Mechanism of Injury-Provoked Poliomyelitis

MATTHIAS GROMEIER* AND ECKARD WIMMER

Department of Molecular Genetics and Microbiology, School of Medicine, State University of New York at Stony Brook, Stony Brook, New York 11794

Received 12 January 1998/Accepted 27 February 1998

Skeletal muscle injury is known to predispose its sufferers to neurological complications of concurrent poliovirus infections. This phenomenon, labeled “provocation poliomyelitis,” continues to cause numerous cases of childhood paralysis due to the administration of unnecessary injections to children in areas where poliovirus is endemic. Recently, it has been reported that intramuscular injections may also increase the likelihood of vaccine-associated paralytic poliomyelitis in recipients of live attenuated poliovirus vaccines. We have studied this important risk factor for paralytic polio in an animal system for poliomyelitis and have determined the pathogenic mechanism linking intramuscular injections and provocation poliomyelitis. Skeletal muscle injury induces retrograde axonal transport of poliovirus and thereby facilitates viral invasion of the central nervous system and the progression of spinal cord damage. The pathogenic mechanism of provocation poliomyelitis may differ from that of polio acquired in the absence of predisposing factors.

Provocation poliomyelitis (PPM) is the phenomenon of poliomyelitis resulting from physical trauma during infection with poliovirus (PV) (15, 17). Typically, PV infects the gastrointestinal tract, causing mild symptoms or no symptoms at all. In 1 to 2% of infections, PV invades the central nervous system (CNS), where it uniquely targets motor neurons for destruction, resulting in flaccid paralysis (15, 17). Poliomyelitis can be controlled by vaccination with inactivated or orally administered, attenuated PV types 1 to 3 (OPV) (15, 16, 25). Indeed, the World Health Organization has targeted PV for global eradication with predominantly OPV by the year 2000 (26). Although considered effective and safe (6), OPV may cause, on very rare occasions, neurological complications (vaccine-associated paralytic poliomyelitis) (2). This has generally been attributed to the emergence of neurovirulent PV variants in OPV recipients (7, 16, 23). However, an accumulation of cases of vaccine-associated paralytic poliomyelitis following vaccination with OPV in Romania has been linked to multiple intramuscular (i.m.) injections of various therapeutic or preventive agents administered to OPV recipients (22). These recent observations have greatly renewed the interest in PPM, a phenomenon that was first recognized in infections with wild-type PVs (1, 10, 11, 14). Occurrences of PPM remain a health problem in developing countries and countries with a preference for i.m. administration in pediatric practice (10, 27). Although a provoking effect of injections could be reproduced in nonhuman primates previously (4), no pathogenic mechanism leading to PPM has been determined.

The worldwide application of OPV involves the administration of hundreds of millions of doses per year, an effort being accelerated by the World Health Organization’s action plan to eradicate PV. The scope of the dissemination of live PV vaccine makes studies of the risk factors for neuroinvasion by this agent necessary. Here, we present experiments with mice transgenic for the human PV receptor (hPVR-tg mice) that have allowed us to identify a risk factor for neurological complications associated with multiple i.m. injections of wild-type PV-infected animals. We found that the pathogenic spread of PV to the CNS in PPM may be fundamentally different from neuropathogenesis in the absence of skeletal muscle trauma. Determining the pathogenic mechanism for PPM may help in the development of delivery systems that target the CNS and may enhance our understanding of the adverse effects of i.m. vaccine administration in general.

MATERIALS AND METHODS

Sciatic nerve transection. All survival surgical procedures were performed in accordance with institutional guidelines on animal welfare. Mice were anesthetized with an intraperitoneal injection of 0.5 mg of xylazine per g of body weight and 0.01 mg of ketamine per g of body weight. The left flanks of sedated animals were shaved in preparation for surgery. A transcutaneous incision of approxi-mately 1/2 in. was made to expose the fascia overlying the biceps femoris and caudofemoralis muscles. A small incision into the fascia served to access the sciatic nerve in the recess between the muscles. An ~1/4-in. segment of the sciatic nerve was removed. The cutaneous defect was treated with three to five sutures with Prolene 0-5 (Ethicon). Mice were treated with an analgesic, buprenorphine hydrochloride, administered intraperitoneally (in 0.012-mg doses) every 8 h. All 36 mice subjected to the procedure survived without any sequelae apart from the neurological defects stemming from the nerve transection. Discontinuity of the sciatic nerve was confirmed by autopsy in all surgically treated mice at the time of sacrifice.

