Pathophysiology of the post-polio syndrome and persistence of poliovirus genomes in polio survivors

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The “invisible group” of polio survivors

Polio survivors form the largest single group of people with physical disabilities.

Polio survivors are “invisible” in the community.

Knowledge about the late consequences of polio (LEOP), and their impact upon the lives of polio survivors and their families, is almost non existent amongst the medical profession, policy makers, the community at large, and indeed the polio survivors themselves.

The LEOP have a dramatic impact on the ability of those polio survivors affected to maintain their mobility and independence and successfully undertake the activities of daily living.

There is a high cost to both polio survivors and the health system in trying to get a diagnosis and adequate treatments.

Thus, the need for in-depth knowledge about the causes and pathogenesis with the aim of developing effective therapies.
Interest in Poliovirus

We investigate viruses as infectious agents that cause disease in their host.

Our subjects are the etiology of viral diseases and mechanisms of viral pathogenensis.

We are interested in Picornaviruses (RNA viruses) whose prototype is Poliovirus (3 types are known to exist).

Picornaviruses (over 100 types) are estimated to infect billions of humans per year, causing a vast array of disease syndromes (paralysis, meningitis, heart disease, hepatitis, common cold, etc.).

Picornaviruses contain a plus-stranded RNA genome that functions as mRNA as soon as the viral particle enters the cell. The viral proteins, which are synthesized, recruit cellular factors. Together, they provide a menu for genome replication and genome encapsidation (i.e. the formation of new viral particles).

Poliovirus

X-ray Structure determination:
J.M. Hogle, M. Chow, D.J. Filman (1985)

Three-dimensional structure of poliovirus at 2.9 Angstroms resolution
Science, 229, 1358

(PDB entry: 2PLV)

Radial depth cue rendering with grasp (A. Nicholls) on Silicon Graphics:
J-Y. Srbo
### Picornavirus classification

<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
<th>Serotypes</th>
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<tbody>
<tr>
<td>Picornaviridae</td>
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<tr>
<td>Enterovirus</td>
<td>Human enterovirus A (17)</td>
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<td>Human enterovirus B (58)</td>
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<td>Human enterovirus D (3)</td>
<td>Enterovirus-68, 70, 94</td>
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<td>Parechovirus</td>
<td>Human parechovirus (14)</td>
<td>HPeV-1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14</td>
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<td>Ljungan virus (4)</td>
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<td>Kobuvirus</td>
<td>Human Aichi virus (1)</td>
<td>AiV</td>
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<td>Bovine Kobuvirus</td>
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<td>Cardiovirus</td>
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<td>animal EMC; Theiler's murine encephalomyelitis virus; Rat Theravirus; Human Vilyuisk encephalomyelitis virus; Human Saffold virus (9)</td>
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<td>Theilovirus (12)</td>
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Pathogenesis of poliomyelitis

In virology, the term "Pathogenesis" refers to mechanisms by which a virus causes disease in a host organism.

Pathogenetic mechanisms are complex and multi level.

Poliovirus infects humans only, but disease can also be produced experimentally in primates and transgenic mice.

The human receptor for poliovirus is a protein designed as CD155. It is the key for pathogenesis as it allows viral entry into cells.

Poliovirus replicates in the oro-gastro-intestinal tract (tonsils, Peyer's patches?) from which it can migrate to the central nervous system where it targets motor neurons.

Destruction of motor neurons causes irreversible paralysis or death, a disease called poliomyelitis.

Poliovirus receptor

CD155 receptor

CD155 bound to poliovirus
Pathogenesis of poliomyelitis

invasion of the CNS though the bloodstream and/or retrograde axonal transport

1. Viral invasion of innervating motor axon at neuromuscular junction.
   - Poliovirus-infected muscle cell.

2. Retrograde axonal transport of virus/receptor complex.
   - Nerve axon (retrograde axonal transport of virus-receptor complex)

3. Viral replication and destruction of motor neuron.
   - Poliovirus-infected motor neuron.
   - Death of the motor neuron.

