# INTRAVENOUS IMMUNOGLOBULIN TREATMENT IN CHRONIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY

**A clinical and immunological study**

(De behandeling van patiënten
met een chronisch inflammatoire demyeliniserende
polyneuropathie met intraveneus immunoglobuline.
Klinisch en immunologisch onderzoek)

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Aan mijn ouders,
en aan Frederique

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*"Common sense would seem to indicate it,*
*but common sense is often betrayed by statistics"*
**Michael H Brooke**

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# ABBREVIATIONS

| | |
|---|---|
| Bb | = Borrelia burgdorferi |
| CIDP | = chronic inflammatory demyelinating polyneuropathy |
| CNS | = central nervous system |
| CSF | = cerebrospinal fluid |
| ELISA | = enzyme-linked immunosorbent assay |
| GBS | = Guillain-Barré syndrome |
| HIV | = human immunodeficiency virus |
| HLA | = human leucocyte antigen |
| IDP | = inflammatory demyelinating polyneuropathy |
| IFA | = immunofluorescence assay |
| MAG | = myelin associated glycoprotein |
| MHC | = major histocompatibility complex |
| MHT | = mixed hemagglutination test |
| NBL | = neuroblastoma cell line |
| NBL-IFA | = neuroblastoma-immunofluorescence assay |
| NCV | = nerve conduction velocity |
| PNS | = peripheral nervous system |
| PNT | = peripheral nervous tissue |
| TLC | = thin layer chromatography |

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# GENERAL INTRODUCTION

Patients with a chronic inflammatory demyelinating polyneuropathy (CIDP) may respond to treatment with corticosteroids and to plasmapheresis, which was demonstrated in controlled clinical studies.[77,81]
In an uncontrolled study it was found that 13/17 CIDP patients had a rapid and clinical important improvement after infusion of Fresh Frozen Plasma (FFP). A beneficial response was also seen after intravenous immunoglobulin (IVIg) treatment.[328]

The aims of this study were:
- to evaluate the clinical effectiveness of IVIg in CIDP patients,
- to define clinical and immunological factors associated with improvement after IVIg in CIDP patients and
- to investigate immunological mechanisms involved in the response to IVIg treatment.

History, clinical signs, symptoms, pathology, differential diagnosis and treatment of CIDP are described in chapter 1.
In chapter 2, the results of a double-blind placebo-controlled crossover study on the effectiveness of IVIg treatment in 7 patients with CIDP are shown.
Various clinical and laboratory factors were analyzed in relation to improvement after IVIg in 52 patients fulfilling the criteria of CIDP (Chapter 3).
The pathogenesis of the chronic inflammatory demyelinating polyneuropathy is unknown. There is evidence that immune-mediated mechanisms play a role, which is discussed in Chapter 4.
To investigate a humoral immune-mediated mechanism of action of IVIg, a mixed hemagglutination assay (MHT) was adapted from Vedeler et al.[325] to detect anti-human peripheral nerve tissue antibodies (Chapter 5).
To study the interaction of auto-antibodies with IVIg, an in vitro model was necessary. An immunofluorescent assay (IFA) detecting circulating anti-neuroblastoma (NBL) cell line antibodies was developed. Different NBL cell lines from various species were tested for reactivity with patients's sera. The mouse-rat NBL 108cc15-IFA showed the highest specificity for the detection of antibodies in patients with an inflammatory demyelinating polyneuropathy (Chapter 6).
This NBL 108cc15 cell line showed partial cross-reactivity with human peripheral nerve tissue. Furthermore it was shown that IVIg could inhibit the interaction between serum from a CIDP patient with this cell line. (Chapter 7).

Susceptibility for immune-mediated diseases may be determined by immune response differences in relation to the major histocompatibility (MHC) antigens. Therefore, HLA antigens in 52 patients with CIDP were compared to healthy controls. Furthermore, the HLA antigens and the presence or absence of neural antibodies in these 52 CIDP patients were related to improvement after IVIg treatment (Chapter 8).
The NBL 108cc15-IFA was used to study the interaction between patients' antibodies and IVIg. These experiments showed anti-idiotypic suppression of antibodies against neural tissue by IVIg (Chapter 9).

Chapter 1

# CHRONIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY

## HISTORY

Targowla was the first who clearly described a patient with recurrent neuritis; the report appeared in 1894.[301] Since then several authors presented case reports on patients with a recurrent sensorimotor polyneuropathy.

The first large study was presented by Austin[16] in 1958, who made a careful description of the disease. Austin selected from the literature 30 cases with a recurrent, symmetrical polyneuropathy involving arms and legs and added 2 more cases. In these 32 patients no known cause of polyneuropathy, such as intoxication (lead, arsenic or thallium), diabetes mellitus, porphyria, obliterative vascular disease or malnutrition was found.

According to Austin, a representative example of a recurrent polyneuropathy is a young adult who develops over many weeks or months, the progressive symptoms and signs of a symmetrical, chiefly distal and predominantly motor polyradiculoneuropathy involving arms and legs. Cranial nerves VII, VI, III, IX and X are occasionally involved and sphincters are almost always spared. There is usually no severe pain, infection, or systemic illness. The cerebrospinal fluid (CSF) protein is usually elevated. Electrophysiologic studies demonstrate a mixture of nerve conduction block and partial denervation with severe motor unit loss.

Generally, the patient gradually improves and often recovers completely. After months or years without symptoms, another bout of recurrence may occur. Each recurrence may be somewhat variable in severity, duration, and residual signs.

In the group of 32 patients there was some male preponderance, the average age of onset was 23 years. The peak of disability was slowly reached, on average after five months from onset. The duration of the recovery phase generally took longer than the duration of progression of muscle weakness. The mean number of bouts in these 32 patients was 3, with intervals of 4 years. Four patients had a definite downhill course and died.

Enlarged and firm peripheral nerves were reported in 11 of 17 cases. Nerve enlargement was either due to collagen and reticulin proliferation or to mucoid interstitial tissue. It was suggested

that patients with enlarged nerves because of interstitial edema are the ones particularly prone to respond to corticosteroids.
In Austin's unique study, one patient with a spontaneously recurrent polyradiculoneuropathy had subsequently 20 recurrences depending on corticosteroid treatment during a study period of 5 years. The patient had a consistent, reproducible and predictable pattern of response to ACTH, cortisone and prednisone which was controlled with intramuscular, intravenous and oral placebo treatment.
These observations showed that recurrences will occur if the corticoid dosage is less than what may be considered as the steroid requirement of the patient at that time. To maintain a good and stable clinical condition, this patient needed up to 300 mg cortisone (~60 mg prednisone) per day. It was the impression that during the course of the disease the corticosteroid requirement increases, that later responses to treatment may be less rapid and that no cure could be achieved. Austin concluded that corticosteroids temporarily suppress the clinical and laboratory manifestations of active neuropathy: CSF protein decreases and enlarged nerves return to normal size. A remarkable feature mentioned by Austin was that fasciculations consistently returned during recovery periods.
In 1967, Hinman and Magee[130] and in 1968, Dyck et al.[73] published reports on this disease. In 1969, Thomas et al.[303] reviewed the literature and found 14 acceptable examples of recurrent acute symmetrical polyneuritis and added 2 more cases. They also described identical clinical signs and symptoms in 3 patients with a progressive phase of at least 4-8 weeks and used the name "recurrent and chronic relapsing Guillain-Barré polyneuritis" which suggests that this might be a chronic recurrent form of the Guillain-Barré syndrome (GBS).
A number of authors reported their experience with the disorder under various names: Matthews et al.[196] "relapsing corticosteroid-dependent polyneuritis", De Vivo and Engel[64] "steroid-responsive recurrent polyneuropathy", Dolman and Allen[68] "relapsing hypertrophic neuritis".
In 1975, Dyck et al.[74] reported their own series of 53 patients and introduced the name: "chronic inflammatory polyradiculoneuropathy" (CIP). Typical features of CIP were described which included: muscle weakness (distal slightly more than proximal), distal sensory disturbances (numbness far more frequently than pain), hyporeflexia or areflexia, elevated CSF protein, slowed motor and sensory nerve conduction velocity (NCV) mostly marked in the proximal part of the nerve, and an associated disease must be excluded. They found a pure motor CIP in 9% and a pure sensory CIP in 6% of the cases.
Later, the name varied again: Prineas and McLeod[246] (1976) used the name "chronic relapsing polyneuritis", Oh[226] (1978) named it "subacute demyelinating polyneuropathy responding to corticosteroid treatment" whereas Dalakas and Engel[62] (1981) used "chronic relapsing (dysimmune) polyneuropathy. In 1982, Dyck et al.[77] reported their prednisone trial in patients with a "chronic inflammatory demyelinating polyradiculoneuropathy" (CIDP), since then, CIDP is the most widely used name. In 1987, McCombe et al.[198] reviewed 92 patients and in 1989, Barohn et al.[23] reviewed 60 patients with a CIDP. Barohn et al. concluded that CIDP is still an heterogenous disorder and proposed diagnostic criteria that allow for the heterogeneity.

## DIAGNOSIS

There are no specific clinical signs and symptoms or laboratory tests that prove the diagnosis of CIDP. Therefore the diagnosis is descriptive referring to clinical signs and laboratory data.

### Signs and symptoms

The onset of CIDP can be in any decade of life.[16,23,74,79,198,246] There appears to be a slight male predominance. Generally the patients have symptoms of muscle weakness and sensory disturbances, especially paresthesias and numbness (Table 1).

Table 1. **CIDP characteristics**

| author | N pat. | male % | female % | onset age (years) | re-lapse % | follow-up (years) | s/m % | m % | s % | cranial nerve involve-ment % | CSF increased prot % | CSF increased cells % | minimal progressive phase (weeks) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Austin[16] | 32 | 69 | 31 | 23 | 100 | 8 | - | - | - | 34 | 87 | 13 | 21 |
| Dyck[74] | 53 | 66 | 34 | 50 | 34 | 7.5 | 84 | 10 | 6 | 10 | 91 | 11 | 26 |
| Prineas[246] | 23 | 61 | 39 | 32 | 82 | 3 | 70 | 30 | 0 | 43 | 87 | 13 | 3 |
| McCombe[198] | 92 | 62 | 38 | 35 | 65 | 10 | 72 | 22 | 6 | 16 | - | - | * |
| Barohn[23] | 60 | 58 | 42 | 48 | 47 | 2.8 | 86 | 14 | 0 | 17 | 95 | 3 | 8 |

* = 16% of the patients had a progressive phase shorter than 4 weeks
s = pure sensory polyneuropathy
m = pure motor polyneuropathy
s/m = combined sensorimotor polyneuropathy
CSF = cerebrospinal fluid
prot = protein
\- = not mentioned

Pain is reported as relatively infrequent[79] but was observed in other studies[198] in 20% of the patients. Tremor is occasionally found,[198,246] however it was reported by Dalakas and Engel in 44% of the CIDP patient.[62] Tremor is neither related to weakness nor to severe proprioceptive loss. Dysautonomia is rare. A pure or almost pure motor polyneuropathy occurs in a minority (10-30%) of the patients, whereas a pure sensory polyneuropathy is relatively rare (0-6%).[23,74,79,198,246] Usually the symptoms are symmetrical and the patients both have proximal and distal muscle weakness.[23,79] Prineas et al.[246] described even a predominance of proximal over distal limb muscle weakness. Weakness is more frequently in the limbs than in the trunk.[79] Cranial nerve involvement is observed in 10-43% of the patients (Table 1).[23,74,79,198,246] A hypo- or areflexia is found in all patients.[23,74,79,198,246] Papilledema may occur and has been attributed to the elevated CSF protein concentration.

### Course

Dyck et al.[79] concluded that the neurological symptoms in CIDP develop slowly and that maximal deficit is reached in many months or years. In his study only patients were included with a progressive phase of at least 6 months.[74] However, the minimal duration of progressive disease before a patient can be diagnosed to have a CIDP is arbitrary. It varies in different

studies: Prineas et al.[246] (3 weeks), McCombe et al.[198] (16% within 4 weeks), Barohn et al.[23] (2 months) and Austin[16] (5 months). Generally, a progression of symptoms of more than 4-6 weeks seems to be a safe interval to separate CIDP from the Guillain-Barré syndrome (GBS).
CIDP may have a course with recurrences, which was essential for the diagnosis in the study of Austin.[16] In large studies,[23,74,198,246] the percentage of patients with remitting disease varies between 34-82% (Table 1). McCombe et al.[198] found that a relapsing course was significantly associated with earlier age of onset and that there was a significant increase in the number of relapses during pregnancy and 3 months postpartum.
Dyck et al.[74] distinguished 4 different courses in 52 patients: a gradually progressive (15%), stepwise progressive (35%), recurrent (35%) and a monophasic (15%) course.

**Laboratory examinations**
The CSF protein is increased in 87-95% of the patients[23,74,79,246] and especially so during relapses.[246] A CSF pleocytosis is found in only 3-13% of the patients[23,74,79,246] (Table 1).
According to Dyck et al.,[74,79] it is a characteristic - although not invariable - finding that the nerve conduction velocities (NCV) of both motor and sensory fibers are slowed. Some authors[23,198,246] included in their studies only patients with a motor NCV below 60-75% of normal values. The amplitude of the action potentials is generally reduced in motor and sensory nerves.[79] Nerve conduction blocks cause a fall in compound muscle action potentials (CMAPS's), but this has to be distinguished from dispersion and loss of motor units. Distal motor latencies are generally delayed.[79] Electrodiagnostic criteria of demyelination in CIDP were recently described by Albers and Kelly.[7]
Nerve biopsies may show a mononuclear, often pericapillary, epi-, peri- or endoneurinal cell infiltrate without evidence of vasculitis. Furthermore there may be some edema, segmental demyelination and remyelination or signs of hypertrophic neuropathy.[79] Stripping of myelin by invading macrophages was observed by electron microscopy in nerve biopsies from 5 CIDP patients.[29,245] Krendel et al.[157] investigated sural nerve biopsies in 14 CIDP patients and found endoneurinal pericapillary cellular infiltration in 4, onion bulbs in 5 and predominant demyelination in 7 patients. These findings are not specific features of CIDP. Barohn et al.[23] investigated sural nerve biopsies in 56 CIDP patients and found predominantly demyelinating changes in 27 (48%), axonal changes in 12 (21%), mixed axonal and demyelinating features in 7 (13%), whereas 10 (18%) biopsies were normal.
The value of nerve biopsies in CIDP patients is questionable. However, in difficult cases it might contribute to the diagnosis, especially by excluding vasculitis and amyloid deposits.

**Absence of associated disorders**
Dyck et al.[74,79] proposed that the diagnosis of CIDP can only be made in the absence of an inherited polyneuropathy, intoxication or demonstrable systemic disease. This opinion has been challenged by Barohn et al.[23] since clinical and laboratory features indistinguishable from CIDP may occur in association with some systemic disorders.
GBS is associated with disorders as Hodgkin's disease, chronic hepatitis and inflammatory bowel disease.[14] Other autoimmune disorders like myasthenia gravis may occur in combination with rheumatoid arthritis, SLE and Graves' disease.[228] Therefore, it's not surprising that CIDP, a disorder of presumed autoimmune pathogenesis, occurs in the setting of other immune-mediated conditions. This means that the presence of an other disorder does not always exclude the diagnosis of CIDP.[23,43,54,55,139,151,198]

McCombe et al.[198] found an associated disease with possible allergic or autoimmune aetiology in 9 of 92 CIDP patients: thyrotoxicosis (4), psoriasis (2), urticaria (1), iritis (1) and eczema (1). Barohn et al.[23] included 8 patients who had a concurrent condition in a series of 60 patients with CIDP: Hodgkin's disease (1), benign monoclonal gammopathy (5), inflammatory bowel disease (1) and chronic hepatitis (1).
Furthermore in some patients with CIDP, clinical features and magnetic resonance imaging (MRI) signs of central nervous system demyelination have been demonstrated.[23,79,93,208,227,261,304,329]

Table 2~

**Proposed Diagnostic Criteria***

**Mandatory Inclusion Criteria:** All patients must have these features
1. Progression of muscle weakness (steady, stepwise or relapsing) for 2 months
2. Symmetrical proximal and distal weakness in upper or lower extremities
3. Areflexia or hyporeflexia

**Mandatory Exclusion Criteria:** Patients must be devoid of these features
1. Clinical features, including pure sensory neuropathy, mutilation of hands or feet, retinitis pigmentosa, ichthyosis, orange tonsils, history of exposure to drugs or toxins known to cause peripheral neuropathy
2. Laboratory findings of low serum cholesterol levels, abnormal porphyrin metabolite values, fasting glucose levels of >7.6 mmol/L, low serum vitamin B12 levels, hypothyroidism, heavy metal intoxication, CSF WBC >50 x $10^6$/L
3. Nerve biopsy specimen with features of vasculitis, neurofilamentous swollen axons, intramyelinic blebs, amyloid deposits, Schwann cells with evidence of storage materials typical for Fabry's disease, adrenal leukodystrophy, metachromatic leukodystrophy, globoid cell leukodystrophy, or Refsum disease
4. Electrodiagnostic features of neuromuscular transmission defect, myopathy, or anterior horn cell disease

**Major Laboratory Criteria**
1. Nerve biopsy specimens with predominant features of demyelination that include segmental demyelination, remyelination, loss of nerve fibers, onion-bulb formation, and perivascular inflammation
2. Nerve conduction studies with features of demyelination, including slowing of conduction velocities in at least 2 motor nerves to <70% of lower limit of normal (2 nerves required to avoid inclusion of patients with focal compression neuropathy)
3. CSF protein level >0.45 g/L

**Diagnostic Categories**

*Definite*
1. Mandatory inclusion criteria
2. Mandatory exclusion criteria
3. All 3 major laboratory criteria

*Probable*
1. Mandatory inclusion criteria
2. Mandatory exclusion criteria
3. 2 of 3 laboratory criteria

*Possible*
1. Mandatory inclusion criteria
2. Mandatory exclusion criteria
3. 1 of 3 laboratory criteria

**Concurrent illness**

Patients in this group have an acquired demyelinating polyneuropathy accompanying another disorder; conditions so far described include thyrotoxicosis, HIV infection, monoclonal gammopathy, hereditary motor and sensory neuropathy, CNS demyelination, chronic active hepatitis, inflammatory bowel disease, Hodgkin's disease

*CSF indicates cerebrospinal fluid; WBC, white blood cell count; HIV, human immunodeficiency virus; and CNS, central nervous system

~ Chronic inflammatory demyelinating polyradiculoneuropathy. Clinical characteristics, course, and recommendations for diagnostic criteria. Arch Neurol 1989;46:878-884. 

It has even been suggested that CIDP may develop in patients with a hereditary neuropathy, which responds to steroids and plasma exchange.[77,211]
All these diverse findings indicate that CIDP is not one condition but a syndrome, which may be the result of different causes with common immunopathogenic mechanisms.[23]
Barohn et al.[23] recently concluded that CIDP is not in that regard different from GBS, a condition for which diagnostic criteria have been established.

In the following sections the new criteria proposed by Barohn et al.[23] (Table 2) will be discussed.

**Mandatory Inclusion Criteria:** All patients must have the following features (Table 2):

1. *Progression of muscle weakness for 2 months* which is probably included to separate CIDP from the acute form, the Guillain-Barré syndrome (GBS).[14] Patients with GBS usually have a monophasic course with a rapid onset and a progressive phase over 1-4 weeks. After a plateau phase, the majority of the patients partially or completely recover in weeks or months.
   Some CIDP patients initially have a typical course of GBS. However, subsequently they have a relapse of muscle weakness or a chronic progressive course without a tendency to spontaneous recovery.[23,198,246,337] This does not mean that all GBS patients with a relapse in fact have CIDP. For instance a biphasic course after plasma exchange has been reported by Osterman et al.[230] in 5 of 23 GBS patients, 2 to 4 weeks after initial improvement and by Ropper et al.[253] in 10 of 94 GBS patients, 1 to 6 weeks after improvement.
   Deterioration after plasma exchange may be explained by persistence of a pathogenic neurotoxic factor and indicates that the treatment was simply too short in these GBS patients.
   A group of patients difficult to classify are those who completely recovered but who had an acute relapse several months or even up to 36 years later.[337] Because of the recurrence they may be classified as CIDP but they can also be considered as patients with a second bout of GBS.

2. *Symmetrical weakness in upper and lower extremities.* Most patients with CIDP have this pattern of weakness, but in some patients there is asymmetry. This has also been described in patients with GBS.[14] However, asymmetry is important in the distinction of CIDP from other neuropathies such as vasculitic neuropathy,[82,214] multifocal demyelinating neuropathy with persistent conduction block[176] and multifocal motor neuropathy.[236]
   In patients with systemic vasculitis and neuropathy,[30,41,209,266,333] distinction from CIDP is usually not difficult, but it is, if the *vasculitis* is *confined to the peripheral nervous system* and the neuropathy is not of the mononeuritis multiplex type. Kissel et al.[147] and Herati et al.[128] more frequently found a distal symmetric sensorimotor polyneuropathy than a mononeuritis multiplex in patients with non-systemic vasculitic neuropathy. Dyck et al[82] investigated 20 such patients. Multiple mononeuropathy was seen in 13 patients, asymmetric neuropathy in 4, distal polyneuropathy in 3, and sensory polyneuropathy in 1 patient. The symptoms included weakness, atrophy, paresthesias, pain and sensory deficits. In an affected nerve in patients with multiple neuropathy (most frequent were the ulnar, peroneal or median nerve), the symptoms generally reached a maximum in hours or days. The site of the lesion is usually not a common site of compression or entrapment.[82] There is evidence of axonal degeneration and segmental demyelination,[128] but axonal degeneration predominates.[82] CSF protein is generally not elevated.[82,128] It was found that patients with increased CSF protein had a better response to immunosuppressive agents.[128]

In *multifocal demyelinating neuropathy with persistent conduction block*,[176] there is a subacute onset with a slow progression of sensorimotor symptoms involving one or two nerves in the arms, usually the median or ulnar nerve. Arms are always more affected than legs and tendon reflexes are generally absent in the arms but preserved in the legs. The sensory disturbances seem to involve primarily large-fibre modalities (vibration, position and touch). These patients have normal motor latencies and there are focal conduction blocks in the nerves which may persist at the same site for months or years. These patients may respond to corticosteroids. *Multifocal motor neuropathy* resembles multifocal demyelinating neuropathy but is confined to motor nerves.[236] These patients have asymmetrical weakness, which develops in one arm and progresses over 2 - 3 years to the other arm, legs and trunk. Initially this polyneuropathy resemble lower motor neuron disease. However, later in the course of the disease multifocal conduction blocks compatible with patchy selective demyelination can be demonstrated in motor but not sensory fibres. In these patients, upper motor neuron and bulbar signs were not seen but reflexes were sometimes relatively brisk in areas of weakness. The patients sometimes had paresthesias but sensory examinations remained normal. Antibodies to GM1 and other gangliosides can be demonstrated in these patients. Pestronk et al.[236] reported that these patients may respond to cyclophosphamide and not to treatment with corticosteroids or plasma exchange. However, antibodies to GM1 are not a specific feature of this disorder since they have been demonstrated in patients with other neuropathies (approximately 20% of patients with GBS or CIDP) and in patients with myasthenia gravis, amyotrophic lateral sclerosis (ALS),[280] polymyositis, systemic lupus (SLE) and rheumatoid arthritis. The question is whether these multifocal neuropathies are variants of the inflammatory neuropathies or are other neuropathies for instance vasculitic neuropathies.

3. *Areflexia* of all tendon reflexes occurs in most patients with CIDP. However, in mild cases or shortly after onset of disease, total areflexia is not obligatory.

**Mandatory Exclusion Criteria:**

1. Patients must be devoid of the following *clinical features* (Table 2)
   - Pure sensory neuropathy. Some other studies reported a sensory neuropathy in 0-6% of patients with CIDP (Table 1). A pure sensory neuropathy may occur in diabetes mellitus, vasculitis, Sjögren syndrome,[190] primary biliary cirrhosis,[44] amyloidosis, pyridoxine abuse, after the use of antibiotics,[288] folate deficiency, HIV infection,[56,85] and in some hereditary sensory neuropathies. Also a paraneoplastic polyneuropathy or a neuropathy induced by chemotherapeutic drugs may be of a pure sensory type. In Fabry's disease, pain is the predominant symptom. In plasmacell dyscrasia's initially there may be only sensory disturbances. Therefore, all these disorders should be considered before the diagnosis pure sensory form of CIDP is made. A neuropathy which resembles the pure sensory form of CIDP is chronic idiopathic ataxic neuropathy or CIAN. CIAN,[63] presents with distal paresthesias and sensory ataxia with slow progression, areflexia, normal strength and a profound loss of proprioceptive and kinesthetic sensation extending up to the most proximal joints. Motor NCV's are normal, but sensory potentials are absent. Some patients have serum monoclonal or polyclonal gammopathy, but only few patients have elevated CSF protein. Immunosuppressive agents or plasma exchange were not effective. During a follow-up period of 17 years, patients continued to worsen, but no signs of systemic illness or malignancy were

found. CIAN has also been referred to as "progressive sensory neuropathy in patients without carcinoma".[144]

- Mutilation of hands or feet; which is not a feature of patients with CIDP but occurs especially in neuropathies with marked impairment of sensation, such as the hereditary sensory neuropathies, but also occurs in patients with diabetic sensory neuropathy, alcoholic neuropathy or sensory neuropathy in carcinoma.
- Retinitis pigmentosa; which can be found in different storage- and degenerative diseases and in disorders associated with inborn errors of metabolism such as phytanic acid storage (Refsum) disease;[252] a rare autosomal recessive disorder due to storage of phytanic acid in the peripheral and central nervous system. Symptoms develop between the ages of 10-30 year, but occasionally later. The course may be relapsing. Clinical features are a chronic distal demyelinating sensorimotor polyneuropathy and usually cerebellar ataxia with pigmentary retinal degeneration. The peripheral nerves may be thickened and NCV's are sometimes, but not always, markedly reduced.
- Ichthyosis; which may occur in patients with phytanic acid storage disease, but also in patients with abetalipoproteinemia (Bassen-Kornzweig), an autosomal recessive disorder with onset of neurological symptoms nearly always by the age of 20.[129] There is ataxia, dysarthria, areflexia, proprioceptive loss, muscle weakness, pes cavus and scoliosis. These patients have low serum cholesterol, phospholipids, free fatty acids and chylomicrons in the absence of low density lipoproteins. The neurological complications may be secondary to vitamin E deficiency.[167]
- Orange tonsils; which have been described in patients with hereditary high density lipoprotein (HDL) deficiency (Tangier's disease).[129]
- Drugs and toxins; that may cause a polyneuropathy. Most of these (acrylamide, carbon disulfide or organophosphates) cause mainly axonal neuropathies.[12] Some drugs and toxins may cause a neuropathy which may mimic CIDP, for instance *amiodarone*, a drug to treat cardiac arrhythmias. Amiodarone may cause a severe chronic sensorimotor polyneuropathy after 6 months to 3 years of treatment. The CSF cell count is usually normal, the CSF protein is slightly or moderately increased and the NCV is generally severely decreased. Nerve biopsies show segmental demyelination,[98,234] but there are also signs of axonal degeneration.[234] Electron microscopy may reveal Schwann cells with numerous lipid containing lysosomal inclusions in Schwann cells. After discontinuing the drug, slow but usually complete recovery occurs. *Perhexilene*, used in the treatment of angina pectoris, may result in a gradual sensorimotor polyneuropathy with slow motor nerve conduction velocity and a raised CSF protein. Segmental demyelination and a variable degree of axonal degeneration is found in sural nerve biopsies together with the presence of lipid body inclusions. *Gold*, used in the treatment of rheumatoid arthritis may induce a sensorimotor or purely motor polyneuropathy. Whether the polyneuropathy was caused by the drug or occurs as complication of the disease can often not be established. The NCV can be severely slowed and the CSF protein is increased in 50% of the patients. Both evidence of demyelinating and axonal disturbances have been found in nerve biopsies. *Dapsone*, used in the treatment of leprosy and in various dermatologic disorders, may induce a predominantly motor polyneuropathy with reduction of the NCV.

*Alcohol* abuse may result in a chronic, sensorimotor polyneuropathy with a distal predominance. The motor NCV is normal or mildly slowed but the amplitude is frequently reduced.

In *hexacarbon* polyneuropathy, secondary demyelination and considerable reduction in nerve conduction velocity has been found. Hexacarbon solvents, are used in industrial and domestic agents e.g. in glue.

2. Patients must be devoided of the following *laboratory findings*:
   - Low cholesterol levels; in patients with a neuropathy this may indicate Tangier's disease.
   - Abnormal porphyrin metabolite values. Porphyrin metabolites are often measured in patients with polyneuropathy but porphyria is rare, the onset is usually acute and peripheral neuropathy seldom occurs in patients without previous abdominal or mental disturbances.
   - Fasting glucose levels of >7.6 mmol/l; which can be found in patients with diabetes mellitus. Usually, axonal disturbances predominate.[166] CSF protein is increased in two thirds of the diabetes patients with a polyneuropathy.[189] Generally, the distinction between CIDP and diabetic polyneuropathy is not difficult, but sometimes diabetes patients may present with a predominantly motor neuropathy, slowed NCV and increased CSF protein.
   - Low Vitamin B12 levels. Vitamin B12 levels are usually measured together with vitamin B1 and B6. Vitamin B1 is important in patients with malnutrition and vitamin B6 deficiency in patients treated with Isoniazid (INH). Intoxication with vitamin B6 may also cause neuropathy. Neuropathy in patients with vitamin B12 deficiency is usually not difficult to distinguish from CIDP, but sometimes a sensorimotor polyneuropathy with a remitting or stepwise progressive course may occur.
   - Hypothyroidism; but this condition rarely presents with a sensorimotor polyneuropathy, slowed NCV and increased CSF protein.[165,249]
   - Heavy metal intoxication. Screens for heavy metals are expensive and very rarely necessary. Segmental demyelination[169] has been found in experimental lead neuropathy, but these features have not been described in human nerves after lead exposure. Other metals mainly cause an axonal neuropathy.
   - CSF white blood count > 50 x $10^6$. In CIDP, the number of CSF cells is usually within normal limits, but it may be slightly increased. If the number of CSF cells is > 50 x $10^6$/l, another diagnosis should be considered, for instance *Lyme borreliosis*.