Virus propagation, virus inoculation, and i.m. injection of experimental animals. PV type 1 (Mahoney) [PV1(M)] was propagated and purified as described elsewhere (8). Defined amounts of virus as determined by plaque assay were suspended in sterile phosphate-buffered saline and inoculated into one of the tail veins of a sedated mouse by using a 27-gauge 1/2-in. hypodermic needle. i.m. injections were performed as follows. A 27-gauge 1/2-in. hypodermic needle was used to inject approximately 10 μl of phosphate-buffered saline into the left gastrocnemius muscle of a sedated mouse. Three times, the needle was pulled back and pushed forward to penetrate a wider area of the gastrocnemius muscle.

Determination of virus load and neuropathology. Tissues of interest from various spinal cord regions and skeletal muscle were removed from sacrificed hPVR-tg mice. Each sample was weighed, placed in 1 ml of minimal essential medium, and homogenized in a Dounce homogenizer (Wheaton). Each homog-enate was serially diluted, and the amount of infectious particles per 3 mg of tissue was analyzed in a plaque assay. Tissue specimens from the spinal cords of sacrificed mice were removed and subjected to fixation and paraffin embedding as described previously (9). The 5-μm sections were cut at a thickness of 10 μm on a rotary microtome and mounted for histological staining. Histological staining with luxol fast blue, periodic acid-Schiff stain, and hematoxylin was performed according to standard protocols (9).

RESULTS

Upon PV infection, hPVR-tg mice develop a paralytic condition clinically and pathologically reminiscent of primate po-
lomyelitis (9, 12, 20). Four experimental groups of hPVR-tg mice were used: group I, untreated hPVR-tg mice; group II, mice that were treated with a left sciatic nerve transection alone (Fig. 1); group III, sham-operated mice that received multiple i.m. injections at intervals indicated in the legend to Fig. 2; and group IV, mice subjected to a left-sciatic-nerve transection that were treated with the same regimen of multiple i.m. injections as group III (Fig. 2).

Sciatic nerve transections were performed in such a manner as to minimize muscle trauma interfering with the experiment (Fig. 1 and Materials and Methods). Sham-operated mice were subjected to the identical procedure, only the nerve transection itself was left out. Seven days after surgery, all mice were inoculated intravenously with $5 \times 10^6$ PFU of PV1(M). Mice from groups III and IV were subsequently given i.m. injections into the left gastrocnemius muscle at regular intervals (Fig. 2).

At 48, 72, and 96 h postinfection (p.i.), four animals from each experimental group were sacrificed, and the following analyses were carried out. (i) At the time of sacrifice, a clinical neurological status was established. (ii) Tissues from the cerebral cortex, cervical spinal cord, lumbar-sacral spinal cord, injected gastrocnemius muscle (where applicable), un.injected skeletal muscle, and serum were obtained. All tissues were homogenized and the virus load in the homogenate was quantified in a plaque assay. (iii) Tissue specimens from four areas of the spinal cord were obtained and analyzed histopathologically.

Clinical evaluation revealed an increased susceptibility to PV-induced neurological complications due to muscle injury inflicted by multiple i.m. injections (Fig. 2). Group III mice developed more severe signs of poliomyelitis earlier than their peers from group I (Fig. 2). The onset of paralysis in animals of group III occurred preferentially in the injected lower extremity, whereas animals of groups I and II as well as from group IV generally developed paraparesis initially (Table 1).

Table 1. Sites of initial paralysis in PV-infected hPVR-tg mice*

<table>
<thead>
<tr>
<th>Exptl group</th>
<th>No. of mice with paralysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left lower extremity</td>
</tr>
<tr>
<td>I</td>
<td>1</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>13</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
</tr>
</tbody>
</table>

* Members of groups of experimental animals comprising 16 mice each were infected by the intravenous route with $5 \times 10^6$ PFU of PV1(M). They were treated according to the experimental protocol outlined in the legend to Fig. 2 and observed clinically twice daily, and it was recorded which limb showed initial signs of weakness.

