From the Division of Rehabilitation Medicine,
Departments of Public Health Sciences
and KI at Danderyd Hospital,
Karolinska Institutet, Stockholm, Sweden

The post-polio syndrome – studies of immunology and immunomodulatory intervention

Henrik Gonzalez

Stockholm 2005

ISBN 91-7140-483-X

To my daughter Annalena

ABSTRACT

Late effects in patients with prior poliomyelitis were first reported in the nineteenth century and have been referred to as the post-polio syndrome (PPS). PPS is characterized by new muscular deterioration, fatigue and pain after a stable period of at least 15 years.

In order to study the pathophysiological role of a possible inflammatory reaction in PPS, four key cytokines were determined with mRNA expression in mononuclear cells from cerebrospinal fluid (CSF) and peripheral blood (PB) of 13 patients. The PPS patients displayed greater numbers of CSF cells expressing mRNA for TNF-α ($p<0.02$), IFN-γ ($p< 0.02$), IL-4 ($p<0.001$) and IL-10 ($p<0.05$), compared to eight controls with other neurological disorders (OND). The level of increase was in the same range as found in seven control patients with multiple sclerosis (MS). There was no cytokine increase in PB. The increase of cytokines in the CSF indicates an inflammatory process in the CNS of PPS patients.

To explore whether the inflammatory process could be down-modulated, 16 patients were treated with high-dose (90g) intravenous immunoglobulins (IVIG). The increase of TNF-α, IFN-γ, IL-10 and IL-4 mRNAs in CSF was reproduced with TaqMan RT-PCR. In PB TNF-α was significantly increased. Six–8 weeks after treatment TNF-α and IFN-γ mRNA levels were significantly reduced, while IL-10 remained unchanged.

To analyze whether down-modulation of the inflammatory process was accompanied by changes of muscle strength, walking performance and Quality of Life, an open clinical trial including 14 PPS patients was performed.

Regarding Quality of Life there was a statistically significant improvement for seven of eight subscales of the SF-36, most obvious in vitality. A trend towards an increase in muscle strength and function was found in the PPS patients when comparing data before and after IVIG treatment.

To further study the clinical effect of IVIG, a randomized double-blinded placebo-controlled study was performed. One hundred forty-two patients were randomly chosen to receive infusion of either 90 g IVIG or placebo, over three consecutive days, which was repeated after three months.

Average muscle strength was increased by 4.3% over baseline in patients receiving IVIG, while the placebo group displayed a 5.8 % decrease ($p=0.029$). Five of eight subscales of the SF 36 improved significantly over baseline in the IVIG group, but none in the placebo group. Two subscales, vitality and general health, revealed a significantly better outcome in favor of IVIG ($p=0.042$ and $p=0.048$, respectively). The Physical Activity Scale of the Elderly (PASE) was improved in the IVIG group compared to the placebo group ($p=0.018$). Patients reporting pain on the VAS scale improved in the IVIG group, but not in the placebo group ($p=0.037$).

It is concluded that there is an increase of cells expressing cytokines in the CSF of PPS patients corresponding to that in MS, a well-known neuroinflammatory disease. This indicates an inflammatory process in the CNS. IVIG treatment leads to a decrease of the inflammatory process which is then followed by an increase of muscle strength and physical activity as well as improved Quality of Life and decrease of pain over six months. This finding may open the way for new therapeutic strategies in the post-polio syndrome.

ISBN 91-7140-483-X

This thesis is based on the following four papers, referred to in the text by their Roman numerals

I. Gonzalez H, Khademi M, Andersson M, Wallström E, Borg K, Olsson T.
Prior poliomyelitis – evidence of cytokine production in the central nervous system
J Neurol Sci. 2002 Dec 15; 205(1): 9-13.

II. Gonzalez H, Khademi M, Andersson M, Piehl F, Wallström E, Borg K, Olsson T.
Prior poliomyelitis – IvIg treatment reduces proinflammatory cytokine production
J Neuroimmunol. 2004 May; 150(1-2): 139-44.

III. Kaponides G. Gonzalez H, Olsson T, Borg K,
The effect of intravenous immunoglobulin in patients with post-polio syndrome – an uncontrolled pilot study
Submitted.

IV. Gonzalez H, Stibrant Sunnerhagen K, Sjöberg I, Kaponides G, Olsson T, Borg K,
Intravenous immunoglobulin for the post-polio syndrome; a randomized controlled trial.
Submitted. .

Contents

1. Introduction

1.1 Acute paralytic poliomyelitis

Acute poliomyelitis is caused by poliovirus, which is an enterovirus. The three serotypes of the virus are named polio 1 (Brunhilde), polio 2 (Lancing) and polio 3 (Leon). Polio 1 is most commonly associated with paralytic polio (Johnson 1995; Howard 2005). The poliovirus is transmitted through the fecal-oral pathway and the most common symptoms are gastrointestinal, with diarrhea, accompanied by generalized symptoms such as fever and illness. Fewer than 1% of infected patients show symptoms from the central nervous system (CNS).
The natural course of acute poliomyelitis varies between individuals. A person who survives the acute phase of poliomyelitis recovers within two weeks. However, the poliovirus affects the anterior horn cells causing paresis or paralysis. There is a recovery of muscle strength, reaching a plateau after 1-2 years, leaving patients with varying degrees of sequelae (Halstead and Rossi 1987; Mulder 1995), from a slight monoparesis to tetraparesis with respiratory insufficiency requiring ventilatory assistance.
The muscle weakness is most often asymmetric and most often found in a lower leg. Some cases are asymptomatic even though they have a significant loss of motorneurons. This type is referred to as non-paralytic poliomyelitis. The “stable period”, that follows initial recovery lasts for decades (Halstead and Rossi 1987).

1.2 History of poliomyelitis

The oldest reference to paralytic poliomyelitis is an Egyptian stele over 3,000 years old (Hunt 2005).

In 1789 the British physician, Michael Underwood, published the first known clinical description of polio:

> "Debility of the Lower Extremities"
> *"The disorder intended here is not noticed by any medical writer within the compass of my reading, or is not a common disorder, I believe, and it seems to occur seldomer in London than in some parts. Nor am I enough acquainted with it to be fully satisfied, either, in regard to the true cause or seat of the disease, either by my own observation, or that of others; and I myself have never had the opportunity of examining the body of any child who has died of the complaint. I shall, therefore, only describe its symptoms, and mention the several means attempted for its cure, in order to induce other practitioners to pay attention to it. It seems to arise from debility, and usually attacks children previously reduced by fever; seldom those under one, or more than four or five years old. The Palsy ... sometimes seizes the upper, and sometimes the lower extremities; in some instances, it takes away the entire use of the limb, and in others, only weakens them."*

In 1840, Heine described the clinical features of poliomyelitis and suggested that the spinal cord was involved, and in 1890 Medin drew attention to the epidemic character of the disease. The poliovirus was identified in 1908 by Landsteiner and Popper (Schwick 1991).
Enders, Weller and Robbins (Weller, Robbins et al. 1949) published a technique for culturing the virus which led to the development of the vaccines in the 1950s.
This generated an eradication of polio in Sweden in the beginning of the 1960s. In Sweden 51 000 cases of polio were reported between 1905 and 1962 with a mortality incidence of 6000 (Axelsson 2004). The last great epidemic took place in 1953 when around 7,000 cases were diagnosed. Worldwide, new cases are still reported. In 2002 approximately 2,000 new cases were reported, mainly in central Africa and in Southeast Asia (WHO 2003).
Due to global vaccination programs, the number of new cases has dramatically decreased during recent years.

1.3 The post-polio syndrome

Development of new symptoms in patients with prior poliomyelitis was first mentioned as early as 1875 by Raymond and Charcot (Raymond 1875). After the “stable period” many patients with prior poliomyelitis exhibit new symptoms from the neuromuscular system as well as other symptoms such as fatigue and pain. This condition has commonly been called the post-polio syndrome (PPS).
Increased muscle weakness, was reported to vary between 12-18%, for “unstable” patients, in a Swedish polio population compared with 4-8% in healthy controls during an eight-year period (Grimby, Stalberg et al. 1998). The reduction in muscle strength may even affect previously weakened

muscles, but new weakness and atrophy may be seen in muscle groups earlier unaffected (McComas, Quartly et al. 1997).
Fatigue is most often expressed as decreased muscle endurance during activities. Another form of fatigue is described as mentally-exaggerated daytime tiredness. Both types of fatigue are accompanied with a prolonged time for recovery (Fischer 1985; Halstead and Rossi 1985; Halstead, Wiechers et al. 1985; Bach, Alba et al. 1987; Cashman, Maselli et al. 1987; Cashman, Maselli et al. 1987; Feldman and Soskolne 1987; Fischer 1987; Bach, Alba et al. 1989; Dunn 1991; Agre, Grimby et al. 1995; Grimby, Tollback et al. 1996; Dalakas 2002; Clavelou 2004).
Pain of either muscular or joint origin is reported to be the second most common complaint from the PPS patients. In an outpatient clinic in Washington 71% of the patients complained of muscle pain and an equal amount suffered from joint pain (Halstead and Rossi 1987).
Some patients also exhibit breathing problems (Hill, Robbins et al. 1983; Fischer 1985; Bach, Alba et al. 1987; Fischer 1987; Bach, Alba et al. 1989) and swallowing difficulties (Sonies and Dalakas 1991; Sonies and Dalakas 1995).