Nerve-muscle junction (synapsis)

Pathogenesis of polio

Viruses reside at the **threshold between dead and living matter**. Poliovirus is a chemical with a life cycle. Its empirical formula is:

\[ \text{C}_{332,652} \text{H}_{492,388} \text{N}_{98,245} \text{O}_{131,196} \text{P}_{7,501} \text{S}_{2,340} \]

Inside the cells, **poliovirus expresses the hallmarks of a living entity**:

1. **Multiplication**,
2. **Heredity**,
3. **Variation** (mutation of the genome sequence),
4. **Recombination** (i.e., exchange of genomic sequences with related viruses such as members of *Picornaviridae*).

**Genetic elements of the viral genome influence neurovirulence.**

We will discuss **poliovirus multiplication, heredity and variation** with regard to the **Post-Polio Syndrome**

From: Eckard Wimmer, Stony Brook University, NY - [http://www.mgm.stonybrook.edu/wimmer/index.shtml](http://www.mgm.stonybrook.edu/wimmer/index.shtml)
Poliovirus RNA genome

Replication of Poliovirus Genome

3D RNA polymerase (critical enzyme for virus replication)

Acute/symptomatic enterovirus infections are widely recognized. Examples include paralytic polio, meningitis, myocarditis, etc.

**Persistent enterovirus infections** are documented in agammaglobulinemic patients. Examples are chronic excretion of poliovirus after natural infection or vaccination with live attenuated virus.

Recently, it has been shown that the **incubation period of polio** can span several years.

In fact, polio developed in an immunodeficient woman who remained **chronically infected for 12 years** after vaccinating her child (N Engl J Med. 2011 Jun 16; 364:2316-23).
Vaccine-Derived Poliomyelitis 12 Years after Infection in Minnesota

Aaron S. DeVries et al.

A 44-year-old woman with long-standing common variable immunodeficiency who was receiving intravenous immune globulin suddenly had paralysis of all four limbs and the respiratory muscles, resulting in death. Type 2 vaccine-derived poliovirus was isolated from stool. The viral capsid protein VP1 region had diverged from the vaccine strain at 12.3% of nucleotide positions, and the two attenuating substitutions had reverted to the wild-type sequence. Infection probably occurred 11.9 years earlier (95% confidence interval [CI], 10.9 to 13.2), when her child received the oral poliovirus vaccine. No secondary cases were identified among close contacts or 2038 screened health care workers. Patients with common variable immunodeficiency can be chronically infected with poliovirus, and poliomyelitis can develop despite treatment with intravenous immune globulin.

We thank the following persons for their efforts during this project: Richard Danila, Kristen Ehresmann, Sara Lowther, Claudia Miller, and Elly Pretzel at the Minnesota Department of Health; Gregory Armstrong, Jane Iber, Olen Kew, Eric Mast, Steven Oberste, Mark Pallansch, and Jane Seward at the Centers for Disease Control and Prevention; Vicki Carlson, Karen Ferrara, Gary Kravitz, and Doris Nordbye at the United Hospital and Clinic; Anita Guelcher, Chris Hendrickson, and Lisa Ide at the University of Minnesota Medical Center, Fairview; and John Modlin at the Dartmouth–Hitchcock Medical Center.
In contrast to paralytic polio, the origin of PPS is unclear.

Different factors have been blamed for: the aging process, the distal degeneration of the residual enlarged motor neurons that are proper of polio, and chronic inflammation.

It has been proposed (but not proven) that persistence of mutated PVs may cause the progressive neuromuscular damage seen in PPS.
Poliomyelitis: CNS areas infected by Polioviruses

- Anterior, lateral and posterior horn neurons
- Motor neurons
- Cortex
- Thalamus
- Hypothalamus
- Basal Ganglia
- Reticular formation
- Cerebellum
- Encephalic trunk neurons (ambiguous V, VII, VIII, XII)
Late effects of polio: progression from poliomyelitis to PPS

Death of spinal motor neurons following infection with poliovirus

Residual enlarged spinal motor neuron that is innervating increased numbers of muscle cells

Distal degeneration of axonal sprouts or loss of entire motor units

A: Normal Status
B: Paralytic poliomyelitis
C: Neurological and functional recovery
D: Neurological and functional stability
E: Onset of PPS

