Disease Focus

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Polio, Still Lurking in the Shadows

Anthony N. van den Pol

Department of Neurosurgery, Yale University School of Medicine, New Haven, Connecticut 06520

Introduction

Iron lungs sucking breath into immobile bodies. Paralysis. Useless limbs. Forced separation of children from their families. Polio conjures up a nightmarish vision, in part due to the rapid and unexpected onset of paralysis, the inability to predict who might succumb, and the absence of any cure for the potentially life-long poliovirus-associated paralysis. Sporadic cases of polio have plagued us for thousands of years. In the last 150 years, epidemics of polio have generated fear and confusion. Ironically, associated with increased sophistication in clean water supplies and sewage disposal in cities, the immunity that had previously been generated from early contact with the virus was delayed until a later stage of development, leading to mass outbreaks of paralytic polio. In 1952 polio caused 3200 deaths, and >21,000 cases of paralysis in the US alone (<http://www.cdc.gov/vaccines/pubs/pinkbook/downloads/polio.pdf>). Rehabilitation centers such as the one shown in the photograph in Figure 1 in 1953 were crowded with young patients.

After smallpox, poliovirus (PV) has been on the road to potentially becoming the second major human virus to be eradicated from the planet. Many countries have not seen a case of poliomyelitis in a decade or longer, raising our confidence

in the eventual elimination of this pathogen. But recent events have reminded us that although the end of polio may be near, it is not gone, and can return unexpectedly. A case in point is found in Tajikistan, a country bordering Afghanistan and China. Similar to the US, Tajikistan had not had a reported case of polio in over a decade from 1997 on. But two years ago (2010), polio showed a dramatic resurgence, with >450 cases of polio-induced paralysis identified (<http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6018a3.htm>). But the spread of the virus was worse than the 450 cases suggest. Fewer than 1% of those (nonimmunized) infected by PV show paralytic symptoms (Mueller et al., 2005; Modlin, 2010), suggesting that >45,000 people may have been infected by the virus within only a few months. The outbreak in Tajikistan may have arisen from a single infected traveler carrying the virus from a region of India where that strain of PV had been endemic. From Tajikistan, the virus spread to Turkmenistan, Kazakhstan, and the Russian Federation. An aggressive and rapid vaccination program successfully contained this unexpected PV outbreak. PV remains endemic in Pakistan and Afghanistan, and is most problematic in Nigeria where civil unrest and suspicion about immunization safety and intent have complicated and arrested critical vaccination programs.

The long-term paralysis that occurs in ~1% of patients is the result of PV infection and irreversible killing of motor neurons in the anterior horn, resulting in flaccid paralysis of denervated muscles, or less commonly killing of motor neurons in the bulbar region of the brainstem, affecting muscles innervated by cranial

nerves and thus interfering with breathing, speaking, or swallowing. Since little can be done to cure paralytic poliomyelitis, most efforts focus on prevention of infection, first by immunization and second by improving sanitation in regions at risk. Intramuscular immunization with inactivated PV (Salk vaccine) is the safest vaccine approach, but requires skilled health professionals for administration, making it problematic in isolated communities lacking medical expertise. The oral (Sabin) vaccine made from attenuated replication-competent PV is easily administered, particularly in regions of the world with a paucity of health care experts, and has played a key role in controlling PV infections in both large urban populations and also in remote villages. A major drawback of the Sabin vaccine, however, is that rare vaccinated individuals can develop and even transmit the disease if the PV genome reverts from attenuated to potentially paralytic wild-type.

Despite the enormous effort in eradicating poliovirus reaching every nation, there are still a surprisingly large number of mysteries related to the basic underlying mechanisms of PV infection and spread to the brain, and particularly to spinal cord motor neurons. What is it about human motor neurons that make them selectively vulnerable to PV destruction, whereas most other neurons, including sensory neurons, show little infection? How does PV get into the brain, and how does it transit to the motor neurons? Why do only a very small percentage (<1%) of those with active infections of type 1 PV show any neurological symptoms? The remaining 99+ % of those infected show either no symptoms at all, or in a small percentage of those infected, mild tran-

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Correspondence should be addressed to Anthony N. van den Pol at the above address. E-mail: anthony.vandenpol@yale.edu.