Currently, three sets of diagnostic criteria are used in parallel: Halstead's and Rossi's definition (PPS) (Halstead and Rossi 1987), progressive post-poliomyelitis muscular atrophy (PPMA) as described by Dalakas (Dalakas 1995) and post-polio muscular dysfunction (PPMD) according to Borg 1996 (Borg 1996) (Table 1).

Some criteria are shared by the three definitions. These are:
- History of acute polio infection.
- Ensuing stable period.
- Development of new muscle weakness and fatigue.
- No other diagnosis explaining the symptoms.

The number of polio patients at risk for developing PPS varies depending on what diagnostic criteria are used, and how. Codd first reported a prevalence of 22% (Windebank, Daube et al. 1987) in a Minnesota cohort, and then four years later reported 68% in the same population (Windebank, Litchy et al. 1991). In contrast, Munsat suggested that all polio survivors will develop PPS in time (Munsat 1991).
In addition, 2003 Farbu et al. presented a somewhat more narrow set of criteria. These criteria were based on those by Halstead and Rossi, but with more emphasis on the decrease of physical function i.e. muscle strength. Using these criteria, and combined with a very careful neurological examination, the percent of patients with PPS were restricted to 26% (Farbu, Rekand et al. 2003). Furthermore, Farbu introduced the term polio related loss of function which constituted 53% of the diagnoses (Farbu, Rekand et al. 2003).

Table 1. The three definitions used for the post polio syndrome

Post-polio syndrome (Halstead and Rossi)	a. A confirmed history of paralytic polio. b. Partial to fairly complete neurologic and functional recovery. c. A period of neurologic and functional stability of at least 15 years' duration. d. The onset of two or more of the following health problems since achieving stability: unaccustomed fatigue, muscle and/or joint pain, new weakness in muscles previously affected and/or unaffected, functional loss, cold intolerance, new atrophy. e. No other medical diagnosis to explain these health problems.
Progressive post-poliomyelitis muscular atrophy (Dalakas)	Used to describe objective signs and symptoms reflecting lower-motor-neuron deterioration. a. New muscular weakness and atrophy. b. Fasciculations. c. Weakness of bulbar muscles. d. New respiratory difficulties. e. Sleep apnea.
Post-polio muscular dysfunction (Borg)	1. History of paralytic polio: Confirmed or not confirmed: Partial or fairly complete functional recovery. 2. After a period of functional stability of at least 15 years, development of new muscle dysfunction: Muscle weakness, muscle atrophy, muscle pain, fatigue. 3. Neurological examination compatible with prior poliomyelitis: Lower motor neuron lesion, decreased or absent tendon reflexes, no sensory loss. Compatible findings on EMG and/or MRI.

1.4 Pathogenesis of PPS

Since the description of PPS the pathogenesis has been discussed. There is substantial consensus regarding the pathophysiology of the neuromuscular symptoms. Denervation is compensated for by a reinnervation leading to an increase of motor unit territory of up to 20 times normal size. The denervation-reinnervation process has an upper limit, and in the end reinnervation cannot keep up with denervation. Denervation uncompensated for leads to decreased muscle strength (Wiechers and Hubbell 1981; Dalakas, Elder et al. 1986; Cashman, Maselli et al. 1987; Ravits, Hallett et al. 1990; Dalakas 1995; Dalakas 1995; Borg 1996; Halstead 1998).

Even though the definite cause of the peripheral denervation is still unclear, several possible mechanisms have been suggested and, as presumed by Jubelt and Cashman (Jubelt and Cashman 1987). These possible etiologies are:

- chronic poliovirus infection
- loss of motor neurons due to normal aging process
- premature aging of cells permanently damaged by poliovirus
- premature aging of remaining normal motor neurons due to increased metabolic demand
- loss of muscle fibers in enlarged, reinnervated motor units
- predisposition to motor neuron degeneration because of glial, vascular, and lymphatic damage caused by poliovirus
  motor units with poliomyelitis-induced vulnerability to secondary insults
- genetic predisposition of motor neurons to both poliomyelitis and premature degeneration
- an immune-mediated mechanism

It is more difficult to explain the central/mental fatigue and the more generalized pain that are also frequent symptoms of PPS. One hypothesis that could explain these symptoms of PPS and also contribute to the motorneuron-degeneration is that of an inflammatory process in the CNS (Dalakas 2002).
This hypothesis includes chronic persistent inflammation that contributes to the subsequent degeneration of anterior horn cells in the central nervous system (CNS). Inflammation in the anterior horn cells was first described in 1879 by Vulpian (Vulpian 1879). Later autopsy studies demonstrated inflammation in the spinal cord, located in the parenchyma. Further, perivascular inflammatory signs were noted, with mononuclear cells indicating an ongoing inflammation (Pezeshkpour and Dalakas 1987; Pezeshkpour and Dalakas 1988; Miller 1995). The hypothesis is supported by findings of an increase in markers of inflammation and antibody responses in the cerebrospinal fluid (CSF) of PPS patients (Ginsberg, Gale et al. 1989; Sharief, Hentges et al. 1991).

The cause of a possible intrathecal inflammatory process is unclear. A suggestion with some support in the literature is the presence of polio virus, presumably, in a mutated form, in the CNS (Calvez, Pelletier et al. 1995; Colbere-Garapin, Duncan et al. 1998; Pelletier, Duncan et al. 1998; Couderc, Girard et al. 2001; Girard, Gosselin et al. 2002; Labadie, Pelletier et al. 2004)
However contradictory reports from other groups are unable to verify a persistent poliovirus (Salazar-Grueso, Grimaldi et al. 1989; Melchers, de Visser et al. 1992; Roivainen, Kinnunen et al. 1994; Jubelt, Salazar-Grueso et al. 1995).
Another possible mechanism is that of a defect in the immune-system which may explain both the susceptibility for poliomyelitis and PPS (Rekand, Langeland et al. 2002; Farbu, Rekand et al. 2003).

1.5 Basal neuroinflammatory aspects

The CNS is considered to be an immune-privileged site, in the sense that immune responses to infections are not so strong as those in other compartments. This is believed to be due to the consequences of an immune reaction in the brain or spinal cord being more dangerous than those in other organs. Hence some pathogens, for example Herpes virus, HIV and Borrelia Burgdorfii, are able to survive for a long time in the CNS.
The existence of the blood brain barrier (BBB) limits the number of lymphocytes patrolling the CNS, while the infrequent expression of Major Histocompatibility Complex (MHC) class II molecules in the brain also indicates the immune-privileged state of the CNS.
Nevertheless, immune responses occur in CNS disorders both in infectious diseases and in autoimmune inflammatory disease such as MS. In neuroinflammation, leukocytes enter the CNS-CSF compartment. Mononuclear cells (MNC) present in the CSF in these conditions are thought to represent the MNCs in the brain and spinal cord. Accordingly, it is possible to study the immune active cells in the CNS compartment by sampling CSF and isolating MNCs for study.
Cytokines are a family of immune mediators secreted from MNCs and other cells acting in a paracrine fashion, exerting many effects. CD4 positive lymphocytes are divided into Th1 and Th2 depending on the type of cytokine they produce. In the present work we analyzed a panel of Th1 and Th2 cytokines. Cytokines often have a very short half-life, for which reason we found it more appropriate to study mRNA expression of different cytokines in MNC than to measure soluble cytokines in the CSF with ELISA (Olsson 1995).

1.6 Intravenous immunoglobulin treatment (IVIG)

Intravenous immunoglobulin is pooled human immunoglobulin used as treatment in many inflammatory disorders including the Guillan-Barre syndrome, myasthenia gravis and multiple sclerosis. The mechanism of action is not fully understood and may vary in different conditions. It has been proposed that the immunoglobulins block Fc receptors on phagocytic cells, suppress cytokine synthesis and neutralize pathogenetic antibodies, all leading to downregulation of immune responses.