Onset: 8 Years
8-35 Years
> 15 Years
The Post-Polio Syndrome: multiple factors in pathogenesis

CO-FACTORS:
- Aging, loss of motor neurons
- Overuse, muscle degeneration
- Disuse, muscle atrophy

DISTAL DEGENERATION HYPOTHESIS:
- Distal degeneration of axonal sprouts in enlarged motor units and/or
- Loss of entire motor units

PERSISTENT VIRAL REPLICATION:
- Virus-induced cell damage
- Chronic inflammatory response
- Immune-mediated injury
- Decreased expression of neurotrophic factors
We investigated whether persistent poliovirus infections were associated with the late consequences of polio.
METHODS
Bioinformatics: Phylogenetic tree of PVs and polio-like EVs
Different genomic regions have been investigated:

- 5’UTR (cloverleaf and entry into ribosomes)
- 5’UTR-VP2 (entry into ribosomes-capsid proteins)
- 3Dpol (RNA polymerase)
Methods: amplification of the 3Dpol region

Primers for the 3Dpol region

Amplification of the 3 poliovirus types
## Immunofluorescence of polioviruses using different antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>CBV1</th>
<th>CBV2</th>
<th>CBV3</th>
<th>CBV4</th>
<th>CBV5</th>
<th>CBV6</th>
<th>CAV2</th>
<th>CAV4</th>
<th>CAV9</th>
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<th>echo11</th>
<th>EV71</th>
<th>PV1</th>
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Procedures for detecting polioviruses in post-polio patients

- RT-PCR, CPE, antigen expression, cytokines
- co-culture with human cell lines
- leukocyte separation

Primary cell culture
- Nerve
- Muscle
- Duodenum

Leukocytes

CSF
Saliva
Urine

RT-PCR
CAN POLIOVIRUS BE DETECTED IN PPS PATIENTS?
### PPS patients (n = 81)

<table>
<thead>
<tr>
<th>Male/Female</th>
<th>Age (years, M ± SD)</th>
<th>Years from APP (years, M ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.36</td>
<td>57.4 ± 7.3</td>
<td>53 ± 7.0</td>
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### Controls (n = 76)

Blood donors (n=26); neurologic patients with non-infectious, autoimmune, or neoplastic disease (n=11); **family members of PPS patients** (n=39)

<table>
<thead>
<tr>
<th>Male/Female</th>
<th>Age (years, M ± SD)</th>
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<tbody>
<tr>
<td>0.67</td>
<td>39.7 ± 13.4</td>
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Year of Acute Paralytic Poliomyelitis

VAPP CASES
Age at onset of Acute Paralytic Poliomyelitis
Age of the investigated PPS patients

Age of PPS patients (years)
PV genome fragment in PPS patients (n=81)

69/81 Positive (84%)
Poliovirus genome fragments in control subjects (n=76)

- Blood donors (0/26)
- Family members of PPS patients (2/39)
- Pathologic controls (1/11)

No. positive: 3/76 (1,3%)
PV detection in primary cultures from PPS patients: skeletal muscle & nerve

Primary culture of surgical samples: virus detection after >30 yrs from the acute event
PV detection in primary cultures from PPS patients: skeletal muscle & nerve

MUSCLE FRAGMENT (RR)

ISCHIATIC NERVE FRAGMENT (CM)

PV antigen
PV detection in primary cultures from PPS patients: duodenal epithelial cells

Polio-1
3Dpol fragment

3D pol (630 bp)
PV antigens in the AV3 cell line co-cultured with leukocytes of PPS patients

A. Uninfected cells
B. Reference PV1 (Chat strain)
C. PPS (RR strain)
D. PPS (LL strain)
PATIENT LL

1933: acute paralytic polio
2007: detection of PV1 in CSF and leukocytes

Detection of PV-1 in a PPS patient 74 years after APP
Multiple mutations and deletions have been detected in the 5`UTR and VP1 regions of PVs obtained from PPS patients. THE 3D POL REGION IS INSTEAD CONSERVED.
Since previous literature could only show “genomic fragments” of polioviruses in PPS patients, we investigated whether fragments from all over the poliovirus genome could be obtained.