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sient symptoms that might include a sore throat, diarrhea, headache, or stiff muscles. Type 2 PV, which may have recently been eradicated, was even less likely to cause paralysis, with <1 in 2000 infected people showing neurological symptoms (Nathanson and Martin, 1979). Some questions related to neurological dysfunction can be approached from a single-cell perspective. In contrast, events leading to paralytic PV-selective infection of spinal cord motor neurons cannot be understood in the absence of a broader perspective of PV infection within its human host, and within the global environment.

Cellular tropism of PV

PV is a member of the picornavirus family and genus *Enterovirus*. Within this genus there are three serotypes of PV (1, 2, and 3), all of which can cause disease, and 60+ human serotypes of enteroviruses, distributed among four virus species (A, B, C, and D). Immunity against one of the three PV serotypes does not protect against the other two, and so historically most effective polio vaccines contained 3 strains of attenuated (Sabin) or inactivated (Salk) virus (Racaniello, 2001). PV is a small (30 nm diameter) virus (Figs. 2, 3) with no surrounding membrane envelope. Its genome is a 7.5 kb single positive-sense strand of RNA (Fig. 2), surrounded and protected by the protein capsid. Translation of the PV genome generates a single polyprotein which is cut by viral proteases to generate four capsid proteins, VP1–VP4, and several nonstructural proteins. Nonstructural proteins here refers to proteins that are expressed in the infected cell, but are not incorporated into the virus particle. PV is one of the smallest viruses that causes human disease; in contrast, the DNA genomes of herpes and pox viruses are 20- and 30-fold larger, respectively, and these DNA viruses are also 5–10 times bigger than PV.

The receptor for poliovirus (PVR) is a member of the Ig superfamily, CD155 (Mendelsohn et al., 1989); all three serotypes of PV use the same PVR. CD155 is necessary, but not sufficient to generate infection. A number of non-neuronal cells express CD155, but show little infection with PV, indicating that other cofactors also play critical roles. PVR is expressed in both human motor neurons, and in muscle end plates (Leon-Monzon et al., 1995). In humans, PVR is expressed in both a membrane-bound and releasable form. The membrane-bound receptor is the critical one for PV entry into a cell. In contrast, the releasable PVR, pres-

Figure 1. Photo showing polio patients at Rancho Los Amigos National Rehabilitation Center in California in 1953. Used with permission of Rancho Los Amigos.

Figure 2. On the left is a schematic representation of the positive strand RNA genome of poliovirus. This is translated into a single polyprotein, which is then cleaved into a number of poliovirus proteins. In the upper right, a model of poliovirus is seen, with the four capsid proteins combining to form the elements of the virus capsid.

Figure 3. A transmission electron micrograph shows the 30 nm poliovirus particles. Courtesy of the US Centers for Disease Control.

ent in CSF and serum, may serve in part as a beneficial decoy, reducing the probability of PV attachment to the membrane-bound receptor (Baury et al., 2003).

Because PV primarily infects human cells, there is no latent reservoir of virus in animals, a key feature in the potential for the global eradication of the virus. Most animals, including mice, are refractory to PV infection due to the absence of a human-type PVR. However, experimental injection of the PV RNA into non-human cells does result in productive generation of the virus (Holland et al., 1959a,b), underlining the importance of the capsid-PVR binding in maintaining species selectivity to humans. Old World primates can also be infected by PV, with a symptomatology not too different from that seen in humans; experimental primate PV infections were a great asset in our initial understanding of PV infections leading to flaccid paralysis and in developing immunization strategies to combat the virus (Bodian, 1954, 1955; Nathanson, 2008).

To enable the use of small animals in studies of PV actions and underlying mechanisms, a number of lines of very useful transgenic mice have been generated that express the human PVR (Mendelsohn et al., 1986; Ren et al., 1990; Koike et al., 1991; Ren and Racaniello, 1992). These mice can be productively infected with PV resulting in paralysis, and have proven a considerable asset in understanding some aspects of PV infection. Limitations of these mouse models relate to the inability to infect these mice

by the oral route (the critical one in humans; Zhang and Racaniello, 1997), to the non-identical expression pattern of the PVR in humans and transgenic mice, and possibly to the lack of diffusible PVR in the mouse. An ideal mouse model would express PVR in both the same cell types and in approximately the same concentration as in humans. Crossing PVR-transgenic mice with mice lacking the type I interferon receptor does generate a mouse model whereby PV can target motor neurons after oral infection (Ida-Hosonuma et al., 2005). This is consistent with the view that despite the underlying importance of the PVR, other factors play crucial roles in allowing or blocking spinal cord infections.