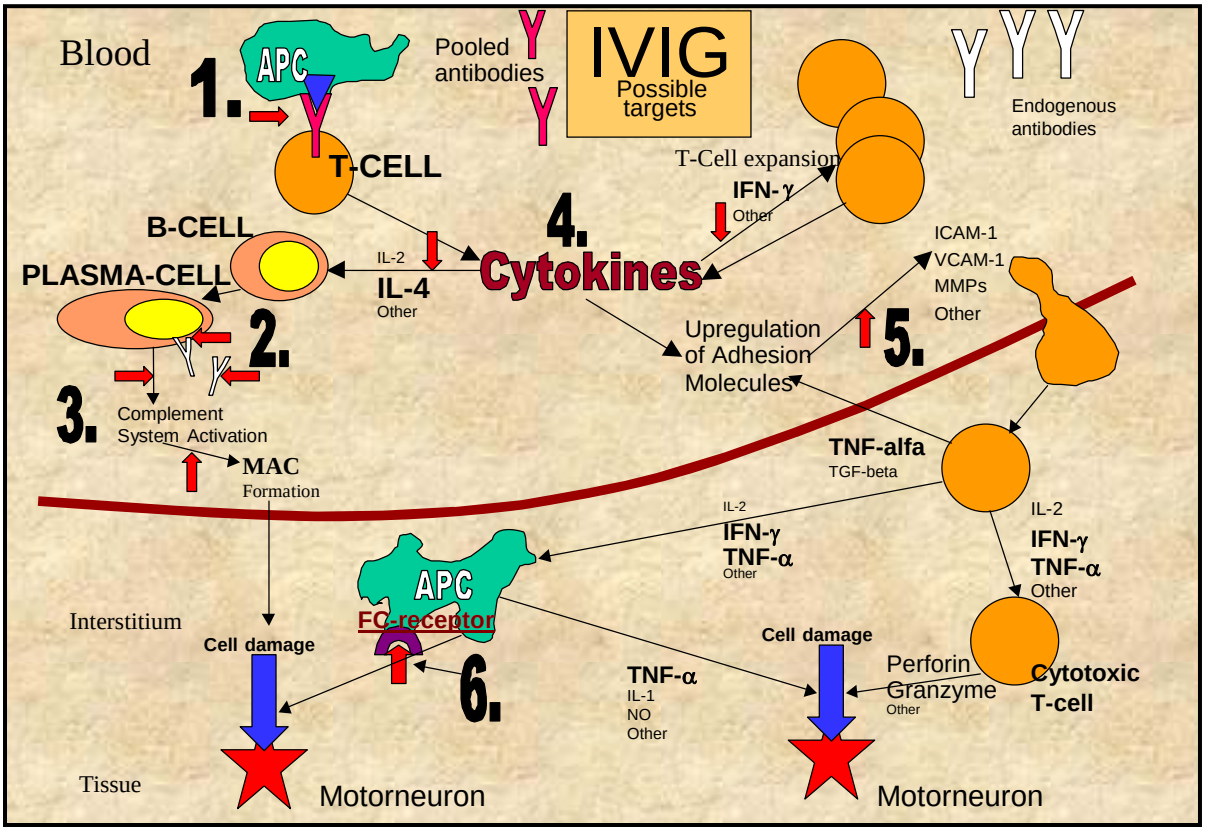

Fig 1. Possible targets for the immune-modulatory effects of IVIG.

1. Interference with co-stimulatory molecules
2. Modulation of antibody production and neutralization of antibodies by anti-idiotypic antibodies
3. Inhibition of complement activation and MAC formation
4. Modulation of inflammatory and anti-inflammatory cytokines and chemokines
5. Modulation of adhesion molecules
6. Modulation of Fc-receptors on macrophages and other effector cells

2. Aims

General aim

The purpose of the present work was to establish and evaluate a posited inflammatory process in the CNS, and to evaluate the outcome of immune-modulation by means of IVIG, in post-polio patients.

Specific aims

Specific aims were:

I. to analyze the level of cytokine mRNA expression of mononuclear cells in CSF and peripheral blood in patients with PPS (I and II),

II. to evaluate the effect of immune-modulation by means of IVIG on the cytokine mRNA expression levels in the CNS of PPS patients (II),

III. to evaluate the effect of immune-modulation by means of IVIG on clinical function and Quality of Life (III, IV), and

IV. to assess side-effects of IVIG in PPS patients (III and IV).

3. Patients and Methods

3.1 Patient selection

All patients were selected from out-patient clinics at Danderyd University Hospital, Karolinska University Hospital and Huddinge University Hospital, Stockholm, Akademiska University Hospital, Uppsala and Sahlgrenska University Hospital, Gothenburg, Sweden (Table 2.).

Table 2. The demographic data of the patients included in studies I-IV.

	Study I	**Study II**	**Study III**	**Study IV**	
Number of patients	13	16	14	135	
				IVIG	Placebo
Female	8	7	6	47	39
Male	5	9	7	20	29
Mean age	62.8	58.5	57.5	61.5	59.0

The patients were aged between 18 and 75 years with a history of polio virus infection followed by restitution or improvement regarding motor function and disabilities after the initial infection. The polio disease should have been confirmed by EMG in the lower extremities in at least two major muscle groups (mm quadriceps, gastrocnemicus, and tibialis anterior). The patients had also experienced increased symptoms such as muscular weakness, discomfort or pain after a period of at least 15 years' functional stability with no other explanation for the symptoms. Thus the patients fulfilled the criteria for the PPS as described by Halstead and Rossi (Halstead and Rossi 1987).
In addition, all the patients had stable weight (maximum change 7 kg) during the previous five years.
In study III, all PPS patients were ambulatory with or without walking aids.
For study IV, additional inclusion and exclusion criteria are presented in Table 3.

Table 3. Additional criteria for inclusion in study IV.

Inclusion Criteria	**Exclusion criteria**
A muscle that had deteriorated within the previous five years, and was judged clinically to have 20-75 % of the muscle strength of a comparable normal population of the same age and gender. Body Mass Index (BMI) ≤ 29 kg/m^2.	Selective IgA deficiency. Diabetes Any active malignancy, history of active malignancy or treatment for malignancy during the previous three years. Subjects who within 12 weeks prior to enrolment received any immunosuppressive/ systemic corticosteroid treatment (topical corticosteroids excluded). Disabling pain from extremities or skeletal system due to previous fracture(s), arthritis; or for other reasons not related to PPS. Hepatitis or HIV disease. Increased liver enzymes (ASAT, ALAT, γGT) above twice upper normal value. Creatine kinase >10 μkat/l. Conditions associated with a risk of poor protocol compliance (e.g. known drug or alcohol abuse).

3.2 Ethical considerations

All enrollments to the studies followed the recommendations of the Declaration of Helsinki (HELSINKI 2000).
The studies were approved by the ethical committee of the Karolinska Institute and the Karolinska University Hospital.

3.3.1 In situ Hybridization (I)

In situ hybridization (ISH) using synthetic oligonucleotide probes can sensitively and specifically detect cellular cytokine mRNA expression, which in turn roughly correlates to protein expression of the corresponding cytokines (Olsson, Bakhiet et al. 1993). It allows examination of cytokine mRNA expression among CSF and PB mononuclear cells.
Briefly, mononuclear cells from CSF and PB were isolated using standard methods and dried onto slides. TNF-α, IFN-γ, IL-4 and IL-10 mRNA expressing cells were detected by ISH as previously described (Link, Soderstrom et al. 1994; Andersson M 1997; Khademi, Wallstrom et al. 2000). With this methodology, we have repeatedly demonstrated increased numbers of cells expressing these cytokines in both PB and CSF of patients with MS (Andersson M 1997) (Khademi, Wallstrom et al. 2000). Coded slides were evaluated with light microscopy and cells with more than 14 autoradiographic silver grains were arbitrarily defined as cells expressing a particular cytokine. Data were expressed as numbers of cells positive per 100 000 plated PB and per 10 000 plated CSF mononuclear cells. For each cytokine, 50 000 cells in duplicate were encompassed with ISH when analyzing PB mononuclear cells. For CSF mononuclear cells, the number of cells plated for each cytokine ranged between 2000 and 10 000. The number of cells recovered from each sample restricts the number of cytokines that can be studied on CSF with this methodology. For this reason we gave priority to four cytokines typical for TH1 and TH2 responses. The coded samples were also evaluated repeatedly to validate the readings. There was negligible variation between the readings. The first evaluation is reported here.

3.3.2 Relative quantification of mRNAs with real-time quantitative TaqMan RT-PCR (II).

The real-time quantitative TaqMan method was developed to minimize laboratory bias while evaluating RNA-levels. The procedure was modified for human cytokines from that described for rat (Hammarberg, Lidman et al. 2000)(Muhallab, Lundberg et al. 2002). It is a computerized technique that permits reproducibility between laboratories. It allows quantification of mRNA from cells or tissues. The term real-time refers to the monitoring of the progress of PCR as it occurs, not the endpoint determination used by conventional systems. This method has the advantages of sensitivity, specificity, rapitidy, and reliability (Gibson, Heid et al. 1996; Heid, Stevens et al. 1996). Altogether, until now, TaqMan RT-PCR has been considered the most powerful method for analyzing intracellular mRNA-expression for cytokines in the CSF and peripheral blood specimens used in the present work.

The TaqMan RT-PCR method is described briefly below:

- Cell pellets were lyzed and total RNA was extracted.
- Reverse transcription was performed.
- The result was amplified with a specific sequence-detection system.
- The PCR products were run on an agarose gel and confined to bands of expected size.
- The bands were sequenced to show the homology to the reported sequences for human cytokines IFN-γ, TNF-α, IL-4 and IL-10.
- Probes were labeled with a reporter dye and a quencher dye.
- Relative quantification of mRNA was performed
- As endogenous control 18S rRNA was used.
- As negative control, samples without added cDNA were used.
- The relative amount of mRNA expression was calculated as the ratio between cytokine mRNA and the 18s rRNA.

3.3.3 Muscle Strength dynamometer test (III,IV)

In study III and IV, dynamic dynamometry were used for evaluating muscle strength before and after the IVIG treatment. For more detailed description see the Method sections in papers III and IV.
In study III two standard muscle groups (quadriceps femoris and hamstrings) were selected.
The subject was seated in the dynamometer chair. The big muscle groups in both legs were evaluated with five repetitions of a combined knee extension/flexion movement pattern in a concentric isokinetic mode.

