Sabin attenuated LSc/2ab strain of poliovirus spreads to the spinal cord from a peripheral nerve in bonnet monkeys (*Macaca radiata*)

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Vaccine-associated paralytic poliomyelitis is a serious concern while using the live attenuated oral polio vaccine for the eradication of poliomyelitis. The bonnet monkey model of poliovirus central nervous system (CNS) infection following experimental inoculation into the ulnar nerve allows the comparative study of wild-type and attenuated poliovirus invasiveness. Dosages $\geq 10^4$ TCID$_{50}$ of Mahoney strain of poliovirus type 1 [PV1(M)] result in paralysis. In contrast, even with $10^7$ TCID$_{50}$ of Sabin attenuated strain of poliovirus type 1 (LSc/2ab), no paralysis occurs, but virus spreads into the CNS where viral RNA is found in spinal cord neurons. While wild-type PV1(M) viral RNA replicates in neurons (and possibly in glial cells) and in cells around vessel walls, which may be mononuclear or endothelial cells, attenuated viral RNA is detected only in neurons. Systemic viraemia and gastrointestinal virus shedding occurs only in PV1(M)-infected animals. While a systemic serologic response is detected in both groups of animals, cerebrospinal fluid antibodies are detected only in animals infected with PV1(M). Both the PV1(M) and LSc/2ab strains spread to the cervical spinal cord and then to the lumbar spinal cord following ulnar nerve inoculation. Neuronophagia and neuronal loss are only seen in PV1(M)-infected monkeys in whom clinical paralysis is observed. Infection with LSc/2ab does not result in neuronophagia, neuronal loss or clinical paralysis. Spread of attenuated poliovirus in spinal cord neurons without causing paralysis following inoculation into the ulnar nerve is an important finding.

Introduction

The killed polio vaccine and the live attenuated polio vaccine have both been used effectively in the progressive eradication of poliomyelitis. However, the risk of vaccine-associated paralytic poliomyelitis (VAPP) is a serious concern when using the live attenuated vaccine. It is common practice to immunize children simultaneously against a number of childhood diseases and, thus, to administer oral polio vaccine (OPV) along with the intramuscular diphtheria–pertussis–tetanus vaccine. Intramuscular injections are known to increase the risk of paralysis caused by poliovirus (Wyatt, 1989). In recent years, provocation paralysis following OPV administration has been observed at a much higher rate in Romania compared with USA (Strebel et al., 1994) and has been strongly associated with injections that have been administered after the OPV. Provocation paralysis is caused by the retrograde axonal transport of poliovirus from the innervating sites of damaged nerves of the injected muscles to the central nervous system (CNS) (Gromeier & Wimmer, 1998).

In conventional OPV neurovirulence testing, primates are inoculated intraspinally. Virus spread within the CNS and histological evaluation of both the spinal cord and the brain are then used to assess the degree of neurovirulence and the extent of virus attenuation. From these studies it is evident that the attenuated strain of poliovirus is capable of replicating within the CNS following direct introduction into the CNS. This finding has been supported by the *in situ* hybridization studies of Couderc et al. (1989). There are several studies that show that wild-type poliovirus spreads to the CNS through a neural route from its primary site of replication (reviewed by Morrison & Fields, 1992). However, the ability of the attenuated strain of virus to spread from a peripheral nerve to the CNS in a primate model has not been studied previously.

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although there is evidence that the attenuated strain of poliovirus spreads to the CNS in the mouse model (Ohka et al., 1998).

