INTRODUCTION

Poliovirus (PV) is a small, non-enveloped virus with a single-stranded, positive-sense genomic RNA; it is known as the causative agent of poliomyelitis and belongs to the family Picornaviridae. The motor neurons are the major target of PV infection in the central nervous system (CNS) (Bodian, 1949). The tropism of PV to the motor neurons is attributable in part to the expression of the PV receptor (PVR) (Crotty et al., 2002; Ida-Hosonuma et al., 2002; Koike et al., 1994; Ren & Racaniello, 1992).

Tissues susceptible to PV infection in the CNS are limited: the brainstem, the roof nuclei of the cerebellum, the precentral gyrus of the cerebrum and the spinal cord (the cervical and lumbar cords) (reviewed by Minor, 1997). Among these tissues in the CNS, the spinal cord seemed to have a high susceptibility to PV infection: PV can adapt to the spinal cord with an increased tropism (Nathanson & Bodian, 1961) and a PV mutant that has a tropism for the spinal cord, but not for the brain, has been isolated (Jia et al., 1999). The adaptation of PV was partly supported by an enhanced efficiency in the uncoating step with decreased thermostability in the virion (Couderc et al., 1999). The lumbar cord supported stable replication of a PV mutant with a severe defect in viral protein synthesis, which showed unstable replication in in vitro-cultured cells and also in the brain (Arita et al., 2004). Experimental infections of other enteroviruses, including coxsackievirus A21 (Dufresne & Gromeier, 2004) and enterovirus 71 (Arita et al., 2005), also suggested that the spinal cord provides a niche for enterovirus infection.

The properties of PV infection in neurons remain controversial. Sabin vaccine strains show decreased levels of viral protein synthesis in a neuroblastoma cell line (SH-SY5Y) or in the cell lysate, compared with the parental virulent strains (Gutiérrez et al., 1997; Haller et al., 1996; Svitkin et al., 1985, 1990). Primary hippocampal neurons of mice produce 100-fold fewer infectious particles than do fibroblasts (Daley et al., 2005), although the growth of PV in a neuroblastoma cell line (SK-N-SH) (Yanagita et al., 2005) or in 293 cells (Campbell et al., 2005), which retained some properties of the neuronal lineage (Shaw et al., 2002), was almost identical to that in HeLa cells. The sensitivity of SK-N-SH cells to cell death was different from that of HeLa cells, and multiple rounds of PV infection were required to cause cytopathic effects (CPE) in SK-N-SH cells (Yanagita et al., 2005). Mice inoculated with PV replicons did not show noticeable
pathogenesis, despite the occurrence of replication and the expression of foreign gene products (luciferase and green fluorescent protein) (Bledsoe et al., 2000), even after repetitive inoculations via the intrathecal route (Jackson et al., 2001). Viral RNA was detected for at least 12 months in the spinal cord of mice in a persistent-infection model of PV (Destombes et al., 1997). Therefore, neurons or cells derived from neural origins could show partial resistance to cell death caused by PV infection.

In this study, we analysed the relationship between PV replication in the spinal cord, damage in the motor neurons and poliomylitis-like paralysis in transgenic mice expressing human PVR (TgPVR21). We performed both biological and histological analyses and estimated the number of critical motor neurons required for severe residual poliomylitis-like paralysis in TgPVR21 mice.

METHODS

Cells, viruses and antibodies. 293T cells (human embryonic kidney cell line 293 expressing the large T antigen of Simian virus 40) (DuBridge et al., 1987) and HEP-2c cells (a human larynx epidermoid carcinoma cell line) were cultured as monolayers in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum (FCS). HEP-2c cells were used for virus titration and measurement of replication kinetics of the PV replicon. 293T cells were used for propagation of PV1 (Sabin strain) and trans-encapsidation of the PV replicon. Rabbit hyperimmune serum against the PV 2C protein (aa 68–329) was a kind gift from Dr Naokazu Takeda, Department of Virology II, National Institute of Infectious Diseases, Japan. pKS435 is a derivative of plasmid pKS435 (a generous gift from Dr Koji Sakai, AIDS Research Center, National Institute of Infectious Diseases, Japan). pKS435 has the puromycin-resistance gene (puri) as a selection marker instead of the blasticidin S deaminase gene in pKS336. The resultant plasmid was designated pKS435-EGFP-PV CAPSID and was used for the transient expression of PV capsid proteins in 293T cells.