   In patients with Lyme borreliosis the neuropathy is often very painful and the type is that of a mononeuritis (multiplex) or a sensorimotor polyneuropathy. The patients usually have a rapid progression of muscle weakness, but a more protracted course during several months has been described.[231] The patients may have areflexia and the CSF protein is often increased. In these patients, there is evidence of both demyelinating[106,289] and axonal[184] disturbances. Peripheral nerve vasculitis has also been reported in patients with Lyme borreliosis.[40] Improvement of NCV was found after penicillin treatment.[114] For the diagnosis, detection of Borrelia burgdorferi antibodies is important but a negative test does not exclude the diagnosis.[273] The demonstration of these antibodies is dependent on the duration of the disease, prior antibiotic treatment, the presence of HLA-DR2 antigen[344] and the technique used to demonstrate these antibodies.[117]

   Other neuropathies which may sometimes resemble CIDP and which are associated with an increased CSF cell count are neuropathies in malignancy e.g. (non-)Hodgkin's disease, or those associated with vasculitis, sarcoidosis, cytomegalovirus (CMV), herpes zoster and HIV infections.[54,65,171]

3. Patients must be devoided of the following features in *nerve biopsies*:
   - vasculitis or neurofilamentous swollen axons; which argue against the diagnosis of CIDP. These neurofilamentous swollen axons have been found in ALS, in giant axonal neuropathy and after intoxication with acrylamide, aluminum chloride, and vincristine.
   - Intramyelinic blebs; which can be found in GBS but may also be an artefact.
   - Amyloid deposits which are not present in biopsies of CIDP patients.
   - Storage materials in Schwann cells; which can be seen in neuropathies other than CIDP and usually can be distinguished from CIDP on clinical criteria without the need of a nerve biopsy. Storage material is present in:

   *Fabry's disease*, an X-linked recessive disorder which develops in childhood or adolescence. Pain is the major feature and may be the presenting symptom. Characteristic are telangiectatic skin lesions.

   *Adrenoleucodystrophy* (ALD), an X-linked recessive disorder, the age of onset is usually in childhood. Polyneuropathy, dementia, tetraplegia, seizures, blindness and adrenal insufficiency are features of this disease. An increased ratio of C26 to C22 fatty acids can be demonstrated in patients and carriers.

   *Adrenomyeloneuropathy*, which is characterized by spastic paraplegia, distal muscle weakness and sensory loss. The features develop in adolescence or adult life.

   *Metachromatic leukodystrophy* (MLD), a heterogenous disorder with at least five distinct autosomal recessive forms. The cause of this storage disease is deficiency of arylsulphatase. The motor NCV is reduced and the amplitude of sensory nerve action potentials is reduced or absent. The tendon reflexes are depressed or absent. The disease is a mixture of cerebellar and pyramidal signs, dementia, optic atrophy and polyneuropathy. The onset is usually before the age of 2, sometimes between 3-20 years and occasionally there may be a adult onset form of MLD. Therefore, this diagnosis should be considered in any adult with a combination of dementia and polyneuropathy.

   *Globoid cell leukodystrophy* (Krabbe's disease), a rare autosomal recessive disease caused by a deficiency of galactosylceramide beta-galactosidase. The onset of this multisystem degenerative fatal demyelinating disease is between 3 and 6 months. It is not difficult to distinguish the disease from CIDP.

   *Refsum's disease*, which is discussed on page 11.

4. Patients should be devoid of the following *electrodiagnostic features* indicating: neuromuscular transmission defect (myasthenia gravis or Eaton-Lambert syndrome) or myopathy, which are not a feature of CIDP. However, fasciculations may be found in patients with CIDP.

**Major laboratory criteria** (Table 2)

1. In *nerve biopsies* of patients with the clinical diagnosis of CIDP, the classical predominant features should be demyelination, remyelination, loss of nerve fibers, onion-bulb formation, and perivascular inflammation, but these are not always present. Barohn et al.[23] found that 18% of 56 CIDP patients had no abnormalities in their sural nerve biopsy, 21% had evidence of axonopathy, whereas 13% had mixed demyelinating and axonal changes.

2. The *nerve conduction velocities* are usually moderately or severely slowed. However, especially shortly after onset of CIDP or in mildly disabled patients, the NCV may be only slightly decreased.
3. The *CSF protein* is usually increased, especially so during relapses,[246] but is normal in 5-13% of the patients.[23,74,79,246]

According to Barohn et al.[23] the diagnosis of CIDP can be made using the presented criteria (Table 2):

**Diagnostic Categories**

*Definite*
1. Mandatory inclusion criteria
2. Mandatory exclusion criteria
3. All 3 major laboratory criteria

*Probable*
1. Mandatory inclusion criteria
2. Mandatory exclusion criteria
3. 2 of 3 laboratory criteria

*Possible*
1. Mandatory inclusion criteria
2. Mandatory exclusion criteria
3. 1 of 3 laboratory criteria

**Concurrent illness**

Patients in this group have an acquired demyelinating polyneuropathy accompanying another disorder; conditions so far described include thyrotoxicosis, HIV infection, monoclonal gammopathy, hereditary motor and sensory neuropathy, CNS demyelination, chronic active hepatitis, inflammatory bowel disease, Hodgkin's disease

The presence of some other associated disorders does not always exclude the diagnosis of CIDP. This has been discussed on page 6.

Other disorders which may cause a chronic, mainly demyelinating polyneuropathy and which should be included in the differential diagnosis of CIDP are:

1. *Plasmacell dyscrasias* such as lytic multiple myeloma (Kahler's disease), Waldenström macroglobulinemia,[162,201] sclerotic myeloma, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal protein and skin changes),[22] B cell lymphoma of the peripheral nerve[138] and cryoglobulinemia.[182,219] These disorders will be identified if in all patients with unexplained neuropathy immuno-electrophoresis or immunofixation is carried out.[350]

2. *Chronic polyneuropathy of undetermined cause* (McLeod neuropathy). McLeod et al.[202] described 67 patients with a chronic symmetrical polyneuropathy which was slowly progressive over many years and in which the cause remained undiagnosed. The mean age of onset was

50 years, total areflexia was uncommon, the mean CSF protein was 0.73 g/l and the NCV in most cases was only mildly slowed. Generally, these patients were only mildly disabled. Distinction from CIDP may be difficult, especially if these patients are seen late in the course of the disease when axonal loss of fast conducting fibres suggest a demyelinating disorder.

## TREATMENT

### Corticosteroids

Austin[16] presented a historical overview, but also a N=1 trial in a CIDP patient who had 20 relapses - spontaneously or induced by drug withdrawal - during a period of 5 years. This patient responded dramatically and repeatedly to corticosteroids. Since then, many uncontrolled studies of corticosteroid treatment in CIDP patients were published (Table 3). The time lapse between initiation of prednisone treatment until onset of clinical improvement varied up to 3.5 months.[64] In an uncontrolled study Dyck et al.[74] treated 38 CIDP patients with different dosages of corticosteroids during variable periods. It was concluded that some patients initially responded favorably to prednisone treatment, but this benefit was often not apparent over protracted periods and there were severe complications such as gastric hemorrhage and perforation, activation of tuberculosis and bronchopneumonia.

Table 3. **Corticosteroid treatment in CIDP patients**

uncontrolled studies

| Author | year | N patients | treatment pos | treatment neg | response dubious |
|---|---|---|---|---|---|
| Thomas[303] | 1969 | 5 | 2 | - | 3 |
| DeVivo[64] | 1970 | 1 | 1 | - | - |
| Matthews[196] | 1970 | 3 | 3 | - | - |
| Dolman[68] | 1973 | 1 | - | - | 1 |
| Dyck[74] | 1975 | 38 | 15 | 23 | - |
| Griggs[109] | 1976 | 1 | 1 | - | - |
| Prineas[246] | 1976 | 21 | 14 | 5 | 2 |
| Oh[226] | 1978 | 10 | 10 | - | - |
| Dalakas*[62] | 1981 | 25 | >12 | - | - |
| Sladky[283] | 1986 | 6 | 6 | - | - |
| McCombe[198] | 1987 | 76 | 49 | 27 | - |
| Barohn[23] | 1989 | 59 | 56 | 3 | - |

* prednisone was "successful in the majority" of patients

Dalakas and Engel[62] reported that in a series of 25 patients with CIDP, daily high-single-dose prednisone (1.5-2 mg/kg body weight), slowly tapered of to an alternated-day program, was very successful in the "majority" of the patients. Side effects which occasionally were seen included: hyperglycemia, hypertension, avascular necrosis of femoral or humeral head, osteoporosis and cataract. Unfortunately no exact percentages or duration of treatment were mentioned.

McCombe et al.[198] treated 76 CIDP patients with corticosteroids, 49 (65%) improved. Of 12 patients who did not respond to steroids, 7 were treated with azathioprine and improved, the other patients were successfully treated with cyclophosphamide.

Barohn et al.[23] reviewed their 10 year experience with 60 CIDP patients. All but one patient received "high-dose" prednisone and 95% initially improved. The mean time to the first signs of improvement was rather long (1.9 months) and maximal improvement was reached not earlier than after 6.6 months. After discontinuing steroids, 59% of the patients with a monophasic course and 18% of the patients with a relapsing course were in remission. An overall complete remission was observed in 30% of the patients. Side effects of treatment were not mentioned. It was concluded that the majority of patients initially showed a beneficial response to steroids, but that relapses will occur in 70% of the patients after discontinuation of steroids.

A controlled clinical trial was performed in 28 CIDP patients by Dyck et al.[77] Prednisone was shown to cause a small but significant improvement over no treatment accessed by a neurological disability scale. No difference in response was found between patients with a progressive course or those with a recurrent one.
The percentage of patients responding to steroids varies in the reported studies, probably because the selection of patients and the evaluation of treatment is different. Unfortunately assessment of treatment effects are not always based on clinically relevant features and the side effects of long term steroid treatment in CIDP and its effect on the disability of these patients has hardly been investigated.
Recently in an editorial on the treatment of CIDP, Dyck[83] emphasized that prolonged corticosteroid use is not safe; mentioned side effects were: weight gain, hypertrichosis, altered appearance, hyperglycemic hyperosmolar coma, psychosis, gastric ulcer, infection, cataract, osteoporosis and aseptic necrosis of hips or shoulders.

**Immunosuppressive drugs**
No clinical trials are available on the therapeutic effect of azathioprine, cyclophosphamide or cyclosporin A (Table 4). Case reports showed promising results, however follow-up was usually short and side effects of long term treatment may be serious.
In 1985 Dyck et al.[80] published a non-blinded, randomized trial in 30 CIDP patients who were treated with alternate day decremental prednisone therapy alone or combined with 2mg/kg azathioprine for 9 months. Three patients were withdrawn from the study because of a change in diagnosis. No statistically significant differences were demonstrated between the 2 groups. Treatment with the anti-lymphocytic drug polyinosinic-polycytidylic acid (poly-ICLC) has been reported to be successful in 2 of 4 CIDP patients[86] and one study in 4 CIDP patients showed beneficial effects of total lymphoid irradiation after other treatments had failed.[257]

Table 4. **Treatment of CIDP with different immunosuppressive agents**

uncontrolled studies

| Author | year | treatment | N patients | treatment pos | treatment neg | response dubious |
|---|---|---|---|---|---|---|
| Heathfield[125] | 1970 | aza | 1 | - | 1 | - |
| Yuill[346] | 1970 | aza | 1 | - | - | 1 |
| Cendrowski[42] | 1977 | aza | 2 | 1 | 1 | - |
| Prusinski[247] | 1978 | aza | 1 | 1 | - | - |
| Walker[330] | 1979 | aza | 2 | 2 | - | - |
| Dalakas[63] | 1981 | aza | 4 | 3 | 1 | - |
| Pentland[235] | 1982 | aza | 5 | 5 | - | - |
| McCombe[198] | 1987 | aza | 7 | 4 | 3 | - |
| Prineas[246] | 1976 | cyclophos | 4 | 4 | - | - |
| Rosen[255] | 1976 | cyclophos | 3 | 3 | - | - |
| Dalakas[63] | 1981 | cyclophos | 1 | 1 | - | - |
| McCombe[198] | 1987 | cyclophos | 5 | 4 | - | - |
| Kolkin[150] | 1986 | cyclosp A | 1 | 1 | - | - |
| Jongen[142] | 1988 | cyclosp A | 2 | 2 | - | - |
| Tindall[307] | 1988 | cyclosp A | 10 | 6 | 4 | - |
| Engel[86] | 1978 | poly-ICLC | 4 | 2 | 2 | - |
| Rosenberg[257] | 1985 | lymphoid irr | 4 | 3 | 1 | - |

aza=azathioprine; cyclophos=cyclophosphamide; cyclosp A=cyclosporin A; poly-ICLC=polyinosinic-polycytidylic acid; lymphoid irr=lymphoid irradiation

**Plasma exchange (PE)**

There are several studies on the effects of PE in patients with CIDP (Table 5). PE is often not the first choice of treatment and is usually applied if side effects of other treatments had occurred, or after unresponsiveness or loss of response to treatment with corticosteroids, azathioprine or cyclophosphamide. In the different series, both patients with remitting and progressive course improved. Some patients needed regular PE to maintain the improved clinical condition; in other patients, addition of immunosuppressive drugs was necessary. Some patients do not respond to PE but respond after prednisone, azathioprine or cyclophosphamide.

Table 5. Plasma exchange (PE) in patients with CIDP

| Author | year | N patients | Treatment response | | |
|---|---|---|---|---|---|
| | | | pos | neg | dubious |
| Fowler[96] | 1979 | 1 | 1 | - | - |
| Levy[175] | 1979 | 1 | 1 | - | - |
| Server[275] | 1979 | 1 | 1 | - | - |
| Cook[49] | 1980 | 3 | 1 | - | 2 |
| Mark[191] | 1980 | 1 | 1 | - | - |
| Server[276] | 1980 | 2 | 2 | - | - |
| Toyka[310] | 1980 | 1 | 1 | - | - |
| Dalakas[62] | 1981 | 3 | 1 | 2 | - |
| Gross[111] | 1981 | 6 | 2 | 4 | - |
| Connor[46] | 1982 | 2 | 2 | - | - |
| Gross[110] | 1982 | 2 | 2 | - | - |
| Tindall[306] | 1982 | 14 | 6 | 8 | - |
| Toyka[311] | 1982 | 1 | 1 | - | - |
| Vedeler[325] | 1982 | 7 | 5 | 2 | - |
| Feasby[92] | 1983 | 5 | 5 | - | - |
| Pollard[239] | 1983 | 5 | 2 | 2 | 1 |
| Valbonesi[316] | 1983 | 4 | 4 | - | - |
| Tandon[298] | 1983 | 11 | 5 | 6 | - |
| Burke[37] | 1985 | 9 | 7 | 2 | - |
| Pollard[240] | 1985 | 8 | 6 | 2 | - |
| McCombe[199] | 1987 | 13 | 10 | 3 | - |
| Kunze[158] | 1988 | 11 | 9 | 2 | - |

In 1986 Dyck et al. reported the results of the only controlled PE study.[81] In a double-blind trial, 15 patients were randomized to PE and 14 patients to sham exchange for three weeks. Plasma was replaced by albumin. A neurological disability score, showed no significant differences between the two groups. However, it was concluded that PE had a beneficial effect on some manifestations of CIDP. Five patients (33%) receiving PE had an improvement in their scores at three weeks, as compared with base-line scores, that exceeded the largest improvement attained by any patient receiving sham exchange. This conclusion was based on the finding of improvement in scores on weakness, deep tendon reflexes and nerve conduction velocities. Improvement was observed both in patients with slowly progressive disease and those with a remitting course. Thirteen of 14 patients who initially had sham PE subsequently received therapeutical PE, 4 patients (31%) improved. In this controlled study, only the initial effects of PE were investigated and not the effects of long-term treatment. The beneficial effects of PE began to fade 10 to 14 days after treatment.

**Intravenous immunoglobulin**

High-dose polyspecific immunoglobulin for intravenous use (IVIg) might be an alternative treatment in patients with CIDP. Several uncontrolled studies have been published. Busch et al.[38] (1982) described the favorable effect of fresh frozen plasma in 2 CIDP patients. Vermeulen et al.[328] (1985) reported the effect of infusion of fresh frozen plasma (FFP) and IVIg (0.4 g/kg body weight for 5 consecutive days) in 17 patients with CIDP. The first signs of improvement were seen within 8 days from onset of treatment and no serious side effects were observed. Improvement was short-lasting in most patients, but none of the patients became refractory for further IVIg treatment. Of the 13 patients who improved, 5 patients were treated with cyclophosphamide and two improved, 3 patients were treated with azathioprine and two

improved. One patient had PE and improved. Of the 4 non-responders, 2 patients were treated with cyclophosphamide and one improved. Albama et al.[6] (1987), Cook et al.[50,51] (1987), Curro Dossi et al.[59] (1987) and recently Faed et al.[87] found similar effects of IVIg. All 9 patients described by Faed et al.[87] responded after treatment with a dosage of 0.4 g IVIg/kg body weight for 3 consecutive days, which indicates that the initial treatment dosage is arbitrary. Results of studies on IVIg treatment are shown in Table 6.

Table 6. **Intravenous immunoglobulin (IVIg) treatment in CIDP patients**

| Author | year | N patients | Treatment response pos | neg |
|---|---|---|---|---|
| Vermeulen[328] | 1985 | 17 | 13 | 4 |
| Cook[50,51] | 1987/8 | 5 | 5 | - |
| Albama[6] | 1987 | 1 | 1 | - |
| Curro-Dossi[59] | 1987 | 4 | 4 | - |
| Faed[87] | 1989 | 9 | 9 | - |

**Comparison of different treatments in CIDP**

The percentage of CIDP patients responding to the various treatments is difficult to estimate. The definition of improvement and the selection of patients vary considerably. In general, most patients initially improve after treatment with corticosteroids, but a stable remission, off therapy, may occur in only 30% of the patients. The results of PE and IVIg treatment are yet not systematically evaluated during long-term follow-up. Variations in response may partially be explained by differences in the selection of patients. The clinical effectiveness of azathioprine, cyclophosphamide, lymphoid irradiation and Poly-ICLC treatment is difficult to estimate because only non-randomized studies in less than 10 patients have been reported. In a randomized study it was shown that addition of azathioprine to prednisone treatment did not result in a superior response or allowing a lower maintenance prednisone dosage. Some patients improve after one type of treatment and not after another treatment. The time-lapse between onset of corticosteroids, PE or IVIg treatment and the beginning of improvement varies considerably. Other factors important in the choice of treatment are the side-effects, convenience of maintenance therapy and costs of long-term treatment.

**Clinical follow up**

The majority of CIDP patients has no tendency for a spontaneous good recovery. Dyck et al.[74] evaluated 53 patients with CIDP with an average follow up of 7.5 years. Complete recovery occurred only infrequently; about 60% of the patients were able to remain ambulatory and to work, 25% became confined to a wheelchair or bedridden, and approximately 10% died from their disease. Of thirty-eight patients who were treated with different dosages of corticosteroids, 3% recovered, 63% had varying neurologic deficits but were ambulatory, and 34% were in a wheelchair or bedridden. McCombe et al.[198] surveyed 92 patients with a CIDP; in 87 patients the follow up was approximately 10 years. Seventy-three percent had made a good recovery and were independent; 12% died, half of them from the disease. Barohn et al.[23] followed 60 CIDP patients for on average 34 months. Twenty-four patients(40%) reached partial or complete remission without continuation of medication, 2 patients (3%) died from complications of the disease and 3 (5%) did not respond to treatment.

These studies indicate that maintenance treatment can not be discontinued in most CIDP patients and that the majority of the patients do not reach complete clinical remission despite immunosuppressive therapy.

Chapter 2

# HIGH-DOSE INTRAVENOUS IMMUNOGLOBULIN TREATMENT IN CHRONIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY

**A double-blind placebo-controlled crossover study**

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## INTRODUCTION

Chronic inflammatory demyelinating polyneuropathy (CIDP) differs in course and prognosis from the Guillain-Barré syndrome (GBS). The course of CIDP may be a continuous or stepwise worsening with fluctuations in severity or a prolonged monophasic deterioration over many months or years. The prognosis of CIDP is worse than of GBS; in a group of 53 patients with CIDP followed for an average of 7.4 years only 2 recovered, 36 had mild to moderately neurological disability, 6 were confined to wheelchair, 6 were dead from the disease, and 3 died from other cause.[74] Patients with CIDP may respond to treatment with corticosteroids[77] or plasmapheresis.[81] The results of non-controlled studies suggest a beneficial effect of high-dose intravenous immunoglobulin treatment (IVIg).[50,59,87,328]
We studied the effect of IVIg in a double-blind placebo-controlled crossover study in patients with CIDP, who we considered to be dependent on regular IVIg treatment.

## PATIENTS AND METHODS

### Criteria for eligibility

Patients with signs and symptoms of CIDP[79] who had, according to their physician, responded to IVIg and needed this treatment at regular intervals to prevent relapse. The patients were not on corticosteroids, other immunosuppressive drugs, or plasmapheresis and showed signs of deterioration after discontinuation of IVIg treatment.

**Randomization**
Regular IVIg treatment was discontinued in 7 patients. Using a sealed envelope technique, patients were randomized, after informed consent, to placebo or IVIg treatment. All patients received once placebo (albumin) and once IVIg. The two treatments could not be distinguished. The first trial treatment was given when the patients' condition had deteriorated and the 2nd trial treatment when on day 8 after the onset of the 1st treatment the clinical condition had further deteriorated. If the patients' clinical condition had not changed, the second treatment was postponed until further deterioration occurred. If the patients' condition had improved after the first treatment, the second treatment started when the patients' condition again deteriorated. The trial code was broken after the results of all patients had been recorded.
The ethics committee of our hospital approved the study.

**Treatment**
The dosage was approximately 0.4 g/kg bodyweight/day for 5 consecutive days and was administered intravenously. The total dosage of freeze dried IVIg was divided in 50 ml bottles, each containing 3 g immunoglobulin. If the treatment was placebo the same number of 50 ml bottles was given each containing 3 g albumin. The daily dose was infused within 2 hours. The immunoglobulin CLB (prepared in the Central Laboratory of the Dutch Red Cross Blood Transfusion Service) is produced from plasma of more than 3.000 Dutch blooddonors by ethanol cryoprecipitation (Cohn fraction II). The resulting product is then treated at pH 4 with traces of pepsin to make it suitable for intravenous use. It's characteristics are similar to those of the immunoglobulin described by Skavaril.[282] It contains 99% IgG, 1% IgA and traces of IgM (protein content 60g/l). IgG subclasses are distributed as follows: IgG1 57,5%, IgG2 28%, IgG3 9%, IgG4 5,5%. At least 85% of the IgG is monomeric, less than 7% dimeric and less than 3% polymeric.
Placebo was prepared from 20% human albumin solution in which no IgG could be detected (less than 0,1%). After pasteurization (10 hours at 60°C), the albumin solution was diluted to 4% and freeze dried.

**Assessment of treatment response**
After discontinuation of IVIg treatment, the patients were seen at regular intervals by two investigators of this study. Treatment responses were assessed with the modified Rankin scale.[319] This scale is shown in Table 1. When they detected at least one point deterioration on the Rankin scale, the 1st trial treatment was started and the investigators recorded the time lapse since the last IVIg treatment. Clinical examination was repeated at day 8 after the onset of treatment. The two investigators together had to record whether the patient had improved (at least one point improvement on the Rankin scale), was unchanged or had deteriorated (at least one point deterioration on the Rankin scale). This was repeated after the 2nd trial treatment.

**Electrophysiological investigations**
Using a Disa 15-c-01 electromyograph, the nerve conduction of the right motor median nerve was measured with disk surface electrodes in the tendon belly montage on the right abductor pollicis brevis muscle and after supramaximal stimulation at the wrist and elbow. Care was taken to place the active electrode at the optimal site for a maximal biphasic compound muscle action potential. The compound muscle action potentials (CMAP's) were recorded after both stimulations. These measurements were carried out on day 1 and day 8 of each trial treatment course.

**Statistical analysis**
Assuming that all the patients would respond to IVIg and none to albumin, it was calculated that 7 patients would be sufficient for this study. The treatment responses were analyzed with the sign test.

Table 1. **Patients characteristics at initial diagnosis**

| study number | age (years) | NCV motor median nerve (m/s) | CSF protein (mg/100ml) | CSF cells ($x10^6$/l) | duration IVIg treatment before trial onset (months) | Rankin Score* before initial IVIg treatment | Rankin Score* after IVIg (before start trial) |
|---|---|---|---|---|---|---|---|
| 1 | 63 | 49 | 341 | 20 | 14 | 3 | 0 |
| 2 | 46 | 37 | 265 | 8 | 19 | 3 | 0 |
| 3 | 10 | 22 | 80 | 1 | 52 | 5 | 1 |
| 4 | 54 | 34 | 251 | 0 | 4 | 5 | 1 |
| 5 | 31 | 19 | 246 | 12 | 52 | 4 | 0 |
| 6 | 56 | 6 | 80 | 6 | 11 | 4 | 1 |
| 7 | 7 | 43 | 55 | 3 | 36 | 3 | 1 |

NCV = nerve conduction velocity (under limit of normal:51 m/s)
CSF = cerebrospinal fluid (upper limit of normal: children 40 mg/100 ml, adults 50 mg/100 ml

**Rankin scale***
0 = asymptomatic
1 = non-disabling symptoms which do not interfere with lifestyle
2 = minor disability symptoms which lead to some restriction of lifestyle, but do not interfere with the patients capacity to look after themselves
3 = moderate disability symptoms which significantly interfere with lifestyle or prevent totally independent existence
4 = moderately severe disability symptoms which clearly prevent independent existence, although patient does not need constant attention day and night
5 = severely disabled, totally dependent requiring constant attention day and night

## RESULTS

The characteristics at initial diagnosis of the 7 patients (4 women and 3 men) are shown in Table 1. All patients had weakness of at least both legs, hypo- or areflexia, slowed nerve conduction velocities and elevated CSF protein level. There were no signs of systemic disease and no kinship history of neuropathy. The disability expressed by the Rankin scale[319] before the initial IVIg treatment and the optimal score after several courses of IVIg are shown in Table 2. All 7 patients were on IVIg treatment at regular intervals to maintain the improved condition (mean treatment duration 27 months, mean treatment interval 2 weeks, mean IVIg dose 0.4g/kg bodyweight/2 weeks). The mean of the time lapses since discontinuation of the regular IVIg treatment until deterioration was 11 weeks (range 4-24). IVIg was the 1st trial treatment in 3 patients and the 2nd in 4.

After IVIg, all 7 patients had improved on day 8 after the onset of treatment. After placebo, none of the patients had improved; the condition had deteriorated in 2 and was unchanged in 5 patients. Three of these 5 patients further deteriorated after 1 week and 2 after 3 weeks. The time lapses since day 8 of the trial treatment until deterioration are shown in Table 2. The mean of the time lapses was 6.4 weeks after IVIg trial treatment and 1.3 weeks after placebo treatment. The time lapses were longer in each patient after IVIg treatment than after placebo treatment. These results show that as a group the IVIg treated patients did better than placebo (sign test $p=0.02$).
Since none of the patients had side effects of treatment, the blinding was not violated.

**Table 2. Time-lapse until clinical deterioration in weeks**

| study number | After IVIg | After Placebo |
|---|---|---|
| 1 | 4 | 0 |
| 2 | 6 | 1 |
| 3 | 8 | 3 |
| 4 | 11 | 1 |
| 5 | 4 | 0 |
| 6 | 9 | 3 |
| 7 | 3 | 1 |
| Mean | 6.4 | 1.3 |

**Electrophysiological measurements**
The mean of the nerve conduction velocity slightly increased from 32 m/s to 37 m/s in the IVIg treatment group and did not change in the placebo group (31 m/s). The mean CMAP after stimulation at the wrist increased (from 5.7 mV to 11.1 mV) in the IVIg group and decreased (from 9.5 mV to 7.8 mV) in the placebo group. The mean CMAP after stimulation of the elbow increased more in the IVIg group (from 4.5 mV to 6.8 mV) than in the placebo group (from 5.2 mV to 5.6 mV) (Figure 1)
The changes in NCV or CMAP's after treatment did not reach statistical significance (Wilcoxon matched-pairs signed-ranks test).

