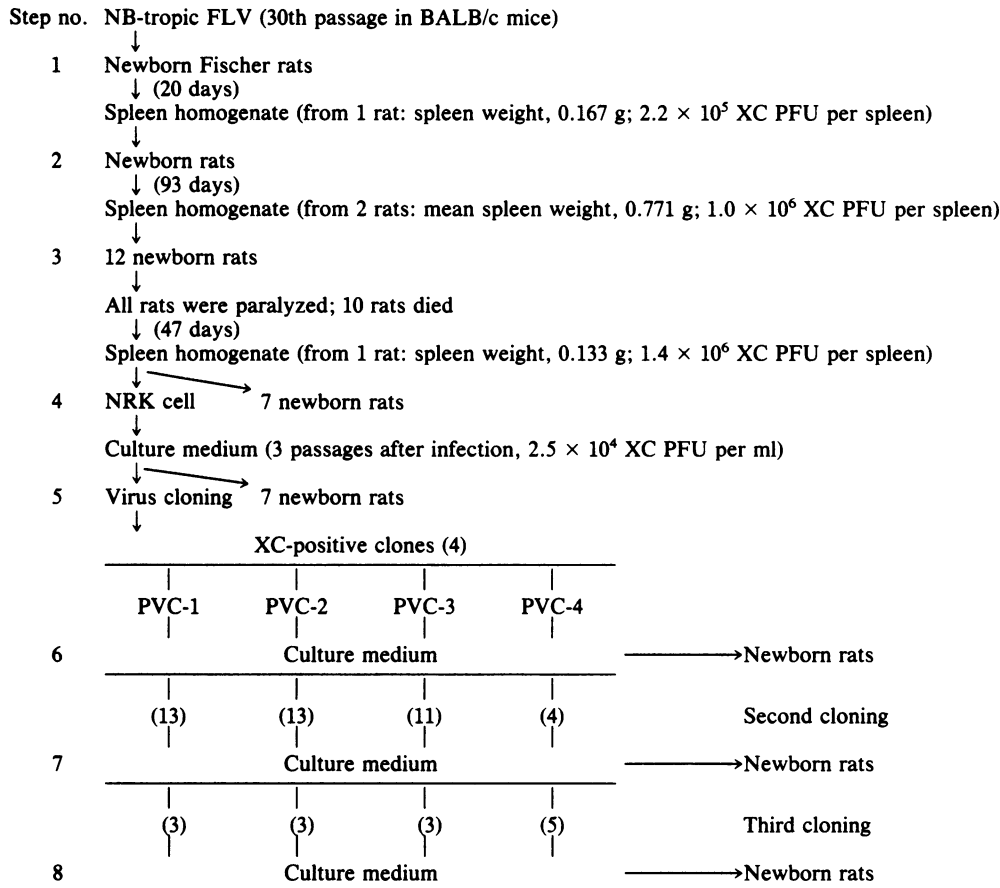

Step no. NB-tropic FLV (30th passage in BALB/c mice)
↓

1 Newborn Fischer rats
   ↓ (20 days)
   Spleen homogenate (from 1 rat: spleen weight, 0.167 g; 2.2 × 10⁵ XC PFU per spleen)
   ↓

2 Newborn rats
   ↓ (93 days)
   Spleen homogenate (from 2 rats: mean spleen weight, 0.771 g; 1.0 × 10⁶ XC PFU per spleen)
   ↓

3 12 newborn rats
   ↓

   All rats were paralyzed; 10 rats died
   ↓ (47 days)
   Spleen homogenate (from 1 rat: spleen weight, 0.133 g; 1.4 × 10⁶ XC PFU per spleen)
   ↓ ⟶

4 NRK cell ⟶ 7 newborn rats
   ↓

   Culture medium (3 passages after infection, 2.5 × 10⁴ XC PFU per ml)
   ↓ ⟶

5 Virus cloning ⟶ 7 newborn rats
   ↓

   XC-positive clones (4)