Construction of the PV replicon. A plasmid encoding the PV replicon with a luciferase reporter was constructed from plasmid PV-139(−) mc (Arita et al., 2004). A cDNA fragment of the PV IRES (internal ribosome entry site) was amplified by PCR with primers PV110 + 5′-GGCGTAATTTCGACAAACCAAGGTTTAC-3′ and PV-Smal− (5′-TAACTCCCCCGGGTTAAAGTTAATGATAC-3′), using plasmid pMah-Sacl as the template. The PCR product was digested by EcoRI and Smal and then cloned into the corresponding sites of PV-139(−) mc. The resultant construct, encoding a PV luciferase replicon (PV-Fluc mc), was designated pPV-Fluc mc.

DNA transfection. A six-well plate (Falcon) with a 30% confluent monolayer of 293T cells was transfected with 1 μg pKS435-EGFP-PV CAPSID DNA per well by using Effectene transfection reagent (Qiagen) and then incubated at 37 °C in 2 ml 10% FCS/DMEM per well. The cells were washed with 10% FCS/DMEM at 24 h post-transfection and then used for trans-encapsulation of the PV replicon.

RNA transfection. RNA transcripts were obtained by using a RibomAX large-scale RNA production system – T7 kit (Promega) with Dral-linearized DNA of pPV-Fluc mc as the template. RNA transcripts were transfected into a monolayer of 293T cells, which were transiently expressing PV capsid proteins, by the DEAE/dextran method (van der Werf et al., 1986).

Western blot analysis. Western blot analysis was performed by using rabbit hyperimmune serum against the PV1 (Mahoney) virion (Arita et al., 1998), which was a kind gift from Dr Akio Nomoto, Department of Microbiology, Graduate School of Medicine, The University of Tokyo, Japan. The samples were subjected to 5–20% polyacrylamide gradient gel electrophoresis (e-PAGEI; Atto Corporation) in a Laemmli buffer system (Laemmli, 1970). The proteins in the gel were transferred to a PVDF filter (Immobilon; Millipore) and blocked in PBS containing 0.1% Tween 20 and 5% non-fat dry milk. The filters were incubated with rabbit hyperimmune serum against PV1 (Mahoney) (1:1000 dilution in PBS containing 0.1% Tween 20 and 0.5% non-fat dry milk) at room temperature for 1 h. The filters were washed by PBS containing 0.1% Tween 20 three times for 5 min each and then incubated with donkey anti-rabbit IgG antibodies conjugated with horseradish peroxidase (Amersham Biosciences) (1:2000 dilution in PBS containing 0.1% Tween 20 and 0.5% non-fat dry milk) at room temperature for 1 h. The filters were washed by PBS containing 0.1% Tween 20 three times for 5 min each, and then treated with the ECL Western blotting analysis system (Amersham Biosciences) for detection of the signal.

Trans-Encapsidation of the PV replicon. For the preparation of seed stocks of trans-encapsidated PV-Fluc mc (TE-PV-Fluc mc), 293T cells in a six-well plate (Falcon) were transfected with pKS435-EGFP-PV CAPSID DNA followed by transfection of the RNA transcript of PV-Fluc mc at 24 h post-transfection, and then incubated at 37 °C in 2 ml 10% FCS/DMEM per well. Cells were harvested when all of the cells showed CPE. For the preparation of TE-PV-Fluc mc, 293T cells transiently expressing PV capsid proteins in a 10 cm diameter dish (Falcon) were inoculated with 100 μL seed stock in 10 ml 10% FCS/DMEM per dish, and were harvested when all of the cells showed CPE (around 48 h post-inoculation (p.i.)). Virus stocks were stored at −70 °C.

For Western blot analysis, TE-PV-Fluc mc was purified from the cell supernatant of infected 293T cells by using DEAE/Sephrose CL-6B (Amersham Biosciences) (Arita et al., 1998), followed by centrifugation at 35,000 r.p.m. for 2.5 h at 4 °C in a Beckman SW41 rotor with 1 ml of
a 30% sucrose cushion. The pellet was washed three times with distilled water and then dissolved in 100 μl PBS at 4°C overnight. Any remaining pellet was disrupted by pipetting and then stored at −70°C.