## DISCUSSION

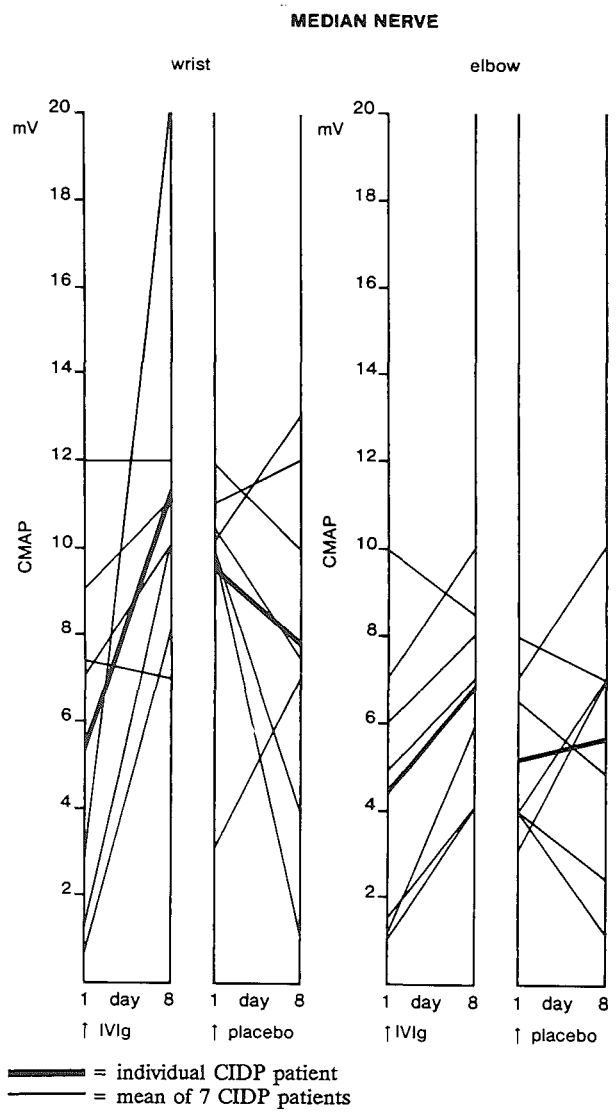
All 7 CIDP patients had deteriorated after discontinuation of maintenance IVIg treatment. Thereafter none of the patients responded to placebo infusion whereas all the patients had a beneficial response to IVIg treatment. The mean time lapse between the end of the trial treatment and the occurrence of deterioration was 6.4 weeks after treatment with IVIg and 1.3 weeks after treatment with placebo.
One may argue that the assessment of the investigators was influenced since they knew that 1 of the treatments was placebo and the other the assumed effective treatment. However, the study was double-blind and the blinding was not violated by side effects. If the investigators had judged the condition of a patient to have improved after 1 treatment and not improved after the other treatment, the probability of finding a treatment response to IVIg by chance is 0.5. However, the probability of finding, by chance, a treatment response to IVIg in 7 consecutive patients is very low: 0.008.

We did not expect the nerve conduction velocity to change within 8 days of treatment. The mean of the nerve conduction velocity in the treatment group slightly increased and did not change in the placebo group. The mean of the CMAP's in the treatment group increased after stimulation at the wrist and decreased in the placebo group. The mean of the CMAP's after stimulation at the elbow increased more in the treatment group than in the placebo group, but all these differences did not reach statistical significance. Changes of the CMAP's were not

always correlated with the clinical condition. This might be explained by fluctuations in dispersion of the conduction velocities, resulting in alterations of the form of the CMAP. Another cause could be the rapid changes in excitability of the nerves which we observed in these patients and which is presently under investigation. The explanation may also be that we only investigated a distal segment of one nerve instead of looking for a conduction block at a more proximal location of the nerve.

In the complete assessment of a treatment, it is not sufficient to demonstrate that the treatment is effective. We need to know whether the effect of treatment has an important clinical effect on disability. We did not investigate this, but a suggestion of how effective this treatment can be, is shown in Table 1. All the studied patients were severely disabled before initial IVIg treatment. After several courses of IVIg treatment, the clinical condition of all these patients had considerably improved. The patients were reluctant to discontinue the treatment and to deteriorate to pretreatment condition. Therefore they gave us permission to carry out this study, provided we started treatment as soon as deterioration became apparent. This prevented us from studying the extent that IVIg treatment may improve disability.

**Figure 1. Compound muscle action potential of median nerve, before and after IVIg or placebo treatment.**




IVIg prepared according to the method described has no serious side effects.[27,170,264] The major drawback of IVIg treatment is its expense. Compared to plasma exchange, IVIg is more convenient to the patient and not more expensive. Corticosteroids are less expensive than IVIg, easier to administer, but the potential longterm side effects must be considered.[228]

Chapter 3

# INTRAVENOUS IMMUNOGLOBULIN TREATMENT IN PATIENTS WITH CHRONIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY

**Clinical and laboratory characteristics associated with improvement**

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## INTRODUCTION

Patients with chronic inflammatory demyelinating polyneuropathy (CIDP) may respond to corticosteroids[77] and to plasma exchange[81] which was shown in controlled studies. Improvement has also been reported after administration of azathioprine,[62,235] cyclophosphamide[62,96] and cyclosporin A.[142,150,307] Recently, several uncontrolled studies described a beneficial response to intravenous immunoglobulin (IVIg) treatment.[6,50,59,87,328] The beneficial effect of IVIg treatment in patients with CIDP was supported by the results of a double blind placebo controlled cross-over study.[322]
In this report we present our experience with IVIg treatment in 52 patients who were judged to have a CIDP. The aims of this study were to investigate what the proportion of CIDP patients is that improved after IVIg treatment, what the effect of IVIg is on the disability of these patients and which clinical and routine laboratory factors are associated with improvement.

## PATIENTS AND METHODS

### Patients

Clinical and laboratory records were reviewed for all patients who were judged to have a CIDP and who were treated with IVIg.
All patients included in this study fulfilled the criteria outlined by Dyck et al.[74]: (1) the course of the disease may be steadily progressive, chronic monophasic, or recurrent; (2) nerve conduction may be normal in motor and afferent fibres, but more commonly (though not

mandatory) it is slowed; (3) cyto-albuminologic dissociation is usually seen at some time during the course of the disease and most of our patients (4) had a tendency to symmetric involvement and to involvement of proximal as well as distal limb muscles.
We tried to exclude patients with the Guillain-Barré syndrome,[15] therefore all patients had the clinical features of a polyneuropathy with a progression of weakness of at least 2 months. Patients with known systemic disease were excluded in our series, except 4 patients with diabetes mellitus and 2 patients with a benign monoclonal gammopathy, type IgG-kappa and IgG-lambda, respectively.

**Clinical assessment**
Muscle strength was tested manually using the Medical Research Council (MRC) scale.[204] At least 6 muscle groups were tested on each side. These were muscles for: shoulder abduction, elbow flexion, wrist extension, hipflexion, knee extension and ankle dorsiflexion. Asymmetric weakness between left and right was defined as a difference of 2 or more grades on the MRC scale between at least 1 of the same muscle groups. Discrepancy in weakness between arms and legs, was defined as a difference in muscle strength of 2 or more grades on the MRC scale between ankle dorsiflexion and wrist extension.
The disability of the patients was assessed using the Rankin scale.[319] This scale estimates functional disability or handicap. Improvement was defined as an increase of at least 1 step on the Rankin disability scale.

**Treatment**
All 52 CIDP patients were treated with high-dose intravenous immunoglobulin (Central Laboratory of the Dutch Red Cross Blood Transfusion Service) in a dosage of 0.4 g/kg body weight/day for 5 consecutive days. If necessary, treatment was repeated. The 52 patients did not receive corticosteroids or other immunosuppressive treatment within the 2 months before, or together with IVIg treatment.

**Statistical analysis**
We investigated the association between treatment response and the following characteristics: sex, age less than 50 years, disease duration less than 1 year, absence of asymmetric weakness, absence of discrepancy in weakness between arms and legs, areflexia legs, areflexia arms, progression of weakness until 4 weeks or less before onset of treatment, remitting or progressive course of the disease, CSF protein more than 0.5 g/l in adults or more than 0.4 in children, CSF cell count $10.10^6$/l or less and slowed nerve conduction velocity (NCV) of the motor median nerve. The NCV of the median nerve was measured over the forearm with standard techniques, slowed NCV was defined as a NCV of less than 80% of the lower limit of normal. The lower limit of normal was 51 m/s.
For analysis of the association between each of the above mentioned characteristics and treatment response, the two-sided Fisher exact probability test was used. A logistic regression analysis was used for analyzing simultaneously the predictive value of all those characteristics which were significantly related with improvement.

## RESULTS

Fifty two patients (33 men and 19 women) were diagnosed as having CIDP over a study period of 8 years. The age ranged from 5 to 82 years (mean 48 years) at onset of CIDP in the group of patients who improved and from 10 to 76 years (mean 46 years) in the group of patients who did not improve.
The mean follow-up in the group of patients who improved was 4.1 years, median 3.1 years (range 8-251 months). The mean follow-up in the group of patients who did not improve was 5.7 years, median 3.6 years (range 16-295 months). Before IVIg was started, 9 of 52 patients (17%) had remissions and exacerbations. The mean and median time between onset of CIDP and IVIg treatment in the patients with a remitting course was 9 months (range 4-18 months). The mean time between onset of CIDP and IVIg treatment in the patients with the progressive form was 25 months, median time 6 months (range 2-247 months). The group of patients with a remitting course has a mean age of 36 years, median 31 years (range 10-64 years). The patient group with the progressive form has a mean age of 49 years, median 54 years (range 5-82 years).
Follow-up revealed lungcancer in 2 patients; in one patient 6 months, in the other patient 2 years after the diagnosis of CIDP was made and IVIg was started. The second patient died. One patient died from mesothelioma after 6 years of IVIg treatment. Two patients died after a myocardial infarction and one patient died from complications after abdominal surgery; these 3 patients received IVIg for a period of 2-3 years. All 6 patients had improved after IVIg, one had a remission after one treatment course, the other five needed repeated infusions to maintain the improved condition.

**Treatment response**
Thirty two (62%) of the 52 patients improved after IVIg. The improved condition could be maintained in 30 (94%) of these patients for a mean follow-up period of 4 years. There was no correlation between the course of CIDP (remitting or progressive) and improvement after IVIg. The mean time after onset of IVIg treatment to the initial signs of improvement was 5 days (range 3-8 days). The mean time to reach maximal improvement was 5 weeks (range 2 - 16 weeks).
The disability before treatment and the maximal score after treatment was measured with the Rankin score (Table 1). Four patients improved 1 grade, 10 patients improved 2 grades, 8 patients improved 3 grades and 10 patients improved 4 grades on the Rankin scale.
Twenty one of the 32 patients who improved (40% of all patients) needed intermittent IVIg treatment to maintain the maximal level of improvement. Initially, most of these patients received 0.4 g IVIg/kg bodyweight every other week. In 9 of these 21 patients the dosage of IVIg could gradually be tapered off to an average dosage of 0.25 g IVIg/kg body weight every other week, without clinical signs of relapse. This maintenance therapy of IVIg was administrated within 1.5 hours at an outpatient department.
In 2 of 32 patients, there was a shortlasting improvement (± 3 weeks) and subsequent IVIg infusion had no effect. Nine of the 32 patients who improved reached complete remission (17% of all patients). The median follow-up in patients in remission was 24 months (range 6-68 months). The only differences between patients who reached complete remission and the other patients who improved was the disease duration before treatment. The median time from the first signs of CIDP to the onset of IVIg in the patients who reached complete remission was

Table 1. **Minimal and maximal clinical grade in 32 CIDP who improved after IVIg treatment**

| Rankin Score | Before IVIg treatment no. | After IVIg treatment no. |
|---|---|---|
| 0 = asymptomatic | - | 7 |
| 1 = non-disabling symptoms which do not interfere with lifestyle | - | 18 |
| 2 = minor disability symptoms which lead to some restriction of lifestyle, but do not interfere with the patients capacity to look after themselves | - | 4 |
| 3 = moderate disability symptoms which significantly interfere with lifestyle or prevent totally independent existence | 12 | 3 |
| 4 = moderately severe disability symptoms which clearly prevent independent existence, although patient does not need constant attention day and night | 13 | - |
| 5 = severely disabled, totally dependent requiring constant attention day and night | 7 | - |

3 months (range 2-8 months), whereas the median time was 7 months (range 2-220 months) in the group of patients who improved but who needed intermittent IVIg treatment.

**Side effects of IVIg treatment**

Minor side effects such as headache nausea and fatigue were sometimes observed, mainly related to the speed of infusion. None of the patients developed hepatitis. Ten patients who had intermittent IVIg infusions for at least 2 years were tested for HIV; all were negative.

**Laboratory data**

The mean conduction velocity of the motor median nerve was 27 m/s (range 6-58 m/s) in the group of patients who improved and 44 m/s (range 11-61 m/s) in patients without improvement. The mean CSF protein was 1.08 g/l (range 0.35-3.41) in the group with improvement and 1.10 g/l (range 0.33-3.44) in the group without improvement. The mean number of cells in the CSF in both groups was $5x10^6$/l, the range in the group with improvement varied between 0-$20x10^6$/l, in the group without improvement it varied between 0-$27x10^6$/l.

Table 2. **Clinical and laboratory characteristics associated with improvement after IVIg treatment in 52 CIDP patients**

| | improved after IVIg | | P value* |
|---|---|---|---|
| | YES N=32 no (%) | NO N=32 no (%) | |
| Sex: male | 20 (63%) | 13 (65%) | ns |
| female | 12 (37%) | 7 (35%) | ns |
| Age <50 years | 12 (38%) | 11 (55%) | ns |
| Disease duration <1 year | 28 (88%) | 8 (40%) | 0.0005 |
| Progression of weakness until <4 weeks before onset of treatment | 32 (100%) | 14 (70%) | 0.002 |
| Remitting course | 6 (19%) | 3 (15%) | ns |
| Absence asymmetric weakness | 30 (94%) | 16 (80%) | ns |
| Absence discrepancy weakness between arms and legs | 30 (94%) | 8 (40%) | 0.0001 |
| Areflexia arms | 27 (84%) | 6 (30%) | 0.0001 |
| Areflexia legs | 28 (88%) | 14 (70%) | ns |
| NCV motor median nerve <80% of normal | 27 (84%) | 8 (40%) | 0.002 |
| CSF protein >0.50 g/l | 30 (94%) | 18 (90%) | ns |
| CSF cells $<10.10^6/l$ | 27 (84%) | 19 (95%) | ns |

* Fisher's exact probability test, 2 sided

**Factors associated with improvement**

Table 2 shows that 5 factors were significantly associated with improvement; disease duration < 1 year, progression of weakness until <4 weeks before onset of treatment, absence of discrepancy in weakness between arms and legs, areflexia of the arms and slowed NCV of the motor median nerve over the forearm.

Since the factor "progression of weakness until onset of treatment" was required for improvement, we excluded this factor from further analysis.

Using logistic regression, the other 4 factors significantly associated with improvement were analyzed and the predictive value of these factors combined was calculated (Table 3). This Table shows that if the factors "absence of discrepancy in weakness between arms and legs, disease duration less than 1 year, areflexia of the arms and slowed NCV over the motor median nerve" are present, the estimated probability of improvement is 93%. If none of these factors

Table 3. **Logistic regression model for chance of improvement after IVIg**

| Factor | | Odds ratio | Logistic coefficient | P-value |
|---|---|---|---|---|
| Absence of discrepancy in weakness between arms and legs | (a) | 6.664 | 1.897 | 0.076 |
| Disease duration less than one year | (b) | 6.234 | 1.830 | 0.041 |
| Areflexia arms | (c) | 4.138 | 1.420 | 0.102 |
| NCV motor median nerve < 80% of normal | (d) | 3.284 | 1.189 | 0.199 |

Probability of improving after IVIg if factors a, b, c and d are all present = $1 / (1 + e^{-y}) = 0.93$
$y = C_0 + C_a + C_b + C_c + C_d = 2.556$
$C_0$ = constant term = -3.780
$C_{a,b,c,d}$ are the logistic coefficients of factors a,b,c, and d

is present, the estimated probability is 2%.
The first 2 factors are the strongest predictive factors, but the last one "slowed motor median NCV" still has a clinically relevant contribution to the probability of improvement, since the Odds ratio is 3.284.

**Patients without improvement**
Twenty (38%) of 52 CIDP patients did not improve after IVIg treatment. Fifteen of these 20 patients were treated with prednisone, and 5 improved. After tapering off prednisone, one of these 5 patients was treated with Azathioprine which was followed by improvement. Five patients were not treated with prednisone; one patient showed a slow spontaneous improvement without further treatment and the 4 other patients were not treated with prednisone because of fear of side effects. One of the 2 patients with short lasting improvement after IVIg had a favorable response to prednisone. The second patient did not respond to prednisone, azathioprine or cyclophosphamide. This patient improved after plasma exchange and treatment with cyclosporin A. After two years, this patient is in Rankin scale 3.
We reviewed the case records and the follow-up information of the 20 patients who had not improved after IVIg, and tried to find a diagnosis other than CIDP. Two patients might have had a polyneuropathy of the type as described by McLeod,[202] and 1 patient might have vasculitis limited to the peripheral nervous system.[82] In 2 patients the polyneuropathy was associated with mild diabetes mellitus and in 1 patient with alcohol abuse.
Three patients had a polyneuropathy which could have been initially a neuropathy as described by Pestronk;[236] in sera of these 3 patients high titers of anti-GM1 antibodies were demonstrated. In one patient a hereditary polyneuropathy was likely. In the 10 other patients, no diagnosis other than CIDP could be established.

## DISCUSSION

This study shows that 38% of patients judged to have a CIDP did not improve after treatment with IVIg. A further 4% had a shortlasting improvement and subsequent infusions had no effect. A complete remission was reached in 17% which might have been caused by treatment but spontaneous remissions do occur and because some patients may have had a GBS like course even after progression of more than 8 weeks. Especially in this group of patients it is difficult to decide whether or not these patients had responded to treatment. A beneficial treatment response is more likely in the patients (40%) who improved after IVIg and who needed intermittent infusions to maintain the improved condition.

IVIg treatment was not accompanied by serious side effects. None of the patients developed hepatitis. In 10 patients who had been treated at regular intervals for at least two years, HIV antibodies could not be demonstrated.

One of the major advantages of treatment with IVIg in comparison with prednisone is that improvement begins immediately. This is clearly demonstrated when we compare our results with that of a study in 60 patients[23] treated with 100 mg prednisone daily for 2 to 4 weeks followed by an alternate-day single-dose of 100 mg until the clinical improvement reached a plateau phase. In that study the mean time for initial improvement was 1.9 months (after IVIg 5 days) and the mean time to reach a clinical plateau was 6.6 months (after IVIg 5 weeks).

To maintain the improved condition, 40% of the patients needed intermittent IVIg infusions every other week. We tried various schedules to reduce the IVIg dosage. We had more favorable results with tapering off the infusion dosage than with increasing the interval between the infusions. In 9 of 21 patients, the dosage could be reduced to 0.25 g IVIg/kg body weight every other week.

Maintenance treatment with IVIg is expensive. This treatment has to be compared with plasma exchange and to prednisone which is the standard treatment in CIDP. There is no study which shows what the maintenance dosage of prednisone usually is and what the side effects are of long term prednisone treatment in CIDP. It is reasonable to assume that the side effects of prednisone treatment in myasthenia gravis are not much different from CIDP. It has been estimated that approximately 25% of patients with myasthenia gravis develop serious side effects within 4 years of treatment with prednisone.[228]

Prolonged plasma exchange has not been associated with any complications except anaemia,[82] is about as expensive as IVIg treatment, but is inconvenient for the patient.

CIDP, according to the criteria outlined by Dyck et al,[74] is difficult to distinguish from some other neuropathies. The first neuropathy to consider has been described by McLeod et al.[202] This neuropathy is a slowly progressive axonal degeneration of undetermined cause. The characteristic history is that of a gradual onset of numbness, tingling, coldness or burning sensation in the feet and hands, weakness of the legs and less commonly weakness of the hands. Neurological examination may demonstrate distal muscle wasting and weakness in the lower limbs which may be quite pronounced. In McLeods' series of patients one third had areflexia of the legs, but total areflexia was less common. The CSF protein was often slightly elevated and the NCV's were slightly reduced and sometimes reached values which suggested a demyelinating neuropathy. If these patients are seen late in the course of the disease, distinction from CIDP may be difficult.

A second neuropathy that may be difficult to distinguish from CIDP is the non-systemic vasculitic neuropathy.[82] The course in these patients may be stepwise progressive and thereafter

static for a time. These patients may have severely affected legs and no or slight involvement of the arms.
Finally, CIDP may be confused with multifocal motor neuropathy. In the patients described by Pestronk et al[236] asymmetrical weakness developed in one arm and progressed over 2 to 3 years to involve the other arm, legs and trunk. High titers of antibodies to GM1 ganglioside were demonstrated in these patients. These patients do not necessarily have areflexia of the arms or slowed NCV of the motor median nerve over the forearms. Three of our patients had initially a similar course and in sera withdrawn at the beginning of the disease we could demonstrate high titers of antibodies to GM1. These three patients did not respond to IVIg.
We conclude that several factors which can be taken together as a progressive neuropathy of relatively short duration with clearly affected arms, are associated with improvement after IVIg treatment and that the presence of these factors may have consequences for the choice of treatment in patients with CIDP.

Chapter 4

# IMMUNOPATHOGENESIS OF CIDP

## INTRODUCTION

It is likely that the inflammatory demyelinating polyneuropathies are precipitated by antecedent illnesses.
In CIDP, the rate of antecedent infectious diseases is generally reported to be lower than in GBS.[14,79] However, Prineas et al.[246] reported various illnesses within a month before onset of neurological symptoms in 15 of 23 (65%) CIDP patients: non-specific upper respiratory tract infections in 11, varicella in 1 and flu-like symptoms in 3 patients. McCombe et al.[198] recently reported that 29 of 92 (32%) CIDP patients had a history of various preceding infectious diseases in the six weeks before onset of neuropathy. In this study a cytomegalovirus (CMV) IgG antibody titer >1:4 was found on 19 of 39 (49%) patients with CIDP, in 11 of 22 (50%) patients with GBS and in 2 of 25 (8%) healthy controls. The mean height of the CMV antibody titer was also found to be significantly increased in CIDP and GBS patients compared with controls. However, high titers of CMV antibodies can also be the result of (endogenous) reactivation or of polyclonal B cell activation elicited by another infection. Other antecedent events reported in CIDP patients are: hepatitis B virus,[139] HIV,[54] rubella,[226] and vaccinations with influenza[96] and tetanus toxoid.[110]
It has been investigated if HLA-linked genetic factors may influence susceptibility to CIDP or GBS. Immunogenetic studies have not demonstrated a human leucocyte antigen (HLA) association in GBS patients,[4,143,164,342] but in two series with a total number of 36 CIDP patients, an association with the HLA-A30/31,B8,DR3 haplotype was found.[5,290] Recently Feeney et al.[94] found increased frequencies of HLA-A3 and B7 in a group of 71 CIDP patients. In 56 CIDP patients, who were typed for DR and DQ they found that HLA-DR2 was increased. Although none of these findings reached statistical significance after correction, it was suggested that CIDP might be associated with HLA-DR2. The linkage between HLA-B7 and DR2 appeared much greater in patients with CIDP than in healthy controls.[94]
An association with other immune-mediated diseases or immune-compromised conditions is observed in both CIDP and GBS patients and has been discussed in Chapter 1.

Controlled studies showed that both CIDP[81] and GBS patients[100,108,302] can improve after plasma exchange, which suggests a pathogenic role for circulating antibodies. In different assays, circulating anti-neural tissue antibodies have been demonstrated. Animal models showed that clinical and laboratory symptoms resembling CIDP and GBS can be induced by both cellular and humoral transfer experiments.
As there is a limited understanding of the underlying events at the molecular level in neuropathy, the definite roles of antibodies and cellular immunity have not been established.

**Immunohistopathology**
The percentage of nerve biopsies showing inflammatory cell infiltrates is low (11-29%).[23,157] Usually, these small mononuclear cell infiltrates are perivascular located in either epi-, peri- or endoneurium. A non-perivascular, predominantly endoneurinal located inflammatory infiltrate may be present in CIDP. These infiltrates however should preferentially be located in the region of the nerve roots[242] and are therefore not often found in sural nerve biopsies.
In nerve biopsies from 6 CIDP patients, it was found that HLA class II antigens were expressed within nerve fascicles and the perineurium,[241] even in the absence of an inflammatory infiltrate in the perineurium.[242] Schwann cells forming onion bulbs were clearly HLA class II positive, suggesting that Schwann cells in patients with CIDP may act as antigen presenting cells.
Immunoglobulin deposits have been shown in peripheral nerve tissue of patients with CIDP.[124,225] Using a direct-immunofluorescence assay (IFA), Dalakas and Engel[60] demonstrated linear deposits of IgM immunoglobulin at the Schwann cell membrane of myelinated fibers in 6 of 9 CIDP patients, IgG was present in 3 of 9 biopsies whereas 10 control biopsies did not show IgG or IgM deposits. The specificity of immunoglobulin deposits in peripheral nerve tissue must be questioned, because these have also been demonstrated in polyneuropathies associated with diabetes mellitus,[53] carcinoma,[52] vasculitis,[124] IgM monoclonal gammopathy,[213] and also in other diseases such as acute hepatitis, idiopathic liver cirrhosis,[313] and in healthy controls.[53,69,84,180,295]

**Humoral immunity**
The presence of anti-neural tissue antibodies in patients with CIDP and GBS has been investigated extensively. As both neuropathies are probably different manifestations of one disease they were studied simultaneously in most assays.
As the putative antigen(s) in GBS and CIDP are still unknown, whole peripheral nerve, myelin, Schwann cells, other neural constituents and neural equivalents as neuroblastoma cell lines have been used for antibody detection.

**Antibodies to peripheral/central nerve tissue**
A technique to demonstrate antibody binding to nerve tissue is an immunofluorescence assay (IFA). In these experiments, patients' serum is incubated with non-pathological human or animal peripheral nerve tissue. In most reports, GBS sera were evaluated. The technique is complicated by aspecific binding of immunoglobulins resulting in a high incidence of positive controls.
Vedeler et al.[324] who first described a mixed hemagglutination test (MHT) to demonstrate anti-human peripheral nerve tissue antibodies, found that a positive MHT in GBS and CIDP patients was associated with a beneficial response to plasma exchange.[325] After studying an extended group of patients, Osterman et al.[229] could not confirm their initial observations.
The results of various assays to detect anti-peripheral nerve antibodies are shown in Table 1.

Table 1. Antibodies in serum directed against peripheral nerve tissue

| Author | year | test | GBS | | CIDP | | controls | | | | | | antigen |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | normals | | pnp | | non-pnp | | |
| | | | N | pos % | N | pos % | N | pos % | N | pos % | N | pos % | |
| Tse[313] | 1971 | IFA | 6 | 67 | - | - | 35 | 3 | - | - | 25 | 8 | h/rat/gp mo/rab |
| Luijten[186] | 1972 | IFA | 6 | 50 | - | - | 1 | 0 | 4 | 0 | - | - | h |
| Dowling[69] | 1973 | IFA | 40 | 70 | - | - | - | 15 | - | - | 37 | 22 | mo |
| Novak[223] | 1973 | IFA | 6 | 83 | - | - | 5 | 20 | - | - | - | - | mo |
| Schott[272] | 1978 | IFA | 20 | 65 | - | - | 23 | 13 | - | - | - | - | h |
| Swash[295] | 1979 | IFA | - | - | 2 | 0 | 1 | 0 | 4 | 50 | - | - | h |
| Dalakas[60] | 1980 | IFA | - | - | 7 | 100 | 1 | 0 | 1 | 0 | - | - | h |
| Nyland[225] | 1981 | IFA | 4 | 25 | 4 | 50 | - | - | - | - | - | - | h |
| Toyka[311] | 1982 | IFA | - | - | 1 | 100 | - | - | - | - | - | - | h |
| Hays[124] | 1988 | IFA | 2 | 50 | 4 | 25 | - | - | 93 | 10 | 2 | 0 | h |
| Nyland[224] | 1978 | GCA | 40 | 38 | 4 | 25 | 40 | 10 | - | - | 22 | 0 | h |
| Vedeler[327] | 1988 | ELI | 90 | 59 | 36 | 19 | 63 | 8 | 42 | 14 | 105 | 8 | h |
| Vedeler[324] | 1982 | MHT | 42 | 36 | 9 | 22 | 20 | 0 | - | - | 12 | 0 | h |
| Vedeler[325] | 1982 | MHT | 11 | 55 | 7 | 71 | 20 | 0 | - | - | - | - | h |
| v Doorn[320] | 1987 | MHT | 16 | 69 | 22 | 50 | 25 | 0 | 34 | 3 | 71 | 11 | h |
| Osterman[229] | 1988 | MHT | 36 | 53 | - | - | 30 | 0 | - | - | - | - | h |

**assays:**
IFA = immunofluorescence assay
GCA = anti-globulin consumption assay or Coombs' consumption test
ELI = enzyme linked immunosorbent assay (ELISA)
MHT = mixed hemagglutination test

**controls:**
normals = healthy controls; pnp = patients with a non-GBS/CIDP polyneuropathy;
non-polyneuropathy = patients with various other diseases; - = not indicated

**antigen:** nerve tissue from h = human; gp = guinea pig; mo = monkey; rab = rabbit

## Complement-activation

The first complement fixation (Cf) assay using nerve tissue was described by Melnick in 1963.[207] Since then, several investigators tested for complement activation with various substrates (Table 2). Koski et al.[153](1985) used a C1q-fixation assay with peripheral nerve myelin extract and found a high percentage of positive reactions in sera from GBS and CIDP patients; controls were also often positive but they generally had lower antibody titers. Since Koski et al.[153] found up to fifty-fold increased titers in GBS sera obtained early during the course of active disease, differences in results among investigators might be related to the timing of serum withdrawal. A terminal complement (C5-C9) fixation assay by Koski et al.[155] showed a similar high incidence of positive results compared with the C1q fixation assay.[153]
The results of complement fixation assays in vitro seem compatible with CSF findings regarding complement activation in vivo. Consumption of C3 and C4 was not found in serum from both GBS[9,269,309,340] and CIDP[269] patients. One publication reports activation of C3a and C5a in CSF and not in serum from GBS patients.[119] Amarenco et al.[9] found C3 consumption in CSF during

Table 2. **Complement fixation (Cf) assays**

| Author | year | complement component measured | GBS | | CIDP | | controls | | | | | | antigen |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | normals | | pnp | | non-pnp | | |
| | | | N | pos % | N | pos % | N | pos % | N | pos % | N | pos % | |
| Melnick[207] | 1963 | CH50 | 38 | 50 | 14 | 43 | 183 | 3 | 34 | 26 | 1035 | 6 | p/c-h |
| Dalakas[60] | 1980 | C3 | - | - | 7 | 86 | 1 | 0 | 1 | 0 | - | - | p-h |
| Latov[163] | 1981 | CH50 | 11 | 27 | 9 | 22 | 8 | 0 | 45 | 9 | 74 | 0 | p-h/rab |
| Nyland[225] | 1981 | C3b | 5 | 40 | 3 | 67 | 10 | 0 | - | - | - | - | p-h |
| Hughes[132] | 1984 | CH50 | 17 | 12 | 11 | 9 | 19 | 0 | 20 | 0 | 15 | 0 | p-h |
| | | | 17 | 29 | - | - | 19 | 0 | 20 | 0 | 15 | 7 | p-gp |
| Ryberg[262] | 1984 | CH50 | 18 | 50 | - | - | - | - | - | - | - | - | c-h |
| | | | 18 | 56 | - | - | - | - | - | - | - | - | p-h |
| Ryberg[263] | 1984 | CH50 | 19 | 37 | - | - | - | - | 12 | 8 | 50 | 2 | c-h |
| | | | 19 | 21 | - | - | - | - | - | - | - | - | p-h |
| | | | 19 | 42 | - | - | - | - | - | - | - | - | p/c-h |
| | | | 19 | 16 | - | - | - | - | - | - | - | - | c-h |
| Koski[153] | 1985 | C1 | 12 | 92 | 4 | 75 | - | - | 12 | 50 | 7 | 43 | p-h |
| Koski[154] | 1986 | C1 | 7 | 100 | - | - | 15 | 20 | 3 | 100 | 25 | 8 | p-h |
| Koski[155] | 1987 | C5b-9 | 19 | 100 | 7 | 86 | 10 | 0 | 5 | 80 | 12 | 42 | p-h |
| Hays [124] | 1988 | C3b | 2 | 100 | 4 | 50 | - | - | 93 | 13 | 2 | 0 | p-h |
| Osterman[229] | 1988 | CH50 | 36 | 83 | - | - | 50 | 0 | - | - | - | - | p-h |
| Winer[340] | 1988 | CH50 | 100 | 7 | - | - | 100 | 1 | - | - | - | - | p-h |

**antigen:**
p=pns; c=cns; h=human; gp=guinea pig; rab=rabbit

the course of GBS and Sanders et al.[270] showed that the terminal attack complex (C5b-C9) was activated in the CSF of GBS patients.
These findings suggest that complement-binding antibodies are in vivo present in GBS and that these antibodies are likely to activate the terminal complement attack complex.
In 1989, Koski et al.[153] reported that C1q-fixating anti-peripheral nerve myelin antibodies are directed against a neutral glycolipid which can be absorbed with the Forssman antigen. This observation may have important implications in further research on the antigen(s) involved in the pathogenesis of the inflammatory demyelinating polyneuropathies.