The most affected leg was analyzed. The leg was identified by visual inspection for muscle atrophy and clinical assessment. The two parameters assessed were peak torque (PT) and total work (TW) for the respective muscle group. The values before and after treatment were compared and the percentage difference was calculated for each muscle group. Peak torque were assessed at 60°/s and 120°/s, total work at 120°/s and 180°/s.

In study IV an alternative approach was used. Each individual principal investigator chose the muscle with the best potential of improvement i.e. the one that displayed strength within the limits of 20%-75% of that of an age- and gender-matched normal population. Thus m. quadriceps, m. gastrocnemius, m. tibialis anterior, hamstrings or hand muscles were chosen as the primary endpoint. Submaximal exercise (50 W) was performed on a bicycle ergometer for 5 min prior to the muscle test. The testing of right or left side and the different muscles was done in a random order, with a rest of 2-2.5 min between each set of tests.
Peak isometric strength Newtonmeters (Nm) was measured three times at a 60º-knee angle during extension and flexion in both legs.

To test the isometric measurement of the dorsal and plantar flexors the person tested was placed in sitting position. The ankle was in a 90° position, which was defined as the point at which the sole of the foot was perpendicular to the axis of the leg and to the axis of rotation of the ankle joint. The order of the two tests was randomized with a resting period interval of 2-2.5 min.
Hand muscle grip strength was measured three times using an electronic grip force sensor (GRIPPIT^{TR}). Peak maximum grip force and the mean value of the 10-second sustained grip (measured in Newtonmeters) for right and left hand were recorded.

3.3.4 Walking ability (III,IV)

Walking ability was measured with the six-minute-walk distance test according to Harada et al (Harada, Chiu et al. 1999). The subjects were asked to walk a measured distance (for example 25 meters or more) as many times as possible at an individually-chosen speed for six minutes. Time and distance covered were reported if the subject broke off the test. At the end of the test, total distance covered and the use of any walking aid were reported.

3.3.5 Functional balance (IV)

Functional balance was assessed using the Timed “Up and Go” test (Lin, Hwang et al. 2004). The subject was asked to rise from an armchair, stand still for a moment, walk to a line on the floor three meters away, turn, return, turn around and sit down again. The subject was scored according to the time in seconds required to complete the task. The two treatment groups were compared regarding the change in time score (seconds) needed to complete the test, comparing baseline and the post-treatment visit after start of treatment.

3.3.6 Quality of Life (III, IV)

Quality of Life was assessed with the SF-36 questionnaire. This self-administered generic measure of health status contains 36 questions (Sullivan and Karlsson 1998; Coons, Rao et al. 2000; Meyer-Rosberg, Burckhardt et al. 2001). Eight multi-item scales measure PF (physical functioning), RE (role physical), BP (bodily pain), GH (general health), VT (vitality), SF (social functioning), RE (role emotional) and MH (mental health). SF-36 as a whole was considered as a primary endpoint while the individual sub-scales were considered as secondary endpoints.

3.3.7 Physical activity of the Elderly (PASE) (IV)

Physical activity was assessed using the Physical Activity of the Elderly questionnaire and the change from baseline to the post-treatment visit was calculated. PASE provides an excellent balance between low respondent burden and high validity, covering all occupational, household and leisure activities. It specifically assesses walking relevant for the group studied before and after the study period (Washburn, Smith et al. 1993; Washburn and Ficker 1999; Washburn, McAuley et al. 1999).

The PASE scores correlate with physiologic and performance characteristics such as balance, peak oxygen uptake, resting heart rate and blood pressure and percent body fat (Washburn, McAuley et al. 1999).

3.3.8 Pain (IV)

To assess the pain level the Visual Analogue Scale (VAS) (Huskisson 1974; Joyce, Zutshi et al. 1975; Price, Bush et al. 1994) was used. It consists of a 100-mm scale where 100 mm stands for the worst imaginable pain and zero stands for no pain at all.

3.3.9 Fatigue (IV)

Fatigue was assessed using the Multidimensional Fatigue Inventory (MFI-20).
The MFI-20 is a self-report instrument. Its 20 statements, which cover different aspects of fatigue, are organized in fives scales: general fatigue, physical fatigue, reduced activity, reduced motivation, and mental fatigue (Smets, Garssen et al. 1995; Smets, Garssen et al. 1996).

3.3.10 Statistical analysis (I-IV)

The statistical analysis used are described in the papers I-IV. For a summary see Table 4.

Table 4. Statistical methods used in the four studies.

	Inter-group comparisons	Intra-group comparisons	Software	
Study I	Mann-Whitney U-test		JMP3.2: (SAS Institute, Cary, NC)	Corrected for multiple comparisons with the Bonferroni method
Study II	Mann-Whitney U-test	Wilcoxon signed rank test	JMP3.2: (SAS Institute, Cary, NC)	Corrected for multiple comparisons with the Bonferroni method
Study III		Paired t-test (muscle tests) Friedman non-parametric test, Wilcoxon signed rank test (SF-36)	SPSS for Windows, Rel. 11.5.1 2002. Chicago: SPSS Inc.	
Study IV	Mann-Whitney U-test	Wilcoxon signed rank test	SPSS for Windows, Rel. 11.5.1 2002. Chicago: SPSS Inc.	

4. Results and Discussion

4.1 Paper I

The 13 PPS patients included in the study had significantly elevated levels of mononuclear cells (MNC) with mRNA expression of cytokines in CSF. Elevation was seen for all four cytokines analyzed, when compared to the control group with patients with other neurological diseases (OND) (Fig 2.). The levels of mRNA for TH-1 and TH-2 cytokines were in the same range as those in the positive control group of MS patients. Compared to the MS patients the PPS showed a tendency towards more cells expressing IL-4 and fewer expressing IL-10.

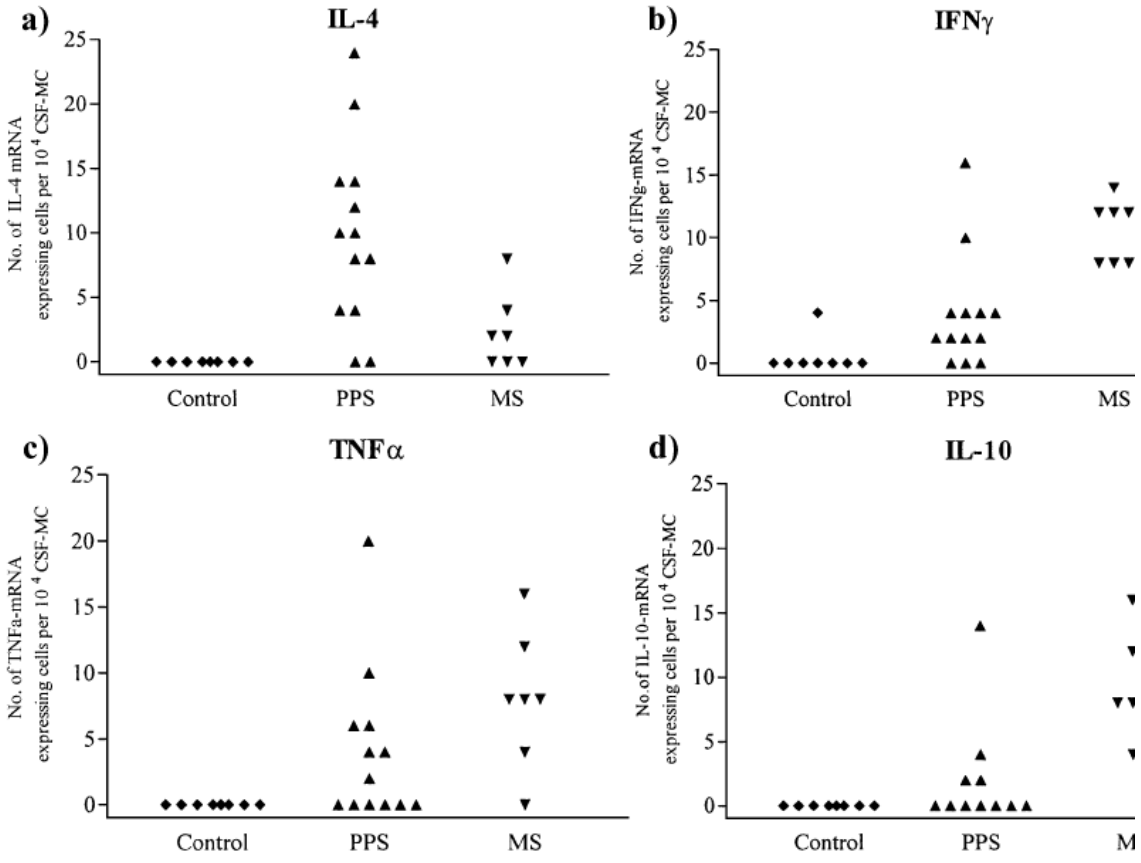

Fig 2. Cellular cytokine mRNA expression among CSF MNCs.

The increase in cytokine expression might have different explanations. The most likely interpretation is that the PPS patients had an inflammation located in the CNS. Further support for an intrathecal inflammatory process is that mononuclear cells in the CSF also were elevated fivefold (unpublished data) compared to the OND patients.
In the light of earlier published data (Dalakas 1995) (Miller 1995), the locus for this inflammation might be the spinal cord parenchyma.
The increased expression of cytokines was found only in the CNS and not in PB, in contrast to the findings in the MS patients. The difference between PPS and MS patients may be a difference in blood brain barrier (BBB). In contrast to the MS patients, there was no BBB damage in PPS patients (unpublished data). Thus, the inflammatory process found in PPS patients was only located intrathecally.
The profiles of cytokine expression in PPS and MS patients differed. IL-4 was more and IL-10 less expressed in the MNC´s in CSF from the PPS patients. This indicates that the neuroinflammation in PPS is specific and clearly separate in comparison with the neuroinflammation present in MS.