**Luciferase assay.** For the measurement of luciferase activity in in vitro-cultured cells, HEp-2c cells in 96-well plates (Falcon) (2·8 × 10^4 cells per well) were infected with 100 μl of the indicated dilution or titre of TE-PV-Fluc mc. The cells were harvested at the time indicated by adding 50 μl passive lysis buffer (Promega) and 10 μl lysisate was used for the measurement of luciferase activity. For the measurement of luciferase activity in the spinal cords of TgPVR21 mice, the spinal cords of inoculated mice were collected around the lumbar area (1·5–2·0 cm) at the time indicated and stored at −70°C. After freezing and thawing of the collected spinal cord samples, were homogenized with 250 μl passive lysis buffer (Promega) and then subjected to centrifugation at 20,000 g for 1 min at 4°C. Part of the supernatant (2 or 10 μl) was used for the measurement of luciferase activity with the Luciferase Assay system (Promega) and a TRITON 1 Microplate luminometer (Applied Biosystems), according to the manufacturers’ instructions.

**Electron microscopy.** Purified TE-PV-Fluc mc was subjected to negative staining in uranyl acetate as described previously (Utagawa et al., 2002). Samples were examined by transmission electron microscopy (JEM-1220; JEOL DATUM) at an acceleration voltage of 80 kV and images were obtained at a magnification of × 50,000.

**Virus titration.** Virus titre was determined by measuring the 50% cell culture infective dose (CCID50) by the microtitration assay in HEp-2c cells (Nagata et al., 2002), and also by measuring the infectious units (IU) by counting the number of infected cells stained by indirect immunofluorescence against the viral antigen (Barclay et al., 1981) expressed in HEp-2c cells (Nagata et al., 2002). Samples were examined by transmission electron microscopy (JEM-1220; JEOL DATUM) at an acceleration voltage of 80 kV and images were obtained at a magnification of × 50,000.

**Intraspinal inoculation and histological analysis of TgPVR21 mice.** All animal procedures were approved by the Committee for Biosafety and Animal Handling and the Committee for Ethical Treatment of Animals of the National Institute of Infectious Diseases, Japan. Animal care, breeding, virus inoculation and observation were performed in accordance with the guidelines of the Committees.

For the histological analysis, the spinal cords of inoculated mice were collected at day 3 p.i. and sections around the lumbar cord were prepared (Nagata et al., 2001). Lesions on the sections were observed after haematoxylin and eosin staining or after Luxol fast blue/cresyl violet staining (Klüver–Barrera method). The lesion scores of the spinal cords were determined according to the procedure recommended by the World Health Organization for the quality control of oral PV vaccine strains (WHO, 1990).

**RESULTS**

**trans-Encapsidation of the PV replicon in 293T cells**

For quantitative analysis of PV replication in the spinal cord of TgPVR21 mice, we trans-encapsidated a PV replicon encoding firefly luciferase in place of the capsid genes (TE-PV-Fluc mc) in 293T cells. 293T cells were transfected with expression vector pKS435-EGFP-PV CAPSID PV, which expresses the PV capsid proteins as a fusion protein with EGFP under the control of the HEF-1x promoter (Fig. 1). The RNA transcript of PV-Fluc mc was then transfected into the 293T cells expressing PV capsid proteins, resulting in the production of pseudovirions (Fig. 1c). The composition of the capsid proteins of trans-encapsidated PV-Fluc mc (TE-PV-Fluc mc) was similar to that of PV1 (Mahoney) virions. However, the VP2 and VP3 proteins in TE-PV-Fluc mc particles were slightly smaller than those of PV1 (Mahoney) (Fig. 1d). These results indicated that the PV replicon was trans-encapsidated efficiently in 293T cells transiently expressing PV capsid proteins.