   | PVC-1 | PVC-2 | PVC-3 | PVC-4 |

6   Culture medium   ⟶ Newborn rats

   | (13) | (13) | (11) | (4) | Second cloning

7   Culture medium   ⟶ Newborn rats

   | (3) | (3) | (3) | (5) | Third cloning

8   Culture medium   ⟶ Newborn rats

FIG. 1. Isolation of paralysis-inducing viruses. NB-tropic FLV prepared from the spleens of infected BALB/c mice (5, 7) was injected intraperitoneally into rats (4 × 10⁴ XC PFU, 4 × 10³ spleen focus-forming units per rat). Rats were killed on the indicated day. Spleen homogenates were prepared from one or two infected rats with 5 ml of culture medium (Eagle minimal essential medium supplemented with 5% newborn calf serum) and stored at −70°C until used. For passages, rats received 0.2 ml of the homogenate. At step 4 the culture medium of infected NRK cells was serially diluted 10-fold and overlaid on NRK cells. The NRK cultures were passaged three times. The culture medium was removed, and the cells were tested with XC cells (12). The culture media from XC-positive as well as XC-negative cultures were tested for paralysis induction (the inoculum [0.2 ml] contained about 3 × 10³ PFU of XC-positive virus in cases of XC-positive culture medium). All of the rats inoculated with XC-positive culture medium developed paralysis and died, whereas those inoculated with XC-negative culture medium did not. The culture medium at step 4 was diluted to the endpoint and overlaid on NRK cultures preseeded in 24-well multidishes (Nunc Co. Ltd., Denmark) (about 0.25 PFU per well). These cultures were tested with XC cells after two or three passages, and the XC-positive culture medium was injected into newborn rats. This cycle of cloning was repeated two more times. Days were counted from infection to time rats were killed. Numbers in parentheses are XC-positive wells per 24 wells tested. The last-mentioned newborn rats were infected as described in the legend to Fig. 2.
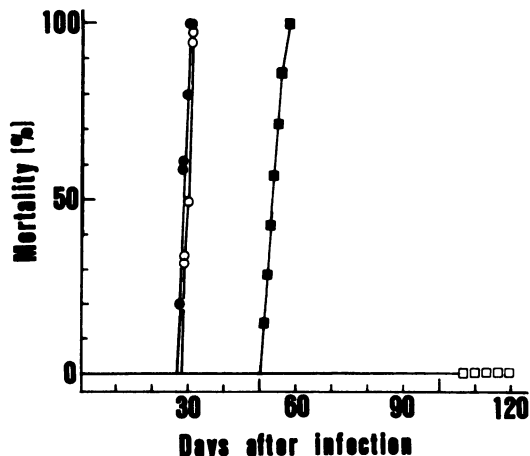

FIG. 2. The mortality of rats infected with cloned viruses. Newborn rats were infected intraperitoneally with PVC-111 (□, 4.7 × 10²

from the CNS tissue (Table 1) was not from the contaminating blood but was produced by the cells in CNS tissue.

Histopathological examination frequently revealed vacuolar degeneration and loss of neurons in the spinal cord of rats paralyzed with PVC-211, PVC-321, or PVC-441 virus (Fig. 4). In two rats which had been infected 130 days previously with PVC-111 virus, the neuronal degeneration was found less frequently in spinal cords although the rats were not paralyzed. In five rats infected with cloned F-MuLV, degenerated neurons were not found by 128 days. In all instances, we observed neither inflammation nor infiltration of leukemic cells.

The PVC viruses as well as F-MuLV showed a one-hit pattern in XC-plaque formation on NIH 3T3, BALB 3T3, and

PFU), PVC-211 (○, 4.5 × 10²), PVC-321 (●, 6.7 × 10²), or PVC-441 (■, 8.4 × 10²). Symbols represent individual rats.

TABLE 1. Virus recovery from various tissues[a]

Rats infected with	Rat no.	Days after infection	Spleen wt (g)	Paralysis	Recovery of virus (PFU/0.1 g of tissue) from			
					Spleen	Brain	Upper s.c.[b]	Lower s.c.
PVC-321	1	28	0.15	+	78,000	3,100	4,000	5,200
	2	28	0.14	+	160,000	5,200	10,000	11,000
PVC-441	1	31	0.20	−	36,000	2,900	3,000	6,800
	2	31	0.20	−	62,000	3,700	2,300	4,200
	3	51	0.17	+	92,000	2,500	4,300	5,500
	4	51	0.28	+	34,000	3,600	4,600	6,600
PVC-111	1	34	0.23	−	21,000	8	33	108
	2	34	0.34	−	18,000	3	43	71
	3	132	0.78	−	27,000	47	130	96
	4	132	0.66	−	23,000	45	58	92
F-MuLV	1	128	0.85	−	110,000	940	3,400	3,500
	2	128	0.52	−	53,000	510	1,500	1,400

[a] Rats were infected intraperitoneally with PVC-321 (6.7×10^2 PFU per rat), PVC-441 (8.4×10^2 PFU per rat), PVC-111 (4.7×10^2 PFU per rat), or F-MuLV (5×10^4 PFU per rat). At the indicated time, the infected rats were killed. Spleens or brains were homogenized with 5 ml of culture medium. Upper or lower spinal cords were homogenized with 1 ml of culture medium. These homogenates were centrifuged at 3,000 rpm for 30 min, and the supernatants were frozen at −70°C until used. Virus titers were determined on NRK cells by XC assay.

[b] The spinal cord was divided into two portions. The half surrounded by thoracic vertebrae was designated the upper s.c., and the other half surrounded by lumbar and sacral vertebrae was designated the lower s.c.

NRK cells but did not induce plaques on mink lung cells. The titers of each virus on NIH 3T3, BALB 3T3, and NRK cells fell within 10-fold differences (data not shown). The NRK cells infected with each of the PVC viruses did not induce foci on S+L− mink cells (11) when tested by infectious center assay (0 focus-forming units per 10^5 infected NRK cells). Thus, the possible contamination of xenotropic, amphotropic, or recombinant virus-like MCF was excluded, and PVC viruses were all ecotropic and NB-tropic.