**Antibodies to cultured cells**

Cell cultures can be used to identify more specifically the pathological events in the inflammatory demyelinating polyneuropathies. Since myelin of the peripheral nervous system is produced by Schwann cells, these cell cultures were considered as models for demyelination. Unfortunately Schwann cell clones or lines are not yet available and cultures are to a variable extend contaminated with collagen tissue or with fibroblasts. Results of studies using various cultured cells are shown in Table 3.
When cultured Schwann cells, obtained from rat peripheral nerves, are incubated with sera from patients with various diseases, weak immunoglobulin staining of both Schwann cells and fibroblasts could only be observed during the first days of culture when 30-40% fibroblasts were present. The meaning of this finding is not clear, but it was suggested that this could be the result of dedifferentiation of Schwann cells, resulting in a change of the antigenic expression

Table 3. **Antibodies and functional investigations against cultured cells**

| Author | year | test | GBS | | CIDP | | controls | | | | | | antigen |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | normals | | pnp | | non-pnp | | |
| | | | N | pos % | N | pos % | N | pos % | N | pos % | N | pos % | |
| **Neuroblastoma cells** | | | | | | | | | | | | | |
| Rosenberg[256] | 1975 | tox | 4 | 100 | 2 | 100 | 6 | 1 | - | - | 16 | 0 | mouse C1300 |
| Tindall[305] | 1980 | tox | 10 | 80 | - | - | 166 | 2 | - | - | 14 | 0 | mouse C1300 |
| Müller[216] | 1984 | RIA | 11 | 55 | - | - | - | - | - | - | 58 | 50* | human SH-SY5Y |
| v Doorn[321] | 1988 | IFA | 48 | 42 | 42 | 43 | 40 | 0 | 43 | 7 | 85 | 5 | rat-mouse 108cc15 |
| **Schwann cells** | | | | | | | | | | | | | rat nerve |
| Kennedy[146] | 1979 | IFA | 5 | 100 | 4 | 100 | 3 | 100 | - | - | 6 | 100 | sw 70% |
| | | | 5 | 0 | 4 | 0 | 3 | 0 | - | - | 6 | 0 | sw 100% |
| **Dorsal root ganglia** | | | | | | | | | | | | | |
| Cook[52] | 1971 | tcd | 31 | 84 | 2 | 0 | 11 | 9 | 16 | 31 | 23 | 39 | mouse |
| Dubois[71] | 1971 | tcd | 6 | 83 | - | - | - | - | - | - | - | - | rodent |
| Hirano[131] | 1971 | tcd | 6 | 100 | - | - | 11 | 9 | - | - | - | - | mouse |
| Birchem[28] | 1987 | tcd | 10 | 40 | - | - | - | - | - | - | - | - | rat |
| **Peripheral nerve** | | | | | | | | | | | | | |
| Arnason[13] | 1969 | tcd | 5 | 0 | - | - | - | - | - | - | - | - | rat |

assays:
tox = cytotoxicity assay
RIA = radio-immuno assay
IFA = immunofluorescence assay
tcd = tissue culture demyelination
sw = Schwann cell culture (70%= 70% Schwann cells and 30% fibroblasts)
* = 21/33 (64%) patients with myasthenia gravis were positive

during culture.[146] The Schwann cell cytotoxicity assay reported by Lisak et al.[181] did not show differences between serum from GBS and controls.
In tissue culture experiments with myelinated rodent spinal ganglia cultures, evidence of a serum factor was found in GBS patients showing a complement-dependent demyelination[71,131] which diminished or disappeared with serum obtained later in the course of GBS.[131] In cultures of mouse dorsal root ganglia, a cytotoxic effect and complement-dependent demyelination was found in serum from 26 of 31 GBS patients.[52] However, such serum factor was not found in 2 patients with CIDP neither was it specific for GBS as it was present in sera from patients with other polyneuropathies and other neurologic disorders as well.[71,131] Complement dependent myelinotoxic activity against rat cerebellar cultures was found in serum from patients with GBS and multiple sclerosis.[31]
In 1987, Birchem et al.[28] showed with electron microscopic studies that serum from 4 of 10 GBS patients in the presence of complement and in the absence of mononuclear cells, caused

a direct myelin-related Schwann cell lysis in cultures containing pure rat Schwann cells and dorsal root ganglion neurons. In addition they found that serum from recovered GBS patients had no cytolytic activity to neurites or Schwann cells.[28] Arnason et al.[13] found demyelination of rat trigeminal nerve cultures after incubation with lymphocytes derived from nerve-immunized rats but also with lymphocytes from 7 of 9 GBS patients, whereas lymphocytes from 5 control patients, sera from 5 GBS patients and sera from nerve immunized rats did not induce demyelination. The results of tissue culture demyelination experiments are rather confusing as is illustrated by Silberberg et al.[281] who found that sera from all 20 normals and even 4 agammaglobulinemic patients induced some demyelination of mouse cerebellum cultures.
Other cells which have been used for the detection of anti-neural tissue antibodies are neuroblastoma (NBL) cells. NBL is a tumor of neural crest origin. Because NBL's are malignant cells, they express various oncofetal antigens. Most primitive neuroblastic cells can still differentiate in Schwann cells and in neuronal cells. Neuroblastomas are a heterogenous group of neoplasms, they usually have the ability of forming neurites, they have electrically excitable membranes, possess neurohormone receptors and produce multiple hormones and enzymes.[115]
Rosenberg et al.[256] using a complement dependent cytotoxicity assay, found a high incidence of antibodies in sera from CIDP and GBS patients directed against a cholinergic and a non-cholinergic neuroblastoma cell line, which were originally cloned from an Ajax C1300 mouse NBL tumor. Immunofluorescence studies showed evidence of both IgM and IgG antibodies. Reactivity was not found against a NBL cell line of human origin. The high incidence of antibodies against these mouse NBL cells was confirmed by Tindall et al.[305] using an antibody dependent cytotoxicity assay. Müller et al. reported that 6 of 11 (55%) of GBS patients[216] and 44 of 109 (40%) of patients with myasthenia gravis[217] had antibodies against the human NBL cell line SH-SY5Y. Many different NBL cell lines - expressing various neural antigens - are available and apparently express antigens showing reactivity with sera from various neurological diseases[217] (Chapter 7).
Hybrid cell lines sometimes express certain antigens even stronger than the parental lines did. We used the hybrid mouse neuroblastoma/rat glioma NBL cell line 108cc15. On the ion excitable membrane of the 108cc15 cell line, many neural properties are expressed.[115]
Clonal cell lines offer the advantage of an unlimited supply of standardized antigens, and are therefore a feasible tool to study the interactions with anti-NBL antibodies and IVIg.

**Antibodies to subcellular fractions of neural tissue**
Many studies have been performed regarding to antigen(s) which might be involved in the pathogenesis of the inflammatory demyelinating polyneuropathies (Table 4).
The peripheral nerve contains glycoproteins and glycolipids that may act as antigenic determinants involved in GBS and CIDP. Three proteins $P_0$, $P_1$, and $P_2$ account for approximately 70% of peripheral nervous system (PNS) myelin proteins. $P_0$ is the major protein and is restricted to the PNS. $P_1$ is probably identical to myelin basic protein (MBP) and is also found in the central nervous system (CNS), but $P_2$ is predominantly restricted to the PNS. Galactocerebroside is a major part of lipid constituents of both CNS and PNS myelin.[336] Other structures of importance are glyco(sfingo)lipids.
Luijten et al.[187] found evidence for $P_2$ specific antibody secreting cells in GBS patients using a plaque forming assay. However, antibody studies with serum from patients with CIDP or GBS against antigenic neural structures such as $P_2$[188,340,351] galactocerebroside,[260,340] or myelin basic protein (MBP)[351] were found incidently positive. Antibodies against the myelin associated

Table 4. **Antibodies against fractions of neural tissue**

| Author | year | test | GBS | | CIDP | | controls | | | | | | antigen |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | normals | | pnp | | non-pnp | | |
| | | | N | pos % | N | pos % | N | pos % | N | pos % | N | pos % | |
| Zweiman[351] | 1983 | ELI | 19 | 5 | 11 | 0 | 12 | 0 | 9 | 0 | - | - | P2-bov MBP-gp |
| Sato[271] | 1986 | ELI | 6 | 50 | 3 | 33 | 18 | 0 | 3 | 67 | 52 | 27 | c-MAG-h |
| Winer[340] | 1988 | ELI | 100 | 34 | - | - | 100 | 17 | - | - | - | - | p-gal-h |
| | | | 1 | 0 | - | - | 10 | 0 | - | - | - | - | P2-h |
| Luijten[187] | 1988 | ELI | 20 | 0 | - | - | - | - | - | - | - | - | P2-h |
| Luijten[186] | 1984 | PFA | 5 | 100 | 2 | 0 | 3 | 0 | - | - | - | - | P2-h |
| Nobile[222] | 1985 | RIA | 9 | 0 | 7 | 0 | 8 | 0 | 30 | 43 | 9 | 0 | c-MAG-h |
| Rostami[261]* | 1987 | RIA | 17 | ? | 11 | ? | 6 | ? | 6 | ? | 18 | ? | c/p-gal |
| | | RIP | 6 | ? | - | - | 16 | ? | - | - | 45 | ? | |
| Ilyas[134] | 1988 | TLC | 26 | 19 | - | - | 10 | 0 | - | - | 19 | 0 | glyc lip h nerve |
| Winer[340] | 1988 | Cf | 100 | 0 | - | - | 100 | 0 | - | - | - | - | gal-h |
| Koski[155] | 1989 | Cf | 12 | 100 | - | - | 25 | 28 | - | - | 11 | 45 | Forssman |

* No difference found between GBS, CIDP and controls; percentage of positive sera is not mentioned

**assays:**
ELI= enzyme linked immunosorbent assay; PFA= plaque forming assay; RIA= radio-immuno assay; RIP= radio-immuno precipitation assay; TLC= thin layer chromatography; Cf = complement fixation assay

**antigen:**
p=pns; c=cns; h=human; bov=bovine; gp=guinea pig; MBP=myelin basic protein; MAG=myelin associated glycoprotein; gal=galactocerebroside; glyc lip=glycolipids;

glycoprotein (MAG) are associated with polyneuropathy in patients with paraproteinemia.[163,222] The carbohydrate-determinant of MAG cross-reacts with the leu 7-antigen (recognized by HNK-1/L2 monoclonal antibodies) that is expressed on natural killer cells and therefore may have functional implications for anti-MAG antibodies in cellular immunity.[67] The MAG antigen belongs to the immunoglobulin superfamily of which several members are expressed exclusively or abundantly in the nervous system and include the N-CAM (neural adhesion molecules). Anti-MAG antibodies have been demonstrated in patients with GBS, CIDP but also in stroke patients and some healthy controls.[200,271] The presence of anti-MAG antibodies in GBS and CIDP could not be confirmed by others.[222] Antibodies to glycolipids[248] and gangliosides[134,236] are often observed in different neuropathies, mainly in those associated with (oligo) clonal IgM production but also in CIDP, as well as in other neurological diseases. Antibodies against gangliosides may arise as the result of (mechanical) nerve damage.[36] Anti-glycosphingolipid antibodies can be detected with thin layer chromatography (TLC) and by Elisa.[135] Using TLC, high levels of IgM antibodies reacting with GD1a and GT1b and IgG antibodies to GD1b gangliosides have been found in 4 of 26 of GBS patients.[135] Antibodies of the IgG and IgM class against GM1 and GD1b have been demonstrated in 1 of 15 CIDP patients.[134,248] Anti-GM1 antibodies have been especially found in other diseases as multifocal motor neuropathy, amyotrophic lateral sclerosis (in lower titer), multiple sclerosis and in systemic lupus.[236,280]

Antibodies of the IgG class to LM1 ganglioside, the major ganglioside of human peripheral nerve myelin, was shown in 1 of 26 GBS patients.[134,248] Antibodies against LM1, GD1a, GD1b and GT1b were neither present in 19 patients with other neurological diseases, including various polyneuropathies nor in 10 normal controls.[135]
Koski et al.[156] reported that the complement fixing antibodies in GBS serum, which are directed against peripheral nerve myelin extract also bind a neutral glycolipid present on nerve tissue. These antibodies could be absorbed by Forssman glycolipid. Forssman antigen is a common non-specific viral and bacterial antigen. Anti-glycolipid antibodies have been demonstrated in many (autoimmune) diseases. Therefore, the pathogenicity of these antibodies in GBS and CIDP remains to be shown.
Cardiolipin antibodies of the IgA class have been found in 23% of patients with GBS.[97] It is suggested that antibodies to cardiolipin cross-react with myelin phospholipids.[97] Anti-cardiolipin antibodies of the IgG and IgM class are often detected in various neurological[32,174] and other autoimmune diseases,[174] but may also be found in GBS.[232] Anti-cardiolipin antibodies were found in 30% of patients with variety of uncomplicated infections. Far reaching conclusions about the pathogenetic significance of cardiolipin antibodies should not be drawn as the antibodies may be an indicator of a preceding infection.

**Immune complexes**
Circulating Immune complexes have been infrequently identified in CIDP patients[320,321] and are analogous to cardiolipin antibodies presumably related to a triggering infectious event.[43]

**Passive transfer of antibodies in animals**
Passive transfer demyelination (ptd) experiments were performed with sera from GBS/CIDP patients, as well as with serum or lymphocytes from animals with (chronic) experimental allergic neuritis.
Intraneural injection of GBS serum into rat sciatic nerve produced microscopic signs of demyelination.[90,91,268] A conduction block was observed after perineural,[118] but not after intraneural injection of serum from GBS patients.[53,183,340] Microscopic signs of demyelination were less pronounced after storage of serum,[183,238,298] but even stored GBS serum showed more distinguished microscopic signs of demyelination than fresh control serum did.[90,91,268]
Nerve conduction studies confirmed that mainly fresh serum of GBS patients obtained during the active phase of the disease blocked nerve conduction in contrast to frozen serum or fresh serum obtained during the recovery phase.[118]
Only selected animals could be used to study the pathogenic role of antibody transfer experiments, which may be related to antigenic differences or variation of the blood-nerve barrier in different animals. Heiniger et al.[126] investigated marmoset monkeys and found that intramuscular injections of immunoglobulins or purified IgG from 5 CIDP patients responding to PE, resulted in a 24-42% reduction of the initial sciatic motor nerve conduction velocity (NCV). Significant reduction of NCV, was only observed with immunoglobulins from the 5 patients responding to PE and not with serum from a non-responding patient. After the injections had been stopped, reduction of NCV was partially reversible. Although NCV decreased, muscle weakness and ultrastructural myelin changes were very mild.
In conclusion, passive transfer demyelination experiments show results depending on the way of transmission and the species studied. Several investigators could find the presence of a myelinotoxic factor in serum which was affected by storage. The cytotoxic effect on myelin was mainly observed with serum derived from GBS patients in the acute stage of the disease.

Transfer experiments with lymphocytes from GBS (and CIDP) were not successful in transmission of the disease.[90]

**Cellular immunity**

Cellular immunity in vitro was evaluated by lymphocyte transformation tests (LTT) upon stimulation with nerve extracts, myelin basic protein (MBP) or the purified myelin $P_2$ protein. After exposure to peripheral nerve protein $P_2$, LTT's showed proliferation of peripheral blood lymphocytes (PBL) withdrawn in the active phase of GBS[3,104,218,277] or during the course of CIDP.[151,177] Continued activity to peripheral nerve antigen was sometimes observed even several years after recovery of GBS,[148] but others found loss of proliferation during recovery of GBS[24] or no stimulation at all of PBL's from CIDP or GBS patients after exposure to $P_2$ or MBP.[133,351] LTT's also showed proliferation in the presence of an extract of peripheral nerve in 19 of 30 (63%) GBS patients[58,105,203] and only in 1 of 14 (7%) from patients with a polyneuropathy of other origin.[58]

Evidence of cell-mediated immunity is mainly derived from animal models for GBS and CIDP.

**Experimental models for CIDP**

There are several animal models for inflammatory demyelinating polyneuropathies. Experimental allergic neuritis (EAN) represents the features of GBS.[14,291] EAN can be produced in a number of species by injection with whole peripheral nerve tissue homogenates,[331] myelin,[284] $P_2$ protein,[35,259] peptides of $P_2$, galactocerebroside[267] or by transfer of a $P_2$ specific T cell line upon Lewis rats.[178]

If myelin is injected in animals to induce EAN, the antibodies that generally arise are directed against galactocerebroside.[36] It was found that EAN rabbits treated with plasma exchange[10,112] developed less severe disease than controls.

Chronic EAN as a model for CIDP could be induced in Lewis rats by inoculation of whole bovine dorsal root with Freund's complete adjuvant.[57] New Zealand white rabbits, inoculated with a single dose purified bovine peripheral nerve myelin and Freund's adjuvant developed a chronic relapsing or progressive EAN with slowed nerve conduction velocity, reduction of the compound muscle action potential (CMAP), and onion bulb formation with signs of active demyelination.[120] Recently, Harvey et al.[122] investigated these New Zealand white rabbits and showed that plasma exchange using IgG immunoabsorption columns resulted in a significant beneficial, but shortlasting (2 days) clinical response. Infusion of rabbit fresh frozen plasma in these animals also resulted in clinical improvement.[123] These findings in chronic EAN suggest at least an additional involvement of pathogenic antibodies.

Leucocyte associated cytokines or ion-channel blocking agents have not been studied intensively in GBS of CIDP. In EAN rats it was shown that the $K^+$ channel blocker quinidine caused a significant reduction in weakness and neural inflammatory infiltrate if it was injected simultaneously with the induction of EAN. Single injections had no effect. Therefore it was suggested that ion channel blocking agents may exert some immunomodulatory effect.[212] Recently it was shown that tumor necrosis factor (TNF) could play a role in the proces of demyelination.[33,274]

**IVIg treatment**

IVIg is effective in many autoimmune diseases. Different immunomodulatory actions of IVIg have been demonstrated which may lead to immediate and long-term effects.[221] In autoimmune hematocytopenia's the increase of peripheral blood cell counts is attributed to the blocking[95]

or loss[159] of Fc-receptors on mononuclear-phagocytic cells. Blocking of these receptors can be mediated by dimers in IVIg,[300] anti-HLA class I and II antibodies,[220] and by anti-monocyte antibodies.[220] Monocyte functions can also be blocked by binding of antibody coated red blood cells.[18,233]
Other immediate effects of IVIg are the neutralization of pathogenic antibodies and circulating immune complexes.[194] This was demonstrated in vivo and in vitro for anti F VIII-c antibodies by Sultan et al.[293,294] In addition, there is evidence of IL-1 inhibition by IVIg treatment.[141] In patients with Kawasaki disease the beneficial effect of IVIg was associated with a reduced release of Il-1 from the patients' monocytes.[173]
Longterm effects induced by IVIg may occur by modulation of the cellular and humoral immune response. Such mechanisms are supported by the observation that the serum IgM immunoglobulin level in the majority of ITP patients significantly but transiently increased after IVIg treatment.[136] A suppressive effect of IVIg by interference with B cells was observed in vitro after pokeweed stimulation to induce antibody production.[66] Interaction with idiotype specific B cells was found in vivo by Sultan et al.[293] who demonstrated that anti-F VIII-c antibody activity disappeared and anti-idiotypic antibodies against F VIII antibodies increased after IVIg treatment. These results demonstrate that IVIg can exert improvement in autoimmune diseases by interference with cellular and humoral immune mechanisms as well as with release of cytokines.

## CONCLUSION

The beneficial response to plasma exchange, the demonstration of anti-neural tissue antibodies, the demyelinating activity of patients' serum in purified cultures of Schwann cells and dorsal root ganglions, together with results of passive transfer experiments in animals, suggest that a primary or secondary humoral immune-mediated response, resulting in complement activation and possibly antibody dependent cellular cytotoxicity (ADCC) is involved in the pathogenesis of the inflammatory demyelinating polyneuropathy. A role for primary cellular immunity leading to secondary antibody formation is not excluded.
Westfall and Bernstein[334] proposed a dual infection hypothesis for post-infectious neuropathies, in which a neuritogenic micro-organism and an infection which produces adjuvant material must act simultaneously to cause nerve damage. In the light of this dual antigen theory, the recent finding of circulating antibodies to glycosfingolipid (GSL) antigens in patients with GBS and CIDP is interesting because GSL are highly expressed on neural tissue and have shared antigens with many micro-organisms. Additional support for a dual antigen hypothesis was obtained from EAN induced by bovine $P_2$, that was enhanced when $P_2$ was combined with bovine gangliosides.[297] These experiments may indicate that gangliosides can serve as natural adjuvant for the induction of inflammatory demyelinating polyneuropathies.
The purpose of our study was to acquire insight in the mechanism of action by which IVIg therapy induces improvement in patients with CIDP. The availability of a clonal cell line (NBL 108cc15) expressing antigens reacting with sera from CIDP patients allowed us to study the interaction of IVIg in vitro.

Chapter 5

# CLINICAL SIGNIFICANCE OF ANTIBODIES AGAINST PERIPHERAL NERVE TISSUE IN INFLAMMATORY POLYNEUROPATHY

## INTRODUCTION

At least a proportion of patients with monophasic Guillain-Barré syndrome (GBS)[302] and some patients with chronic inflammatory demyelinating polyneuropathy (CIDP)[81] show a beneficial response to plasmapheresis. This might indicate that a subgroup of these inflammatory demyelinating polyneuropathies (IDP) are antibody-mediated disorders. Using various techniques, antibodies against peripheral nerve tissue (PNT) have been demonstrated in sera from GBS patients, especially during the first four weeks after onset of the neurological symptoms.[152,224,305,313,326,324] However, some of these antibody assays are not specific for inflammatory polyneuropathies, as antibody activity was also detected in patients with other polyneuropathies.[52,69,107,132,163,181,207,272] This suggests that antibodies may also occur as the result of nerve tissue damage exposing immunogenic structures. Vedeler et al.[325] using a Mixed Hemagglutination Test (MHT) found antibodies against PNT in 6 of 11 GBS and in 5 of 7 CIDP patients. Only patients with these antibodies responded to plasmapheresis. The antibodies detected by this technique apparently have a pathogenic role, but this MHT has not been investigated in extended populations of patients. We tested the sera of patients with various causes of polyneuropathy, of patients with neurological disorders other than polyneuropathy, of patients with immune-mediated diseases without nervous system complications and of healthy controls. We also investigated whether antibodies detected by the MHT are exclusively directed against PNT. In addition, we evaluated whether a positive test result predicts a beneficial response to treatment with intravenous immunoglobulin (IVIg) in CIDP patients.

## PATIENTS AND METHODS

**Mixed hemagglutination test (MHT)**
The MHT, described by Tönder[308] and Portanova[243] was slightly modified. The procedure was as follows:

a. *Tissue*
Human post-mortem sciatic nerve, brain, liver, striated muscle and renal tissue was obtained within twelve hours of cardiac death in patients who had no evidence of other diseases. Pieces were snap frozen in liquid nitrogen and stored at -70°C until cryostat tissue sections were cut. No fixatives were used.

b. *Indicator system (EApA)*
The following cells and reagents were used. Sheep red blood cells (SRBC or E), rabbit anti-SRBC antibodies (A) (Sera-lab, Sussex, England) and protein-A (pA) (Pharmacia, Woerden, the Netherlands). All incubations and washings were carried out at room temperature (20-25°C). All dilutions were performed with phosphate buffered saline (PBS) pH 7.2. 10 ml 1% washed SRBC was incubated with 1/4 of the agglutination titer of rabbit anti-SRBC (A) for 30 min., thereafter the EA were washed. EA 1% were incubated with an excess of pA (50 µg/10 ml EA 1 %) for 30 min. and thereafter washed and resuspended to 0.75% EApA.

c. *Incubation tissue section with serum dilutions*
Before testing, sera were heat inactivated for 30 min. at 56°C. Tissue sections were incubated in a moist chamber for 30 min. with 25 microliter of serum dilutions and subsequently carefully washed three times with excess PBS.

d. *Incubation with indicator system (EApA)*
± 0.5 ml EApA 0.75% was incubated with the presensitized tissue sections for 30 min. in a closed chamber allowing the EApA indicator system to sediment on the tissue section. The closed chamber was then carefully turned upside down to allow unbound EApA to sediment to the bottom of the glass well. Microscopic evaluation of adsorbed EApA was performed after 30 min.

e. *Evaluation*
Scoring was performed with a light-microscope (magnification 10 x 40). The MHT assays were always read without knowledge of the diagnosis and without knowledge of the treatment response. The MHT was considered positive, in accordance with Vedeler,[325] when distinct agglutinates could be identified on the tissue section using serum dilutions 1:16.

**Blocking studies**
Nerve tissue sections were pre-incubated for 30 min. with dilutions of purified heat-aggregated IgG immune complexes or sera containing high levels of Rheumatic Factor (IgG-IgM immune complexes). Subsequently the sections were washed and the MHT was carried out with patient or control sera as described above. 10 MHT positive and 10 MHT negative sera were tested.

**Immune complex assays**
Circulating immune complex analysis was carried out with the $^{125}$I C1q-Binding Assay,[349] the IgM Polyethylene glycol (Peg),[19] and the IgA Peg Assay.[317]

**Patients**
Using the MHT, sera from the following patient groups were tested for the presence of antibodies against peripheral nerve tissue: Patients with inflammatory polyneuropathy, polyneuropathy of other causes, neurological disorders other than polyneuropathy, immune complex and/or antibody-mediated diseases without neurological complications and blood bank donors.
"Inflammatory demyelinating polyneuropathy" was defined as a sensorimotor polyneuropathy in the absence of diabetes, uremia, paraproteinemia, connective tissue disease, porphyria, heavy metal intoxication and malignancy. The family history had to be negative for hereditary polyneuropathy. We distinguished acute monophasic Guillain-Barré syndrome (GBS)[14,15] from Chronic and/or Relapsing Inflammatory Demyelinating Polyneuropathy (CIDP).[74,79] In GBS the progression of weakness did not exceed four weeks, in CIDP the progression of weakness exceeded four weeks and/or had a relapsing course. Sera were withdrawn during the active stage of the disease and in CIDP patients before treatment was started.