We measured the titre of TE-PV-Fluc mc by counting the numbers of infected cells stained by indirect immunofluorescence using rabbit hyperimmune serum against the PV non-structural protein 2C (Fig. 2a). The titre of TE-PV-Fluc mc was 6·3 ± 10^3 IU ml⁻¹ and the estimated CCID50 was 3·0 × 10^8 ml⁻¹, which was calculated from that of the PV1 (Sabin) strain (6·7 ± 10^4 IU ml⁻¹ and 3·2 ± 10^8 CCID50 ml⁻¹, 4·8 CCID50 IU⁻¹). PV1 (Mahoney) showed a fourfold higher titre than PV1 (Sabin); however, the CCID50 IU⁻¹ value was similar to that of PV1 (Sabin) (data not shown). Therefore, the estimated CCID50 values of TE-PV-Fluc mc from the titres of attenuated or virulent strains were the same.

Next, we aimed to detect viable viruses that could emerge during trans-encapsidation in the preparations of TE-PV-Fluc mc. We performed virus isolation from 6·3 ± 10^4 IU (corresponding to 3·0 × 10^7 estimated CCID50) TE-PV-Fluc mc. Quantitative control of viable virus in TE-PV-Fluc mc was performed by adding different titres of PV1 (Sabin) (from 3·2 to 3·2 × 10^5 CCID50) to the preparation of TE-PV-Fluc mc (Fig. 2b). We observed substantial CPE in the inoculated cells at the first passage of all samples (Fig. 2b). During the second passage, no CPE was observed in cells inoculated with TE-PV-Fluc mc, in contrast to the complete CPE observed in the cells inoculated with TE-PV-Fluc mc mixed with PV1 (Sabin) at a titre as low as 3·2 CCID50. In the third passage of TE-PV-Fluc mc, the cells showed no CPE. We
further examined the samples collected in the virus isolation for viable virus (Fig. 2c). Complete cell lysis was only observed in the cells inoculated with PV1 (Sabin) or with a high m.o.i. of TE-PV-Fluc mc (at an m.o.i. of 22), but not with diluted TE-PV-Fluc mc. Residual luciferase activity was observed in the samples of the first and second passages, but not in those of the third passage (Fig. 2c). The capsid proteins were not detected in these samples by Western blot analysis (data not shown). Consequently, we could not detect viable virus in the preparation of TE-PV-Fluc mc produced in 293T cells, as reported in the previously established trans-encapsidation system of PV replicon (Jackson et al., 2001).

Replication of TE-PV-Fluc mc in HEp-2c cells and in the spinal cord of TgPVR21 mice

We measured the replication kinetics of TE-PV-Fluc mc in in vitro-cultured cells (HEp-2c cells) and in the spinal cord of TgPVR21 mice (Fig. 3). For the measurement of replication kinetics in vitro, HEp-2c cells were infected with TE-PV-Fluc mc at an m.o.i. of 0.024, 0.24 or 24. Luciferase activity in HEp-2c cells reached a peak level at as early as 6–10 h p.i., depending on the inoculated titre. The number of infected cells inoculated at an m.o.i. of 0.024, 0.24 and 24 was measured by indirect immunofluorescence and was 6·1 × 10², 4·8 × 10³ and 2·6 × 10⁴, respectively. For the measurement of replication kinetics in spinal cords, TgPVR21 mice were inoculated with 3·2 × 10⁵ or 4·1 × 10⁶ IU TE-PV-Fluc mc via the intraspinal route. Maximum luciferase activities in the spinal cords were observed at 10 h p.i. (Fig. 3b). A close correlation between the inoculated titre and the maximum luciferase activity at 10 h p.i. in the spinal cords was observed for a range of titres from 10⁴ to 10⁷ IU TE-PV-Fluc mc (Fig. 3c). These results suggested that the properties of replication of TE-PV-Fluc mc in the
spinal cord of TgPVR21 mice were similar to those in HEp-2c cells, although with a slight delay.