Bonnet monkeys (Macaca radiata), which are found abundantly in India, are effective models of poliovirus infection simulating human disease, as both the intestinal tract and the CNS are susceptible to poliovirus infection. These animals have microfold (M) cells in the small intestine (Samuel et al., 1992), similar to the type of paralysis described in humans (Scinski et al., 1990). After oral inoculation of the wild-type (Mahoney strain) poliovirus type 1 [PV1(M)], virus is shed from the gastrointestinal tract consistently for up to 22 days and paralysis occurs in a small proportion of monkeys, similar to that occurring in humans. In order to consistently create a CNS infection, PV1(M) was inoculated directly into the ulnar nerves of bonnet monkeys; paralytic poliomyelitis occurred in a small proportion of monkeys, similar to that occurring in humans. In order to consistently create a CNS infection, PV1(M) was inoculated directly into the ulnar nerves of bonnet monkeys; paralytic poliomyelitis occurred in all animals inoculated with \( 10^5 \) TCID\(_{50}\) and poliovirus was recoverable from the spinal cord of paralysed animals (John et al., 1992). Characteristic histological changes of myelitis were also observed (Samuel et al., 1993). Following peripheral intraneural inoculation, the virus spreads through a neural route into and within the spinal cord (Ponnuraj et al., 1998). Bonnet monkeys are also susceptible to oral infection with the Sabin attenuated strain of poliovirus type 1 (LSc\(2ab\)). However, peripheral intraneural inoculation of LSc\(2ab\) does not result in paralysis. There is, nevertheless, a seropositive response, suggesting that infection has taken place (John et al., 1992). As intraneural inoculation of PV1(M) can consistently cause CNS infection, this model was used to determine if the attenuated virus can spread to the CNS from a peripheral nerve. We test the hypothesis that LSc\(2ab\) spreads into both the CNS and the extraneural tissue after inoculation into the ulnar nerve.

### Methods

- **Monkeys.** Wild juvenile bonnet monkeys, weighing 1–2 kg and testing seronegative for all three serotypes of poliovirus, were studied after 6 weeks of quarantine. All experiments received prior approval by both the Animal Welfare and Safety Committee of the Christian Medical College, Vellore, India and the Animal Care and Use Committee of the University of Colorado Health Sciences Center, Denver, USA.

- **Virus and cell cultures.** Stock viruses were grown in HEp-2 (Cincinnati) cells. The supernatant of fresh uninoculated monolayers of HEp-2 cell cultures that were harvested by three freeze–thaw cycles and centrifuged for 10 min at 2250 g at 4°C was used as control antigen.

- Poliovirus strain LSc\(2ab\) was obtained from the National Institute for Biological Standards and Control, UK. Three passages of this virus were made at 33°C in HEp-2 cells to prepare a virus stock of \( 10^5 \) TCID\(_{50}\) and poliovirus was detectable by hybridization for the detection of viral mRNA. Samples were also tested with those from 11 monkeys (used in the second experiment and in previous studies) infected with PV1(M). Isolation of poliovirus was attempted on both plasma and Buffy coat samples. In order to understand the host response to both the intra- and the extraneural spread of virus in this model, neutralizing antibody studies were done. Serum was collected on days 0 and 7 p.i. and on the day of necropsy to determine poliovirus-neutralizing antibody titres. A cerebrospinal fluid (CSF) sample was obtained by cisternal puncture at necropsy. For necropsy studies of the spinal cord, laminectomy was performed after exsanguination and spinal cord samples were obtained from the upper and lower segments of the cervical, thoracic and lumbar regions. Samples were assessed by histological staining and by in situ hybridization for the detection of viral mRNA. Samples were also tested for virus isolation, as described earlier (Ponnuraj et al., 1998).

- **Virus inoculation.** Intraneural inoculation into the ulnar nerve at the right elbow was carried out as described previously (Ponnuraj et al., 1998). To determine if there were histological changes in the spinal cord and whether virus replicated in the spinal cord, two experiments were conducted (Table 1).

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Observation period (days)</th>
<th>LSc(2ab)-infected</th>
<th>Mock-infected*</th>
<th>PV1(M)-infected†</th>
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<tr>
<td>1</td>
<td>10–12</td>
<td>3</td>
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<tr>
<td>Previously published†</td>
<td>6–14</td>
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<td>9</td>
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</table>

* Mock-infected monkeys were inoculated with either HEp-2 cell culture supernatant (experiment 1) or MEM (experiment 2).
† Data from Ponnuraj et al. (1998) and Samuel et al. (1992, 1993) are included to provide additional data on histology, viraemia and antibody levels.
Virus culture and estimation of neutralizing antibody titre. Primary monkey kidney cell cultures were used for virus isolation from all specimens and spinal cord suspensions were titrated by standard methods using HEp-2 cells (WHO, 1992). Virus isolates were typed using poliovirus type 1 antiserum by a modification of the microneutralization assay (WHO, 1992). For the determination of neutralizing antibody in the serum and CSF samples, a standard quantal microtitre assay with varying serum dilutions and standard amount of virus (10⁴ TCID₅₀) was carried out on HEp-2 cells (WHO, 1992).