To this end, a collaboration with the Institute of Human Virology (Baltimore, Maryland) was established.
Full-length amplification of persistent PV isolates from PPS patients

Large fragments obtained so far

P03LLVR and P19RRVA: 1248bp fragment

P03LLVR: 2580bp fragment

PV-specific primer pairs
It came out that fragments spanning the entire PV genome can be amplified in samples of PPS patients.

However - due to the extremely small amounts of poliovirus in samples from PPS patients – difficulties were encountered in sequencing the whole genome of persisting poliovirus.
Together with our previous results, the data produced in Baltimore demonstrate that amplified fragments of the poliovirus can be obtained from almost all genome regions (red boxes).

The 2A and 2B regions have not been detected yet.
IS THERE ANY RESIDUAL BIOLOGICAL ACTIVITY IN PERSISTENT POLIOVIRUS OF PPS PATIENTS?
Cytopathic effect and expression of poliovirus antigen in cell lines exposed to persistent poliovirus of PPS patients
Cytokines elicited by persistent poliovirus of PPS patients

Persistent poliovirus from PPS patients (n=18)

ACUTE PV1 INFECTION (Chat strain, 30 hr p.i.)

multi-analyte ELISA array
Thus, persistent Poliovirus from PPS patients produces the following effects in human cell lines:

1) slight cytopathic effect;
2) expression of viral antigens within infected cells;
3) stimulation of cytokine production, especially MCP-1.

MCP1 is a chemokine (chemotactic factor for monocytes and basophils, but not neutrophils or eosinophils) that has been implicated in chronic diseases with monocytic infiltrates (e.g., psoriasis, rheumatoid arthritis, atherosclerosis).

For comparison, infection with vaccine Poliovirus-1 causes:

1) strong cytopathic effect;
2) expression of viral antigens within infected cells;
3) stimulation of cytokine production, especially IL-12 and RANTES.

IL12 stimulates IFN-γ and TNF-α production and reduces IL4 mediated suppression of IFN-γ. It enhances the cytotoxic activity of NK cells and CD8+ T lymphocytes. RANTES is a chemoattractant for monocytes, memory T-helper cells and eosinophils.
POSSIBLE PPS TREATMENTS
I. Post-polio patients have increased cytokine levels in CSF (CNS inflammation)

II. Inflammation is possibly down-modulated by normal Hu immunoglobulin

III. Down-modulated inflammation is associated with increased muscle strength and better quality of life

IV. Unfortunately, results are only temporary and repeated treatment may be required

Chen Z, Chumakov K, Dragunsky E, Kouiavskaya D, Makiya M, Neverov A, Rezapkin G, Sebrell A, Purcell R. National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

Six poliovirus-neutralizing Fabs were recovered from a combinatorial Fab phage display library constructed from bone marrow-derived lymphocytes of immunized chimpanzees. The chimeric chimpanzee-human full-length IgGs (hereinafter called monoclonal antibodies [MAbs]) were generated by combining a chimpanzee IgG light chain and a variable domain of heavy chain with a human constant Fc region. The six MAbs neutralized vaccine strains and virulent strains of poliovirus. Five MAbs were serotype specific, while one MAb cross-neutralized serotypes 1 and 2. Epitope mapping performed by selecting and sequencing antibody-resistant viral variants indicated that the cross-neutralizing MAb bound between antigenic sites 1 and 2, thereby covering the canyon region containing the receptor-binding site. Another serotype 1-specific MAb recognized a region located between antigenic sites 2 and 3 that included parts of capsid proteins VP1 and VP3. Both serotype 2-specific antibodies recognized antigenic site 1. No escape mutants to serotype 3-specific MAbs could be generated. The administration of a serotype 1-specific MAb to transgenic mice susceptible to poliovirus at a dose of 5 μg/mouse completely protected them from paralysis after challenge with a lethal dose of wild-type poliovirus. Moreover, MAb injection 6 or 12 h after virus infection provided significant protection.