**Poliomyelitis-like paralysis in TgPVR21 mice inoculated with TE-PV-Fluc mc**

We then prepared a range of titres of TE-PV-Fluc mc for inoculation into TgPVR21 mice via the intraspinal route and characterized the induced paralysis of the inoculated mice. At 1 day p.i., flaccid paralysis of the hindlimb of TgPVR21 mice inoculated with $3 \times 10^6$ IU (estimated CCID$_{50}$ $1 \times 10^6$) TE-PV-Fluc mc was observed (Fig. 4a). TgPVR21 mice inoculated with the PV1 (Sabin) strain via the intraspinal route showed paralysis at day 1, 2 or 3 p.i., dependent on the titres of inoculated virus (Abe et al., 1995) (Fig. 4b). All of the TgPVR21 mice inoculated with PV1 (Sabin) succumbed to lethal paralysis, in contrast to non-lethal paralysis of those inoculated with TE-PV-Fluc mc.

The paralysis induced by the inoculation of TE-PV-Fluc mc was mostly limited to the left hindlimb, corresponding to the inoculated site in the spinal cord (Fig. 5a). However, some mice inoculated with high doses of TE-PV-Fluc mc ($>3 \times 10^6$ IU) showed paralysis of both hindlimbs and this could even result in lethal paralysis (four out of 18 inoculated mice; Table 1), although no viable viruses were isolated from the CNS (data not shown). Severity of the induced paralysis was classified into four levels according to the symptoms observed in the hindlimb: (i) a decline in grip strength, (ii) weakness of the hindlimb, (iii) partial flaccid paralysis and (iv) complete flaccid paralysis (Table 1). Paralysis could be observed at as early as 10 h p.i. in the mice inoculated with $3 \times 10^6$ IU TE-PV-Fluc mc and the number of mice showing severe paralysis (i.e. partial to complete flaccid paralysis of the hindlimb) reached a plateau at 16 h p.i. (Fig. 4c). The PD$_{50}$ value of TE-PV-Fluc mc in TgPVR21 mice was determined as $3 \times 10^6$ IU ($1 \times 10^5$ estimated CCID$_{50}$). The lowest titre of TE-PV-Fluc mc required for the induction of severe paralysis was $1 \times 10^5$ IU, where half of the inoculated mice showed severe paralysis (Table 1). We observed mild paralysis (i.e. a decline in grip strength and weakness of the hindlimb) in
mice inoculated with $3 \cdot 2 \times 10^4$ IU, but no apparent clinical symptoms were observed with inoculation of $3 \cdot 2 \times 10^3$ IU TE-PV-Fluc mc. Therefore, transient replication of TE-PV-Fluc mc in the spinal cord of TgPVR21 mice caused non-lethal poliomyelitis-like paralysis with different severity in a dose-dependent manner.

Under the conditions examined, we estimated the number of infected cells in the spinal cords, from a value of the maximum luciferase activity per single HEp-2c cell infected by TE-PV-Fluc mc, to be at most $3 \cdot 9 \times 10^4$ cells ($\pm 1\cdot0 \times 10^4$) cells (Bjugn et al., 1997), suggesting that a substantial population of the motor neurons was infected by TE-PV-Fluc mc under the conditions examined.

### DISCUSSION

In this study, we developed a new trans-encapsidation system of a PV replicon in 293T cells. To date, trans-encapsidation systems of PV by helper PV (Barclay et al., 1998; Hagine-Yamagishi & Nomoto, 1989), by recombinant vaccinia virus expressing the capsid proteins (Ansardi et al., 1993) or by plasmid expression vector in combination with recombinant vaccinia virus expressing T7 RNA polymerase...
(Jia et al., 1998), have been established. 293T cells allow a high-level expression of protein (DuBridge et al., 1987) and have been utilized for trans-encapsulation systems of retrovirus (Evans et al., 2004; Pear et al., 1993) and also for increasing the titre of papillomavirus (Pyeon et al., 2005).

TE-PV-Fluc mc particles had a similar composition of capsid proteins to that of wild-type virus; however, apparent sizes of the VP2 and VP3 proteins were smaller than those of wild-type virus (Fig. 1c). TE-PV-Fluc mc was neutralized completely by anti-PV1 antiserum, but not by anti-PV2 or -PV3 antisera (data not shown), suggesting that TE-PV-Fluc mc particles retained the antigenicity of the original PV1. The properties of capsid proteins in pseudovirions remain to be further studied.