Histology. Coded spinal cord samples collected in Carnoy's fixative were embedded in paraplast. Multiple 5 μm sections were stained with Harris's haematoxylin and eosin (H&E) and 10 μm sections with cresyl violet (CV). Histological examination of the spinal cord was carried out for each monkey on upper and lower segments of each of the cervical, thoracic and lumbar regions. From each of the six segments, ten sections were examined and histological changes were scored as before (Ponnuraj et al, 1982). The mean of the scores derived from the upper and lower segments of each anatomical region was used in the analysis.

In situ hybridization. Spinal cord samples were fixed in paraformaldehyde–lysine–periodate fixative and embedded in paraplast. Sections of 10 μm were used for in situ hybridization. Replicative intermediates of poliovirus were detected with positive-sense riboprobes specific for the 5' end of poliovirus type 1 (nucleotides 1–1809), which were made using digoxigenin-labelled UTP (Boehringer Mannheim). In situ hybridization was carried out as described previously (Ponnuraj et al., 1998). In brief, sections were deparaffinized, hydrated, pretreated with 0.2 M HCl at room temperature for 20 min and proteinase K (0.38 mg/100 ml) at 37 °C for 20 min prior to prehybridization for 2 h at 42 °C. Prehybridization mix consisted of 50% deionized formamide, 4 × SSC, 1 × Denhardt's solution, 250 μg/ml yeast tRNA and 10% dextran sulphate. Sections were hybridized with positive-sense probes overnight at 42 °C. Following hybridization, sections were washed with gradually increasing stringency buffers (2 ×, 1 × and 0.5 × SSC) and treated with RNase. The hybridized digoxigenin-labelled probe was detected using the anti-digoxigenin antibody tagged to alkaline phosphatase (Boehringer Mannheim). Bound antibody was detected using NBT/BCIP (Boehringer Mannheim) as a substrate.

Estimation of albumin index. Serum and CSF collected simultaneously were used for the estimation of albumin levels using the Brom Cresyl green method (Peters et al., 1982). In order to establish the normal values of albumin index, serum and CSF samples from 15 normal animals were tested. Albumin indices were calculated using the following formula: albumin index = albumin quotient × 1000 where albumin quotient = CSF albumin/serum albumin (Peters & Bowman, 1992). Albumin indices from experimental animals were compared against values obtained for normal animals and between experimental groups.

Statistical methods. Histological scores of spinal cords from the three anatomical sites of the two groups of animals were compared using the Wilcoxon rank sum test. Serum-neutralizing antibody levels against poliovirus in monkeys inoculated with PV1(M) and LSc/2ab were also analysed using the Wilcoxon rank sum test. Variance analysis was used to find out if the albumin index was elevated in experimental groups compared with the albumin index of normal controls.

Results

LSc/2ab viral RNA demonstrated in the spinal cords of monkeys after intraneural inoculation

In situ hybridization revealed poliovirus RNA in cervical and lumbar regions of the spinal cord of monkeys inoculated with LSc/2ab. RNA was distributed in neurons of the anterior, intermediate and posterior columns of the cervical region. In the lumbar region, some of the anterior horn neurons were not infected. The intensity of signals and the number of neurons in animals infected with LSc/2ab (Fig. 1a) in each section observed were lower than those in PV1(M)-infected animals (Fig. 1b). Due to the increased number of cells in the parenchyma of the grey matter, microscopic areas around the neurons had a darker hue in the spinal cord of monkeys inoculated with PV1(M) than those observed in either controls or LSc/2ab-infected monkeys. In situ hybridization on control animals inoculated with medium did not have any signal, illustrating the specificity of the assay; these data were published previously (Ponnuraj et al., 1998). No counter stain was used in the in situ hybridization as it is fairly easy to appreciate the cellular morphology within the spinal cord. Since virus was inoculated in the ulnar nerve, which originates from the lower cervical and upper thoracic cord, the presence of polioviral RNA in the lumbar region in addition to the
region of origin of the ulnar nerve not only shows that poliovirus replicates in the neurons but also shows that it spreads from neuron to neuron.