**Intravenous immunoglobulin (IVIg) treatment**
21 patients with CIDP were treated with IVIg (human immunoglobulin, 90% monomeric IgG, for intravenous use, Central Laboratory Dutch Red Cross, Amsterdam): 0.4 g/kg body weight/day for five consecutive days. All patients were in a stable or deteriorating neurological condition for at least eight weeks

**Table 1. Antibodies against human sciatic nerve tissue, demonstrated by the Mixed Hemagglutination Test(MHT)**

| Diagnosis | N patients tested | No. patients with antibodies |
|---|---|---|
| Inflammatory Polyneuropathy | | |
| GBS | 16 | 11 (69%) |
| CIDP | 22 | 11 (50%) |
| Total | 38 | 22 (58%) |
| Polyneuropathy of other origin | | |
| Diabetes | 6 | 0 |
| Alcoholic | 4 | 0 |
| IgG-benign gammopathy | 2 | 0 |
| Paraneoplastic (Oatcell) | 1 | 0 |
| Borrelia burgdorferi | 1 | 1 |
| Unknown | 19 | 0 |
| Total | 34 | 1 (3%) |
| Neurological disorders, other than polyneuropathy | | |
| Multiple Sclerosis | 5 | 0 |
| Myasthenia Gravis | 12 | 2 |
| Lambert-Eaton Syndrome | 1 | 1 |
| Viral encephalitis | 1 | 0 |
| Dystrophia Myotonica | 1 | 0 |
| Amyotrophic Lateral Sclerosis | 2 | 0 |
| Miscellaneous (headache, arm or back pain) | 10 | 0 |
| Total | 32 | 3 (9%) |
| Immune complex and/or antibody mediated diseases without neurological complications | | |
| Rheumatoid Arthritis | 9 | 1 |
| Felty Syndrome | 11 | 4 |
| IgA vasculitis | 2 | 0 |
| Systemic Lupus Erythematodus | 2 | 0 |
| Type II Cryoglobulinemia | 1 | 0 |
| Goodpasture | 2 | 0 |
| M. Wegener | 1 | 0 |
| Multiple Myeloma | 4 | 0 |
| Other paraproteinemia's | 7 | 0 |
| Total | 39 | 5 (13%) |
| Blood Bank donors | 25 | 0 |

before IVIg was administered. The patients had no immunosuppressive treatment before IVIg infusion was started. A positive treatment response was recorded when the patient's neurological condition improved within three weeks after the onset of treatment and a negative response when there was no change or deterioration.

## RESULTS

The MHT on sciatic nerve tissue was carried out with sera from 38 patients with inflammatory polyneuropathy, 34 patients with other polyneuropathies, 32 patients with neurological disorders other than polyneuropathy,39 patients with immune complex and/or antibody mediated diseases without neurological complications and 25 healthy blood bank donors. The results are shown in Table 1.

In GBS patients with a positive test, the median duration between the onset of the disease and the bloodsample withdrawal time was 3 weeks (range 0,5 - 27 weeks) versus 4 weeks (range 2 - 15 weeks) in patients with a negative test.

In patients with a CIDP, this median time was 9 months (range 1 - 120 months) in patients with a positive test and 24 months (range 2 - 240 months) in patients with a negative test. These differences are not significant.

**Table 2. MHT against various tissues using sera from patients with an inflammatory demyelinating polyneuropathy.**

| | N patients | Test results: Sciatic Nerve | Brain | Kidney | Liver | Muscle |
|---|---|---|---|---|---|---|
| GBS | 3 | + | - | - | - | - |
| | 1 | + | - | + | - | - |
| | 1 | + | - | + | + | + |
| | 2 | + | - | + | + | - |
| | 4 | + | + | + | + | + |
| | 5 | - | - | - | - | - |
| Total | 16 | 11 | 4 | 8 | 7 | 5 |
| CIDP | 11 | + | - | - | - | - |
| | 11 | - | - | - | - | - |
| Total | 22 | 11 | 0 | 0 | 0 | 0 |

+=positive; -=negative

Table 2 shows the results of the same hemagglutination technique with the sera from 38 patients with inflammatory polyneuropathy when tested against other tissues. None of the 22 patients with CIDP had positive reactions against brain, liver, kidney or striated muscle, neither had the five patients with GBS without antibodies against sciatic nerve. In contrast, 8 of 11 GBS sera that showed a positive MHT with sciatic nerve also had positive reactions with one or more other organ tissues.

**Blocking studies**

Blocking experiments were performed to investigate whether Fc- or C3-receptors present on peripheral nerve tissue played a role in the positive reactions with sera from patients with inflammatory polyneuropathy and in the positive reactions with sera from patients with Felty syndrome. Preincubation of PNT with purified aggregated IgG or with sera containing high levels IgG-IgM immune complexes (Rheumatoid Factor) neither changed the results of 10 MHT positive GBS or CIDP sera nor the results of 10 negative sera (not shown).

**Immune complex assays**

One of the 16 GBS sera and none of the 22 CIDP sera had circulating immune complexes as detected by the solid phase C1q-Binding assay, the IgM Polyethylene glycol (Peg) and the IgA Peg assay.

**Relation between MHT and response to IVIg treatment**

21 of 22 CIDP patients were treated with IVIg. Eight of these 21 patients have been described before.[328] 11 patients had a beneficial response. The relationship between MHT results and the response to IVIg treatment is shown in Table 3. Sera of 7 MHT positive CIDP patients were retested after IVIg treatment. In patients who responded to treatment, the test was negative in 5 and weaker positive in 1 patient. The MHT results were unchanged in the remaining, non-responding patient. A significant relationship could be demonstrated between MHT results and IVIg treatment using Fisher exact 't'-test.

Table 3. **Relationship between antibodies against PNT and clinical response to IVIg treatment in 21 CIDP patients**

| Clinical response to IVIg | Antibodies against PNT | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Positive | 8 | 3 | 11 |
| Negative | 2 | 8 | 10 |
| Total | 10 | 11 | 21 |

Fisher's exact p = 0.045 (2 sided)

## DISCUSSION

Using a mixed hemagglutination test (MHT), antibodies against human peripheral nerve tissue were demonstrated in sera of 50% (11/22) of CIDP and in 69% (11/16) of GBS patients. We found 1 positive serum among 34 patients with a polyneuropathy of various other causes. This patient had a polyneuropathy caused by Lyme borreliosis (Bannwarth's Syndrome).[231] In this disorder neuropathy is probably immune-mediated.[262] The negative results in 33 patients indicate that the antibodies detected in the MHT are not a result of nerve damage. Three sera of a group of 32 patients without neuropathy showed a positive reaction; two patients with Myasthenia Gravis and one patient with Lambert-Eaton Syndrome. Antibodies against various tissues have been described previously in patients with Myasthenia Gravis[192,265] or Lambert-Eaton Syndrome.[172]

Sera from patients with immune-mediated diseases without nervous system involvement were selected for the presence of circulating IgG, IgM and IgA immune complexes, anti-DNA antibodies, anti-HLA antibodies and antibodies against glomerular basement membrane. Four patients with Felty syndrome and one patient with Rheumatoid Arthritis showed a positive MHT. These positive reactions may be the result of the binding of the immune complexes to $C_3$ or Fc-receptors present on PNT. To test this assumption, we preincubated the sciatic nerve sections with aggregated IgG or IgG-IgM immune complexes which should bind to such receptors. No positive MHT results occurred and there was no interference with the positive reactions of sera from patients with inflammatory polyneuropathy. Therefore, $C_3$ or Fc-receptor structures on the human peripheral nerve appear not to be important for antibody binding in this assay. The positive MHT results in Felty syndrome or in Rheumatoid Arthritis are more likely caused by antibodies against the connective tissue of the nerve sections. The immune complex assays were positive in only 1 of 38 IDP sera. Therefore, it is unlikely that circulating

immune complexes play a role in the MHT results. The low percentage of sera containing immune complexes is in contrast with the results of some other studies that showed immune complexes in a higher percentage of the patients with GBS.[11,53,105,296] An explanation may be that the meantime between the onset of the disease and the bloodsample withdrawal time was longer in our group of patients than in some other studies.
In agreement with others[180,207,262,263,313,335] we found antibodies against various other tissues in sera of some GBS patients. Reactivity with other tissues was neither seen in any of the CIDP patients nor in GBS patients who lacked antibodies against PNT, but were present in a high number (8/11) of GBS patients with antibodies against PNT. This reactivity with other tissues is difficult to explain. It is not invariably caused by immune complexes and it is a frequent finding in autoimmune diseases.
A proportion of the patients with an inflammatory demyelinating polyneuropathy showed a negative MHT. An explanation could be that some false-negative reactions occurred because protein A mainly selects for the IgG subclasses $IgG_1$, $IgG_2$ and $IgG_4$, which means that it is certainly not an optimal assay for the detection of $IgG_3$, IgM and IgA antibodies. Another explanation may be that a different pathogenic mechanism causes nerve-damage in antibody-negative patients, which may have consequences for the choice of therapy.
Eleven of 21 patients with CIDP responded to intravenous gammaglobulin infusion. For three reasons we attribute the improvement to IVIg. Firstly, signs of improvement to IVIg were noticed within three weeks after the onset of treatment, while patients were in a stable or deteriorating condition at least 2 months prior to treatment. Secondly, the rapid increase in muscle strength contrasted with the more gradual increase which may occur during the natural course. Thirdly, patients deteriorated on average three weeks after the IVIg infusion and improvement could be achieved by re-institution of the IVIg infusions. However, the response to IVIg treatment has to be confirmed in a randomized clinical trial. Such a trial is in progress. It is likely that the same subgroup of patients with CIDP who will improve after plasmapheresis, removing pathogenic antibodies, will also respond to IVIg, interfering with the binding of antibodies with PNT. An in vitro assay demonstrating pathogenic antibodies would be useful for the selection of patients, who are likely to respond to treatment. We investigated whether there is a relationship between the presence of antibodies against PNT and the response to treatment with IVIg infusion in CIDP. Patients with a positive MHT responded significantly more often to this treatment than patients without antibodies against PNT. Moreover, after IVIg infusion, in 6 CIDP patients responding to IVIg treatment, the initial positive MHT became negative in 5 and weakly positive in the other patient. Antibodies remained positive in a non-responsive patient. These results suggest that the antibodies against PNT were neutralized by the infused IVIg in patients who improved after treatment. The relationship with treatment response suggests that antibodies against peripheral nerve tissue detected by means of the MHT, play a pathogenic role in CIDP.

Chapter 6

# INFLAMMATORY DEMYELINATING POLYNEUROPATHY AND LYME BORRELIOSIS

**Serological similarity in anti-neuroblastoma cell line antibodies**

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## INTRODUCTION

Inflammatory demyelinating polyneuropathies (IDP) can be distinguished in the Guillain-Barré syndrome (GBS) and the chronic inflammatory demyelinating polyneuropathy (CIDP).[14,79] The etiology of IDP is unknown. Both cellular and humoral immune mechanisms have been demonstrated in these polyneuropathies.[14,79,312] At least half of the GBS patients have a prodromal infectious episode one to three weeks before the onset of neurological symptoms.[14] An increased frequency of infections preceding CIDP[198] has also been found. Therefore, IDP may be considered as a post-infectious immune-mediated disease.
The beneficial effect of plasma exchange[81,302] favours a pathogenic role of circulating antibodies. Furthermore, in tissue culture experiments, demyelination has been observed with serum from IDP patients in the absence of inflammatory cells or lymphocytes.[28] The target (auto)antigen(s) involved in IDP are still unknown. Some possible antigenic target structures on nerve, such as $P_1$, $P_2$[351] and galactocerebroside[260] have been excluded, while antibodies reactive to fosfolipids, such as cardiolipin[97] and glycosfingolipids: Forssman related neutral glycolipids[153] and gangliosides[135] have been demonstrated in some patients with IDP.
Anti-neural tissue antibodies have been detected using various assays, such as complement fixation,[154] immunofluorescence,[186,313] mixed hemagglutination,[320,324] and ELISA[163] with native human or bovine peripheral nerve or nerve extracts as antigen. Equivalents for neural tissue have also been applied for antibody detection. In a cytotoxicity assay, antibodies against an Ajax-C1300 mouse neuroblastoma (NBL) cell line were found in serum from patients with GBS and CIDP.[256,305] Using an immunofluorescence assay (IFA), we found antibodies against the mouse-rat hybrid neuroblastoma cell line (NBL) 108cc15 in serum from patients with GBS and CIDP.[321]
In this study we evaluated the use of three neuroblastoma cell lines from different species, for the specificity and sensitivity of antibody detection in patients with various neuropathies. We

found that antibody reactivity in serum from patients with GBS, CIDP and Lyme neuroborreliosis was similar.
There is growing evidence that neuropathy in Lyme borreliosis can be considered as an immune-mediated neuropathy due to cross-reactive antibodies against antigenic determinants present on Borrelia burgdorferi and human tissue.[2,103,279] Similarly, anti-neural antibodies in patients with IDP may cross-react with shared antigenic determinants on other microorganisms. We investigated whether the anti-NBL antibodies in patients with Lyme disease and IDP recognize the same or different structures on NBL-cells.

## PATIENTS AND METHODS

### Sera

Sera were obtained from 71 patients with GBS during acute stage of disease (median 1 week from onset), 57 patients with CIDP during active phase of disease and before treatment was started, 23 patients with Lyme neuro-borreliosis (anti-Borrelia burgdorferi IFA >1:120), 6 patients with erythema chronicum migrans (ECM) without neurological signs or symptoms, 68 patients with various other polyneuropathies, 97 patients with other neurological or immune-mediated diseases and 40 healthy individuals. All sera were stored at -30°C until tested.

### Neuroblastoma cell lines (NBL)

The mouse neuroblastoma-rat glioma 108cc15 cell line was provided by Prof B Hamprecht, Physiological Chemical Institute, University Tübingen, FRG.[116] The mouse NBL cell line N1E115, was provided by dr H Vijverberg, dept Veterinary physiology, pharmacology and toxicology, State University Utrecht, the Netherlands.[8] The human NBL cell line CHP 212 was provided by dr AE Evans, div Oncology, the Children's Hospital of Philadelphia, Pennsylvania, USA.[347] All NBL cell lines were cultured in Dulbecco's modified Eagle's medium with 10% fetal calf serum at 37°C, 5% $CO_2$.

### Neuroblastoma immunofluorescence assay (NBL-IFA)

The NBL-IFA has been described in detail previously.[321] In short: 0.5 X $10^6$NBL cells were incubated with 50 microliter patient serum or mouse monoclonal antibodies. After washings, the resuspended pellet was incubated with goat anti-human-IgG or IgM FITC and with rabbit anti-mouse Ig FITC, respectively. After washings, the NBL cells were resuspended in glycerol and examined on a microscope slide, using an IF microscope. Two hundred cells were counted, a percentage of >20% IF stained cells was considered as a positive test result.

### Absorptions with 108cc15 and N1E115 cell lines

One volume of serum was incubated for 1 hour at room temperature with an equal volume of a washed dry pellet NBL cells, while periodically resuspended. The mixture was then centrifuged for 10 min at 1800 x g; the supernatant was collected and stored at -30°C without addition of conservatives.

### Borrelia burgdorferi (Bb)-IFA

Strain B31 was used in the Bb-IFA.[286] The test was considered "positive" if immunofluorescence was obtained with serum diluted 1:120 or more.

**Borrelia burgdorferi Western Blot analysis**
Western Blot analysis was performed according to the protocol of Kolk et al.[149] with the German skin-isolate PKo.[338] The spirochetes were sonificated, centrifuged and the cell-wall fraction was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Patients' sera were diluted 1:200.

**Monoclonal antibodies (MoAb) against Borrelia burgdorferi**
The MoAb H5332 and H9724 were kindly provided by Dr AG Barbour, Div Infectious diseases, dept Medicine, University of Texas, USA. H5332 is specific for the OspA protein (30-32kd) of Borrelia burgdorferi,[20] but it does not react with all European Borrelia burgdorferi isolates.[338] H9724 is specific for the (41kd) flagellin protein of all Borrelia species.[21] These MoAb were tested in the NBL 108cc15-IFA, using rabbit anti-mouse Ig-FITC.

**Absorption with Borrelia burgdorferi**
Spirochetes were isolated from the cerebrospinal fluid of a Dutch patient with Lyme neuroborreliosis (strain A01CSF).[285] Serum from this patient was absorbed with her Borrelia burgdorferi isolate (H9724 positive and H5332 negative) according to the protocol of Shigal et al.[279] The same procedure was performed using serum from a patient with CIDP.

**Detection of antibodies against glycosfingolipids**
Round bottom polyvinyl plates were coated with 50 microliter of either a crude ganglioside mixture (40 microgram/ml) or with a mixture of neutral glycolipids (10 microgram/ml) containing ceramide hexoside, ceramide dehexoside, ceramide trihexoside, globoside and Forssman glycolipids (Biocarb) dissolved in methanol, for 2 hours at room temperature. After drying, the plates were incubated at room temperature for 30 min with 200 microliter PBS containing 1% gelatine and washed with PBS. Serial dilutions of patient's sera were incubated for 2 hours at room temperature. After washings with PBS/Tween 0,02%, 50 microliter of optimal dilutions of peroxidase labeled rat-anti-human Ig (1:2000), IgG (1:2000) or IgM (1:1000) antibodies (Dakopatts) were incubated overnight at 4°C. Between each incubation step all washings were very extensive. The enzyme reaction was developed for 30 min. at room temperature using O-phenylenediamine (Sigma) as a substrate. The reaction was stopped by adding sodium dodecyl sulphate 10% (Sigma). The optical density was subsequently measured at 450nm.

## RESULTS

The results of the NBL-IFA are shown in Table 1. Antibodies against the NBL 108cc15 cell line were found in 48% of the patients with an IDP, in 52% of the patients with Lyme neuroborreliosis, and in 7% of the patients with a polyneuropathy of other origin, other neurological diseases or other immunological disorders. Patients and healthy individuals with a negative test result had a mean percentage of 2 (0-15%) IF positive 108cc15 cells.
The human cell line CHP 212 gave positive test results in 3/68 (4%) of patients with IDP, in 2/5 multiple sclerosis patients and in 3/12 of myasthenia gravis patients, whereas all other sera were negative.

The large number of positive IFA results with both the 108cc15 and N1E115 cell line was similar for GBS, CIDP and Lyme borreliosis patients. However, the number of sera from patients with other diseases that showed positive NBL-IFA reactions was much higher with the N1E115 than with the 108cc15 cell line. Almost all sera showing 108cc15-IFA antibodies were also positive in the N1E115-IFA.

Absorption experiments with serum from CIDP patients showed that the N1E115 cell line absorbs the anti-108cc15 antibodies while the 108cc15 cell line removes the N1E115 antibodies. These cross-absorptions were not extended to other non-polyneuropathy patient groups which had more discrepancies in NBL-IFA results between the two cell lines.

All sera were tested separately for the presence of IgG and IgM anti-NBL antibodies. In all groups of patients, anti-NBL antibodies were more frequently of the IgM than of the IgG immunoglobulin class.

Table 1. **IgG and/or IgM Antibodies against neuroblastoma (NBL) cell lines in serum from patients with an inflammatory demyelinating polyneuropathy, Lyme borreliosis and various controls**

| Disease | Positive Test Results | | | | | |
|---|---|---|---|---|---|---|
| | 108cc15-IFA | | N1E115-IFA | | CHP212-IFA | |
| | no./N | % | no./N | % | no./N | % |
| Inflammatory demyelinating pnp | 61/128 | 48 | 44/78 | 56 | 3/68 | 4 |
| GBS | 31/71 | 44 | 25/43 | 58 | 1/34 | 3 |
| CIDP | 30/57 | 53 | 19/35 | 54 | 2/34 | 6 |
| Lyme neuro-borreliosis | 12/23 | 52 | 15/22 | 68 | 0/22 | 0 |
| PNP other origin | 5/68 | 7 | 17/44 | 39 | 0/30 | 0 |
| diabetes, alcohol | 0/12 | 0 | 0/8 | 0 | 0/10 | 0 |
| paraprotein | 2/14 | 14 | 5/9 | 56 | 0/4 | 0 |
| miscellaneous | 3/42 | 7 | 12/27 | 44 | 0/16 | 0 |
| Other neurological disorders | 4/56 | 7 | 11/46 | 24 | 5/35 | 14 |
| Non-neurological immune mediated disorders | 3/41 | 7 | 8/33 | 24 | 0/33 | 0 |
| Healthy individuals | 0/40 | 0 | 1/25 | 4 | 0/25 | 0 |

Table 2. **Anti-Borrelia burgdorferi and anti-NBL antibodies in serum from a Lyme and a CIDP patient before and after absorption with Bb and NBL.**

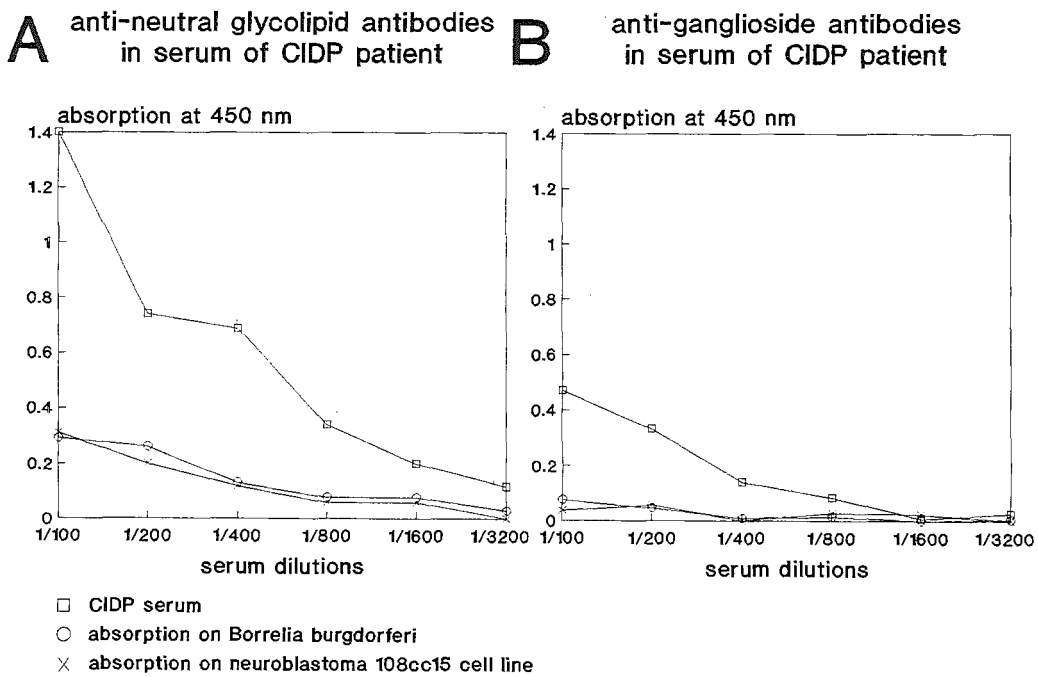
| | NBL 108cc15-IFA | | Borrelia burgdorferi-IFA | |
|---|---|---|---|---|
| | Lyme | CIDP | Lyme | CIDP |
| before absorption | 1:4 | 1:128 | 1:128 | neg |
| after absorption Bb | neg | 1:4 | neg | neg |
| after absorption NBL | neg | neg | 1:128 | neg |

Values are given in titers which represents the highest dilution that gives a positive test result

Serum from patients with Lyme neuro-borreliosis was sampled between 1 and 39 months from onset of disease (median 10 months). Differences in NBL-IFA results were not related to time lapse from onset of Lyme borreliosis and serum sampling date. Furthermore, no relationship was found between severity of clinical signs and symptoms in patients with Lyme neuro-borreliosis and the presence of anti-NBL antibodies. The pattern of NBL-IFA reactivity in serum from patients with Lyme neuro-borreliosis was surprisingly similar to that of patients with GBS or CIDP. In order to evaluate whether anti-NBL antibodies in Lyme borreliosis were related to the neuropathy, we included 6

patients with erythema chronicum migrans (ECM). Although these ECM patients did not develop neurological symptoms during a follow-up period of 6-18 months (median 6 months) from onset of disease, anti-108cc15 antibodies were found in 3 out of 6 sera.
We subsequently investigated if the reaction of GBS and CIDP sera with the NBL 108cc15 could be explained by the presence of anti-Borrelia burgdorferi (Bb) antibodies. Sera from twenty randomly chosen GBS/CIDP patients (10 with, and 10 without anti-NBL 108cc15 antibodies) had a negative Bb-IFA (titer <1:20). The MoAb's H5332 and H9724 were negative in the NBL 108cc15-IFA, whereas the Bb spirochetes used for absorption experiments were H9724 positive and H5332 negative.
Serum from a Lyme neuro-borreliosis patient was absorbed with Bb, this resulted in a removal of anti-Bb and anti-NBL antibodies (Table 2). Absorption of this serum with NBL 108cc15 removed anti-NBL antibodies, whereas anti-Bb antibodies activity remained present both detected in the Bb-IFA and the Bb Western Blotting analysis. Borrelia burgdorferi also removed anti-NBL reactivity from the serum of a CIDP patient (Table 2).

Figure 1. *Anti-neutral glycolipid (A) and anti-ganglioside (B) antibodies in serum of a CIDP patient, before and after absorption with neuroblastoma cell line (NBL) 108cc15 and Borrelia burgdorferi.*




Anti-glycosfingolipid antibodies present in the serum of this CIDP patient were removed after absorption with Bb and NBL 108cc15 (Figure 1, A and B). The antibodies against neutral

glycolipids were preferentially removed by absorption with NBL 108cc15 and Borrelia burgdorferi.

## DISCUSSION

The pattern of antibody reactivity in sera from patients with GBS, CIDP and Lyme neuro-borreliosis was similar for all 3 NBL cell lines tested. The NBL N1E115 cell line showed reactivity with sera from patients with various disorders, whereas the CHP212 mainly showed reactivity with sera from patients with myasthenia gravis and multiple sclerosis. The NBL 108cc15-IFA showed a high percentage of positive reactions with sera from GBS, CIDP and Lyme borreliosis patients.
Using a radio-immuno assay, Müller et al.[217] demonstrated that 44 out of 109 (40%) myasthenia gravis patients had circulating antibodies against the human NBL cell line SH-SY5Y. The differences with our results are likely the result of variation in the antigen expression on the NBL cell lines tested.
The reactivity patterns between the NBL-108cc15 and NIE115 cell line suggest sharing of at least one antigen. Cross-absorption studies with these cell lines indicate that IDP sera recognize the same antigen(s) on both cell lines. Whether the N1E115 cell line expresses an epitope not present on 108cc15 resulting in reactivity with non-IDP sera was not further investigated.
In Lyme borreliosis, antibodies against central[262,292] and peripheral[2,279,320] nervous tissue have been demonstrated. The neuropathy in Lyme borreliosis can be caused by axonal disturbances[318] which can be the result of vasculitis,[40] but there are also signs of demyelination in this disease[106,289] and improvement of peripheral nerve conduction velocity was observed following penicillin treatment.[114] The anti-flagellin (41kd) antibody cross-reacts with axons,[279] myelin and CNS tissue[2,103] as well as with synovia and heart muscle cells.[2] Since anti-flagellin antibodies can be demonstrated in nearly all patients with Lyme borreliosis, other factors must contribute alone or together with anti-flagellin antibodies to cause tissue damage. The negative NBL-IFA with the anti-flagellin Moab H9724 combined with the Bb-IFA results with serum from 20 GBS/CIDP patients excludes the flagellin protein as the target for the cross-reactive antibodies in IDP and Lyme borreliosis patients.
In Lyme borreliosis, polyclonal B cell activation[278,339] and cellular immunity to several host antigens has been demonstrated.[193] The NBL antibodies we encountered in both Lyme and IDP patients could indeed be the result of polyclonal B cell activation caused by Borrelia burgdorferi and by infections preceding the manifestations of IDP. However, Borrelia burgdorferi absorbed the anti-NBL antibodies both from serum of a Lyme neuro-borreliosis and a CIDP patient. This rather points towards an antigenic relationship between NBL antigens, Bb and presumed infectious agents preceding IDP and it argues against non-specific B cell activation.
The finding that CIDP sera were only low-reactive in the Bb-IFA while Bb absorbed anti-NBL activity from CIDP serum is compatible with a very low antigenic expression resulting in negative serology although there is still a possibility for absorption of antibodies. The observation that the anti-Bb antibodies were not removed by absorption with NBL 108cc15 cells, suggests that the shared epitope may not be one of the proteins contributing to Bb-IFA or Bb-Western blotting. We therefore investigated whether other antigenic structures could explain the results. Patients with IDP but also patients with other immune-mediated diseases may have antibodies against glycosfingolipids (GSL).[248] These GSL's are present in many - e.g.

neural - tissues and can be divided in glycolipids and in the sialic acid containing gangliosides. Western-blot analysis is not a proper assay for the detection of anti-GSL antibodies which may explain our Western-Blot results.
The serum from the CIDP patient indeed showed antibodies to various glycosfingolipids (GSL) and we found that anti-GSL antibodies were absorbed by both Bb and the NBL 108cc15 cell line.
Antibodies against GSL epitopes may be induced by a spectrum of micro-organisms that stimulate cross-reactive antibodies to host-related antigens. Whether such antibodies have any relationship with the pathogenesis of CIDP is unknown, but the presence of anti-NBL antibodies in patients with Lyme borreliosis without polyneuropathy may argue against a pathogenic role. We conclude that serological similarities between GBS, CIDP and Lyme borreliosis exist and we suggest that the neuroblastoma 108cc15-IFA may identify a group of post-infectious immune-mediated polyneuropathies from polyneuropathies due to another origin.