The replication kinetics of TE-PV-Fluc mc in the spinal cord of TgPVR21 mice were similar to those in HEp-2c cells, but with a slight delay, as observed previously (Fig. 3) (Bledsoe et al., 2000; Porter et al., 1998). The maximum number of viral genomes of PV found in degenerating motor neurons of cynomolgus monkeys was comparable to that observed in HEp-2c cells (Couderc et al., 1989). However, the efficiency of TE-PV-Fluc mc infection in the spinal cord was lower than that observed in HEp-2c cells, probably because of the limited accessibility of the virion to the target neurons (Table 1). We estimated that a mean of $1 \times 10^5$ IU (or $8 \times 10^2$ estimated CCID$_{50}$) TE-PV-Fluc mc was required for the infection of a single susceptible cell in the spinal cord from the maximum luciferase activity observed in a single infected HEp-2c cell. The PD$_{50}$ values of PV1 (Sabin) and PV1 (Mahoney) in TgPVR21 mice by intraspinal inoculation were $10^{3.4}$ CCID$_{50}$ (Abe et al., 1995) and $<10^{1.3}$ CCID$_{50}$ (N. Nagata, unpublished result), respectively. Assuming that PV infection in a single cell results in the paralysis of inoculated mice, the infectivity of TE-PV-Fluc mc was intermediate between those of the virulent and attenuated strains.

TgPVR21 mice inoculated with TE-PV-Fluc mc showed a wide range of paralysis symptoms (from a decline in grip strength to complete flaccid paralysis of the hindlimb), with histological features typical of PV infection (infiltration of neutrophils, neuronophagia and neuronal loss) (Bodian, 1949; Bodian & Howe, 1941) (Fig. 4; Table 1). The pathological features of TE-PV-Fluc mc were virus-specific, because UV-treated TE-PV-Fluc mc did not cause any clinical symptoms in inoculated mice (data not shown). These observations are inconsistent with previous reports on PV replicons, where no clinical symptoms or pathological features were observed in inoculated mice (Bledsoe et al., 2000; Jackson et al., 2001). Differences in the structure of the replicon [e.g. form of the luciferase protein,
genomic structure of the replicon, length of the poly(A) tail, restriction-enzyme sites and/or unidentified epigenetic modifications (Brown et al., 2005; DeJesus et al., 2005; Porter et al., 1998), the transgenic mice (e.g. strain and age) (Abe et al., 1995; Crotty et al., 2002) and the titration procedure of the trans-encapsidated PV replicon could be critical determinants of the apparent pathogenicity of the PV replicon. TE-PV-Fluc mc showed faster replication kinetics (peak at as early as 6–10 h p.i. with an m.o.i. ranging from 0-024 to 24) compared with those of a previously reported PV replicon (peak at 12 h p.i. at an m.o.i. of 10) (Porter et al., 1998). The replication efficiency of PV was proportional to the size of the deletion in the genome (Kaplan & Racaniello, 1988), and coding sequences of the reporter gene could affect protein-synthesis activity in Hepatitis C virus (reviewed by Lemon & Honda, 1997). The factors required for the pathogenesis of the PV replicon system remain to be further elucidated.

We observed a time lag between the peak of replication of TE-PV-Fluc mc in the spinal cord (10 h p.i.) and the appearance of paralysis (which reached a plateau at 16 h p.i.) (Figs 3b, 4c). PV-induced apoptosis has been observed both in vitro and in vivo (Girard et al., 1999; Romanova et al., 2005; Tolskaya et al., 1996), and Couderc et al. (2002) showed a time lag between the peak of virus growth (8 h p.i.) and the development of apoptosis (28 h p.i.) in a mixed primary nerve-cell culture. These findings suggest a direct
link between in vivo apoptosis and functional loss of motor neurons during the transient replication of TE-PV-Fluc mc. The biological characteristics of the in vivo cell death induced by the PV replicon remain to be further studied.