Viral RNA observed in cells around vessel walls and in glial cells in the spinal cord of PV(1)M-infected but not LSc/2ab-infected monkeys

No viral RNA was detected within glial cells or in vessel walls from monkeys inoculated with LSc/2ab (Fig. 2a). Viral RNA was demonstrated, however, both in the cells around enlarged blood vessels (arrow in Fig. 2b), which could be endothelial cells or invading mononuclear phagocytic cells, and glial cells in the parenchyma (arrowheads in Fig. 2b) in monkeys inoculated with PV1(M). Animals inoculated with control medium, which served as a negative control for in situ hybridization, did not have any signals (data not shown).

Infectious virus was not detectable in the spinal cords from animals inoculated with LSc/2ab despite the presence of RNA

Virus was isolated from all monkeys inoculated with PV1(M) and necropsied between days 6 and 14 p.i. However, no virus was isolated from any of the monkeys inoculated with LSc/2ab and necropsied between days 7 and 11 p.i., although viral RNA was present in the samples of spinal cord collected for in situ hybridization.
Minimal neuronal damage occurs in the spinal cords from monkeys inoculated with LSc/2ab

Animals inoculated with control medium had normal neurons and vessels (Fig. 3a). Monkeys inoculated with the attenuated LSc/2ab strain showed few damaged neurons and an occasional vessel with mild vasculitis. A representative
neuron is seen with hyperchromatic nuclei in Fig. 3(b) (small arrow) along with a vessel with mild vasculitis (Fig. 3b) (large arrow). Arrowheads indicate the increased perineuronal reactivity that is occasionally seen (Fig. 3b). In contrast, numerous neurons with absent Nissl substance were observed in monkeys inoculated with PV1(M) and vessels were enlarged, with severe vasculitis (Fig. 3c). Damaged neurons were found to undergo neuronophagia. Damaged neurons were seen not only by H&E staining but also by CV staining (Fig. 4a). In monkeys inoculated with PV1(M) there was obvious neuronal damage with perineuronal reactivity (Fig. 4b arrowheads), increase in cellularity of the parenchyma and vascular and perivascular reactivity, as seen by CV staining.

To quantify the extent of these qualitative histological findings, ten sections from the upper and lower segment of each region of the spinal cord from each monkey were examined. The mean histological scores of lesions in each of the regions of the spinal cord from five monkeys inoculated with LSc/2ab and those from 11 monkeys inoculated with PV1(M) are shown in relation to the day p.i. in Fig. 5. There was no increase in histological scores with time between day 6 and 14 of observation in this experiment. Since there was no progression of the histological scores with time, all monkeys autopsied at various time-points were grouped together and the average scores were analysed (Fig. 6a, b).

The histological scores in the lumbar region were significantly higher in monkeys inoculated with PV1(M) (mean \( \pm \) SEM, 3.43 \( \pm \) 0.19) when compared with those inoculated with LSc/2ab (1.8 \( \pm \) 0.2) (\( P < 0.003 \)). The histological scores of the thoracic region were also significantly higher in monkeys inoculated with PV1(M) (2.75 \( \pm \) 0.191) when compared with those inoculated with LSc/2ab (1.5 \( \pm \) 0.22) (\( P < 0.005 \)). Histological scores of the cervical cord were not significantly different between the two groups of animals. The mean of cumulative scores of all three regions was significantly higher (8.04 \( \pm \) 0.5) in monkeys inoculated with PV1(M) compared with monkeys inoculated with LSc/2ab (5.3 \( \pm \) 0.37) (\( P < 0.006 \)).