4.2 Paper II

In this second material of 16 PPS patients the finding of increased intrathecal cytokine expression was verified using a different technique.
In all the PPS patients studied, a significantly elevated mRNA expression for the TH-2 cytokine IL-10, and also for the TH-1 cytokines TNF-α and INF-γ, was found compared with those from the 26 OND patients.
However, the increase in IL-4 found in study I could not be reproduced.
Further, in PB there was also a significant increase of TNF-α in the PPS patients compared to OND patients, a difference from study I.
The differences between the two studies may be due to the low number of patients included. However, the most likely explanation is differences in the laboratory techniques**.**
In situ hybridization enables the possibility to find few but active cells in contrast to RT Taqman PCR that enables findings of a small amount of mRNA in a homogenate. Another possibility is that IL-4 mRNA is more sensitive for the preparation-technique when preparing for PCR (Olsson,T. personal communication, 2005).
The second purpose of study II was to evaluate the effect of immunomodulation by means of IVIG.
After an infusion of 90g IVIG, the expression of TNF-α and IFN-γ was significantly decreased, in some patients to an undetectable level (Figure 2).
The expression of IL-10 was not significantly altered after the infusion.
It was thus shown that it was possible to downmodulate the inflammatory process in the PPS patients.
In view of the potentially detrimental effects of proinflammatory cytokines on the nervous system, as further discussed in chapter 5.1.1, it is assumed that the IVIG treatment effects are beneficial to PPS patients**.**

4.3 Paper III

In the 14 PPS patients assessed in study III a statistically significant improvement in quality of life after two and six months after the infusion of IVIG was demonstrated as is seen in Table 5. The most pronounced improvement was for the subscale vitality.

Table 5. SF-36, data before treatment, two and six months after treatment with IVIG (Study III).

		Mean	**95% CI**	**p***	**p****
Physical Functioning	Before	33.5	21.0 – 45.9	0.015	0.007
	2 months	40.0	26.3 – 53.7		
	6 months	43.5	29.3 – 57.6		
Role-Physical	Before	55.8	33.4 – 78.1	0.018	0.011
	2 months	69.2	45.2 – 93.2		
	6 months	82.7	63.8 – 101.6		
Bodily Pain	Before	56.1	37.0 – 75.1	0.006	0.004
	2 months	74.7	64.9 – 84.5		
	6 months	72.4	57.9 – 86.8		
General Health	Before	51.1	39.0 – 63.1	0.013	0.039
	2 months	67.5	56.0 – 79.0		
	6 months	62.0	53.1 – 70.9		
Vitality	Before	30.4	18.5 – 42.2	0.002	0.001
	2 months	69.6	59.2 – 80.0		
	6 months	61.9	49.3 – 74.5		
Social Functioning	Before	67.3	50.3 – 84.3	0.001	0.005
	2 months	87.5	78.2 – 96.8		
	6 months	91.3	83.6 – 99.1		
Role-Emotional	Before	82.1	62.6 – 101.5	0.105	0.109
	2 months	92.3	75.5 – 109.1		
	6 months	97.4	91.8 – 103.0		
Mental Health	Before	70.5	61.4 – 79.5	0.001	0.004
	2 months	87.4	82.2 – 92.6		
	6 months	82.2	72.8 – 91.5		

*Before, 2 months and 6 months
**Before and 6 months

The assessment of muscle strength revealed a slight increase in both quadriceps and hamstrings muscles. However, there was no statistically significant difference between the pre and post-treatment values.
In the six-minute-walk distance test, an increase in distance was found although this was not statistically significant. The result of this study indicated that treatment with IVIG is beneficial for quality of life in the short-term (6 months). The other measurements of motor function and walking ability were not statistically altered after the treatment, although there were trends towards an increase of both muscle strength and walking distance. The lack of significance is most likely due to the limited number of patients in the study.
Thus, studies including a higher number of patients, and placebo controls and a longer follow-up time, are needed to evaluate the changes after IVIG treatment.

The secondary objective was to consider any detrimental effect of IVIG in PPS. No serious adverse effects were noted during the study. From the results of this study it is concluded that IVIG is safe and further trials with IVIG in PPS are feasible.

4.4 Paper IV

This Randomized Clinical Trial (RCT) was performed in order to evaluate the clinical effects of IVIG treatment in 142 PPS patients.
Muscle strength improved by 4.3 % in the IVIG-treated group while a decrease of 5.8 % was found in the placebo group ($p = 0.029$).
Quality of Life according to the SF-36 showed statistically significant improvements in the subscales vitality ($p=0,042$) and general health ($p=0,048$).
Physical activity level assessed with the PASE questionnaire showed a significant improvement in the treated group compared to placebo ($p=0.018$), while MFI-20 scores did not change significantly in either group.
The IVIG-treated group displayed a decrease in pain, visualized as a decrease on the VAS of 2.5 ± 20.0 mm, while the placebo group displayed an increase of 3.5 ± 22.2 mm. This difference between the groups was not statistically significant. When studying a sub-population of patients displaying 20.0 mm or more on the VAS at baseline, patients receiving IVIG had a statistically significant ($p=0.037$) pain reduction compared to the placebo-treated group.

These data provide evidence for an increase of muscle strength and activity levels in persons with PPS treated with IVIG. An effect on Quality of Life measures was found as well.
The degree of decline in motor-function noted for the placebo-treated group, during 6 months, was greater than in previous descriptions of the natural course in untreated persons with PPS. Earlier long-term studies have reported the decline to vary between 1%-2.5%/year (Agre, Grimby et al. 1995; Grimby, Stalberg et al. 1998).
It is suggested that this is due to the selection of patients for study. First, our patients had truly progressive PPS. Secondly, all the investigators

selected the muscle that was judged clinically as the one with the best potential of improvement, most often the quadriceps muscle group. This is also the muscle group earlier shown to have the fastest deterioration among ambulatory patients (Agre, Grimby et al. 1995; Sandberg and Stalberg 2004).

It is not excluded that effects on pain and vitality might lead to better performance in muscle strength tests. However, there was no correlation between changes in pain and the performance in the muscle strength test. This points to a direct effect of IVIG on motor function.

The improvement in several sub-scales of SF-36 in the IVIG group indicates increased Quality of Life during treatment. The most prominent improvement was on the sub-scales vitality and general health, for which there were significant differences also when compared with the placebo-treated group.
No serious adverse advents were reported; thus it is concluded that IVIG is safe and has beneficial effects in the PPS.

5. General discussion and future directions

5.1.1 Summary of results

The main findings of the study were that:

A. PPS patients have MNCs with mRNA expression of both TH-1 and TH-2 cytokines, mainly in the CNS.
B. the elevated MNC levels with expression of cytokines were down-modulated with IVIG.
C. A clinical effect with increase in muscle strength, physical activity and improved Quality of Life, mainly for general health and vitality, as well as a decrease of pain, followed the down-modulation of the cytokine expression. There were only modest side-effects.

5.1.2 Neuro-inflammation in PPS

An increase in MNCs with mRNA expression of cytokines in CSF was found with In situ hybridization, indicating an inflammatory reaction in PPS patients. The expression of mRNA in MNCs in CSF is in the same range as in MS. Applying TaqMan RT-PCR, these signs of intrathecal inflammation were reproduced. In addition an elevated expression of TNF-α was found in PB with the TaqMan RT-PCR.
In other inflammatory diseases of the nervous system it is believed that CSF inflammatory cells originate from parenchymatous lesions**.** Detectable MNCs are believed to be located close to the CSF. In PPS there are autopsy reports of inflammatory lesions within the spinal cord (Pezeshkpour and Dalakas 1987; Pezeshkpour and Dalakas 1988; Dalakas 1995; Miller 1995).
Thus, the findings of CSF intrathecal cellular cytokine mRNA expression in PPS patients would be consistent with a leakage of activated leukocytes from an inflammatory process primarily located in the spinal cord parenchyma. Intra-parenchymatous cytokine production may conceivably be richer than can be judged from the CSF cell data.

There are several hypothetical reasons for the persistent cytokine production in the CNS of PPS patients:

- an immune response against a persistent, perhaps mutated, polio virus infection
- defective down-regulation of the original antiviral response
- a polio-virus-induced autoimmune response directed against unknown neuronal, or non-neuronal, autoantigens
- an immune response secondary to neurodegeneration.