The susceptibility of Fischer rats to paralysis caused by PVC-321 virus is age dependent. When rats were infected with PVC-321 virus within 2 or 4 days of birth, they developed paralysis and died 28 to 76 days after infection, whereas those infected at 6 to 7 days of age looked healthy during 5 months of observation (data not shown).

The paralysis observed by us apparently differs from that observed by Dawson et al. (1). PVC-2, PVC-3, PVC-4, and their subclones always induced paralysis without leukemia development and killed the infected rats within one or two months. So far, the cloned MuLVs which are paralysis-inducing in rats are not reported. Some MuLVs are paralysis-inducing in mice. The paralytogenic ecotropic viruses derived from wild mice are N-tropic and have a long latent period (2 to 5 months) (3, 8), whereas our viruses are NB-tropic and have a short latent period. In mice paralyzed by the ecotropic virus of wild mice, viral antigens are found not only in glia cells but also in neurons (9), whereas the antigens are found rarely in neurons in our instances (Fig. 3). One of the temperature-sensitive mutants of Moloney MuLV is paralysis-inducing in mice but not in inbred W/Fu rats (6).

Our results suggest that at least two factors are critical for paralysis induction. Since the paralysis-inducing PVC-321 and PVC-441 viruses grew in the CNS tissue better than
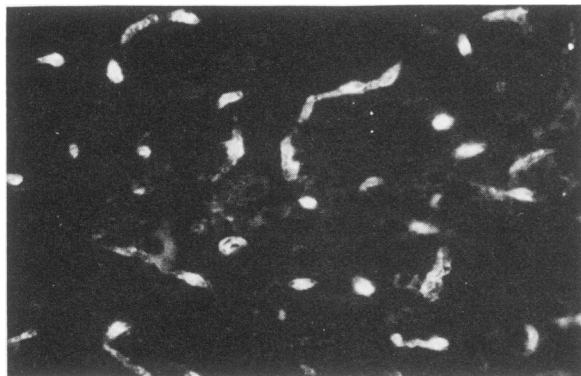

FIG. 3. Detection of viral antigens in the cells of spinal cord by IFA. The spinal cord was removed from a rat which had been infected with PVC-321 virus (6.7×10^2 PFU per rat) 28 days previously and was fixed by the method of Sainte-Marie (13). The cord was thin sectioned and treated with rabbit antiserum to Rauscher MuLV (14) and fluorescein isothiocyanate-conjugated goat immunoglobin G to rabbit immunoglobin G (Cappel Laboratories). (Magnification, ×250.)
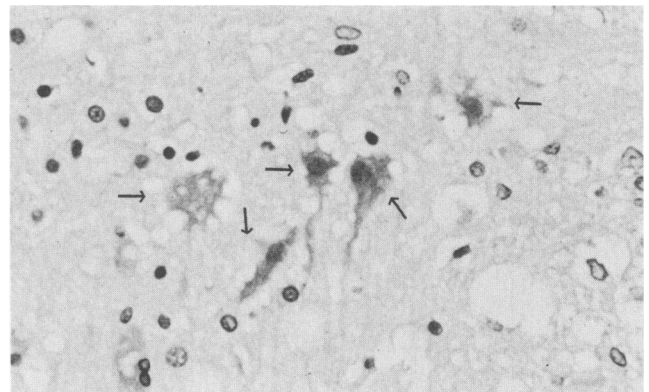

FIG. 4. Histological examination of the spinal cord of a paralyzed rat infected with PVC-321 virus. A portion of the same spinal cord as that used for Fig. 3 was fixed with 10% Formalin in phosphate-buffered saline (pH 7.2) and embedded in paraffin. The thin-sectioned samples were stained with hematoxylin and eosin. Arrows show the degenerated neurons. (Magnification, ×450.)

PVC-111, which is not a paralysis-inducing virus, growth in CNS tissue may be a factor. However, cloned F-MuLV grew considerably well in the CNS tissue. The F-MuLV, when tested with six newborn rats, did not induce paralysis but induced the enlargement of thymus and spleen in one rat during 4 months of observation. Neuronal degeneration may be another factor. We did not find degenerated neurons in F-MuLV-infected rats despite fairly good viral growth. However, PVC-111 (not paralysis-inducing) induced neuronal degeneration, as did PVC-211, PVC-321, and PVC-441.

Neuronal degeneration was observed throughout the CNS tissue of paralyzed rats as observed previously in paralyzed mice (9). We could not determine the lesions that were causally related to paralysis of lower but not upper limbs. In any case, more detailed pathological studies are needed.

Here we obtained four unique MuLV clones which are different in pathogenicity from each other. These viruses may be useful to study a viral gene which is related to paralysis induction.

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