**LSc/2ab was not recovered from extraneural sites**

Virus was not isolated from any of the buffy coats or plasma samples drawn from 1 to 7 days p.i. after inoculation with LSc/2ab. Poliovirus was not found in throat or stool samples from monkeys inoculated with LSc/2ab or in samples from control monkeys inoculated with either the supernatant from HEP-2 cell culture lysate or MEM. Fig. 7(a) is a representation of virus titres in plasma from PV1(M)-infected animals (\( n = 11 \)). Viraemia was detectable between 2 and 4 h after intraneural inoculation of PV1(M) in 63% of monkeys. After 2 days p.i., all monkeys were viraemic and by day 4 p.i., 80–90% of monkeys had viraemia (titres of \( 10^4–10^5 \) TCID\(_{50}\)/ml). On days 5 and 6 p.i., viraemia was present in about 40% of monkeys and by day 7 p.i., viraemia was detected only in 11% of monkeys.

In animals inoculated with PV1(M), poliovirus was isolated from the throat of two monkeys and from a stool sample in one
monkey 1 day after intraneural inoculation. On day 2 p.i., only two monkeys were shedding virus from the gastrointestinal tract. By day 3 p.i., shedding was seen in 8 of the 11 (72%) monkeys used in this analysis and, thereafter, the number of monkeys that continued to shed poliovirus varied between 5 and 7 out of the 11 (45–63%) monkeys examined.

Serum antibody responses occurred in LSc/2ab-infected animals despite the lack of demonstrable virus in extraneural sites

All five animals inoculated with LSc/2ab exhibited serum antibody responses. Virus titres in the serum ranged from 8 to 512 (182 ± 94) on the day of necropsy (from 7 to 11 days p.i.), but no monkey had detectable antibodies in the CSF. Many of the animals inoculated with PV1(M) not only had antibodies in serum, but also in their CSF (serum titre 1115 ± 736; CSF titre 23 ± 13) on the day of necropsy (between 6 and 14 days p.i.). The difference in serum antibody titres in the two groups of animals was not statistically significant.

Albumin indices

Normal control monkeys had albumin indices ranging from 1.2 to 6.3 with a mean of 3.7 (n = 15). In animals infected with PV1(M), albumin indices ranged from 5.26 to 37.24 (n = 12), which was significantly higher than normal controls (P < 0.0003), indicating a breach in the blood–brain barrier. In LSc/2ab-infected animals, albumin indices ranged from 3.9 to 5.5 (n = 3) and were significantly different from the PV1(M)-infected animals (P < 0.03), but were not different from normal controls (Fig. 7b).

Discussion

Previous work demonstrated that wild-type PV1(M) spread to the spinal cord after experimental virus inoculation into the ulnar nerve of bonnet monkeys (Ponnuraj et al., 1998). As the ulnar nerve fibres relate to C7, C8 and T1 levels of the spinal cord, the inoculated virus was transported to the cervical cord, replicated and spread caudally with associated paralysis of the lower extremities (Ponnuraj et al., 1998). In contrast, similar intraneural inoculation of bonnet monkeys with the attenuated poliovirus strain LSc/2ab did not cause paralysis, although a seropositive response indicated that the virus had replicated within the infected hosts (John et al., 1992). Current studies have shown that the site of attenuated viral RNA replication is within the CNS.

The preliminary experiment with the attenuated virus causing rare damaged neurons suggestive of virus cytopathic effect at various cord levels without associated infectious virus prompted us to look for viral RNA by in situ hybridization. The presence of viral RNA in cervical and lumbar neurons within the spinal cords of LSc/2ab-infected animals confirms the spread of virus from the initial inoculation site to subsequent sites of replication in the spinal cord.

Following intraneural inoculation, distinct differences were seen in the spread of the wild and attenuated strains with respect to cell types in which viral RNA was distributed, the number of cells containing viral RNA, the intensity of signals in the cells, the number of damaged neurons and the recovery of virus. The presence of the attenuated strain can only be demonstrated by in situ hybridization and not by virus isolation in tissue culture. Identification of viral RNA in lumbar spinal cord tissues provides evidence that infectious virus particles have spread from one spinal cord level to another. Infection of the lumbar neurons could have occurred via the axons and dendritic processes of infected neurons at an upper level though either a haematogenous route or a retrograde spread from damaged nerve endings created by the intramuscular injections. It is well known that skeletal muscle injury induces retrograde axonal transport of poliovirus and thereby facilitates virus invasion of the CNS (Gromeier & Wimmer, 1998). The possibility of an undetectable level of viraemia seeding damaged nerve endings that may have been created by the intramuscular injections at the time of inoculation cannot be ruled out.