Histological analysis showed a correlation between the severity of the clinical symptoms and the lesion scores in most mice inoculated with TE-PV-Fluc mc (Table 2). However, for mice with a partial loss of the motor neurons

### Table 1. Clinical symptoms of TgPVR21 mice inoculated with TE-PV-Fluc mc

<table>
<thead>
<tr>
<th>Inoculated titre of TE-PV-Fluc mc (IU)</th>
<th>Severity of paralysis of the hindlimb*</th>
<th>Estimated no. infected cells†</th>
<th>Inoculated titre (IU) per infected cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5 × 10^7</td>
<td>0</td>
<td>3-9 × 10^4 (100)</td>
<td>3-8 × 10^2</td>
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<tr>
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<td>0</td>
<td>2-6 × 10^4 (66)</td>
<td>1-6 × 10^2</td>
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<td>ND</td>
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<td>0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1-6 × 10^5</td>
<td>1/6</td>
<td>2-4 × 10^3 (1-4)</td>
<td>3-0 × 10^2</td>
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</tr>
<tr>
<td>3-2 × 10^3</td>
<td>3/3</td>
<td>6-6 × 10^1 (0-17)</td>
<td>4-9 × 10</td>
</tr>
</tbody>
</table>

*–, No symptoms; +, decline in grip strength; ++, weakness of the hindlimb; ++++, partial flaccid paralysis; ++++, complete flaccid paralysis. Number of mice showing indicated symptoms over total number of inoculated mice is shown. Number of mice with lethal paralysis is shown in parentheses.
†Number of infected cells was estimated from the total luciferase activity recovered from the spinal cord of inoculated mice. Number in parentheses represents percentage of infected cells, where the highest number of infected cells observed under the examined conditions (3-9 × 10^4 cells) is taken as 100 %. ND, Not determined.

Histological analysis showed a correlation between the severity of the clinical symptoms and the lesion scores in most mice inoculated with TE-PV-Fluc mc (Table 2). However, for mice with a partial loss of the motor neurons

### Table 2. Clinical symptoms and lesion score of the spinal cords of TgPVR21 mice inoculated with TE-PV-Fluc mc

<table>
<thead>
<tr>
<th>Mouse no.</th>
<th>Inoculated titre of TE-PV-Fluc mc (IU)</th>
<th>Paralysis*</th>
<th>Lesion score of the spinal cord†</th>
</tr>
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<tr>
<td></td>
<td></td>
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<td>Position +1</td>
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<tr>
<td></td>
<td></td>
<td>L</td>
<td>R</td>
</tr>
<tr>
<td>1</td>
<td>1-5 × 10^7</td>
<td>L + + + , R +</td>
<td>2</td>
</tr>
<tr>
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<tr>
<td>12</td>
<td>3-2 × 10^3</td>
<td>–</td>
<td>0</td>
</tr>
</tbody>
</table>

*Mice inoculated with the indicated titre of TE-PV-Fluc mc were subjected to histological analysis at day 3 p.i. –, No symptoms; +, decline in grip strength; ++, weakness of the hindlimb; ++++, partial flaccid paralysis; ++++, complete flaccid paralysis; L, left hindlimb; R, right hindlimb.
†Positions of the section examined are represented as 0 (inoculation site), +1 (3 mm cephalad from the inoculation site) and −1 (3 mm caudad from the inoculation site). Lesion of the spinal cord was scored as: 0, no lesion; 1, inflammation (represented by an infiltration of neutrophils); 2, inflammation with partial degeneration of the motor neurons; 3, inflammation with severe degeneration and a partial loss of the motor neurons; 4, severe inflammation and complete loss of the motor neurons. L, Left side of anterior horn; R, right side of anterior horn.

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(with a lesion score of < 3), it was difficult to make a correct inference for the severe paralysis from the overall lesion scores only. We estimated that the proportion of critical motor neurons was, at most, 1·4 % of susceptible neurons in the lumbar cord (Table 1). Limbs affected by poliomyelitis in humans showed a mean of 40·8 % remaining motor units (McComas et al., 1997). Therefore, a small population of the motor neurons in the lumbar cord seemed to be critical for severe paralysis in TgPVR21 mice.

In summary, we have developed a trans-encapsidation system for a PV replicon in 293T cells and analysed the poliomyelitis-like paralysis of TgPVR21 mice induced by the PV replicon. This model would be useful for the analysis of in vivo cell death induced by PV infection and for the development of effective therapies for poliomyelitis (Dodd et al., 2005).

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Quantitative analysis of poliomyelitis-like paralysis