Another line of research has also raised some interesting questions: by injection of PV into the normal brain, mutant PVs have been recovered that show a selective infection and destruction of normal mouse motor neurons in the absence of PVR binding (Jubelt et al., 1980a,b; Ford et al., 2002), or more generalized infection of the CNS with mouse brain-derived mutant PV (Gromeier et al., 1995). A single six amino acid sequence in the VP1 capsid protein may enable PV to infect non-PVR mouse motor neurons (Martin et al., 1988; Murray et al., 1988). The results suggesting selective motor neuron death support the view that PVR expression by motor neurons is not the only factor in cytolytic motor neuron targeting by the virus.

What mechanism underlies the PV-mediated death of infected cells? First, PV reduces synthesis of cellular proteins in favor of virus proteins. Furthermore, the nontranslated 5' end of the PV genome contains an internal ribosome entry site (IRES) sequence which directs translation of the PV RNA genome. The PV IRES sequence appears to play a critical role in neurotoxicity. Replacing the PV IRES with an IRES from another enterovirus attenuates the neurovirulence of the recombinant PV without blocking PV translation or virus replication (Gromeier et al., 1996). PV may also kill cells when PV 2A, 2C, and 3C proteins induce apoptosis (Calandria et al., 2004; Buenz and Howe, 2006; Autret et al., 2007). Finally, PV can infect some cells which release progeny virus without generating a cytopathic effect (Morrison et al., 1994).

Infection of the host organism and spread to motoneurons

The virus enters the body by an oral route; unlike many other infectious organisms, PV appears resistant to stomach acid, and

initially infects the throat, including the tonsils, and gut. From the gut, the virus may penetrate the lymph nodes. A number of models of PV penetration of the CNS are based on the view that an intermediate step between gut and spinal cord infection requires the virus to move from the gut, possibly via the lymphatic system, into the blood stream (viremia). From there it might directly cross the blood–brain barrier (BBB) into the CNS and initiate infection of neurons; it is, however, unusual for a virus, even a small one such as PV, to diffuse across the BBB, although minor transient injury may enhance penetration of some viruses into the CNS (van den Pol, 2009a,b). Other potential mechanisms of PV movement across the BBB include direct infection of the endothelial cells, transendothelial transport, a Trojan horse infection of macrophage or dendritic cells that express the PVR and can be productively infected (Wahid et al., 2005) or other immune cells that subsequently enter the brain, or entry through one of the regions of the brain such as the area postrema or median eminence with weak BBBs and subsequent diffusion or transport within the brain (Couderc et al., 1990; Freistadt et al., 1993; Tyler and Nathanson, 2001; Pfeiffer, 2010).

Although infection of the gut is generally seen as a prelude to more widespread dissemination of the virus, when incompletely inactivated (Salk) PV vaccine was given by intramuscular injection to humans (Cutter incident), PV was subsequently found in the gut and was secreted fecally, causing additional cases of paralytic poliomyelitis (Nathanson and Langmuir, 1963a,b; Offit, 2005). This indicates that the gut can become infected either by direct oral ingestion or by an indirect route from other regions of the body. The gut plays a key role in virus dissemination in two contexts. It is here that the virus gains an entry into the body, and is also the source of infectious PV fecally released into the environment. The identity of the lower gut cells that productively generate PV for release into the gut lumen is not clear, but epithelial cells, cells of Peyer's patch, and lymphatic cells have been implicated (Nathanson, 2008). After the initial infection, PV may be shed from the gut into feces for 2–8 weeks leading to virus dispersal to other individuals.