The inability to recover virus from the spinal cord of LSc/2ab-infected animals could be due to a number of reasons. Low virus yield due to defects in replicative mechanisms have been shown in several in vitro studies using tissue culture cell lines (Kanda & Melnick, 1959; Tershak & Makkar, 1988; Agol et al., 1989; Monica & Racaniello, 1989) and cell-free systems (Svitkin et al., 1985, 1988). Cell cultures with similar amounts of virus inocula, when 100 p.f.u. per cell is released from wild-type poliovirus type 1 infection, only 1 p.f.u. per cell is released with LSc/2ab (Agol et al., 1989). In the bonnet monkey model, a higher titre of attenuated virus is necessary to effectively infect the monkeys by the oral route (T. J. John, unpublished observations). Replicative defects that result in lower virus titres in vitro may have similar effects in vivo and, therefore, could have resulted in lower titres. These lower virus titres coupled with the sensitivity of the isolation technique are probably the reasons for failure to isolate virus.

Another plausible explanation for the absence of infectious virus particles in spite of the presence of viral RNA by in situ hybridization could be the formation of incomplete virus particles due to the inherent defects in the translation process of the Sabin attenuated strain. Wild-type and Sabin attenuated poliovirus type 1 differ by 55 mutations across the entire genome (Racaniello, 1988). Studies by V. I. Agol and collaborators (Svitkin et al., 1985, 1988) show that translation of mRNAs from attenuated strains of poliovirus types 1 and 3 is less efficient than that of their neurovirulent counterparts. The defect in translation may explain the presence of viral RNA and the very low numbers of infectious particles, which were not detectable by isolation. The isolation method for infectious virus could detect only 50 infectious units or more.
per 1 g of tissue. In monkeys inoculated with PV1(M), the highest titres of virus were found in the lumbar cord and ranged from 50 to 300,000 TCID_{50} in animals necropsied between days 6 and 12 following intraneural inoculation, with the highest titres on day 6 p.i. and the lowest titres on day 12 p.i.

As we necropsied animals between 7 and 12 days following intraneural inoculation of LSc/2ab, there is a possibility that virus titres could have been declining. Declining titres, in combination with the fact that a 100-fold lower titre is generally observed with the LSc/2ab strain, could have contributed to negative results in isolation studies.

This model demonstrates another interesting difference in the viral RNA replication and neuronal damage in the spinal cord. With PV1(M) infection, viral RNA is detected in nearly all of the neurons, along with a similar proportion of neurons being found damaged in tissue sections stained with CV. In contrast, although a large number of neurons were found to contain polioviral RNA, CV staining showed only an occasional damaged neuron following inoculation with LSc/2ab. Loss of Nissl substance is detected by CV staining and the presence of Nissl substance is an indication of intact host cell protein synthesis. The lack of inhibition of host cell protein synthesis by attenuating mutations may be the reason for neurons appearing normal when stained with CV. The attenuating mutations are known not to turn off host cell protein synthesis (Bernstein et al., 1985).

If the anticipated titres in the LSc/2ab-infected animals are 100-fold lower than those found after PV1(M) inoculation (10^{2.45–10^{4.65}} TCID_{50}) then viraemia should have been detected by the method used: the detection limit is 10 TCID_{50}/ml of plasma. Therefore, failure to detect viraemia in animals infected with LSc/2ab is most probably a true finding and not a false negative finding due to the insensitivity of isolation technique.

In this animal model, PV1(M) but not LSc/2ab is detectable in systemic circulation following intraneural inoculation. In the bonnet monkey, viraemia following oral infection and either intramuscular or intravenous inoculation lasts between 24 and 48 h (T. J. John, unpublished observations). However, the virus is persistent for several days following intraneural inoculation. Results from the present study show an interesting link between viraemia and replication of poliovirus in the cells around vessels. Failure to detect viraemia following LSc/2ab inoculation accompanies the lack of viral RNA in cells around spinal cord vasculature and minimal vascular reactivity. In contrast, prolonged viraemia following PV1(M) inoculation is accompanied by viral RNA in the endothelial cells of the vasculature and increased vascular reactivity. Therefore, it is possible that endothelial cells may be shedding virus into the bloodstream.