Presently, it is not possible to distinguish between the hypotheses for the increased cytokine expression. The most explored hypothesis is the first, i.e. a persistent polio virus infection. While polio virus itself mostly gives a lytic monophasic infection, several other non-retroviral RNA viruses can persist in their hosts. Depending on cell-type and viral strain, long-term persistent

infections can be obtained experimentally both *in vitro* and *in vivo* (Colbere-Garapin, Duncan et al. 1998). Further, in humans with PPS, some reports give both serological and PCR-based evidence for a persistent polio virus infection (Dalakas 1995) (Julien, Leparc-Goffart et al. 1999). However, in several other studies no persistent polio virus infection has been demonstrated (Melchers, de Visser et al. 1992; Roivainen, Kinnunen et al. 1994; Jubelt, Salazar-Grueso et al. 1995).
A Swedish study of 37 PPS patients, in collaboration with the Swedish Institute for Infectious Disease Control (SMI), detected no signs of a persistent polio virus infection (Gonzalez et al 2005, unpublished data). Thus, there are conflicting results concerning the existence of a persistent polio-virus infection in PPS patients. Our recent study (Gonzalez et al 2005, unpublished data) indicates that a persistent polio-virus infection seems unlikely. However, further data are needed.

5.1.3 Neurotoxicity of cytokines

Cytokine production is a reflection of the immunologically active cells and a powerful tool to maneuver threats of all kinds. Cytokines trigger the immune system when needed to defend the host from infections. They modulate the system to be active at an accurate level when a trauma occurs. If expressed and produced inaccurately they may harm the host's own cells. Several cytokines have been transgenically overexpressed in the CNS of mice, resulting in neurodegeneration after several months in many cases (Campbell 1995).

In the PPS patients there was a global increase of several cytokines, and thus all could be involved in the neurodegenerative process. Further, considerable neuronal death and axonal damage was recently described in CNS inflammation due to an immune response against a non-neuronal target, e.g. myelin basic- protein-induced experimental autoimmune encephalomyelitis (Smith, Groom et al. 2000). For IFN-γ and TNF-α, a series of possible mechanisms may harm motorneurons.
Thus, based on the findings of the present studies and others, it is hypothesized that intrathecal cytokine production contributes to the clinical manifestations of PPS. The data are consistent with a detrimental role of cytokine production and inflammation in causing late and slow neurodegeneration in PPS. IFN-γ activates microglia and macrophages, in turn involving a series of noxious effector mechanisms such as nitric oxide products/peroxynitrite, protease production and glutamate production (Smith, Groom et al. 2000). The same holds true for TNF-α (Sun, Newman et al. 2004). In PPS the cytokine increase including IFN-γ and TNF-α might play an important role in a neurodegenerative process.
The cause of the ongoing motor neuron deterioration in PPS patients is not fully understood. However, it is clear that most, if not all, the post-polio stigmata are reflections of a deterioration in the neuromuscular unit. The other symptoms, such as pain and fatigue, are often secondary to the combination of deterioration of the neuromuscular unit and a normal degenerative process. Thus, pain of musculoskeletal origin may be due to excessive strain on the joints and tendons and physical fatigue might be

caused by large motor units with under-dimensioned capillary and metabolic capability (Einarsson, Grimby et al. 1990; Borg and Henriksson 1991).

5.1.4 Immunomodulation in PPS

Immunomodulation in PPS patients is not a new issue. The Salk polio vaccine using the sublingual route in eighty post-polio patients, resulted in clinically marked reduction in muscular pain, headache and/or fatigue (Bailey 1985). High-dose prednisone treatment has been tried in a small number of PPS patients (Dinsmore, Dambrosia et al. 1995). The trial showed a slight increase in muscle strength at the three months interval, in a double-blinded randomized study. The improvement had disappeared by the six-month follow-up when the prednisone was discontinued (Dinsmore, Dambrosia et al. 1995). Alpha 2-interferon has been given with no effect (Dalakas, Aksamit et al. 1986).

The use of IVIG as an immune-modulating agent seemed reasonable since IVIG is a well-known agent with documented effect in neuroinflammatory disorders and has relatively few side effects. IVIG reduces both TH-1 and TH-2 lymphokines, as well as monokines (Andersson, Skansen-Saphir et al. 1996). Furthermore, IVIG is effective in moderating inflammatory reactions in Guillain Barre Syndrome (Hughes 1997).
It is concluded that IVIG dampens the inflammatory reaction in PPS patients as well. The mechanisms for the reduction of cytokines in neuroinflammatory disorders, as well as in PPS, are unknown. There is, however, one big difference between PPS and other neuroinflammatory disorders: the PPS patients had an acute infection caused by a known agent i.e. poliovirus.
Thus it is necessary first to consider a direct effect of pooled antibodies directed to a chronic poliovirus infection or defective downregulation of the original viral response.
As discussed earlier, a persistent virus infection seems unlikely and therefore the possible option for the IVIG effect is non-specific, i.e. the same possible general targets as discussed earlier (chapter 1.7).

5.1.5 Clinical effects of immunomodulation in PPS

Assessment of the clinical effect of IVIG treatment showed that the PPS patients had increased muscle strength and physical activity as well as increased Quality of Life scores, mainly for vitality and general health. This indicates that IVIG has effects predominantly on the motor functions. However, there are other symptoms that are not primarily considered to be connected to the motor functions. An effect on pain was also found. One could speculate that pain decreases as the patients become more active. Thus an effect on pain could be secondary to the increased activity. One has however to consider the opposite i.e. the decreased pain may lead to increased activity.
Mental fatigue is a common complaint in PPS patients. One would imagine that SF-36 subscale vitality reflects mental fatigue. However the questions in SF-36 could be interpreted as questions directed to assess physical fatigue.

Furthermore, in a study by Östlund and collaborators (2005, unpublished data), comparing data from SF-36 and MFI-20 showed that mental fatigue does not significantly contribute to vitality. This means that vitality in PPS patients is within the physical domain. Thus, this points to that the most prominent effects of IVIG are on the motor functions.

5.2 Future directions

Of great interest is to evaluate whether the inflammatory process is chronic: does it start at the acute infection or at the onset of PPS?
Another important issue is to further analyze the background of the inflammation. Is it due to a chronic virus infection, to neurodegeneration or to an unknown factor? In this context it is of special interest to consider immune-genetic factors that may contribute to making the individual susceptible to poliomyelitis in the first place and later on to develop PPS.

To study the duration of the IVIG effects the patients that were treated should be followed clinically, and CSF cytokine levels should be determined. From the present data it is obvious that the duration exceeds six months. Unpublished data (Gonzalez et al 2005) from a one-year follow up in a smaller subgroup of PPS patients (n=41) show that the effect may exceed one year.
Data are needed for analyzing the correlation between clinical effects and cytokine levels. In addition, other cytokines not analyzed in the present study should be evaluated as possible generators of PPS. Optimal would be to find a cytokine correlated with the PPS symptoms appearing in the PB, making it easy to assess patients and the effects of agents with immune-modulation.

Of importance is to determine the optimal dose of IVIG as well as to evaluate the effects of a combination of IVIG and conventional physiotherapy. When more is known about the etiology and pathophysiology, more selective immune-modifying agents may be considered. These may include interference with mechanisms such as the homing of inflammatory cells to the CNS, and local cytokine production; i.e. agents that become available in other neuroinflammatory diseases.

6. Conclusions

The post-polio syndrome is characterized by new muscle weakness, pain and fatigue developing decades after the acute poliomyelitis. Different etiologies for PPS have been discussed.

The focus of the present work was the immunological aspects. An elevated expression of cytokines in CSF mononuclear cells was found, indicating an inflammatory reaction in CNS of PPS patients. The inflammation was downregulated by means of IVIG. With the downregulation, an increase in motor function e.g. muscle strength, and a higher activity level led to a better Quality of Life especially for vitality, and less pain.

The mechanism for the IVIG remains unknown. However, irrespective of cytokine production, the present findings will be of importance for further development of immune-mediated therapies in PPS.

7. Acknowledgements

I take this opportunity to give special thanks to some special people, without whom I would not have been able to succeed with my work. Most important, I would not be the person I am today!

The patients. First of all I would like to thank all patients that continuously have had a great patience with me and the research team. You have inspired me more than any other.

To my principal supervisor Kristian Borg, thanks! You led me into this field of medicine. You have always been encouraging and reachable at all times. You really give the kind of support that makes people grow! I do appreciate you as supervisor and true friend combined.

My second supervisor Magnus Andersson: thanks for all your support and friendship. I appreciate that you have encouraged me in good times and bad.

Tomas Olsson: even though you never have been my “official” supervisor, where would we be now without you? You’ve a big heart and extraordinary skills. Your enthusiasm is infectious.

Marie-Louise Östensson, a great hug to you! Even though you never really believed in my thesis, you have without exception shown that you trusted me. You’ve given me the practical opportunity to complete this work.

Seija Lund, you have been a very important research partner with your talent for performing projects with accuracy.

The big talent Mohsen Khademi who has performed the laboratory work with patience and skill. You have taught me a lot about neuro-immunology. Always positive and alert.

Lisbet Broman, the warmest and deepest thanks for all of the work that you have performed during these years. You are a wonderful person.

Berit! You have followed me from the first weeks while I worked as a physician at the Neurological Clinic. You have always been supportive and our discussions about life has always been fruitful. Now you also have checked my thesis with enthusiasm. I like you!

Georgios, my friend and research colleague. I whish you as great or better ideas and findings in the field of post-polio than I have encountered. You have a great courage.

Erik Wallström, Fredrik Piehl for excellent support with ideas, statistical advice and friendship. I suppose that both of you will achieve amazing scientific results in the duty of mankind…..

Professor Katharina Stibrant-Sunnerhagen. I want to thank you for your always good advice in clinical research methods and your positive thinking regarding our mutual work with the randomized trial.