Chapter 7

# ANTI-NEUROBLASTOMA CELL LINE ANTIBODIES IN INFLAMMATORY DEMYELINATING POLYNEUROPATHY

**Inhibition - in vitro and in vivo - by intravenous immunoglobulin**

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## INTRODUCTION

The Guillain-Barré syndrome (GBS)[15] and the chronic inflammatory demyelinating polyneuropathy (CIDP)[74] are considered to be immunologically mediated disorders of the peripheral nervous system. The successful treatment by plasmapheresis of some patients with GBS or CIDP suggests that circulating anti-nerve antibodies might be of importance. Several assays are available to detect antibodies against neural determinants in sera from patients with GBS and CIDP.

We recently studied the occurrence of antibodies in GBS and CIDP, using a mixed hemagglutination test (MHT) against tissue sections of sciatic nerve as originally described by Vedeler.[324] The MHT detected antibodies in about 50% of the patients with GBS and CIDP, whereas positive test results were rarely observed in sera from patients with other polyneuropathies or other disorders.[320] In CIDP patients, these antibodies disappeared or decreased after intravenous immunoglobulin (IVIg) treatment. Patients showing these antibodies in pretreatment serum samples more often improved after IVIg. These results suggest that antibodies detected with the MHT are of pathogenetic significance. To study the in vitro interaction of IVIg and antibodies against nerve tissue, the MHT is not a suitable technique. Rosenberg et al.[256] and Müller et al.[216] demonstrated antibodies against a neuroblastoma cell line (NBL) in sera from patients with various neurological diseases. Since a technique using cells instead of tissue-sections would enable us to study the interaction between IVIg and antibodies in sera from patients with CIDP, we investigated if antibodies against neuroblastoma cell lines can be detected in sera of these patients. Using an immunofluorescence assay (IFA) we found antibodies against three neuroblastoma cell lines (CHP 212, N1E 115 and 108cc15) in patients with GBS or CIDP. The 108cc15 appeared to be the most specific cell line.

The aims of this study were to compare NBL-IFA (108cc15) and MHT in terms of sensitivity and specificity and to investigate the in vivo and in vitro interaction of IVIg with antibodies detected by this IFA.

## PATIENTS AND METHODS

### Sera

Sera were obtained from 48 patients with GBS and 42 patients with CIDP. The diagnosis of GBS[15] and of CIDP[74] was made by clinical criteria. Sera from patients with GBS were sampled within a median period of 1 week of onset of the neurological symptoms and in patients with CIDP during an active phase of the disease. Sera from 43 patients with a polyneuropathy of other or unknown causes and 46 sera from patients with various neurological diseases without polyneuropathy were used as controls. Finally, sera from 39 patients with non-neurological immune-mediated diseases (rheumatoid arthritis N=9, Felty syndrome N=11, vasculitis N=1, SLE N=2, cryoglobulinemia N=1, Wegener's granulomatosis N=1, Goodpasture N=2, multiple myeloma N=5, other dysproteinemia's N=7) and 40 healthy bloodbank donors were tested. All sera were heat-inactivated for 30 minutes at 56°C before use. The sera were numbered and the technician who performed the tests did not know the diagnosis of the patients.

### The mixed hemagglutination test (MHT)

The MHT was carried out as described before.[320]

### Neuroblastoma cell line (NBL)

The rat-mouse NBL cell line 108cc15[116] was kindly provided by Prof B Hamprecht, Physiological-chemical Institute University Tübingen, Western Germany. The cell line is cultured in Dulbecco's Modified Eagles Medium (DMEM) with 10% fetal calf serum (FCS) at 37°C 5% $CO_2$.

### Neuroblastoma cell line immunofluorescence assay (NBL-IFA)

0.5 x $10^6$ NBL cells were incubated with 50 microliter serum for 90 minutes at 4°C (for the optimal detection of IgM-antibodies) and 60 minutes at 22°C (for the optimal detection of IgG-antibodies). After incubation, the cells were washed two times in phosphate buffered saline (PBS-BSA 1%, pH 7,4) and the pellet was resuspended to a concentration of 0,5 x $10^6$ NBL cells/100 microliter and incubated with goat anti-human IgG for 30 minutes at 22°C (Go-anti-Hu IgG-FITC, Nordic, Tilburg, the Netherlands) or 45 minutes at 4°C with goat anti-human IgM (Go-anti- Hu IgM-FITC, Nordic).
The antisera were diluted in PBS (anti-Hu IgG 1:7, anti-Hu IgM 1:9). After washing in PBS three times, the cells were mounted in glycerol and examined with a Leitz IF microscope (8x63) with a 495 nm excitation and a 525 nm filter. The proportion of fluorescent cells of 200 counted cells is determined and expressed as a percentage. A percentage of >20% IF stained cells was considered as a positive test result (Figure 1).

### Absorption studies

100 microliter serum was incubated for 5 hrs with 20 x $10^6$ NBL 108cc15 cells at 22°C, while periodically resuspended, then centrifuged for 10 minutes at 1800 x g and the supernatant was

harvested. The supernatant was absorbed once more with 20 x $10^6$ fresh NBL cells. After absorption the serum was diluted with PBS to the same protein concentration as the pre-absorption serum. All the sera became negative in the NBL-108cc15 IFA after one or two absorptions.

**In vitro-incubation with intravenous immunoglobulin**
This was assayed with a CIDP serum containing IgM-anti NBL 108cc15 antibodies. IVIg was diluted to a 6% solution in distilled water. Fifty microliter of different serum/IVIg solution ratio's (range 0.1-0.9) was incubated with 1 x $10^6$ NBL 108cc15 cells for 90 minutes at 4°C. After washing (2 times in PBS-BSA 1%) the cells were incubated for 45 minutes Goat-anti-human IgM (1:9). Then the cells were washed again and read as described above. The results are expressed as % IF stained NBL 108cc15 cells.
Incubations were also performed and compared to serum/PBS dilutions (range 0.1-0.9).

**Immune complex assays**
Rheumatoid factor was analyzed, using an Elisa assay, in serum of 37 GBS or CIDP patients who had a positive MHT and/or NBL 108cc15-IFA.
Circulating immune complex analysis was carried out in sera from 40 CIDP or GBS patients (29/40 sera showed a positive MHT and/or NBL-IFA) using the $^{125}$I C1q-Binding Assay,[349] the IgM Polyethylene glycol (peg)[19] and the IgA Peg Assay.[317]

Table 1. **Comparison of MHT and NBL 108cc15-IFA**

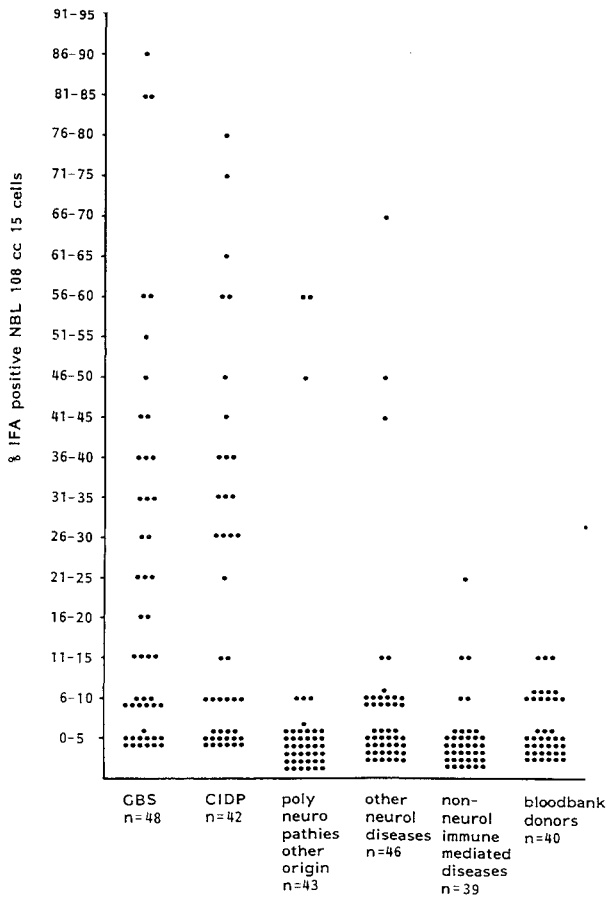
| Disease | N sera | MHT pos no. | NBL 108cc15-IFA IgG pos no. | NBL 108cc15-IFA IgM pos no. | NBL 108cc15-IFA IgG and/or IgM pos no. |
|---|---|---|---|---|---|
| Inflammatory polyneuropathy | | | | | |
| GBS | 48 | 23 | 6 | 17 | 20 |
| CIDP | 42 | 17 | 8 | 12 | 18 |
| total | 90 | 40 | 14 | 29 | 38 |
| Other polyneuropathies | | | | | |
| diabetes | 6 | 0 | 0 | 0 | 0 |
| alcohol | 4 | 0 | 0 | 0 | 0 |
| paraprotein | 5 | 0 | 0 | 0 | 0 |
| miscellaneous | 28 | 2 | 1 | 3 | 3 |
| total | 43 | 2 | 1 | 3 | 3 |
| Other neurological disorders | | | | | |
| multiple sclerosis | 6 | 0 | 0 | 0 | 0 |
| myasthenia gravis | 12 | 2 | 1 | 1 | 1 |
| Lambert-Eaton | 2 | 1 | 0 | 0 | 0 |
| Amyotrophic Lateral Sclerosis | 5 | 0 | 0 | 0 | 0 |
| miscellaneous | 21 | 0 | 1 | 2 | 2 |
| total | 46 | 3 | 2 | 3 | 3 |
| Non-neurological, immune-mediated disorders | | | | | |
| rheumatoid arthritis or Felty syndrome | 20 | 5 | 1 | 0 | 1 |
| miscellaneous | 19 | 0 | 0 | 0 | 0 |
| total | 39 | 5 | 1 | 0 | 1 |
| Bloodbank donors | 40 | 0 | 0 | 0 | 0 |

## RESULTS

**Sensitivity and specifity of the NBL-IFA**
The comparison of the antibodies detected with two techniques, the MHT and the NBL-IFA, is shown in the Table 1. Antibodies against the NBL cell line 108cc15 (IgG, IgM or both) were present in 20/48 (42%) of the patients with GBS and in 18/42 (43%) of the patients with CIDP. Three of 43 patients with polyneuropathy of other causes showed antibodies against this NBL cell line. These were: one

patient with Non-Hodgkin Lymphoma, one patient with Systemic Lupus Erythematosus and one patient with polyneuropathy of unknown origin. One of the 12 patients with myasthenia gravis and two of 34 patients with other neurological diseases showed positive reactions with the NBL 108cc15 cell line, these were a patient with amyotrophic plexus neuritis and one patient with dementia and an oatcell carcinoma. Of 39 patients with various immune-mediated disorders one patient with Felty disease showed a positive test. Normal donors had a mean fluorescence of 4% cells (Figure 1).

Figure 1. *Antibodies against NBL 108cc15 cell line in inflammatory demyelinating polyneuropathy and control sera.*




**Immunoglobulin class and immune complexes**

The majority of the antibodies against the 108cc15 cell line in patients with GBS or CIDP were of the IgM class only (n=24), 3 patients with GBS and 2 with CIDP showed both IgM and IgG antibodies and 3 of the patients with GBS and 6 of the CIDP patients showed IgG antibodies alone.

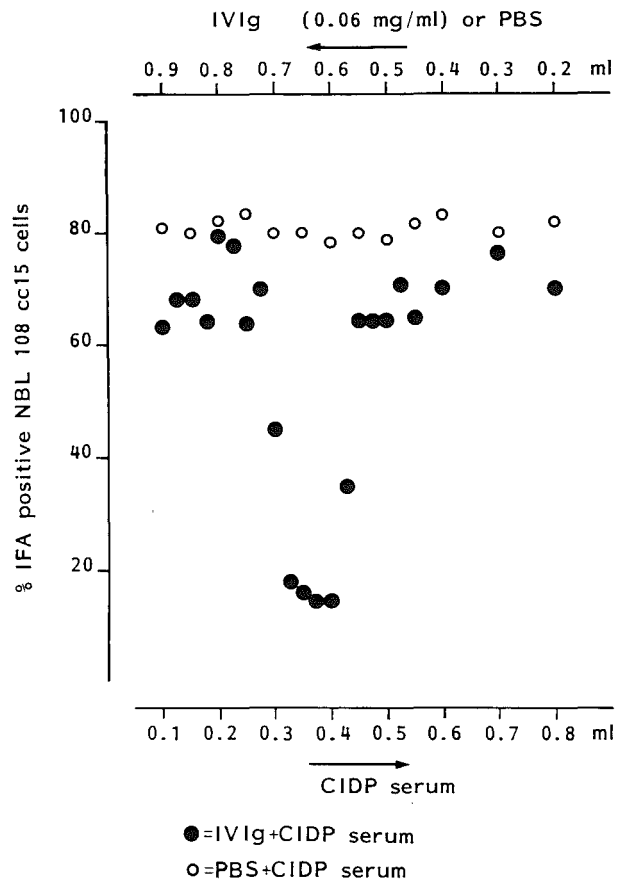
There was no difference between GBS and CIDP patients in respect of the antibody class distribution.

One of the 17 tested GBS, (14/17 showed a positive MHT and/or NBL-IFA), and none of 23 tested CIDP sera, (15/23 showed a positive MHT and/or NBL-IFA), had circulating immune complexes as detected by the solid phase C1q-Binding assay, the IgM Peg or the IgA Peg assay. Rheumatoid factor (Rf) was analyzed in the 37 GBS or CIDP patients with a positive MHT and/or NBL-IFA. Sixteen patients had weakly positive (n=4) or positive (n=12) Rf activity (IgG, IgM and/or IgA). Seven patients had IgM.Rf, 3 IgG.Rf and 6 IgA.Rf. Of the 20 patients with IgM-NBL antibodies 4 had a positive IgM.Rf.

**Relation between MHT and NBL techniques**

In these 90 patients with GBS or CIDP, 40 had a positive MHT, 38 a positive NBL-IFA and 23 patients had both a positive MHT and a positive NBL-IFA. To investigate if the NBL line expresses antigens present on human sciatic nerve, sera from 13/23 patients who had both a positive MHT and NBL-IFA were absorbed with excess NBL 108cc15 cells. Three patients sera showed a negative MHT after absorption of the NBL antibodies with the NBL 108cc15 cell line, 6 patients showed a clear decrease in agglutinates against the sciatic nerve compared with the unabsorbed serum but remained positive in the MHT and 4 sera showed an unchanged MHT before and after absorption with the NBL 108cc15.

Figure 2. *Antibodies against NBL 108cc15 cell line (NBL-IFA) using different serum/IVIg ratio's.*




**Relation between NBL antibodies and clinical response to IVIg**

The relation between antibodies against the NBL 108cc15 cell line and the clinical response to IVIg was investigated in 10 CIDP patients who had NBL-IFA antibodies in pretreatment serum samples. After IVIg treatment 7/10 patients improved. All these 7 patients became NBL-IFA negative. Three patients did not improve: one became NBL-IFA antibody negative and 2 had persisting NBL-IFA antibodies.

**In vitro interaction between IVIg and NBL-antibodies**

The in vitro interaction between IVIg and NBL 108cc15-IFA antibodies was studied with serum from a patient with a CIDP who responded to IVIg treatment. As can be seen (Figure 2) there is an optimal range of IgG-concentration showing maximal inhibition whereas despite increasing the concentration of IgG, the degree of inhibition is less.

## DISCUSSION

Using an immunofluorescence assay, antibodies against the neuroblastoma (NBL) cell line 108cc15 were detected in 42% of sera from patients with GBS or CIDP whereas these antibodies were found in 5% of sera from patients with other disorders and in none of the 40 bloodbank donors. Antibodies against peripheral nerve tissue (MHT) were detected in 44% of the GBS or CIDP patients, in 8% of sera from patients with other disorders and in none of the bloodbank donors. After absorption the NBL 108cc15 cell line removed anti-sciatic nerve activity from 9 of the 13 GBS or CIDP sera.

These absorption studies and the number of concordant and discordant reactions when both techniques are compared suggest that there is a partial sharing of antigenic determinants relevant for inflammatory demyelinating polyneuropathy on the NBL 108cc15 cell line and human sciatic nerve.

The MHT and the NBL 108cc15-IFA detect antibodies in 42-44% of the patients with inflammatory demyelinating polyneuropathy whereas 61% of the patients show antibodies in one of the two assays, and 26% of the patients are positive in both assays. Although both assays have a low percentage of positive reactions in polyneuropathies of other origin and in other neurological diseases, the pathogenic role of the antibodies described is still speculative and an epiphenomenon is not excluded. The observation that antibodies detected with both techniques become undetectable or considerably decrease in titer in patients improving after IVIg is suggestive for pathogenic antibodies. This raises the question whether patients with 'seronegative' inflammatory polyneuropathy have antibodies of higher avidity resulting in non-detectable free serum antibodies or have polyneuropathy due to a different pathogenesis for instance cell mediated immunity.

A beneficial effect of IVIg has been reported in disorders such as autoimmune thrombocytopenia[39,136] autoimmune leukocytopenia,[237] acquired factor VIII deficiency[293,294,348] and myasthenia gravis.[89,102,140] These presumably antibody mediated diseases have in common a variable response to corticosteroids, alkylating immunosuppressive drugs and temporary improvement on plasma exchange. Recently a beneficial response to IVIg has also been observed in CIDP patients,[6,50,328] which is now investigated in an randomized controlled clinical trial.

The mechanism of the beneficial effect of IVIg is unknown. IVIg blocks Fc-receptor mediated immunological functions. This mechanism may play a role in CIDP, as macrophages have been demonstrated in nerve biopsies of these patients. Blockade of these cells might prevent the cytopathological effect of anti-neural antibodies mediated by macrophages. The decrease of the anti-NBL antibodies after IVIg treatment suggests a different mechanism. In chronic idiopathic thrombocytopenic purpura it has been shown that after IVIg, con A induced antibody production diminishes.[66] Although such an effect could explain the decrease of antibodies in CIDP patients improving after IVIg, an alternative mechanism is that IgG binds to and neutralizes auto-antibodies. This was demonstrated by Sultan et al.[294] for acquired anti-F VIII antibodies. In in vitro experiments, they demonstrated that there was an optimal range for the inhibition of anti-F VIII antibodies by IVIg.

In this study the in vitro effect of IVIg was investigated with serum from a CIDP patient with high titred IgM-anti NBL antibodies. These antibodies were significantly inhibited when a certain amount of IVIg was added. The prozone effect in these in vitro experiments, which was also found by Sultan et al.[294] suggests that it is an interaction between antibodies. Further

studies are needed to test the hypothesis that IVIg contains anti-idiotypic antibodies reacting with pathogenic auto-antibodies in patients with inflammatory demyelinating polyneuropathy. In these studies an immunofluorescence assay to demonstrate NBL 108cc15 antibodies, which are rather specific for this polyneuropathy and which disappear in patients improving after IVIg, may be a useful technique.

Chapter 8

# HLA ANTIGENS IN PATIENTS WITH CHRONIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY

## INTRODUCTION

Chronic inflammatory demyelinating polyneuropathy (CIDP) is a clinically heterogenous disorder.[23] Clinical trials have shown that patients with CIDP can be treated successfully with prednisone,[77] plasma exchange[81] and intravenous immunoglobulin (IVIg).[322] However, not all of the patients respond to one of these different methods of treatment. The variability in clinical response may have a genetic component. HLA typing in patients with Guillain-Barré syndrome (GBS) did not reveal any significant differences between HLA antigen frequencies in GBS patients and in controls.[4,164,143,342,290] However, Winer et al.[342] reported that HLA-DR2 was significantly increased in 12 GBS patients with most profound muscle weakness compared with 77 other GBS patients studied.

Stewart et al.[290] tested 16 patients with a chronic relapsing polyneuropathy and 8 patients with a subacute polyneuropathy (which could be distinguished from GBS) and found an association with HLA-A30/31 and also with HLA-B8 and DR3. Adams et al.[4] suggested an association with HLA-A1, B8 and DR3 by testing 14 CIDP patients.

Recently Feeney et al.[94] found increased frequencies of HLA-A3 and B7 in a group of 71 CIDP patients. In the 56 CIDP patients who were typed for HLA-DR, they found that HLA-DR2 was increased. Although none of these findings reached statistical significance after correction for the number of tested antigens, it was suggested that CIDP might be associated with the HLA-DR2 antigen.

The aims of our study concerning a group of 52 CIDP patients were to investigate firstly, whether there are significant associations with any of the HLA-A, B, C, DR or DQ antigens and if so, do they confirm the results of other studies; secondly, if there is a relationship between improvement after high-dose IVIg and HLA antigens; and thirdly, if the presence of

anti-neural tissue antibodies is associated with certain HLA antigens or with improvement after IVIg.

## PATIENTS AND METHODS

### Patients

All of the 52 patients included in this study were judged to have a CIDP and fulfilled the diagnostic criteria outlined by Dyck et al.:[79] (1) the course of the disease may be steadily progressive, chronic monophasic, or recurrent; (2) nerve conduction may be normal in motor and afferent fibres, but more commonly (though not mandatory) it is slowed; (3) cyto-albuminologic dissociation is usually seen at some time during the course of the disease and (4) there is a tendency to symmetric involvement of proximal as well as distal limb muscles. To exclude patients with the Guillain-Barré syndrome, all patients had the clinical features of a polyneuropathy with a progression of weakness of at least 2 months.

### IVIg treatment

All 52 CIDP patients were treated with high-dose intravenous immunoglobulin (IVIg) in a dosage of 0.4 g/kg body weight/day for 5 consecutive days. IVIg was obtained from the Central Laboratory of the Dutch Red Cross Blood Transfusion Service.

### Clinical assessment

The disability of the patients before and several times after IVIg treatment was assessed by using the modified Rankin scale.[319] This scale estimates functional disability or handicap. Improvement is defined as an increase of at least 1 step on the Rankin disability scale.

### Anti-neural tissue antibody assays

Serum from all 52 patients was investigated for the presence of anti-human sciatic nerve tissue antibodies by means of a mixed hemagglutination assay (MHT) and by the rat-mouse neuroblastoma cell line 108cc15 immunofluorescence assay (NBL-IFA). Both assays have been described before.[320,321]

### HLA typing

Typing for the HLA-A, B and C (Class I antigens) was performed with the standard NIH lymphocytotoxity test, and typing for HLA-DR and DQ (Class II antigens) with the two-color fluorescence test[323] using sets of well characterized and highly selected allo-antisera and monoclonal antibodies.

### Statistical analysis

Differences between groups were tested with a chi-square test. Significant p values were corrected for the 44 broad antigens in the HLA-A, B, C, DR and DQ loci. Controls consisted of 504 unrelated healthy Dutch blood donors. Antigenic splits were not analyzed because of the relatively small numbers of cases. Relative risks (RR) were calculated using the formulae suggested by Woolf[345] and Haldane.[113]

**Table 1. HLA phenotypes, neural antibodies and improvement after IVIg treatment in 52 CIDP patients**

| Pat | Sex | HLA-A | HLA-B | HLA-C | HLA-DR | HLA-DRw52/53 | HLA-DQ | improvement after IVIg | NBL-IFA | MHT |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 28, 31 | 35, - | w2, w4 | w13, w15 | w52 | w6, - | + | - | + |
| 2 | F | 1, 3 | B7, W57 | w6, w7 | 1, 7 | w53 | w5, w9 | + | + | + |
| 3 | M | 3, 25 | 7, 35 | w4, w7 | w13, w15 | w52 | w6, - | + | + | + |
| 4 | F | 11, 29 | 51, w55 | w3, - | w15, - | - | w6, - | - | + | - |
| 5 | F | 2, w33 | 44, w58 | w3, w5 | 3, 7 | w52, w53 | w2, w9 | - | - | - |
| 6 | F | 3, 30 | 13, 18 | w6, w7 | 7, w10 | w53 | w2, w5 | - | - | + |
| 7 | F | 28, 11 | 51, 8 | w7, - | 3, 7 | w52, w53 | w2, w9 | - | - | - |
| 8 | M | 2, 23 | w50, - | w6, - | 4, w13 | w52, w53 | w6, w8 | + | - | - |
| 9 | M | 2, 3 | 7, 44 | w7, - | 3, w13 | w52 | w2, w6 | + | + | - |
| 10 | M | 2, 28 | 7, - | w7, - | w15, - | - | w6, - | + | - | - |
| 11 | F | 29, 30 | 44, 13 | w6, - | 4, 7 | w53 | w2, w7 | + | - | + |
| 12 | M | 2, 28 | 51, 7 | w2, - | 4, - | w53 | w7, - | + | + | + |
| 13 | M | 2, 3 | 44, 35 | w4, - | 4, 7 | w53 | w2, w7 | + | + | - |
| 14 | F | 2, 31 | 51, w60 | w3, - | w15, - | - | w6, - | + | + | - |
| 15 | F | 2, - | 7, 27 | w2, w7 | 4, w13 | w52, w53 | w6, w8 | + | + | + |
| 16 | M | 1, 24 | 51, 27 | w2, - | w15, w11 | w52 | w6, w7 | + | - | - |
| 17 | M | 1, 2 | 51, 8 | w4, w7 | 3, w14 | w52 | w5, w2 | + | + | + |
| 18 | F | 23, 24 | 39, w60 | w3, - | 4, w8 | w52, w53 | w8, - | + | + | + |
| 19 | F | 26, 11 | 8, 38 | w3, w7 | 3, w11 | w52 | w2, w7 | + | - | - |
| 20 | M | 2, 11 | 7, w62 | w3, w7 | w13, 9 | w52, w53 | w6, w9 | - | + | + |
| 21 | F | 3, 24 | 51, 35 | w4, - | 4, w11 | w52, w53 | w7, - | - | - | + |
| 22 | M | 1, 3 | 8, 35 | w4, w7 | 1, 3 | w52 | w5, w2 | + | + | + |
| 23 | F | 3, 31 | 35, w60 | w3, w4 | 1, - | - | w5, - | - | - | - |
| 24 | M | 1, 2 | 8, 39 | w7, - | 3, w8 | w52 | w2, w4 | - | + | - |
| 25 | F | 1, 29 | 44, 13 | w5, w6 | w15, 7 | w53 | w2, w6 | + | + | - |
| 26 | M | 2, 24 | 18, - | w7, - | w11, w14 | w52 | w5, w7 | + | + | - |
| 27 | M | 2, 11 | 8, 44 | w7, - | 1, 7 | w53 | w2, w5 | - | + | - |
| 28 | M | 1, 29 | 44, 38 | -, - | w13, 7 | w52, w53 | w2, w6 | + | + | - |
| 29 | M | 2, - | 7, 39 | w7, - | 4, w12 | w52, w53 | w7, w8 | - | - | + |
| 30 | F | 1, 2 | 8, w57 | w6, w7 | 3, 7 | w52, w53 | w2, w9 | + | - | - |
| 31 | M | 1, 2 | 38, w60 | w3, - | w13, w8 | w52 | w6, w4 | + | + | - |
| 32 | M | 2, 3 | 38, w60 | w3, - | w13, - | w52 | w6, - | + | - | + |
| 33 | M | 3, 24 | w62, 27 | w2, w3 | 1, w11 | w52 | w5, w7 | - | - | + |
| 34 | M | 3, - | 7, 27 | w2, w7 | 4, w13 | w52, w53 | w6, w7 | + | - | - |
| 35 | M | 3, - | 51, 7 | w7, - | w15, w11 | w52 | w6, w7 | + | - | - |
| 36 | M | 2, - | 8, w60 | w3, w7 | w15, 3 | w52 | w6, w2 | + | + | - |
| 37 | F | 2, 3 | w62, - | w3, - | 4, w13 | w52, w53 | w6, w8 | - | - | - |
| 38 | M | 1, 2 | 8, w62 | w3, w7 | w15, 3 | w52 | w6, w2 | - | + | - |
| 39 | M | 3, 24 | 7, 8 | w3, w7 | w15, - | - | w6, w5 | - | - | + |
| 40 | M | 2, - | 44, w62 | w2, w3 | w16, 4 | w53 | w5, w8 | - | - | - |
| 41 | F | 2, 3 | 38, w55 | w3, - | w15, 4 | w53 | w6, - | + | - | + |
| 42 | M | 2, 3 | 44, w56 | w1, w4 | 7, - | w53 | w6, w5 | - | - | - |
| 43 | F | 2, 3 | 7, - | w7, - | w15, w8 | w52 | w6, w4 | - | + | + |
| 44 | F | 3, 32 | 39, w56, | - , - | w15, 4 | w53 | w6, w8 | + | - | - |
| 45 | M | 3, - | 7, 35 | w4, w7 | 1, w15 | - | w5, w6 | - | - | + |
| 46 | M | 1, 2 | 44, w57 | w6, - | 7, - | w53 | w2, w9 | + | - | + |
| 47 | F | 1, 3 | 51, 8 | w2, w7 | 3, 7 | w52, w53 | w2, - | + | - | - |
| 48 | M | 2, 3 | w55, 27 | w2, w3 | 4, - | w53 | w7, w8 | - | + | - |
| 49 | F | 2, - | 7, w62 | w3, w7 | w15, - | - | w6, w5 | + | - | - |
| 50 | M | 3, 30 | 8, 18 | w5, w7 | 3, - | w52 | w2, - | - | + | - |
| 51 | M | 2, - | 44, - | w5, - | 4, w6 | w52, w53 | w7, - | + | + | + |
| 52 | M | 1, 2 | 51, w62, | w3, - | 1, 4 | w53 | w5, w8 | + | - | + |

## RESULTS

The results of the HLA typings in the group of 52 CIDP patients, together with the clinical course after IVIg treatment and the results of the anti-neural antibody assays are shown in Table 1.
When the 52 CIDP patients were compared to controls, significantly different frequencies were found for the HLA antigens B5, B16 and Cw2 (Table 2). No significant values were found for HLA-A, DR and DQ antigens .

