Background

In the first half of the 20th century, poliomyelitis was widely feared. It was contagious and affected mainly young people, resulting in paralyzing or even permanent paralysis or death. This condition is caused by three different poliovirus (PV) types. The control of poliomyelitis through mass vaccination has led to an remarkable story of scientific and social progress. After a period of prolonged stability, 50-70% of patients with residual impairments following paralytic poliomyelitis develop a condition designated as "post-polio syndrome" (PPS). These late-onset cases are reported as progressive muscular atrophy, new disabilities and weaknesses, pain and chronic fatigue, cold intolerance [5, 9, 16, 19]. Among experts, there is the view that the neuromuscular rearrangements manifest as impairments of daily living, mobility, upper limb function, and respiratory capacity. The nature of PPS remains controversial, with definitions continuing to indicate that the new symptoms and signs should be unrelated to any orthopedic, neurological, respiratory disturbances. Studies suggested that, with increasing age, the new wasting and weakness is due to "the dual degeneration" of the motor units that had been affected by poliomyelitis [3, 6, 9, 16, 18] (Figure 1).

Methods

PPVs have been searched for in cerebrospinal fluid (CSF) and blood samples taken from 64 adult PPS patients diagnosed with PPS at the Department of Neurology, University of Verona. According to the colleague of Faru [2], healthy blood donors, family members of PPS patients, and neurological patients (with noninfectious, non-neoplastic, non-autoimmune pathologies) were used as controls (Table 1). As shown in Figure 3, PV search was performed by co-infecting CSF, blood leukocytes or other tonsilexamples with susceptible cell lines, MRC-5 (5-6 passages). Cell-free supernatants were tested for PV genomes using specific PCR primers aimed at the SUVR, 3D, and 2D UV regions. Figure 4 shows a specific type-specific PV primers aimed at the 3D region. Original PCR ampalas capable of detecting (≥50 EU) were used. PCR amplicons were directly sequenced using Applied Biosystems fluorescent sequencing kits. Cell lines exposed to samples from PPS patients were also stained with PV-specific mAbs specific against anti-PV nucleoprotein and anti-PV capsid protein. To address putative copies of PV, multiple infections and detections were detected especially in the SUVR 2D and UV regions. The negative regions could be sequences using primers designed on the basis of protease rhinotropism. Multiple copies arrays showed that the release of MCV-1 (monocytic chorioretinitis protein) was significantly enhanced in HeLa cell cultures that had been exposed to PPS patients (Figure 5). Taken together, the data show that the PV genome fragments obtained from PPS patients revealed some neurotropic biological activity, as production of viral capsid proteins and pro-inflammatory cytokines.

Conclusions

The results indicate that low-level PV activity can persist for decades in the majority of polio survivors. The results, however, do not provide a complete explanation of the development of PPS. The present study is investigating the role of the PV genome propogation of altered cellular populations observed from PPS patients. The hope is to understand their possible pathogenic contribution and to devise novel therapeutic approaches.

References

1. [1] A. Bai, J. Maccarin, L. Bertolasi, S. Monaco, A. Q. Toniole; 1. 1. Univ. of Insubria and Ossepoli di Ciorico, Varace, Italy; 2. 2. Univ. of Verona Medical School Verona, Italy

Figure 1. Dual degeneration

Viruses studies suggest that PPS may be related to the low-level persistence of poliovirus "genomic fragments" in the central nervous system [2] and other tissues [14, 15, 16, 18] and to the associated chronically inflammatory responses [5, 9, 10]. In this regard, it should be reminded that PPS are capable of replicating in human macrophages and dendritic cells [20].

Figure 2. Pathogenesis of PPS

Figure 3. Alignment of SUVR, VP1, and 3D UVR sequences

The amounts of PV genome fragments were extremely low in all patients and in different specimens (i.e., ≥500 copies/ml). This made direct sequencing extremely difficult. Hence, a partial sequencing of the VP1 type 1 genome from a PPS patient compared to the reference PV-1 (Bachmann strain). Multiple mutations and deletions were detected especially in the SUVR and VP1 regions. The latter regions could be sequenced using primers designed on the basis of protease rhinotropism. Multiple copies arrays showed that the release of MCV-1 (monocytic chorioretinitis protein) was significantly enhanced in HeLa cell cultures that had been exposed to PPS patients (Figure 5). Taken together, the data show that the PV genome fragments obtained from PPS patients revealed some neurotropic biological activity, as production of viral capsid proteins and pro-inflammatory cytokines.

Figure 4. Type-specific PV primers

Figure 5. Pathology culture of surgical samples: detection of capsid antigens