Although animals infected with PV1(M) had higher antibody levels than those infected with LSc/2ab, the difference was not statistically significant. With PV1(M) infection, persistent viraemia would be expected to produce a good neutralizing antibody response. However, serum antibodies following LSc/2ab infection could be induced (i) by viraemia that was too low to be detected by the isolation method but was sufficient to invoke an immunological response or (ii) by antigenic stimulus occurring in the lymph nodes that drain the CNS.

Antibodies in CSF following PV1(M) inoculation could be due to de novo synthesis by B cells within the CNS and/or due to a breach in the blood–brain barrier. The absence of antibodies in the CSF despite the presence of viral RNA in the spinal cord in LSc/2ab-infected monkeys argues against de novo antibody synthesis. Elevated albumin indices in PV1(M)-infected animals show that there is a breach in the blood–brain barrier.

In recent years, the availability of transgenic mice that express the human poliovirus receptor TgPVR (Koike et al., 1991; Ren et al., 1990) has made several studies possible. Studies with TgPVR have shown that poliovirus travels through a neural route from the muscle (Ren & Racaniello, 1992) and that spread of the virus to the CNS is through fast axonal transport (Okha et al., 1998). Thus, both our monkey model and the mouse model favour transmission of virus to the CNS though a neural route. In the mouse model, one in ten animals developed paralysis following inoculation of Sabin poliovirus type 1 (Okha et al., 1998). However, neurons from the monkey model showed viral RNA but showed neither extensive inflammation nor paralysis. Another major difference in the two models is that when poliovirus is inoculated intravenously, circulating poliovirus crosses the blood–brain barrier independently of the expression of the poliovirus receptor in the mouse (Yang et al., 1997), whereas in the monkey model, virus is rarely detected in the CSF even when there is persistent viraemia. Despite the breach in the blood–brain barrier in the monkey model, virus was rarely detected in CSF samples, whereas in the mouse model, virus accumulation in the CNS was 100 times higher than that of albumin. Despite the presence of poliovirus receptors in enterocytes and M cells in the mouse model, poliovirus does not replicate in the gut (Zhang & Racaniello, 1997), while in the bonnet monkey, replication of poliovirus in the gut is a consistent finding (as in chimpanzees) and CNS infection following oral infection is rare (as in humans). Although other monkeys have a susceptible CNS but not a susceptible gut, and chimpanzees have a highly susceptible gut and CNS, the bonnet monkey model may be the best model to mimic human poliovirus infection and, therefore, an interesting model to study the extent of CNS damage with viruses of varying degrees of neurovirulence.

There is an increasing concern about VAPP, especially with the elimination of wild-type poliovirus circulation in developed countries. In our monkey model, although only a few monkeys were studied, we discovered that the attenuated virus consistently spreads from a peripheral nerve to the spinal cord.
neurons and is capable of replicating in the neurons. However, we did not find neuronophagia, neuronal loss or clinical paralysis, which is a very important finding in this study. In humans, VAPP is a rare event occurring in 1 out of 530,000 primary immunizations with OPV (Minor, 1997). VAPP due to poliovirus types 2 and 3 has been attributed to reversion caused by single point mutations in the genomes of these serotypes. However, cases of VAPP due to poliovirus type 1 have been encountered with strains that have maintained the putative ‘non-neurovirulent genotypic mutations’, i.e. G1480, U525 and G6205 (Friedrich et al., 1996; Georgescu et al., 1994). Experiments with this monkey model have shown that the poliovirus type 1 attenuated strain can replicate in neurons causing minimal damage, minimal inflammation and no neuronophagia.

Although poliomyelitis has been eradicated from a number of developed countries, there have been reports of VAPP from neuronophagia. causing minimal damage, minimal inflammation and no

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