Table 2. **Significantly different HLA antigen frequencies in a group of 52 CIDP patients typed for HLA-A, B, C, DR and DQ antigens**

| HLA antigen | Patients | | | Controls | | | RR | chi square | p | p corrected* |
|---|---|---|---|---|---|---|---|---|---|---|
| | N | positive no. | % | N | positive no. | % | | | | |
| **CIDP patients** | | | | | | | | | | |
| B5 | 52 | 10 | 19 | 504 | 53 | 11 | 2.085 | 4.002 | 0.043 | ns |
| B16 | 52 | 9 | 17 | 504 | 36 | 7 | 2.797 | 6.966 | 0.008 | ns |
| Cw2 | 52 | 9 | 17 | 504 | 45 | 9 | 2.138 | 3.937 | 0.045 | ns |
| **Improvement after IVIg** | | | | | | | | | | |
| B5 | 32 | 7 | 22 | 504 | 53 | 11 | 2.482 | 4.487 | 0.032 | ns |
| B16 | 32 | 7 | 22 | 504 | 36 | 7 | 3.767 | 9.132 | 0.003 | ns |
| Cw2 | 32 | 6 | 19 | 504 | 45 | 9 | 2.402 | 3.765 | 0.049 | ns |
| **No improvement after IVIg** | | | | | | | | | | |
| A1 | 20 | 2 | 10 | 504 | 170 | 34 | 0.265 | 4.464 | 0.033 | ns |
| A3 | 20 | 11 | 55 | 504 | 162 | 32 | 2.551 | 4.560 | 0.031 | ns |
| Bw22 | 20 | 3 | 15 | 504 | 25 | 5 | 3.989 | 5.478 | 0.018 | ns |

* *p* is corrected for 44 antigens tested in HLA-A, B, C, DR and DQ loci

All 52 CIDP patients were treated with IVIg, of which 32 (62%) improved and 20 (38%) patients did not. Sex and age were equally distributed among CIDP patients who improved after IVIg (N=32) and those who did not (N=20).
Because there might be a difference in HLA antigen frequency between these two groups, both were compared to the control group of healthy donors and they were tested against each other. The group of 32 CIDP patients who improved after IVIg treatment showed significantly different frequencies for the HLA antigens B5, B16 and Cw2. The group of 20 CIDP patients who did not improve had significantly different frequencies for the HLA antigens A1, A3, and Bw22.
However, after correction for all 44 tested antigens in the HLA-A, B, C, DR and DQ loci, the results were no longer significant. The group of CIDP patients who improved after IVIg and the patients who did not improve were also compared to each other. HLA-A11 was found in 4 of 20 (20%) patients who did not improve and only in 1 of 32 (3%) patients who improved, HLA-DRw6 was found in 12 of 32 (38%) patients who improved and only in 2 of 18 (10%) patients who did not improve after IVIg treatment. The phenotype frequency in the control

group of 504 donors for HLA-A11 is 12%, and 31% for HLA-DRw6. However, no statistically different p values were found after correction.
Of 52 CIDP patients, 22 (42%) had a positive MHT, 24 (46%) a positive NBL-IFA and 36 (69%) a positive MHT and/or NBL-IFA (Table 1).
The presence of a positive MHT or NBL-IFA was not significantly associated with improvement after IVIg treatment. The MHT results were not significantly correlated with any of the HLA antigens tested for. HLA-DRw8 was more frequently ($p=0.008$) observed in patients with anti-NBL antibodies (4/24) than in patients without anti-NBL antibodies (0/28), but this again is not significant after correction.

## DISCUSSION

This study shows that the HLA antigens B5, B16 and Cw2 are more frequently found in patients with CIDP than in the normal population. Nevertheless, we hesitate to suggest an association between CIDP and genetic factors, since when the p values are corrected for the number of antigens tested in the different HLA loci, the results were no longer significant.
Adams et al.[4] investigated 14 patients with recurrent or chronic relapsing inflammatory polyneuropathy. This HLA typing included recognition of the DR1-DRw8 antigens. The frequency of HLA-B8 was found to be increased, however the p value was not corrected.
Stewart et al.[290] performed HLA typing in 22 CIDP patients, whereas typing for HLA-DR antigens was only performed for DR1, DR2 and DR3. They found a significant association with HLA-A30/31 after correction for the number of tested antigens, but the "probable" association with HLA-B8 and DR3 was not significant.
The recent study by Feeney et al.[94] included the CIDP patients which had been described by Stewart et al.[290] and revealed increased frequencies of the HLA-A3, B7 and DR2 antigens, both in patients with a chronic progressive and a chronic relapsing course of disease. A decreased frequency was found for the HLA-B44 and DR7 antigens. However none of these findings reached statistical significance. Feeney et al.[94] found that the joint occurrence of HLA-B7 and DR2 was much greater than would be seen normally. In our group of 52 patients, 50% of HLA-B7 positive patients are DR2 positive and 29% of the HLA-DR2 positive patients are B7 positive, indicating that the frequencies were not increased as compared to controls.
Therefore we have no arguments for an HLA-DR2 association in patients with CIDP as was suggested by Feeney et al.[94]
A comparison between increased frequencies of certain HLA antigens as reported in other studies and the results in our group of CIDP patients is shown in Table 3.
We could not demonstrate a significant HLA association between the group of CIDP patients improving after IVIg or those who did not.
The presence of MHT or anti-NBL antibodies was not associated with improvement, being in contrast to an earlier study[320] in which we found an association between the presence of anti-peripheral nerve antibodies as demonstrated by a MHT, and the improvement after IVIg in a smaller series of patients. Our present MHT results are compatible with findings by Osterman et al.[229] who could not demonstrate an association between the presence of MHT antibodies and a favourable response to plasma exchange in 36 GBS patients, although such an association initially was reported by the same group in a series of 7 CIDP and 11 GBS patients.[325]

Table 3. HLA typing for Class I and II antigens in CIDP patients performed by different groups.

| | Increased HLA frequency | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Adams B8, DR3[1] | | | Stewart A30/31[2] (B8, DR3)[3] | | | Feeney (A3, B7, B44, DR2, DR7)[4] | | | vDoorn (B5, B16, Cw2)[3] | | |
| | patients | | controls | patients | | controls | patients | | controls | patients | | controls |
| HLA | N = 14 | | N = 571 | N = 22 | | N = 322 | N= 71/56 | | N = 1058 | N = 52 | | N = 504 |
| antigen | pos | % | % pos | pos | % | % pos | pos | % | % pos | pos | % | % pos |
| A3 | 7 | 50 | 29 | 3 | 23 | 29 | 24 | 34 | 27 | 22 | 42 | 32 |
| A30/31 | 1 | 7 | 3 | 7 | 32 | 5 | 8 | 11 | 9 | 6 | 11 | 8 |
| B5 | 2 | 14 | 8 | 2 | 23 | 10 | 10 | 14 | 10 | 10 | 19 | 11 |
| B7 | 3 | 21 | 24 | 6 | 27 | 26 | 25 | 35 | 27 | 14 | 27 | 29 |
| B8 | 7 | 50 | 25 | 9 | 41 | 26 | 18 | 25 | 27 | 12 | 23 | 23 |
| B44 | 1 | 7 | - | nd | nd | nd | 13 | 18 | 29 | 11 | 21 | 22 |
| B16 | 0 | 0 | 7 | 1 | 5 | 1 | 4 | 6 | 6 | 9 | 17 | 7 |
| Cw2 | 1 | 14* | - | nd | nd | nd | nd | nd | nd | 9 | 17 | 9 |
| DR2 | 1 | 8 | 21 | 5/18 | 28 | 21 | 23 | 32 | 29 | 17 | 33 | 28 |
| DR3 | 7 | 50 | 17 | 8/18 | 44 | 14 | 23 | 32 | 29 | 12 | 23 | 24 |
| DR7 | 3 | 23 | 27 | nd | nd | nd | 10 | 14 | 26 | 13 | 25 | 22 |

[1] = significant *p* values, not corrected
[2] = significant *p* values after correction
[3] = significant *p* values before, but not after correction
[4] = "increased, but non-significant *p* values after correction"

* = presumably 7 patients tested, population frequency not known
nd = not tested

In conclusion, in this study we could not detect nor confirm any significant associations between CIDP and HLA-A, B, C, DR and DQ antigens, nor did we find any significant HLA association with the presence of neural antibodies nor with the improvement after IVIg treatment. However, it must be emphasized that all the HLA studies in CIDP patients concern relatively small numbers of cases.

Chapter 9

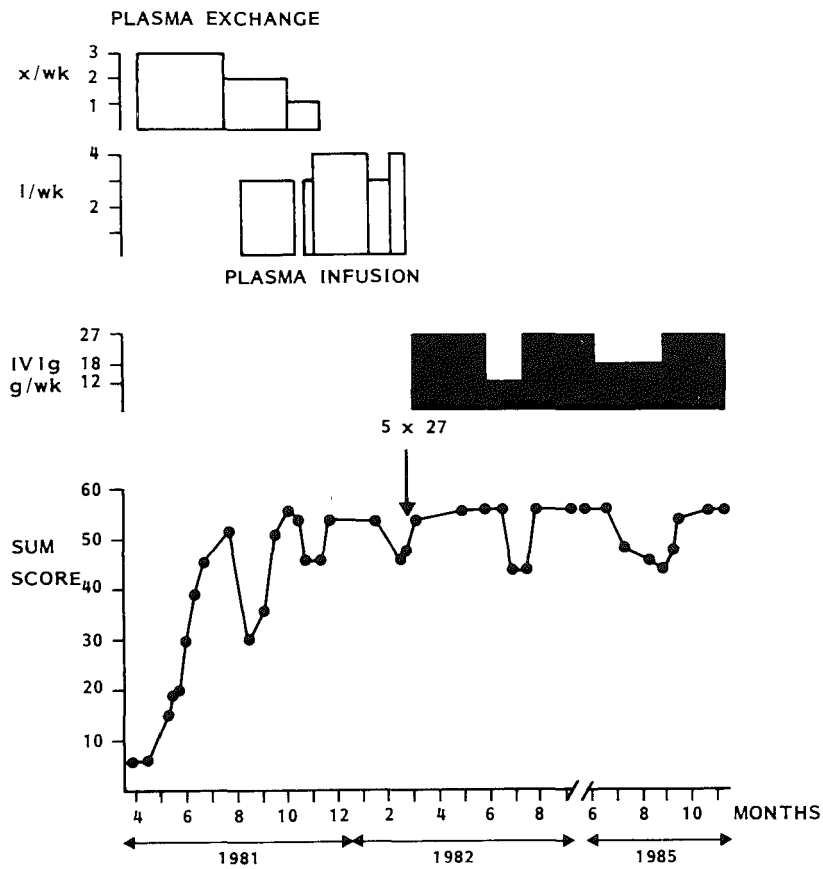
# ON THE MECHANISM OF HIGH-DOSE INTRAVENOUS IMMUNOGLOBULIN TREATMENT OF PATIENTS WITH CHRONIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY

## INTRODUCTION

Acute inflammatory demyelinating polyneuropathy or Guillain-Barré syndrome (GBS) is preceded in over half of the cases by an infectious illness[14] and is self-limiting.[15] Randomized clinical studies demonstrated that the extent and duration of paralysis is favourably influenced by plasma exchange.[100,302] Patients with chronic inflammatory demyelinating polyneuropathy (CIDP),[79,78] may also benefit from plasma exchange, although only transient clinical improvement has been seen with this treatment.[81] In several uncontrolled studies a beneficial response to normal polyspecific immunoglobulins (IVIg) in CIDP was found.[6,50,54,59,87,328] A double-blind crossover study in seven CIDP patients, who were considered to be dependent on IVIg, showed that all patients responded to IVIg and none to placebo infusions.[322] Although the immune mechanism and the antigens involved in CIDP are not yet determined, there is growing evidence that anti-neural antibodies contribute to the demyelination process in GBS and CIDP.[28,52,126,154,153,287,320,321] We previously found that the sera of about half of the patients with GBS and CIDP show antibodies against human sciatic nerve.[320] These antibodies cross-react with a membrane antigen expressed by the 108cc15 neuroblastoma cell line (NBL) allowing their detection by an indirect immunofluorescence assay. The anti-NBL antibodies are often of the IgM-class and decreased or disappeared in patients retested after improvement following IVIg.[321] In vitro studies showed that IVIg inhibits the reaction between serum from a CIDP patient and the NBL cell line.

The aims of the present study were to investigate whether this inhibitory effect can also be obtained with $F(ab')_2$ fragments of IVIg, which would support the suggestion that inhibition of anti-neural tissue antibodies is due to neutralization by specific antibodies present in IVIg. Secondly, we investigated if the IgG fraction of postrecovery GBS serum inhibits anti-NBL activity in autologous prerecovery serum which would suggest that GBS is selflimiting by suppression of auto-antibodies. Thirdly the degree of cross-reactivity between various individuals including recovered GBS patients and healthy donors against a particular anti-NBL antibody from a CIDP patient was evaluated.

Figure 1. *The course of the neurological sum-score in a patient with CIDP upon treatment with plasma-exchange, infusions of FFP and infusions of IVIg. The sum-score is the total MRC score of the following muscles on either side of the body: deltoideus, biceps, extensors of the hand, iliopsoas, quadriceps and tibialis anterior.*



# PATIENTS AND METHODS

**CIDP patient (A)**
A 14 year old boy presented in 1980 with slowly progressive paralysis over a period of two months. He had total paralysis of the legs and arms (MRC 0).[204] Nerve conduction velocities (NCV) of the right motor median and peroneal nerve were decreased and H-reflexes were absent. CSF protein concentration was increased (0.97 g/L). A sural nerve biopsy showed widening of the subperineural space and signs of demyelination. There was no systemic disease, intoxication or family history of hereditary polyneuropathy. The patient did not respond to a 3 months course of prednisone and azathioprine. Plasmapheresis (4 litre exchanges weekly) resulted in rapid, always transient improvement but had to be discontinued after a few months as recurrent shunt-thrombosis caused problems in vascular access. The patient was subsequently treated with transfusions of cryo-supernatant plasma (100 ml/kg body weight weekly) which was as effective as plasmapheresis. When plasma treatment was discontinued clinical deterioration occurred within 2-4 weeks. A similar beneficial effect was observed after IVIg infusion. IVIg 0.4 g/kg bodyweight for 5 consecutive days resulted in maximal improvement after 8 days. Thereafter the patient's condition remained stable for about 2 weeks when subjective and objective signs of muscular weakness reappeared. A regular IVIg treatment regimen with empirically established dosages was continued for 6 years with several unsuccessful attempts to taper off (Figure 1). At the present time the patient receives 21 g IVIg weekly (0.3 g/kg body weight). Currently, pre-infusion serum immunoglobulin concentrations are 14.3 g/L for IgG; 3 g/L for IgM, and 2 g/L for IgA. Liver function tests are normal. There are no subjective and objective side effects of IVIg infusions. This CIDP patient is still able to perform normal daily activities.

**GBS patient (B)**
A 35 year old man developed sensory disturbances and progressive muscle weakness over a period of three weeks, resulting in paralysis of the legs (MRC O) and paresis of the arms (MRC 3). Four weeks from onset of weakness improvement started and resulted in complete recovery after three months.

**Anti-neuroblastoma cell line (NBL) antibodies**
The neuroblastoma (NBL) 108ccl5 cell line was kindly provided by Prof B Hamprecht (Physiological-Chemical Institute, University of Tübingen, FRG). The cells were cultured in Dulbecco's Modified Eagles Medium supplemented with 10% heated fetal calf serum in a humidified atmosphere containing 5% CO2 at 37°C. The indirect immunofluorescence assay (IFA) was previously described.[321] The number of fluorescent cells of 200 counted cells was determined and expressed as a percentage. Forty normal blood bank donors showed a mean of 4% (range 0-15%) positive NBL cells. A percentage of fluorescent cells above 20% was considered positive. The titer of a positive serum was defined as the last dilution step which gave a positive (>20%) NBL-IFA. In the active phase of the disease, patient A (CIDP) had positive anti-NBL antibodies. These antibodies were of the IgM-class and were present in a titer of 1/64. During therapeutically induced remission with IVIg the antibody titer varied between 0 and 1/2. From patient B (GBS) on day 6 after onset of muscle weakness, serum was withdrawn. Anti-NBL antibodies of the IgM class were present in a titer of 1/32. One year after uneventful recovery, serum contained no anti-NBL antibodies of the IgM or IgG-class.

**Sera and immunoglobulin preparations**
Serum samples were also obtained from 25 other GBS patients who had recovered since maximally 5 years. Serum samples from 26 donors from the Leiden Red Cross Blood Transfusion Service were obtained after consent. All sera were stored at -20°C. Plasma was clotted with $CaCl_2$ and subsequently stored at -20°C.
Normal polyspecific immunoglobulin for intravenous use, derived from a large pool of donor plasma (IVIg) was obtained from the Central Laboratory of the Dutch Red Cross Blood Transfusion Service.
The IgM fraction of the CIDP patient's (A) pretreatment serum was prepared by euglobulin--precipitation, and chromatography on Sepharose G200. The high molecular weight fractions containing IgM and no detectable IgG in immunodiffusion were pooled and concentrated with polyethylene glycol 15-20,000 MW to 1.74 mg/ml. IgG fractions from the serum of one of the 26 Blood bank donors and post-recovery serum from the GBS patient (B') were prepared by ammonium sulphate precipitation and chromatography on DE-52 cellulose (Whatman). IgG and IgM concentrations were measured by radial immunodiffusion using monospecific rabbit anti-human IgG and IgM antibodies (Behringwerke, Marburg, FRG).
$F(ab')_2$ fragments were prepared from IVIg and from the post-recovery IgG from the GBS patient by pepsin-digestion (2%, w/w) and chromatography on protein-A sepharose (Pharmacia).
Polyvalent IVIg, postrecovery GBS serum and control serum were checked for rheumatoid factor activity and anti-Ig allotype antibodies.[258]

**Inhibition of NBL-IFA antibodies**
NBL-antibodies: serum of patient A derived during the active phase of the disease was used for inhibition studies in a titer of 1:4 (calculated IgM-level in this dilution step was 0.3 mg/ml). The percentage fluorescent NBL cells with serum from patient A was 87% in a dilution of 1:4 and 78% in a dilution of 1:8. The IgM-fraction of this serum used for inhibition studies contained 0.87 mg/ml. Prerecovery serum of patient B was tested in a dilution 1:1 (calculated IgM level in this dilution step was 2.02 mg/ml). The percentage immunofluorescent NBL cells with prerecovery serum from patient B was 82 % in a dilution of 1:1 and 88 % in a dilution of 1:2.
Inhibitors: serum from patient B from the prerecovery phase was absorbed on NBL-cells until the NBL-IFA was negative. From serum, of patient B obtained one year after uneventful recovery (B'), IgG and $F(ab)_2$ fragments were prepared. Sera from 25 recovered GBS patients, derived within 5 years after recovery and 26 blood bank donors were tested as inhibitors. Polyvalent IVIg was tested as such and after preparation of $F(ab)_2$ fragments.
Inhibition assay: for inhibition experiments, the inhibitor serum, IgG or $F(ab')_2$ fragments were titrated in phosphate buffered saline (PBS) pH 7.4.
One volume (50 microliter) of the antibody containing sera or immunoglobulin-fractions of patient A or B was mixed with one volume of PBS containing various concentrations of inhibitor. The mixture was immediately incubated with $0,5 \times 10^6$ NBL cells and further processed for NBL-IFA. For each mixture, the percentage of inhibition of the NBL-IFA and the corresponding molar ratio is calculated. The molar ratio is defined as the amount of the patients' prerecovery IgM divided by the amount of inhibitory IgG or $F(ab')_2$ and is expressed in mol/mol.

## RESULTS

Incubation of pretreatment serum from the CIDP patient (A) with various concentrations of IVIg and $F(ab')_2$ fragments of IVIg in vitro resulted in dose dependent inhibition of the binding of anti-NBL antibodies to the NBL l08cc15 cells of which an example is shown in Figure 2.

Maximal inhibition occurred with intact IVIg and $F(ab')_2$ fragments of IVIg at a calculated molar ratio of patient's IgM to IVIg and patient's IgM to IVIg-$F(ab')_2$ of about 0,003; the inhibition curves showed a prozone. Similar results were obtained when inhibition of anti-NBL activity was assessed with the IgM fraction of the serum from patient A (Table 1).

Thus $F(ab')_2$ fragments of IVIg contains antibody specificities which interact with the anti-NBL antibodies in the patient's serum. Since anti-NBL antibodies are found in the serum of patients with CIDP and in patients with the self-limiting GBS, we examined whether the serum from patients who recovered from GBS contained antibodies which could inhibit anti-NBL activity in CIDP serum. Table 2 shows that anti-NBL activity in the CIDP patient's (A) serum was inhibited by IgG and $F(ab')_2$ fragments from postrecovery serum from a patient with GBS (B'). The prerecovery GBS serum (B) did not inhibit patients' A anti-NBL antibodies. This prerecovery serum was tested after absorption on NBL cells to remove anti-NBL activity. The IgG fraction and $F(ab')_2$ fragments from post-

Figure 2. *In vitro inhibition of the binding to NBL 108cc15 cells of IgM-anti-NBL antibodies from the serum of a patient with CIDP by IVIg (—) and $F(ab')_2$ fragments prepared from IVIg (- -).*

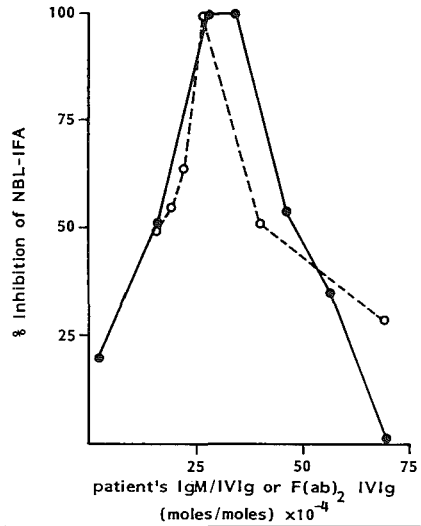



Table 1. **Inhibition of anti-NBL activity in serum and in the IgM-fraction from a patient with CIDP (A) by IVIg and $F(ab')_2$ fragments of IVIg.**

| | Serum | | IgM fraction | |
|---|---|---|---|---|
| | % inhibition* | molar ratio** | % inhibition* | molar ratio** |
| IVIg | 86 ± 3 | 0.0047 (0.0003-0.036) | 81 ± 15 | 0.0021 (0.0005-0.01) |
| $F(ab')_2$ IVIg | 78 ± 9 | 0.0044 (0.0003-0.016) | 82 ± 6 | 0.0011 (0.0005-0.01) |

* Maximal inhibition (mean value ± SD from at least three experiments) of anti-NBL activity obtained by mixing patient's serum or IgM fraction with IVIg or $F(ab')_2$ of IVIg.

** Indicated are molar ratio's between patient's IgM and IVIg or $F(ab')_2$ fragments of IVIg with which maximal inhibition was obtained.
Parentheses indicate the range of molar ratio's that were tested.

Table 2. **Inhibition of anti-NBL antibodies from a patient with CIDP (A) and a patient with GBS (B) by postrecovery (B') GBS-IgG and $F(ab')_2$ fragments, and IgG from a normal donor**

| | CIDP serum (A) | | GBS serum (B) | |
|---|---|---|---|---|
| | % inhibition* | molar ratio | % inhibition* | molar ratio |
| Postrecovery B' GBS-IgG | 69** | 0.036 (0.0071-3.652) | 100 | 0.75 (0.032-16.43) |
| Postrecovery B' GBS $F(ab')_2$-IgG | 65 | 0.02 (0.0048-0.064) | 100 | 0.28 (0.0088-4.493) |
| Normal Donor IgG | 20 ± 2 | 0.005 (0.0025-0.65) | nd | |

nd=not done

* Indicated is the maximal inhibition that was obtained and the molar ratio between patient's IgM and inhibitory IgG or $F(ab')_2$ fragments at which maximal inhibition was achieved.

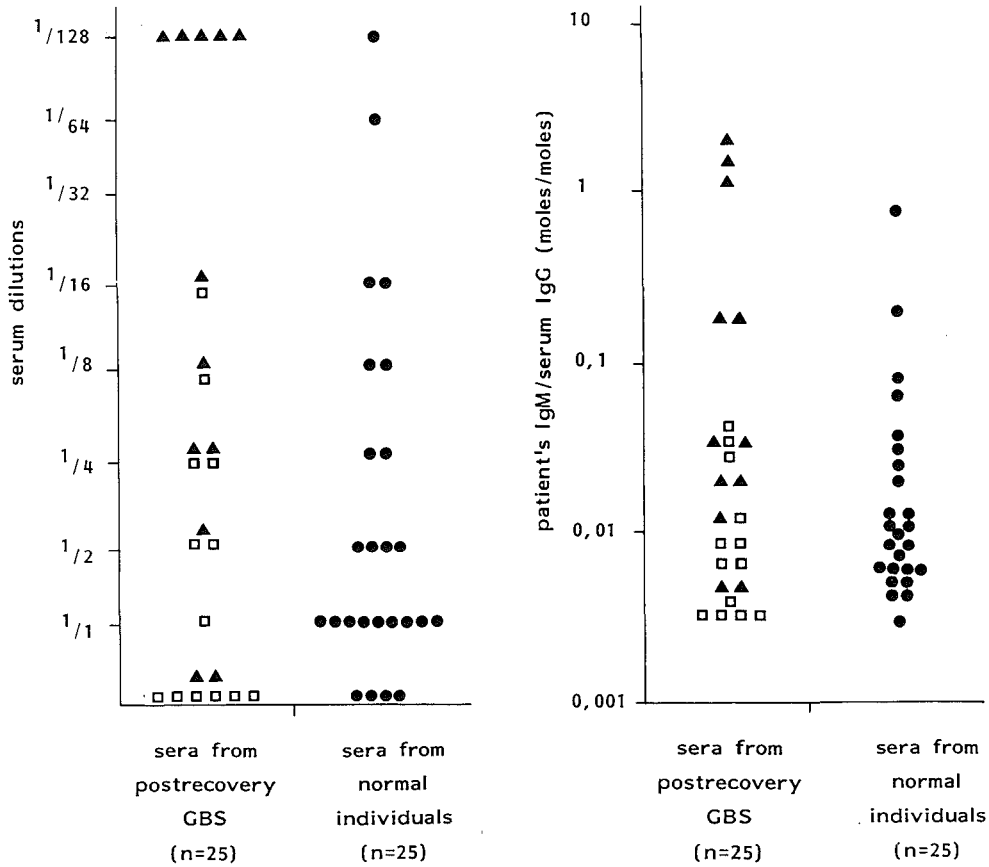
** Results are expressed as mean inhibition (± SD, when at least three experiments were performed), parentheses indicate the range of molar ratio's tested. Due to shortage of prerecovery GBS serum (B) only a few experiments could be performed.

recovery serum of the GBS patient also inhibited anti-NBL activity in autologous prerecovery serum. In all instances, inhibition was dose dependent with an optimum at a specific molar ratio of patients' IgM to inhibitor IgG or $F(ab')_2$ fragments. IgG and $F(ab')_2$ fragments from GBS postrecovery serum (B') were 25 fold more effective in inhibiting autologous prerecovery anti--NBL antibodies compared to the inhibition of the anti-NBL antibodies of the CIDP patient. Inhibition of anti-NBL activity was also found in other postrecovery GBS sera and blood bank donor sera upon incubation with the serum of the CIDP patient. The dilution in which normal donor and post recovery GBS sera were still able to cause 75% inhibition of the CIDP anti-NBL-IFA is shown in Figure 3 (left side). The calculated molar ratio's are shown on the right side of Figure 3. The mean molar ratio of the CIDP patients' (A) IgM to IgG in the inhibitory serum was 0,0589 in normal individuals and 0,2517 in postrecovery sera, this difference did not reach statistical significance. GBS sera obtained within 3 years of recovery were more effective in inhibiting anti-NBL activity than those obtained more than 3 years after recovery. Five out of 12 sera from recovered GBS patients obtained within 3 years after recovery, inhibited anti-NBL antibodies in a dilution >1/64 corresponding with a calculated molar ratio of >0.1. Two out of 25 blood donor sera showed a similar strong inhibition. IVIg and post-recovery GBS sera contained no anti-NBL antibodies, no rheumatoid factor activity and no detectable anti-allotype antibodies against the G1m(1) G1m(3), G1m(4), G1m(17) and Km(1) allotypes expressed in the heavy and light chain regions of human IgG.