An alternative to the hypothesis that virus accesses the CNS via the bloodstream is the possibility that virus may be retrogradely transported back to motor neurons from a muscle group by innervating axons. Direct injection of PV unilaterally

ally into limb muscles results in the ensuing paralysis of that limb, suggesting selective direct retrograde axonal transport of the PV from the injected muscle back to the innervating motor neuron pool; this has been described in humans injected with an incompletely inactivated Salk vaccine (Nathanson and Langmuir, 1963a,b), in experimental monkeys (Bodian, 1954, 1955), and in PVR-transgenic mice (Ohka et al., 2009). Infection of autonomic ganglia leading to axonal transport into the brain has also been postulated (Sabin, 1956). After PV injection into a leg, transection of the sciatic nerve blocked the ensuing paralysis in PVR-mice, indicating at least in this model, that retrograde virus transport is one viable mechanism for PV entry into the spinal cord (Ohka et al., 1998). One early school of thought suggested an olfactory nerve site of entry of PV into the brain; although PV can enter the brain through the olfactory nerve (Bodian, 1959), this does not appear to be its normal gate of access to the CNS in humans.

Infection of motor neurons may be enhanced by the association of the cytoplasmic domain of PVR with Tctex-1, a subunit of the dynein motor complex (Mueller et al., 2002) which may underlie retrograde transport from axon terminals back to the cell body; however, this view may require revision given more recent data suggesting that the dynein-Tctex-1 complex may not bind cargo (Williams et al., 2007).

Provocation poliomyelitis refers to the greater incidence of PV motor neuron targeting and subsequent paralysis that occurs after muscle injury (Bodian, 1954). An underlying mechanism may be enhanced PV retrograde transport into the brain, possibly by a mechanism different from the one normally underlying PV entry into the CNS (Gromeier and Wimmer, 1998). Injury to muscle or injection of unrelated antigens into a limb can enhance the probability of ensuing paralysis after oral PV inoculation. In Romania, if children received unrelated inoculations, generally of antibiotics and often multiple times, soon after receiving the oral PV vaccine, but not before the PV vaccine, the likelihood of a paralytic response was enhanced (Strebel et al., 1995). Similarly, after intravenous PV inoculation into PVR-transgenic mice, muscle injury increased the likelihood of paralysis of that leg, and the paralysis was attenuated by experimental sciatic nerve damage; furthermore, there was an increased virus titer in the injured leg (Gromeier and Wimmer,

1998). Muscle injury may enhance PV exit from the blood, may enhance retrograde axonal transport of the virus, or may increase local virus replication. Another possibility is that injury acts as a beacon for cells of the systemic immune system and enhances PV infection of immune cells with subsequent entry of the infected immune cell into the brain, followed by PV release from the immune cell. Factors that reduce PV entrance into the brain may include limited transport and replication in peripheral neurons, and the awakening of the intrinsic immune system by PV infection, resulting in an up-regulation and release of interferon (IFN) locally which subsequently activates a large number of antiviral genes (Lancaster and Pfeiffer, 2010).

Both the systemic and retrograde axonal transport mechanisms may be viable, and a key as to which mechanism underlies a particular case of polio may relate to different genotypes/substrains of virus. In an elegant set of experiments, Nathanson and Bodian (1961, 1962) used two strains of PV, one neuroadapted strain with a neurotropic tendency, and another with a viremic tendency. Injection of the neurotropic strain into a non-human primate limb caused a high probability of ensuing paralysis of that specific limb (suggesting a mechanism based on direct retrograde axonal transport), whereas injection of the viremic strain also caused paralysis, but with no strong association of paralysis with the injected limb (arguing against axonal transport from the infected muscle). Furthermore, experimental sciatic nerve damage attenuated general paralysis caused by the neurotropic strain, but did not attenuate paralysis generated by the viremic strain.

Immune response

Local PV infection activates an IFN response, in part through activation of TLR3 receptors (Oshiumi et al., 2011; Abe et al., 2012). IFN release, activation of local IFN receptors, and subsequent up-regulation of the expression of scores of IFN-stimulated genes is the first line of immune defense against PV. PV is ultimately cleared from the body by antibodies against the virus that arise after the systemic immune system is activated. The antibodies generated against the PV are the critical factor in preventing poliomyelitis. The attenuated PV used in oral vaccines shows little neurotoxicity in the brain, but maintains a strong replication potential in the gut (Sabin et al., 1954; Sabin, 1956). Interestingly, the antibody re-

sponse to inactivated PV blocks viremia and CNS infections with subsequent exposure to live PV, but may not block enteric infection and production of the virus (Nathanson, 2008).