The MAbs described here could be tested in clinical trials to determine whether they might be useful for treatment of immunocompromised chronic virus excretors and for emergency protection of contacts of a paralytic poliomyelitis case.
In 1988, the World Health Assembly launched the Global Polio Eradication Initiative, which aimed to use largescale vaccination with the oral vaccine to eradicate polio worldwide by the year 2000. Although important progress has been made, polio remains endemic in several countries. Also, the current control measures will likely be inadequate to deal with problems that may arise in the postpolio era. A panel convoked by the National Research Council concluded that the use of antiviral drugs may be essential in the polio eradication strategy. We here report on a comparative study of the antipoliovirus activity of a selection of molecules that have previously been reported to be inhibitors of picornavirus replication and discuss their potential use, alone or in combination, for the treatment or prophylaxis of poliovirus infection.
Positive-strand RNA viruses include a large number of human and animal pathogens whose essential RNA-dependent RNA polymerases (RdRPs) share a structurally homologous core with an encircled active site. RdRPs are targets for antiviral drug development, but these efforts are hindered by limited structural information about the RdRP catalytic cycle. To further our understanding of RdRP function, we assembled, purified, and then crystallized poliovirus elongation complexes after multiple rounds of nucleotide incorporation. Here we present structures capturing the active polymerase and its nucleotide triphosphate complexes in four distinct states, leading us to propose a six-state catalytic cycle involving residues that are highly conserved among positive-strand RNA virus RdRPs. The structures indicate that RdRPs use a fully prepositioned templating base for nucleotide recognition and close their active sites for catalysis using a novel structural rearrangement in the palm domain. The data also suggest that translocation by RDRPs may not be directly linked to the conformational changes responsible for active site closure and reopening.

Fig. 1. Poliovirus 3DPol elongation complex. (A) Crystal packing showing staggered coaxial stacking of upstream template-product duplexes resulting in two nonequivalent pairs of ECs (A&E vs. I&M). Product strand is shown in green, template in cyan, and downstream nontemplate in purple. (B) EC structure showing up- and downstream RNA duplexes as the template strand (cyan) threads through the active site and the red arrow indicates trajectory of downstream RNA duplex. (C) Top view of EC showing the single stranded conformation of the +1, +2, and +3 downstream template nucleotides and the protein clamp of the upstream duplex. Palm domain is in gray, thumb in blue, and the individual fingers (19) are colored with index in green, middle in orange, ring in yellow, and the pinky in pink. (D) 3,500 K composite simulated-annealing omit maps contoured at 1.5σ showing quality of active site electron density for the native (EC) and Mg^{2+}-CTP (EC + CTP) complexes. The pyrophosphate (ppi) in the CTP complex is shown in orange and the presence of metals ions was confirmed by an essentially identical Mn^{2+}-CTP structure (see Figs. S3 and S4).
THE FIFTH AWARD (2009)

PHI award to a team from University of Insubria (Varese, Italy) led by Antonio Toniolo, MD. The study, Persisting Noninfectious Fragments of Poliovirus in PPS Patients: Virus Detection and Susceptibility to Antiviral Drugs, will set up methods for detecting polioviruses in PPS patients and sequencing their genome.
We detected genomic fragments of PVs in 69/81 PPS patients: **84%**

Persisting PVs carry **multiple mutations and deletions**

Average documented persistence of PVs in PPS patients: **53 yrs**

Thus, mutated PVs with modest ability to replicate **can persist for decades in polio survivors**

Persisting polioviruses are endowed with **residual biologic activity expressed, for instance, as inflammatory stimuli**

Persisting PVs were not found in family members of PPS patients. This result demonstrates that **transmission of these “defective viruses” does not occur within the families of PPS patients**

Therefore, these **mutated polioviruses are not transmissible**
In polio survivors, the persistence of residual polioviral activity can be associated with chronic inflammation. Viral diagnosis may pave the way to treating patients with antibodies and/or antiviral drugs in order to stop the progression of virus-associated cell damage. Current experiments in vitro show that neutralizing antibodies and antiviral drugs under development are effective against polioviruses. Polio meetings like this one in Copenhagen will help collecting ideas and will promote the development of effective treatments for PPS patients.
Thanks to the colleagues who introduced us to this area of research:

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