Inger Sjöberg, as one of the Principal Investigators, you were important for the planning and carrying out the randomized study. Always smiling!

Professor Jörgen Borg, as a scientific and clinical teacher, you have given me inspiration and time for this research.

Britta Eklund, Tina Schüldt and Jan Ekholm. As an important part of the institution you all have given me support. Thanks for living the place up!

Mikael Bender, the true enthusiast. If you as the representative for Pharmalink AB had not trusted in my ideas, this whole project would not have been able to accomplish. I also value your constructive ideas and pioneer-ship.

Grifols, the supplier for the immunoglobulin. The company with Cunyat Zapater Jordi as my contact person, has unhesitatingly supplied the product.

To Tim Crosfield for checking the English.

All colleagues at the department of Rehabilitation Medicine, Danderyd University Hospital, as well as at the department of Neurology, Karolinska University Hospital, for understanding the fact that research takes time and giving me support in my clinical work.

Anita Sundelöf, A great thank for the never ending fruitful assistance and support.

Sven-Erik (the promising surgeon), our recurring weeks along the Scandinavian rivers in a never ending hunt for the big salmons. Although the result is not as good as we have wanted, I suppose that it is nice to have more goals to achieve in life. Even after this project. Thank you for your friendship.

And a great thank to all of you not mentioned, but indeed have a huge place in my heart. I'm sure that you know that I think of you with respect and love.

My late mother, who was unable to wait for the day when my thesis was to be presented. I love you.

Finally, but most importantly, my daughter Annalena. You give me strength to go further.

8. References

Agre, J. C., G. Grimby, et al. (1995). "A comparison of symptoms between Swedish and American post-polio individuals and assessment of lower limb strength--a four-year cohort study." Scand J Rehabil Med **27**(3): 183-92.

Andersson, J., U. Skansen-Saphir, et al. (1996). "Intravenous immune globulin affects cytokine production in T lymphocytes and monocytes/macrophages." Clin Exp Immunol **104 Suppl 1**: 10-20.

Andersson M, K. M., Wallstrom E, Olsson T. (1997). "Cytokine profile in interferon-b treated multiple sclerosis patients: reduction of interleukin-

10 mRNA expressing cells in peripheral blood." Eur J Neurol(4): 567- 71.

Axelsson, P. (2004). "The Autumn Ghost; The history of Polioepidemics in Sweden." Doctoral thesis.

Bach, J. R., A. S. Alba, et al. (1987). "Glossopharyngeal breathing and noninvasive aids in the management of post-polio respiratory insufficiency." Birth Defects Orig Artic Ser **23**(4): 99-113.

Bach, J. R., A. S. Alba, et al. (1989). "Management alternatives for post-polio respiratory insufficiency. Assisted ventilation by nasal or oral-nasal interface." Am J Phys Med Rehabil **68**(6): 264-71.

Bailey, A. (1985). "Post-Polio Pain: Treatment by Sublingual Immunotherapy." Symposia Foundation, Miami, Florida: 181-184.

Borg, K. (1996). "Post-polio muscle dysfunction 29th ENMC workshop 14-16 October 1994, Naarden, the Netherlands." Neuromuscul Disord **6**(1): 75-80.

Borg, K. and J. Henriksson (1991). "Prior poliomyelitis-reduced capillary supply and metabolic enzyme content in hypertrophic slow-twitch (type I) muscle fibres." J Neurol Neurosurg Psychiatry **54**(3): 236-40.

Calvez, V., I. Pelletier, et al. (1995). "Persistent poliovirus infection: development of new models with cell lines." Ann N Y Acad Sci **753**: 370-3.

Campbell, I. L. (1995). "Neuropathogenic actions of cytokines assessed in transgenic mice." Int J Dev Neurosci **13**(3-4): 275-84.

Cashman, N. R., R. Maselli, et al. (1987). "Late denervation in patients with antecedent paralytic poliomyelitis." N Engl J Med **317**(1): 7-12.

Cashman, N. R., R. Maselli, et al. (1987). "Post-poliomyelitis syndrome: evidence of ongoing denervation in symptomatic and asymptomatic patients." Birth Defects Orig Artic Ser **23**(4): 237-9.

Clavelou, P. (2004). "[Post-polyiomyelitis syndrome]." Rev Neurol (Paris) **160**(2): 229-33.

Colbere-Garapin, F., G. Duncan, et al. (1998). "An approach to understanding the mechanisms of poliovirus persistence in infected cells of neural or non-neural origin." Clin Diagn Virol **9**(2-3): 107-13.

Coons, S. J., S. Rao, et al. (2000). "A comparative review of generic quality-of-life instruments." Pharmacoeconomics **17**(1): 13-35.

Couderc, T., S. Girard, et al. (2001). "Inhibition of poliovirus RNA synthesis as a molecular mechanism contributing to viral persistence in the mouse central nervous system." Dev Biol (Basel) **105**: 225-30.

Dalakas, M. C. (1995). "Pathogenetic mechanisms of post-polio syndrome: morphological, electrophysiological, virological, and immunological correlations." Ann N Y Acad Sci **753**: 167-85.

Dalakas, M. C. (1995). "The post-polio syndrome as an evolved clinical entity. Definition and clinical description." Ann N Y Acad Sci **753**: 68-80.

Dalakas, M. C. (2002). "Pro-inflammatory cytokines and motor neuron dysfunction: is there a connection in post-polio syndrome?" J Neurol Sci **205**(1): 5-8.

Dalakas, M. C., A. J. Aksamit, et al. (1986). "Administration of recombinant human leukocyte alpha 2-interferon in patients with amyotrophic lateral sclerosis." Arch Neurol **43**(9): 933-5.

Dalakas, M. C., G. Elder, et al. (1986). "A long-term follow-up study of patients with post-poliomyelitis neuromuscular symptoms." N Engl J Med **314**(15): 959-63.

Dinsmore, S., J. Dambrosia, et al. (1995). "A double-blind, placebo-controlled trial of high-dose prednisone for the treatment of post-poliomyelitis syndrome." Ann N Y Acad Sci **753**: 303-13.

Dunn, M. G. (1991). "Post-polio fatigue treated with amantadine." Arch Neurol **48**(6): 570.

Einarsson, G., G. Grimby, et al. (1990). "Electromyographic and morphological functional compensation in late poliomyelitis." Muscle Nerve **13**(2): 165-71.

Farbu, E., T. Rekand, et al. (2003). "Post-polio syndrome and total health status in a prospective hospital study." Eur J Neurol **10**(4): 407-13.

Farbu, E., T. Rekand, et al. (2003). "GM1 antibodies in post-polio syndrome and previous paralytic polio." J Neuroimmunol **139**(1-2): 141-4.

Feldman, R. M. and C. L. Soskolne (1987). "The use of nonfatiguing strengthening exercises in post-polio syndrome." Birth Defects Orig Artic Ser **23**(4): 335-41.

Fischer, D. A. (1985). "Poliomyelitis: late respiratory complications and management." Orthopedics **8**(7): 891-4.

Fischer, D. A. (1987). "Sleep-disordered breathing as a late effect of poliomyelitis." Birth Defects Orig Artic Ser **23**(4): 115-20.