Neuronal plasticity and polio

Additional questions relate to the mechanisms underlying long-term aftereffects of polio, termed the post-polio syndrome. Three to four decades after the initial PV infection and signs of paralysis, ~25% of poliomyelitis survivors show a recurrence of the paralytic symptoms or muscle weakening and fatigue, often affecting muscle groups similar to those initially involved. Although post-polio syndrome has been suggested to be caused by a resurgence of latent virus, there is no substantive support for this perspective (Pallansch and Roos, 2001). Another possibility that may be relevant to post-polio syndrome relates to mechanisms of short-term recovery after the initial acute infection. Paralysis in some patients is temporary, and over days or weeks, some recovery from partial paralysis ensues. The temporary nature of the paralysis here could be due to temporary silencing of neurons by transient virus infection, neuronal response to transient inflammation, or to transient release of inhibitory neuroactive agents from diseased or dying neurons. Another potential scenario is that only a subset of motor neurons innervating a particular set of muscles is killed, and the ensuing functional recovery is due to local plasticity that allows remaining uninfected motor neurons to substitute for the ones that were lost by cytolytic virus infection. This plasticity may involve lateral growth of axons to reinnervate local denervated muscles resulting in motor neurons with expanded muscle fields, and may also involve some central reorganization of systems signaling motor neurons. One reason given for loss of motor neurons years after PV infection is that the cell is “overworked” and wears out. But what the actual mechanism is behind this is not clear; understanding this may give us insight not only into post-polio syndrome, but also into other motor neuron diseases.

The recovery found in some cases of poliomyelitis touches on a key feature of many neurological illnesses involving neuronal degeneration, that redundant cells and systems are available to take over functionally when some neurons are lost. But there is a limit to the redundancy, and after a certain percentage of neurons are lost, symptoms become progressively more evident. A classic example of this is Parkin-

son's disease, where substantia nigra dopamine neurons are lost; in contrast to the flaccid paralysis associated with PV, loss of dopamine neurons leads to muscle tremor and rigidity. Similarly, the loss of hypocretin neurons in narcolepsy can lead to transient flaccid paralysis during cataplexy. Loss of less than ~50–60% of the critical neurons may be asymptomatic, but beyond that, the severity of symptoms shows a positive correlation with the number of neurons lost. In this context, post-polio syndrome may be the manifestation of the combination of cells initially killed by PV plus the ongoing loss of neurons that occurs with aging, resulting in an increased probability that insufficient motor neurons are available to allow continued normal functioning of those muscles initially affected by PV infection. An alternate possibility is a PV-initiated low-grade autoimmune targeting of motor neurons.

A number of viruses, for instance, cytomegalovirus, are more dangerous to the developing human brain than to the adult. In contrast, despite the enhanced maturation of the systemic immune system, PV is more likely to cause a debilitating paralysis in adults than in young children. Why? The alternate name for poliomyelitis of "infantile paralysis" was due to the fact that adults had a greater probability of having previous contact with, and immunity from, the virus, whereas an encounter with PV in young children was more likely to be the first contact, thereby increasing the probability of paralysis. However, if one compares the severity of an encounter with PV, nonvaccinated adults are more likely to show some form of paralysis than young children. The reason behind this developmental difference in susceptibility is not well understood, but may in part relate to a greater degree of plasticity in the developing brain after PV-induced damage, enhancing the potential for recovery from symptoms.

Poliomyelitis and other neuromuscular syndromes

Motor neurons are sometimes targeted by completely unrelated viruses, for reasons that are not clear, but may relate to some specific sensitivity of motor neurons to some factor associated with infection. Vesicular stomatitis virus (VSV), a membrane-bound negative strand RNA virus, binds to receptors found on most cells, and can be neurotoxic. But in a subset of rodents infected with VSV, hindlimb paralysis after motor neuron infection is a prom-

inent symptom (Rabinowitz et al., 1976; Brown et al., 2009). Similarly, the unrelated positive strand RNA virus Sindbis virus also can bind to many different cells in the rodent brain; in addition to other symptoms, Sindbis frequently causes hindlimb paralysis due to motor neuron lytic infection (Kerr et al., 2002).