Clinical evaluation revealed an increased susceptibility to PV-induced neurological complications due to muscle injury inflicted by multiple i.m. injections (Fig. 2). Group III mice developed more severe signs of poliomyelitis earlier than their peers from group I (Fig. 2). The onset of paralysis in animals of group III occurred preferentially in the injected lower extremity, whereas animals of groups I and II as well as from group IV generally developed paraparesis initially (Table 1).

Table 1. Sites of initial paralysis in PV-infected hPVR-tg mice*

<table>
<thead>
<tr>
<th>Exptl group</th>
<th>No. of mice with paralysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left lower extremity</td>
</tr>
<tr>
<td>I</td>
<td>1</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>13</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
</tr>
</tbody>
</table>

* Members of groups of experimental animals comprising 16 mice each were infected by the intravenous route with $5 \times 10^6$ PFU of PV1(M). They were treated according to the experimental protocol outlined in the legend to Fig. 2 and observed clinically twice daily, and it was recorded which limb showed initial signs of weakness.
transport induced accidentally by muscle injury may shorten the incubation period, localize the initial paralytic symptoms, and hasten the progress of virus replication during PPM. This is strongly supported by the observation that peripheral nerve transection interrupting the neural connection between injured muscle and the spinal cord protects mice from PPM (Fig. 2). We observed an increase in viral replication within injured skeletal muscle; in this muscle only small quantities of virus were produced, but the increase may have enhanced the efficiency of PV uptake into the peripheral nerve. Although muscle injury in animals from group IV caused an increase in viral replication, the enhancement of proliferation did not extend to the spinal cord (Fig. 3B).

The conclusions based on clinical observations and quantitation of viral replication in specific tissues were confirmed by histopathological examination. Spinal cord tissue specimens were harvested from sacrificed mice at given time points and processed for histopathological analysis (Fig. 4). The sequence of images demonstrates the progression of poliomyelitic lesions within the lumbosacral spinal anterior horn in hPVR-tg mice. Mice from experimental groups I and II developed lesions typically seen in poliomyelitis only after 96 h p.i. (Fig. 4, panel 1C). Treatment of mice with multiple i.m. injections accelerated the progression of spinal cord lesions induced by PV. In mice from experimental group III, complete destruction of the motor neuron population was already achieved by 72 h p.i. (Fig. 4, panel 2B). At this time, no histopathological damage was evident in the control group (Fig. 4, panel 1B). The increase in the rate of appearance of histological lesions due to repeated i.m. injections covaried with the aggravated clinical course as well as the elevation of virus titers in tissues targeted by PV in mice of group III. As is evident, sciatic nerve transection protected hPVR-tg mice from the aggravated progression of poliomyelitic lesions. In mice from group IV, the pace of advance of poliomyelitic pathology was synchronous with the rate of progression in the control group (Fig. 4; compare panels 1A to C with panels 3A to C). Sections obtained from thoracocervical spinal segments showed a less evident advance of lesion progression in mice from group III (data not shown). This corresponded to the minor increase in viral replication in cervical regions of the spinal cords of mice from group III (Fig. 3C).

**DISCUSSION**

Muscle injury due to injection of vaccines or therapeutic agents is common in medical practice. It has been observed that, if concurrent with PV infection, such injury may increase the risk of neurological complications (13, 22). Using a mouse model developed for the study of poliomyelitis, we have shown that muscular trauma induced by multiple injections can lead to rapid progression of PV-induced paralysis, upregulation of viral replication in certain tissues, and acceleration of the progression of histopathological lesions. Thus, our data provide direct experimental evidence for the concept of PPM.

What is the mechanism by which PPM is induced? Two different routes of PV invasion of the CNS have been observed in experimental infections: traversal of the blood-brain barrier (28) and retrograde axonal transport (3, 21). In 1954, Bodian (4) described experiments with monkeys to mimic PPM where the outcome of provocation in the form of i.m. injections was measured by clinical observations alone. It was speculated that entry of PV into the CNS may be facilitated by localized alterations of vascular permeability in the CNS induced by muscle trauma (4, 23).