## DISCUSSION

A 14 year old patient with severe chronic inflammatory demyelinating polyneuropathy (CIDP) responded to plasmapheresis, infusions of fresh frozen plasma and infusions of IVIg. The

Figure 3. *Inhibition of anti-NBL activity in the serum from a patient with CIDP by postrecovery sera from patients with GBS and sera from normal individuals. The left side presents the lowest dilution of serum able to result in at least 75% inhibition of NBL-immunofluorescence. The right side shows the calculated molar ratios between IgM in the serum of the patient with CIDP and IgG in the inhibitory sera. Normal individuals (●), sera obtained less than 3 years postrecovery from GBS (▲), and sera obtained more than 3 years postrecovery from GBS (□).*




duration of the clinical response did not exceed 4 weeks following a single infusion of IVIg. The serum from this CIDP patient (A) contained anti-neuroblastoma 108cc15 (NBL) antibodies of the IgM-class in a titer of 1/64 as assessed by immunofluorescence. Anti-NBL activity in patient's sera decreased after infusions of IVIg, and reappeared in association with weakness. Incubation of the patient's pretreatment IgM fraction with intact IVIg or with $F(ab')_2$ fragments from IVIg in vitro resulted in a dose dependent inhibition of anti-NBL activity that reached in various assays 80-100% at specific molar ratios between patient's IgM and competitor IgG. The prozone pattern of the inhibition curve was similar to that which had previously been observed with other auto-antibodies neutralized by IVIg.[145,293] Since the inhibitory effect was observed with $F(ab')_2$ fragments from IVIg and since rheumatoid factor activity, anti-Ig allotype antibodies and anti-NBL antibodies, could not be demonstrated in IVIg, these observations suggest

that IVIg contains antibodies against idiotypic determinants expressed on anti-NBL antibodies. Thus the beneficial effect of IVIg in the CIDP patient may be dependent on IVIg mediated suppression of auto-antibodies in a similar fashion to anti-idiotypic suppression of auto-antibodies observed with IVIg in patients with anti-Factor VIII:c auto-antibodies[293,294] and in a patient with autoimmune pure red cell aplasia.[197]

Anti-NBL activity in CIDP patients' serum was inhibited both by IgG and $F(ab')_2$-fragments from IgG of a patient who recovered from GBS (B'). The prerecovery serum from patient B contained - after absorption of the specific NBL antibodies on NBL cell line cells - no inhibitory activity against the CIDP patients' anti-NBL antibodies. Post-recovery IgG from this patient with GBS also inhibited anti-NBL activity in autologous prerecovery serum suggesting that recovery from GBS is associated with the generation of anti-idiotypic antibodies. Spontaneously occurring anti-idiotypes have been found in the serum of patients with SLE in remission,[1] myasthenia gravis,[72] and in the serum of a patient who spontaneously recovered from auto-antibodies to factor VIII.[294] Our observations suggest that anti-idiotypic IgG antibodies against auto-antibodies develop during recovery from GBS and that these anti-idiotypic antibodies cross-react with antibodies in a CIDP patient. The higher ability of the postrecovery serum from the GBS patient (B') to inhibit anti-NBL in his own prerecovery serum (B) points into the direction of higher affinity of the private compared to the cross-reactive idiotypic determinants. To investigate the incidence of the presence of private and cross-reactive anti-idiotypic antibodies, more autologous versus allogeneic combinations must be compared, as we tested only two patients and made only one pre- and postrecovery comparison.

Sera from 5/12 patients recovered within 3 years from GBS but also 2/25 sera from healthy individuals showed strong (>75%) inhibitory anti-NBL activity against the CIDP serum. The remaining sera from recovered GBS and random blood donors showed equal inhibitory activity of the anti NBL antibodies of this particular CIDP patient tested.

Although the mechanism of action of IVIg in antibody-mediated organ-specific autoimmune diseases is still the subject of hypothesis and research,[215] evidence has been provided for IVIg mediated anti-idiotypic suppression in patients with anti-FVIII antibodies and anti BFU-c antibodies.[197,293] It has however not yet been established whether anti-NBL antibodies present in the sera from patients with GBS and CIDP represent pathogenic auto-antibodies.[256] When anti-NBL antibodies are present in CIDP patients, they decrease or disappear in association with rapid clinical improvement after IVIg suggesting that they have in individual patients a pathogenic role.

Our experiments were limited to the anti-NBL antibodies of one CIDP patient and one GBS patient in the acute phase of their disease and need to be extended to confirm the presence as well as the degree of cross-reactivity between GBS and CIDP antibodies. The beneficial effect of IVIg in patients with CIDP and the self-limiting character of GBS, in association with the development of novel anti-NBL antibody-inhibitory activity, may represent additional examples of therapeutic and spontaneous anti-idiotypic suppression of auto-antibodies in human autoimmune diseases.

# GENERAL DISCUSSION

Inflammatory demyelinating polyneuropathies have been divided into an acute and a chronic variety because of their difference in onset, course and prognosis. Criteria have been developed for the diagnosis of the acute form[15] and Barohn et al.[23] suggested that there is a need for criteria for the chronic form as well. Recently this view has been challenged by Dyck[83] who commented that such consensus criteria have a tendency to be thought of as truth, and that if criteria are developed prematurely they may inhibit needed thought and study. He concluded that for the present, patients with a chronic symmetrical polyradiculoneuropathy with low nerve conduction velocity and raised CSF protein without associated monoclonal gammopathy or disease association should be separated from those associated with these disorders.

After intravenous immunoglobulin (IVIg) treatment, 62% of the patients who had been judged to have chronic inflammatory demyelinating polyneuropathy (CIDP) improved (Chapter 3). The first question is whether these patients really responded to this treatment. In a crossover study in IVIg responders it was shown that patients responded to IVIg and not to placebo (Chapter 2), therefore at least some CIDP patients respond to IVIg. The second question is why not all patients improve. One explanation could be that patients who did not improve have a different neuropathy and this seems a likely explanation in some patients. Another explanation could be that at least some of the non-responders did not had an active form of the disease but actually had fixed neurological deficit. Analysis of clinical features which were associated with treatment response showed that patients with CIDP had a chance of over 90% to improve after IVIg if the following factors were present: 1) disease duration of at least 1 year, 2) progression of weakness until treatment, 3) absence of discrepancy between weakness of arms and legs, 4) areflexia of the arms and 5) slowed nerve conduction velocity of the motor median nerve. Patients without all these factors have a low chance of improving after IVIg treatment and may have a different neuropathy. The most important other neuropathies to consider are the chronic axonal neuropathy of undetermined cause, vasculitic neuropathy without systemic vasculitis, multifocal demyelinating neuropathy with persistent conduction block and multifocal motor neuropathy. These neuropathies have been described in the introduction (Chapter 1). The onset of the chronic axonal polyneuropathy of undetermined cause is usually not exactly known and is in many cases thought to be more recent than it actually was. When the neuropathy exists for many years the conduction velocity especially in the legs may have slowed considerably and the CSF protein may have increased over the years. Therefore this neuropathy may be difficult to distinguish from CIDP. A long history of the disease, a very slowly progressive course and slight

involvement of the arms should raise the suspicion of this mysterious neuropathy which unfortunately does not respond to any treatment. If there is a clear difference between weakness of arms and legs, especially if there was asymmetry at onset, vasculitic neuropathy and the multifocal neuropathies should be considered. Until now, IVIg treatment in these patients was disappointing.
Recently it has been reported that patients with IgM monoclonal gammopathy of unknown significance (MGUS) may respond to IVIg treatment.[48] No reports are available on IVIg treatment in patients with clinical features of CIDP accompanied by another disease such as inflammatory bowel disease, chronic active hepatitis, thyrotoxicosis, HIV infection, Hodgkin disease and CNS demyelination. Barohn et al.[23] stressed that the neuropathy in these patients may be indistinguishable from CIDP without another disorder and suggested that CIDP should be considered as a syndrome with diverse causes. It would be interesting to investigate IVIg treatment response in those CIDP patients who have an accompanying disorder and to see if there is any difference compared to CIDP patients without an associated disease.

Should patients with CIDP be treated with IVIg? The crossover study in selected patients with CIDP showed that these patients may benefit from this treatment. If this can be confirmed by the results of a randomized double blind placebo controlled study, IVIg treatment has to be preferred in comparison with other treatments (prednisone and plasma exchange) that were shown to be effective in clinical trials.[81,76] IVIg treatment is safe in contrast to prednisone. There is no need for a trial comparing IVIg and prednisone; if a patient responds to IVIg this treatment can be continued, if not, plasma exchange or prednisone treatment has to be considered. In this decision the inconvenience of regular plasma exchange treatment has to be weighed against the side effects of prednisone. In the treatment of the Guillain-Barré syndrome the situation is completely different because in this disorder the most effective treatment has to be given at once and there is no chance to try a second treatment if the first has failed.

The main advantages of IVIg treatment are that clinical response starts much earlier than after prednisone treatment and that it is without serious side effects, but maintenance treatment generally requires IVIg infusion every week or every other week which is very expensive and which takes about two hours. To develop preparations which are cheaper and which can be administered in smaller volume we need to know more about the pathogenesis of CIDP and about the mechanism of improvement after IVIg.

The therapeutic mechanism of IVIg in CIDP is unknown. This mechanism was investigated in vitro in two CIDP patients producing IgM and IgG antibodies respectively against the neuroblastoma cell line (NBL) 108cc15. IgG and $F(ab)_2$ fragments of IgG from the IVIg pool neutralized the purified IgM anti-NBL antibodies of the first patient as well as the IgG or $F(ab)_2$ fragments of the anti-NBL antibodies of the second patient.[185] Neutralizing antibodies were also present in sera from healthy individuals and in sera from patients who recovered from the GBS. Sera from recovered GBS patients which were obtained within three years from onset of GBS had a stronger neutralizing capacity than sera that were obtained after this period of time. In the one GBS patient tested, neutralizing antibodies were present only in the recovery phase and not in the active phase of the disease. The antibodies present during recovery effectively neutralized autologous anti-NBL antibodies (Chapter 9).

These findings suggest that autologous and therapeutic recovery from GBS and CIDP respectively, occurs by anti-idiotypic antibodies which neutralize pathogenic anti-neural antibodies. Several aspects must be further investigated to establish this hypothesis and to improve the present therapeutical approach. An important aspect is the degree of cross-reactivity between the various anti-idiotypic antibodies especially in relation to inhibition of antibodies from responding and non-responding patients to IVIg treatment. Possibly, non-responders have auto-antibodies that express no cross-reactive idiotypes neutralized by anti-idiotypic antibodies present in IVIg derived from a large donor pool. Studies on this cross-reactivity may subsequently lead to the pretreatment selection of IVIg responding patients. The question is whether or not antibodies of responding patients express a single or multiple cross-reacting idiotypes. In case of a single or limited cross-reacting idiotype, an IVIg preparation with increased effectivity can be prepared from plasma of one or a few individuals with a high titer of neutralizing antibodies against NBL-antibodies. Ultimately when the degree of cross-reactivity is established, it can be decided whether it is justified to prepare human monoclonal anti-idiotypic antibodies for the treatment of CIDP, since such an approach is not feasible when a particular patient needs a unique anti-idiotypic monoclonal antibody.

Other intriguing observations are that 9 of 52 (17%) CIDP patients recovered after one or more IVIg treatment courses and that in some patients the duration of clinical improvement exceeded three half-life periods of IVIg. Permanent remission after IVIg treatment has also been observed in patients with autoimmune thrombocytopenia (ITP). This was mainly described in children,[136] in which it is particularly difficult to distinguish between a therapeutical response and a spontaneous course of ITP. A definite IVIg treatment response however has also been reported in patients with chronic ITP.[137] Similarly, spontaneous remissions in CIDP patients may be difficult to distinguish from remissions induced by IVIg, especially since CIDP patients may even have a rapid spontaneous improvement after a progression of muscle weakness over several months.
Prolonged improvement in CIDP and ITP patients might also be the result of inhibition of auto-antibody production due to interaction between IVIg and antibody presenting - or antibody producing cells. This is presumably not a specific inhibition of particular auto-antibodies, since inhibition of pokeweed induced antibody production in vitro was observed in studies on B cells derived from patients treated with IVIg.[66] Furthermore, in one preliminary study, the clinical effectiveness of IVIg in ITP patients was associated with an increase in the T helper-suppressor subset.[66,314]

A crucial question is whether the anti-NBL antibodies in CIDP and GBS patients have a pathogenic role. It was suggested that anti-nerve antibodies could be the result from nerve damage exposing immunogenic structures.[53] This is unlikely for the anti-NBL 108cc15 antibodies since they were rarely found in patients with other neuropathies and since they were also present in patients with Lyme borreliosis who had no clinical signs of nerve damage.
The anti-NBL antibodies were shown to cross-react with human peripheral nerve tissue, but also with Borrelia burgdorferi, which led to the hypothesis that CIDP and GBS are preceded by infections with various micro-organisms sharing antigenic epitopes with nerve tissue.

The reason why not all patients with circulating anti-NBL antibodies develop a neuropathy is not known. Careful investigations regarding the antibody titer, the immunoglobulin (sub)classes and the ability of complement activation may be helpful to answer this question. Another

possibility to consider is that anti-NBL antibodies itself are non-pathogenic, but that they need another co-factor to cause nerve damage.
Patients with CIDP have in 50% of the cases no anti-NBL antibodies. This may be explained by the insensitivity of the antibody demonstrating technique, another possibility is that in patients without demonstrable anti-NBL antibodies, a neural antigen is involved that is not expressed on the NBL 108cc15 cell line. Clinical differences or a different treatment response between patients with or without anti-NBL antibodies could not be demonstrated. This is in agreement with results in patients with idiopathic thrombocytopenic purpura (ITP), in which circulating auto-antibodies can not be detected in all patients and even the presence of detectable auto-antibodies against platelets is not significantly associated with treatment response.

In conclusion, patients with CIDP may improve after treatment with IVIg. The possible mechanism of action of IVIg was studied by investigating the interactions between IVIg and anti-NBL antibodies. In vitro and in vivo observations suggest that improvement after IVIg treatment occurs by inhibition of auto-antibodies reacting with NBL cells. However, other biological effects of IVIg such as blocking and modulation of Fc-gamma-receptors, enhancement of removal of persisting micro-organisms and immune complexes, and the interference with the production of cytokines cannot be excluded and may act synergistic with the neutralizing antibodies in IVIg.

# SUMMARY

The inflammatory demyelinating polyneuropathies can be distinguished in the Guillain-Barré syndrome (GBS) and in the chronic variety CIDP.
The subject of this thesis is high-dose intravenous immunoglobulin (IVIg) treatment in CIDP.

In Chapter 1, clinical and laboratory features of CIDP were described. A remitting inflammatory polyneuropathy was already reported at the end of the last century. The name of the disease varied several times, currently the most frequently used name is "chronic inflammatory demyelinating poly(radiculo)neuropathy". CIDP generally consists of a sensorimotor polyneuropathy resulting in symmetrical muscle weakness and sensory disturbances with a hypo- or areflexia. Usually the protein in the CSF is increased and nerve conduction velocities are slowed. There is no definite test for the diagnosis of CIDP. Recently Barohn et al.[23] proposed diagnostic criteria for CIDP which were discussed in Chapter 1.
Controlled studies showed that CIDP patients may improve after plasma exchange[81] and prednisone treatment.[76] The observation that CIDP patients may also improve after treatment with IVIg was investigated in a double-blind placebo-controlled crossover study (Chapter 2). In this study, regular IVIg treatment was discontinued after informed consent in 7 patients with CIDP who seemed to have responded to IVIg. After discontinuation of IVIg, all patients deteriorated. Subsequently the patients were randomized to IVIg or placebo (albumin) infusions. The clinical condition of all patients improved after IVIg and did not improve after placebo treatment ($p=0.02$). The mean time lapse from the end of trial treatment to the occurrence of deterioration was 6.4 weeks after treatment with IVIg and 1.3 weeks after treatment with placebo. This selected group of CIDP patients had a beneficial response to IVIg.
In Chapter 3, clinical and laboratory data are presented of a study in 52 patients who were judged to have a CIDP. All patients were treated with IVIg, 20 (38%) patients did not improve after IVIg treatment, 2 (4%) patients had a short lasting improvement and subsequent infusions had no effect, 9 (17%) patients reached a spontaneously or therapeutically induced complete remission and 21 (40%) patients needed intermittent infusions to maintain improvement. All patients who improved initially had symptoms which at least significantly interfered with lifestyle. After IVIg treatment, 90% of these patients were independent in their daily activities. Significantly associated with improvement were: disease duration of less than 1 year, progression of weakness until treatment, absence of discrepancy in weakness between arms and legs, areflexia of the arms and slowed nerve conduction velocity of the motor median nerve. The probability of improvement after IVIg treatment if all these features are present is 93%.

In Chapter 4, immunohistopathology, humoral and cellular immunity, various neural antigens, experimental models and IVIg treatment mechanism in relation to the inflammatory demyelinating polyneuropathies are discussed. An interesting hypothesis is that inflammatory neuropathies may be caused by a dual infection in which one of the micro-organisms is neurotropic and another micro-organism provides adjuvants. Both infections must act simultaneously to cause nerve damage. In the light of this hypothesis it is interesting that antibodies to glycosfingolipids have been demonstrated in GBS and CIDP patients. These antigens are highly expressed on neural tissue and also on micro-organisms. Furthermore, in animal experiments it has been shown that gangliosides may act as adjuvant material.
Using a mixed hemagglutination test (MHT) circulating anti-human peripheral nerve tissue antibodies could be demonstrated in 22 of 38 (58%) patients with GBS or CIDP (Chapter 5). These antibodies were found in only 1 of 34 (3%) patients with polyneuropathy of other origin and in 3 of 32 (9%) patients without polyneuropathy. The presence of these antibodies was related with improvement after IVIg, but this could not be confirmed in a larger number of patients (Chapter 6).
Subsequently the results of a neuroblastoma immunofluorescence assay (NBL-IFA) were presented (Chapter 6). Three neuroblastoma cell lines (NBL) from different species were evaluated for the detection of antibodies in patients with various polyneuropathies. The pattern of antibody reactivity in GBS, CIDP and in patients with Lyme neuro-borreliosis was similar for all 3 NBL cell lines. The NBL N1E115-IFA showed reactivity with sera from patients with many different groups of disorders, whereas the NBL CHP 212-IFA generally gave negative results. The NBL 108cc15-IFA more exclusively gave positive reactions in CIDP, GBS and Lyme borreliosis patients. In Lyme borreliosis, the presence of these antibodies was not related with neuropathy. Analysis of antibody specificity revealed that absorption of CIDP patients' serum on both NBL 108cc15 and Borrelia burgdorferi (the infective agent in Lyme disease) removed anti-glycosfingolipid (GSL) antibodies. It was hypothesized that anti-NBL antibodies may be the result of various infections that induce cross-reacting antibodies against shared auto-antigenic related structures.
The antibodies against the NBL 108cc15 in patients with inflammatory demyelinating polyneuropathy were mainly of the IgM immunoglobulin class; they disappeared in all seven CIDP patients retested after improvement following IVIg. Absorption studies showed partial homology between the NBL 108cc15 and human sciatic nerve tissue. In vitro studies showed that IgG from normal donors (IVIg) inhibits the reaction between serum from a CIDP patient and the NBL cell line. This inhibition may be due to neutralization of auto-antibodies against nervous tissue by anti-idiotypic antibodies in IVIg (Chapter 7).
The development of CIDP, the presence of anti-NBL antibodies and the treatment response to IVIg may be dependent on immunogenetic factors. In the literature an association between CIDP and the HLA-A1, B7, DR3 haplotype and recently with the HLA-DR2 antigen was suggested. HLA typing was carried out in the group of 52 CIDP patients who were all treated with IVIg (Chapter 8). We could neither demonstrate a statistical significant HLA association in CIDP patients, nor in the subgroup of patients improving or not improving after IVIg treatment. No significant HLA association was found between patients with or without anti-neural antibodies.
Finally, further studies on the mechanism of IVIg in CIDP were described in Chapter 9. Purified IgM anti-NBL 108cc15 antibodies from a CIDP patient were inhibited by $F(ab)_2$ fragments of IVIg and by $F(ab)_2$ of IgG of a patient recovered from GBS. Inhibition of anti-NBL antibodies was also found among sera from patients recovered from GBS and among sera

from normal individuals. The inhibition of anti-neural antibodies may be mediated by anti-idiotypes present in sera from recovered GBS patients and in the sera from the normal donor population contributing to IVIg. These findings suggest that the self-limiting character of GBS and the therapeutic effect of IVIg in CIDP are dependent on inhibition of auto-antibodies.

# SAMENVATTING

De inflammatoire demyeliniserende polyneuropathiën kunnen worden onderscheiden in het Guillain-Barré syndroom (GBS) en in de chronische variant CIDP.
Het onderwerp van dit proefschrift is de behandeling van patiënten met een CIDP met hoge dosering intraveneus immunoglobuline (IVIg).

In hoofdstuk 1 worden zowel de klinische verschijnselen als het laboratorium onderzoek bij de CIDP beschreven. De remitterende inflammatoire polyneuropathie werd reeds aan het eind van de vorige eeuw beschreven. De naam van de ziekte veranderde menigmaal, momenteel is de meest gebruikte naam "chronisch inflammatoire demyeliniserende poly(radiculo)neuropathie". De CIDP is meestal een gemengd motorisch-sensibele polyneuropathie die wordt gekenmerkt door een relatief symmetrische spierzwakte, gevoelsstoornissen en verlaagde of afwezige reflexen. Het liquor eiwit is meestal verhoogd en de zenuwgeleidingssnelheid is in het algemeen vertraagd. Er is geen definitieve test voor de diagnose CIDP. Barohn et al.[23] stelde onlangs diagnostische criteria voor, welke worden besproken in hoofdstuk 1. Gecontroleerde studies toonden aan dat CIDP patiënten kunnen verbeteren na plasmaferese[81] en na behandeling met prednison.[76] De observatie dat CIDP patiënten kunnen verbeteren na toediening van IVIg werd onderzocht in een dubbel-blinde, placebo gecontroleerde 'crossover' studie (hoofdstuk 2). In deze studie werd na verkregen toestemming bij 7 CIDP patiënten die waarschijnlijk hadden gereageerd op IVIg, de onderhoudsbehandeling met IVIg gestopt. Na deze onderbreking van de IVIg behandeling gingen alle patiënten achteruit. Vervolgens werden de patiënten gerandomiseerd voor IVIg of placebo (albumine) infusies. De klinische conditie van alle patiënten verbeterde na IVIg, maar de patiënten verbeterden niet na toediening van placebo ($p = 0.02$). De gemiddelde tijdsduur tussen het einde van de trial medicatie en het begin van verslechtering was 6.4 weken na IVIg behandeling en 1.3 weken na placebo toediening. Deze geselecteerde groep patiënten met een CIDP hadden een gunstige reaktie op IVIg.
In hoofdstuk 3, worden klinische en laboratorium parameters besproken van 52 patiënten die voldeden aan de CIDP criteria. Alle patiënten werden met IVIg behandeld, 20 (38%) patiënten verbeterden niet na IVIg toediening, 2 (4%) patiënten verbeterden kortdurend en bij hen hadden de volgende infusies geen effect, 9 (17%) patiënten bereikten een spontane of therapeutisch geïnduceerde complete remissie en 21 (40%) patiënten hadden geregelde infusies nodig om deze verbetering te behouden. Aanvankelijk hadden alle patiënten die verbeterden symptomen die duidelijk interfereerden met hun dagelijkse bezigheden. Na behandeling met IVIg werd 90% van deze patiënten onafhankelijk wat betreft hun dagelijkse aktiviteiten.

Significant geassocieerd met een verbetering na IVIg behandeling waren: ziekteduur korter dan 1 jaar, progressie van de ziekte tot aan de behandeling, afwezigheid van een discrepantie tussen spierzwakte in armen en benen, areflexie van de armen en een vertraagde zenuwgeleidingssnelheid van de motore nervus medianus in de onderarm. De kans om te verbeteren na IVIg behandeling indien al deze factoren aanwezig zijn is 93%.
In hoofdstuk 4, worden de immunohistopathologie, de humorale en cellulaire immuniteit, verschillende antigenen, de diermodellen en het mechanisme van IVIg behandeling besproken in relatie tot de inflammatoire demyeliniserende polyneuropathieën. Een interessante hypothese is dat inflammatoire neuropathieën veroorzaakt zouden worden door een "dubbel-infectie", waarbij een van de micro-organismen neurotroop is en de andere het adjuvants levert. Beide infecties moeten samenwerken om zenuwbeschadiging te veroorzaken. In het licht van deze hypothese is het interessant dat antilichamen tegen glycosfingolipiden (GSL) zijn aangetoond bij GBS en CIDP patiënten. Deze antigenen zijn in hoge mate aanwezig op zenuwweefsel maar ook op verscheidene micro-organismen. Bovendien is er in diermodellen aangetoond dat gangliosiden als adjuvants kunnen werken.

Voordat het mechanisme van IVIg behandeling werd onderzocht, werden testen ontwikkeld voor het aantonen van anti-perifeer zenuwweefsel antilichamen.
Met een hemagglutinatie test (MHT) konden antistoffen tegen humaan perifeer zenuwweefsel worden aangetoond in 22 van de 38 (58%) sera van patiënten met CIDP of GBS (hoofdstuk 5). Deze antilichamen werden slechts bij 1 van de 34 (3%) patiënten met een andere polyneuropathie en bij 3 van de 32 (9%) patiënten zonder polyneuropathie gevonden. De aanwezigheid van deze antistoffen was gecorreleerd met verbetering op IVIg therapie, maar dit kon niet worden bevestigd in een grotere groep CIDP patiënten (hoofdstuk 6).
Vervolgens worden in hoofdstuk 6 de resultaten beschreven van een neuroblastoma immunofluorescentie test (NBL-IFA). Drie NBL cellijnen van verschillende species werden onderzocht op het aantonen van antilichamen bij patiënten met uiteenlopende polyneuropathieën. Het patroon van antilichaam reactiviteit met de drie cellijnen was overeenkomstig voor patiënten met CIDP, GBS en Lyme neuroborreliose. De NBL N1E115-IFA toonde reactiviteit met sera van patiënten met vele verschillende aandoeningen, terwijl de NBL CHP212-IFA vrijwel alleen negatieve resultaten gaf. De NBL 108cc15-IFA gaf vrijwel uitsluitend positieve resultaten met sera van patiënten met CIDP, GBS en Lyme borreliose. Bij patiënten met Lyme borreliose was de aanwezigheid van deze anti-NBL antistoffen niet gerelateerd aan de aanwezigheid van een neuropathie. Absorptie experimenten toonden aan dat zowel de NBL 108cc15 als de Borrelia burgdorferi (de verwekker van de ziekte van Lyme) antistoffen tegen glycosfingolipiden (GSL) verwijderden uit het serum van een patiënt met CIDP. Dit leverde de hypothese op dat de anti-NBL 108cc15 antilichamen kunnen ontstaan door infecties met verscheidene micro-organismen en dat deze antilichamen kunnen kruisreageren met antigenen op zenuwweefsel.
De antilichamen tegen de NBL 108cc15 bij patiënten met een inflammatoire demyeliniserende polyneuropathie waren voornamelijk van de IgM immunoglobuline klasse, zij verdwenen bij de 7 geteste CIDP patiënten die verbeterden na IVIg therapie. Absorptie experimenten gaven aanwijzingen voor partiële homologie tussen de NBL 108cc15 en humaan ischiadicus zenuwweefsel. In in vitro experimenten werd aangetoond dat IgG van normale donoren (IVIg) de reactie tussen het serum van een CIDP patiënt en de NBL cellijn inhibeerde. De inhibitie van deze autoantilichamen tegen zenuwweefsel zou veroorzaakt kunnen worden door neutralisatie door antilichamen aanwezig in IVIg (hoofdstuk 7).

Het ontstaan van CIDP, de aanwezigheid van anti-NBL antistoffen en de respons na behandeling met IVIg kan afhankelijk zijn van immunogenetische factoren. In de literatuur wordt er bij CIDP patiënten een associatie verondersteld met het HLA-A1, B7, DR3 haplotype en recent met het HLA-DR2 antigeen. In hoofdstuk 8, worden de resultaten beschreven van de HLA typering bij de 52 CIDP patiënten, die met IVIg werden behandeld. Geen statistisch significante verschillen werden gevonden in de totale groep CIDP patiënten, noch in de subgroep die wel, of juist niet, verbeterde na IVIg therapie. Evenmin werd een significante HLA associatie gevonden met de aan- of afwezigheid van anti-zenuwweefsel antistoffen bij CIDP patiënten.
Tenslotte wordt nader onderzoek naar het werkingsmechanisme van IVIg bij CIDP beschreven in hoofdstuk 9. Gezuiverde IgM anti-NBL antilichamen van een CIDP patiënt werden geinhibeerd door $F(ab)_2$ fragmenten van IVIg en door $F(ab)_2$ fragmenten van IgG van een patiënt die hersteld was van het GBS. Inhibitie van anti-NBL antilichamen werd ook gevonden met sera van sommige herstelde GBS patiënten en met sera van enkele bloedbank donoren. De remming van anti-neurale antilichamen zou kunnen worden veroorzaakt door de aanwezigheid van anti-idiotypische antilichamen in het serum van herstelde GBS patiënten en in het serum van individuele donoren die bijdragen tot de plasmapool waaruit IVIg wordt gemaakt. Deze bevindingen suggereren dat het 'self-limiting' karakter van het GBS en het therapeutisch effect van IVIg bij patiënten met een CIDP wordt veroorzaakt door remming van autoantilichamen.

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# CURRICULUM VITAE

De auteur van dit proefschrift werd op 23 januari 1959 te Utrecht geboren. Hij bezocht in Utrecht het College Blaucapel, waar hij in 1977 het Atheneum B diploma behaalde. In hetzelfde jaar begon hij met de studie geneeskunde aan de Erasmus Universiteit te Rotterdam, hetgeen in mei 1984 werd afgesloten met het artsexamen. Vervolgens was hij werkzaam als onderzoeker op de afdeling Bloedbank en Immunohematologie van het Academisch Ziekenhuis te Leiden (hoofd: Prof Dr JJ van Rood), waar de basis werd gelegd voor het huidige proefschrift. Sedert oktober 1985 is hij als arts assistent in opleiding tot neuroloog werkzaam in het Academisch Ziekenhuis Dijkzigt te Rotterdam (opleider: Prof Dr A Staal).