PV serves as an important example that helps us appreciate the complexity of determining causation of neurological deterioration after viral infections. Similar to PV, other viruses that cause CNS dysfunction seldom show a consistent CNS effect. For instance, West Nile virus is associated with CNS disease in less than one in a hundred of those infected, and can cause a poliomyelitis-like response in a minority of infected patients (Campbell et al., 2002). PV selectively infects motor neurons, and diagnosis is relatively straightforward, based on paralysis and detection of the virus by PCR. On the other hand, viruses that infect the limbic system, with the potential to cause shifts in mental state, depression, mood, and anxiety disorders with a disease-causing potential similar to PV of <1%, would be substantially more difficult to detect and interpret. For instance, Borna disease virus (BDV) infects the limbic system and causes a large number of behavioral disturbances in a wide variety of mammalian and avian species (Lipkin et al., 2011). BDV has been associated with mental disease in humans, but whether this is merely a correlation or causation is not yet clear. Importantly, the fact that the majority of humans can be infected by a particular virus and not show neurological symptoms cannot be used as an argument that a particular virus does not cause neurologic or psychiatric dysfunction in others, as PV so eloquently shows us.

Other enteroviruses besides PV cause paralysis as well. As we hopefully near the final chapter of endemic PV, attention is turning to other viruses closely related to PV that may also cause paralysis in humans, possibly based on mechanisms similar to those used by PV. One potentially problematic virus is enterovirus 71 which causes hand, foot, and mouth disease which, similar to PV, may cause only minor or no symptoms, but in some cases can cause flaccid paralysis after motor neuron infection, as well as encephalitis or meningitis. Interestingly, this virus does not bind to the PVR, but instead uses different receptors, including human P-selectin glycoprotein ligand-1 (Nishimura et al., 2009). Enterovirus 71 has caused a number of localized epidemics in many regions of

the world over the last few decades, and with the epidemics have come a paralytic disease. During an outbreak of infectious enterovirus 71 in Bulgaria in 1975, paralytic disease occurred in ~21% of 700 patients (Shindarov et al., 1979). E71 can also cause poliomyelitis-like paralysis in non-human primates (Hashimoto and Hagiwara, 1982; Johnson, 1998). In rare cases, related viruses including E70, coxsackie A7, B3, and others can cause paralytic disease in humans by mechanisms that are not yet clear (Grist et al., 1978; Wadia et al., 1983; Pallansch and Roos, 2001). In mice coxsackie A can cause a flaccid paralysis, and coxsackie B, a spastic paralysis (Pallansch and Roos, 2001).

Amyotrophic lateral sclerosis (ALS) is a disease of unknown origin in which motor neurons are selectively lost. The cause of ALS remains unclear. Some studies have reported an elevated prevalence of enterovirus RNA in patients with ALS (Berger et al., 2000; Giraud et al., 2001; Woodall and Graham, 2004; Vandenberghe et al., 2010); however, in other studies enterovirus RNA has not been found in the spinal cord or motor cortex of patients with ALS (Swanson et al., 1995; Walker et al., 2001; Nix et al., 2004). Thus the possible role of enteroviruses in ALS remains unclear. Although no specific virus has been identified as contributing to the pathogenesis of ALS, the parallels between poliomyelitis/post-polio syndrome and ALS may present opportunities for cross-fertilization of information relating to mechanisms underlying both diseases. With polio, the paralytic symptoms of poliomyelitis appear relatively fast (days), and are related primarily to infection and destruction of spinal motor neurons. In contrast, ALS is a slow and progressive disease that leads to continued motor neuron deterioration over years, and includes both spinal and upper motor neurons.

Another symptomatically related syndrome is primary lateral sclerosis which affects the corticospinal neurons, and deterioration over several years leads to spasticity and muscle weakness. Stiff-person syndrome also affects motor neurons, and may be caused by a poorly understood autoimmune dysfunction. In this disorder, a progressive intermittent muscle rigidity can be associated with a heightened sensitivity to a number of sensory inputs, some leading to muscle spasms. Stiff-person syndrome is associated with antibodies against the GABA-synthesizing enzyme, glutamate decarboxylase, and synaptic molecules synaptophysin, amphiphysin, synaptobrevins, and gephyrin (Butler et

al., 1993, 2000; Levy et al., 1999; Geis et al., 2009). Whether stiff-person syndrome is caused by an immune response to a single epitope or multiple targeted antigens is not clear. The immune involvement in stiff-person syndrome also raises the question of whether post-polio syndrome could also arise from a latent immune memory of PV-infected motor neurons.