It has been repeatedly observed in experimental infections...
of nonhuman primates and transgenic mice inoculated i.m. with PV that the onset of paralytic symptoms appears in the injected limb. i.m. administration of improperly prepared inactivated polio vaccine in the Cutter incident of 1955 tragically confirmed these observations; afflicted children developed initial signs of paralysis in the injected arm (19). These circumstantial reports as well as experimental evidence (21) suggested that retrograde axonal transport is responsible for PV invasion of the CNS after i.m. administration of virus.

In this study, we show that retrograde axonal transport of PV can be induced in viremic animals through skeletal muscle injury. Thereby, we simulated the natural course of PPM in humans, since the onset of neurological disease in PV-infected patients is regularly preceded by viremia (3). The augmentation associated with PPM of viral replication rates in the spinal cord could be prevented by sciatic nerve dissection ipsilateral to the site of i.m. injection prior to infection. Localization of initial paralytic symptoms to the limb treated with multiple i.m. injections could be prevented by implementing a neural block through monolateral sciatic nerve transaction (compare panels 2A to 3A with panels 3A to C).

FIG. 4. Histopathological evaluation of PV-induced damage within the lumbosacral segments of the spinal cords of mice from experimental groups I (panels 1A to C), III (panels 2A to C), and IV (panels 3A to C). The results of histopathological analysis of tissue from mice of group II have been omitted because the pace of progression of spinal lesions was indistinguishable from that in group I. Spinal cord tissue specimens were obtained from mice sacrificed 48 h (panels A), 72 h (panels B), and 96 h (panels C) following virus inoculation and were processed as described previously (9). Mice that had not been treated with multiple i.m. injections developed minor signs of virus-induced lesions within 96 h p.i. (panel 1C; arrow indicates motor neurons with characteristic cytopathic changes). In contrast, the spinal cords of animals that had been treated with the regimen of i.m. injections displayed complete destruction of the motor neuron population only 72 h p.i. (panel 2B). The acceleration of the progression of spinal lesions caused by multiple i.m. injections could be prevented by implementing a neural block through monolateral sciatic nerve transaction (compare panels 2A to 3A with panels 3A to C). Bar, 250 μm.
We have thus provided direct evidence that retrograde axonal transport accounts for facilitated access of PV to the CNS via peripheral nerves, resulting in PPM. As in experimental animals treated with i.m. injections, a shortened incubation period and initial paralysis localized to the injected limb were described for victims of the Cutter incident (19). Thus, the pathogenic mechanism for PPM determined in this study may underlie the Cutter incident, which never could be adequately explained (19). The pronounced influence of i.m. injections on localization of paralysis in transgenic mice was not observed in primates (18), probably reflecting the significant extent of extraneural proliferation of PV in the latter.

Upon the induction of muscle trauma, we observed an upregulation of PV propagation within injured muscle tissue that may indicate a pathogenic link between i.m. injections and the induction of retrograde axonal transport of PV. The stimulation of PV replication may rely on the upregulation of the cellular binding site for PV or viral replication within invading inflammatory cells. We did not detect upregulation of the human PV receptor by Northern blotting or immunohistochemical analysis of skeletal muscle tissue. However, the extremely low levels of extraneural PV production, even after stimulation through injury, hinder the evaluation of the role of local extraneural replication during pathogenesis.

Several human enteroviruses other than PV have the propensity to cause poliomyelitis, although the mechanism by which these viruses reach and enter motor neurons is currently unknown (15, 17). It is appealing to speculate that provoking factors may also play a role in the etiology of non-PV-induced poliomyelitis.

ACKNOWLEDGMENTS

Transgenic mice were a generous gift from A. Nomoto, the University of Tokyo, Tokyo, Japan. We are grateful to R. Sutter and P. Strebel, Polio Eradication Activity, National Immunization Program, Centers for Disease Control and Prevention, Atlanta, Ga., for critical reviews of the manuscript. This work was supported by contract 95-200-0948 with the CDC and NIH grant RO1AI39485-01.

REFERENCES