Gibson, U. E., C. A. Heid, et al. (1996). "A novel method for real time quantitative RT-PCR." Genome Res **6**(10): 995-1001.
Ginsberg, A. H., M. J. Gale, Jr., et al. (1989). "T-cell alterations in late postpoliomyelitis." Arch Neurol **46**(5): 497-501.
Girard, S., A. S. Gosselin, et al. (2002). "Restriction of poliovirus RNA replication in persistently infected nerve cells." J Gen Virol **83**(Pt 5): 1087-93.
Grimby, G., E. Stalberg, et al. (1998). "An 8-year longitudinal study of muscle strength, muscle fiber size, and dynamic electromyogram in individuals with late polio." Muscle Nerve **21**(11): 1428-37.
Grimby, L., A. Tollback, et al. (1996). "Fatigue of chronically overused motor units in prior polio patients." Muscle Nerve **19**(6): 728-37.
Halstead, L. S. (1998). "Post-polio syndrome." Sci Am **278**(4): 42-7.
Halstead, L. S. and C. D. Rossi (1985). "New problems in old polio patients: results of a survey of 539 polio survivors." Orthopedics **8**(7): 845-50.
Halstead, L. S. and C. D. Rossi (1987). "Post-polio syndrome: clinical experience with 132 consecutive outpatients." Birth Defects Orig Artic Ser **23**(4): 13-26.
Halstead, L. S., D. O. Wiechers, et al. (1985). "Results of a survey of 201 polio survivors." South Med J **78**(11): 1281-7.
Hammarberg, H., O. Lidman, et al. (2000). "Neuroprotection by encephalomyelitis: rescue of mechanically injured neurons and neurotrophin production by CNS-infiltrating T and natural killer cells." J Neurosci **20**(14): 5283-91.
Harada, N. D., V. Chiu, et al. (1999). "Mobility-related function in older adults: assessment with a 6-minute walk test." Arch Phys Med Rehabil **80**(7): 837-41.
Heid, C. A., J. Stevens, et al. (1996). "Real time quantitative PCR." Genome Res **6**(10): 986-94.
HELSINKI, W. M. A. D. O. (2000). "Ethical Principles for Medical Research Involving Human Subjects."
Hill, R., A. W. Robbins, et al. (1983). "Sleep apnea syndrome after poliomyelitis." Am Rev Respir Dis **127**(1): 129-31.
Howard, R. S. (2005). "Poliomyelitis and the postpolio syndrome." Bmj **330**(7503): 1314-8.
Hughes, R. A. (1997). "Plasma exchange versus intravenous immunoglobulin for Guillain-Barre syndrome." Ther Apher **1**(2): 129-30.
Hunt, R. (2005). "Virology-chapter ten." Microbiology and Immunology On-line **University of South Carolina**.
Huskisson, E. C. (1974). "Measurement of pain." Lancet **2**(7889): 1127-31.
Johnson, R. T. (1995). "Pathogenesis of poliovirus infections." Ann N Y Acad Sci **753**: 361-5.
Joyce, C. R., D. W. Zutshi, et al. (1975). "Comparison of fixed interval and visual analogue scales for rating chronic pain." Eur J Clin Pharmacol **8**(6): 415-20.
Jubelt, B. and N. R. Cashman (1987). "Neurological manifestations of the post-polio syndrome." Crit Rev Neurobiol **3**(3): 199-220.
Jubelt, B., E. F. Salazar-Grueso, et al. (1995). "Antibody titer to the poliovirus in blood and cerebrospinal fluid of patients with post-polio syndrome." Ann N Y Acad Sci **753**: 201-7.
Julien, J., I. Leparc-Goffart, et al. (1999). "Postpolio syndrome: poliovirus persistence is involved in the pathogenesis." J Neurol **246**(6): 472-6.
Khademi, M., E. Wallstrom, et al. (2000). "Reduction of both pro- and anti-inflammatory cytokines after 6 months of interferon beta-1a treatment of multiple sclerosis." J Neuroimmunol **103**(2): 202-10.
Labadie, K., I. Pelletier, et al. (2004). "Poliovirus mutants excreted by a chronically infected hypogammaglobulinemic patient establish persistent infections in human intestinal cells." Virology **318**(1): 66-78.
Lin, M. R., H. F. Hwang, et al. (2004). "Psychometric comparisons of the timed up and go, one-leg stand, functional reach, and Tinetti balance measures in community-dwelling older people." J Am Geriatr Soc **52**(8): 1343-8.
Link, J., M. Soderstrom, et al. (1994). "Increased transforming growth factor-beta, interleukin-4, and interferon-gamma in multiple sclerosis." Ann Neurol **36**(3): 379-86.
McComas, A. J., C. Quartly, et al. (1997). "Early and late losses of motor units after poliomyelitis." Brain **120 (Pt 8)**: 1415-21.
Melchers, W., M. de Visser, et al. (1992). "The postpolio syndrome: no evidence for poliovirus persistence." Ann Neurol **32**(6): 728-32.
Meyer-Rosberg, K., C. S. Burckhardt, et al. (2001). "A comparison of the SF-36 and Nottingham Health Profile in patients with chronic neuropathic pain." Eur J Pain **5**(4): 391-403.
Miller, D. C. (1995). "Post-polio syndrome spinal cord pathology. Case report with immunopathology." Ann N Y Acad Sci **753**: 186-93.

Muhallab, S., C. Lundberg, et al. (2002). "Differential expression of neurotrophic factors and inflammatory cytokines by myelin basic protein-specific and other recruited T cells infiltrating the central nervous system during experimental autoimmune encephalomyelitis." Scand J Immunol **55**(3): 264-73.
Mulder, D. W. (1995). "Clinical observations on acute poliomyelitis." Ann N Y Acad Sci **753**: 1-10.
Munsat, T. L. (1991). "Poliomyelitis--new problems with an old disease." N Engl J Med **324**(17): 1206-7.
Olsson, T. (1995). "Cytokine-producing cells in experimental autoimmune encephalomyelitis and multiple sclerosis." Neurology **45**(6 Suppl 6): S11-5.
Olsson, T., M. Bakhiet, et al. (1993). "CD8 is critically involved in lymphocyte activation by a T. brucei brucei-released molecule." Cell **72**(5): 715-27.
Pelletier, I., G. Duncan, et al. (1998). "Molecular mechanisms of poliovirus persistence: key role of capsid determinants during the establishment phase." Cell Mol Life Sci **54**(12): 1385-402.
Pezeshkpour, G. H. and M. C. Dalakas (1987). "Pathology of spinal cord in post-poliomyelitis muscular atrophy." Birth Defects Orig Artic Ser **23**(4): 229-36.
Pezeshkpour, G. H. and M. C. Dalakas (1988). "Long-term changes in the spinal cords of patients with old poliomyelitis. Signs of continuous disease activity." Arch Neurol **45**(5): 505-8.
Price, D. D., F. M. Bush, et al. (1994). "A comparison of pain measurement characteristics of mechanical visual analogue and simple numerical rating scales." Pain **56**(2): 217-26.
Ravits, J., M. Hallett, et al. (1990). "Clinical and electromyographic studies of postpoliomyelitis muscular atrophy." Muscle Nerve **13**(8): 667-74.
Raymond, M. (1875). "Paralysie essentielle de lénfance, atrophie musculaire consecutive." C.R.Soc.Biol. **27**: 158.
Rekand, T., N. Langeland, et al. (2002). "Fcgamma receptor IIIA polymorphism as a risk factor for acute poliomyelitis." J Infect Dis **186**(12): 1840-3.
Roivainen, M., E. Kinnunen, et al. (1994). "Twenty-one patients with strictly defined postpoliomyelitis syndrome: no poliovirus-specific IgM antibodies in the cerebrospinal fluid." Ann Neurol **36**(1): 115-6.
Salazar-Grueso, E. F., L. M. Grimaldi, et al. (1989). "Isoelectric focusing studies of serum and cerebrospinal fluid in patients with antecedent poliomyelitis." Ann Neurol **26**(6): 709-13.
Sandberg, A. and E. Stalberg (2004). "Changes in macro electromyography over time in patients with a history of polio: a comparison of 2 muscles." Arch Phys Med Rehabil **85**(7): 1174-82.
Schwick, H. G. (1991). "[Poliomyelitis in Landsteiner's time and today]." Wien Klin Wochenschr **103**(5): 136-40.
Sharief, M. K., R. Hentges, et al. (1991). "Intrathecal immune response in patients with the post-polio syndrome." N Engl J Med **325**(11): 749-55.
Smets, E. M., B. Garssen, et al. (1995). "The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue." J Psychosom Res **39**(3): 315-25.
Smets, E. M., B. Garssen, et al. (1996). "Application of the multidimensional fatigue inventory (MFI-20) in cancer patients receiving radiotherapy." Br J Cancer **73**(2): 241-5.
Smith, T., A. Groom, et al. (2000). "Autoimmune encephalomyelitis ameliorated by AMPA antagonists." Nat Med **6**(1): 62-6.
Sonies, B. C. and M. C. Dalakas (1991). "Dysphagia in patients with the post-polio syndrome." N Engl J Med **324**(17): 1162-7.
Sonies, B. C. and M. C. Dalakas (1995). "Progression of oral-motor and swallowing symptoms in the post-polio syndrome." Ann N Y Acad Sci **753**: 87-95.
Sullivan, M. and J. Karlsson (1998). "The Swedish SF-36 Health Survey III. Evaluation of criterion-based validity: results from normative population." J Clin Epidemiol **51**(11): 1105-13.
Sun, D., T. A. Newman, et al. (2004). "Cytokine-induced enhancement of autoimmune inflammation in the brain and spinal cord: implications for multiple sclerosis." Neuropathol Appl Neurobiol **30**(4): 374-84.
Washburn, R. A. and J. L. Ficker (1999). "Physical Activity Scale for the Elderly (PASE): the relationship with activity measured by a portable accelerometer." J Sports Med Phys Fitness **39**(4): 336-40.
Washburn, R. A., E. McAuley, et al. (1999). "The physical activity scale for the elderly (PASE): evidence for validity." J Clin Epidemiol **52**(7): 643-51.
Washburn, R. A., K. W. Smith, et al. (1993). "The Physical Activity Scale for the Elderly (PASE): development and evaluation." J Clin Epidemiol **46**(2): 153-62.
Weller, T. H., F. C. Robbins, et al. (1949). "Cultivation of poliomyelitis virus in cultures of human foreskin and embryonic tissues." Proc Soc Exp Biol Med **72**(1): 153-5.
WHO (2003). "Fact sheet."
Wiechers, D. O. and S. L. Hubbell (1981). "Late changes in the motor unit after acute poliomyelitis." Muscle Nerve **4**(6): 524-8.
Windebank, A. J., J. R. Daube, et al. (1987). "Late sequelae of paralytic poliomyelitis in Olmsted County, Minnesota." Birth Defects Orig Artic Ser **23**(4): 27-38.

Windebank, A. J., W. J. Litchy, et al. (1991). "Late effects of paralytic poliomyelitis in Olmsted County, Minnesota." Neurology **41**(4): 501-7.
Vulpian, A. (1879). "Paralysie atrophique de lénfance, ayant dans son evolution proce´de´ par pousseés succesives. Surcharge adipeuse dans les re´gions envahies par látrophique." Clin.Med.de l´hospital de la charite´. 778.