Therapeutic uses of PV

One positive twist that merits further exploration is the finding that the PVR, CD155, is also expressed on several forms of cancer cells, including glioblastoma, medulloblastoma, and colorectal cancer. The PVR may play an important role in cancer invasiveness and glioma migration, a key problem in eliminating high-grade gliomas from the brain. CD155 is reported to be recruited to the leading edge of migrating cells where it colocalizes with actin; experimental reduction in the expression of CD155 reduces migration of glioma cells *in vitro* (Sloan et al., 2004, 2005); PVR expression is upregulated by sonic hedgehog, a possible clue to the role of PVR during development (Solecki et al., 2002). If PVR can be downregulated in glioma *in vivo*, this may possibly reduce the neuroinvasive potential of these tumor cells and potentially increase the longevity of the patient. That brain tumors express the PVR raises the possibility that recombinant oncolytic PV strains lacking neurotoxicity may prove useful in combating some forms of brain cancer, a promising path for future study (Gromeier et al., 2000). Replacing the PV IRES with a human rhinovirus type 2 IRES blocks PV neurotoxicity, but does not block the ability of the recombinant PV to infect human glioma (Gromeier et al., 1996; Merrill et al., 2004; Goetz and Gromeier, 2010).

PV replicons, which contain a partial virus genome without the genes coding for the capsid, may have promise for selective gene delivery to the same motor neurons or other cells that active PV infects. The capsid protein is provided in *trans* during vector production, thereby restricting infection and replication to a single generation of cells, and allowing the transient amplified expression of genes of interest in the infected cells (Bledsoe et al., 2000; Jackson et al., 2003).

Eradication of PV

Although the oral vaccine has carried us a large step toward eliminating PV infections, as we approach the potential global eradication of polio, two risk factors of the oral vaccine have emerged. First, similar

to other positive stranded RNA viruses, PV replication is based on an error-prone RNA polymerase, leading to frequent mutations in the PV genome. Attenuated active PV can revert back to wild-type infectious neurotropic virus, in rare cases infecting motor neurons and causing poliomyelitis in either the inoculated individual, or in others in contact with the immunized person (Kew et al., 2002).

A second risk factor is the potential for recombination of the attenuated PV genome with genomes from other enteroviruses, particularly human enterovirus C/coxsackie viruses. Such a recombination can result in novel recombinant viruses with disease potential in causing paralysis (Rakoto-Andrianarivelo et al., 2008; Combela et al., 2011), and viruses derived from recombination of attenuated vaccine PV with coxsackie A17 are now circulating in some regions of the world (Jegouic et al., 2009). In patients orally inoculated with all 3 serotypes of PV, recombinant PVs are commonly generated, with some single recombinants containing unique sequences from all 3 parent PVs (Cammack et al., 1988). Recombination is most common among viruses with the most similar sequence homologies. The mechanism may be related to RNA polymerase copying the 3' end of one parental positive RNA strand, after which the polymerase switches copying to a second parental template, resulting in a chimeric negative strand antigenome (Kirkegaard and Baltimore, 1986; Racaniello, 2001). It is difficult to fully predict the final disease-phenotype of PV-coxsackie recombinations even if the nucleotide sequence can be determined.

As we approach the end of wild-type PV infections across the globe, there remain some complications that merit consideration. First, even after some regions of the world have been without active PV infections, PV has still been recovered from local water, suggesting either a latency of virus deterioration, or that some immunized or asymptomatic humans are still releasing active PV in feces. Inactivated PV immunization may protect against viremia and motor neuron-mediated paralysis without completely blocking PV infection and release in the gut. A further concern is that rare individuals with Ig deficiencies (agammaglobulinemia) that may not be able to eliminate PV completely may show ongoing fecal release of PV for years (Minor, 2004; Martín, 2006), potentially acting as a source for PV resurgence. Another concern is that at some point PV in laboratories may

need to be destroyed or raised to a high biohazard level to prevent accidental reintroduction of the virus from experimental stocks, or from possible clinical use in cancer treatment. Elimination of attenuated PV vaccine would prevent accidental reintroduction into the environment, but the vaccine may still be needed in the event that another unexpected PV outbreak occurs from an unexpected source.

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