

ABSTRACTS (T-Z)

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MYELIN WIDENINGS AND MGUS-IGA: AN IMMUNOELECTRON MICROSCOPIC STUDY

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A few studies have reported a variety of nonspecific histological lesions in patients with IgA monoclonal gammopathies and polyneuropathy. In our case, using electron microscopy, we observed widenings of the myelin lamellae similar to those commonly described in IgM neuropathies with anti-myelin-associated glycoprotein activity. Using immunoelectron microscopy, we demonstrated a direct involvement of IgA in myelin lesions. In this patient, who suffered from pure sensory neuropathy with no tremor and no detectable anti-MAG or anti-glycolipid activity in serum, the electrophysiological abnormalities did not resemble those typically observed in neuropathies with anti-MAG antibodies. The indices of terminal latencies were not reduced significantly. Furthermore, the temporal dispersion of motor responses favored peripheral neuropathy of the chronic inflammatory demyelinating type. The search for a direct link between monoclonal dysglobulinemia, regardless of type, and polyneuropathy is important and may influence treatment.

TIME COURSE OBSERVATION USING ANTI-MAG/SGPG IgM ANTIBODY ON CULTURED SENSORY NERVE: CONFOCAL AND ELECTRON MICROSCOPIC APPROACH

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PURPOSE: To clarify the pathogenic mechanism in anti-myelin-associated glycoprotein (MAG)/sulfoglucuronosyl paragloboside (SGPG) IgM antibody-associated polyneuropathy, we analyzed time course demyelination using serum with anti-MAG/SGPG IgM antibody on myelinating dorsal root ganglion culture. **METHODS:** Cultures of explant dorsal root ganglion neurons from E15 rats were prepared. After a 2-week treatment with myelination medium, myelin segments were visualized by light microscopy. Cultures were treated with medium containing Bodipy-C5 (Molecular Probes, Leiden, Netherlands), 10% of rat complement, and 50% of serum with anti-MAG/SGPG IgM antibody. These sensory nerve fibers were analyzed with confocal microscopy in time course. The length of 50 nodes in the cultures was compared between normal cultures, cultures treated with anti-MAG/SGPG IgM antibody with or without complement, and cultures treated with complement only. The Fisher's PLSD test was used for statistical analysis. The plastic section of the demyelinating cultures was also examined by electron microscopy. **RESULTS:** The nodes became significantly wider in the cultures 7 days after incubation in anti-MAG/SGPG IgM antibody with complement (7.8 ± 4.8 [mean \pm SD] m), when compared to normal cultures (3.4 ± 1.9 m) ($p = 2.4E-10$), cultures treated with medium containing complement only (5.6 ± 3.1 m) ($p = 0.0009$), or anti-MAG/SGPG IgM antibody without complement (4.5 ± 2.7 m) ($p = 1.7E-06$). Electron microscopy showed demyelination in the culture treated with anti-MAG/SGPG IgM antibody with complement. **CONCLUSION:** These results support a role of anti-MAG/SGPG IgM antibody and complement in demyelination. Sponsor: Japan-North America Medical Exchange Foundation (JANAMEF) and Mochida Memorial Foundation.

EXPERIMENTAL STUDY OF NERVE REGENERATION IN NERVE GAP MODEL

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INTRODUCTION: In this study, we designed an original nerve gap model in a rabbit and histologically evaluated the importance of accompanying arteries, veins and fascia of muscles on the nerve regeneration. **MATERIALS AND METHODS:** Deep peroneal nerves, anterior tibial arteries and veins in rabbit were exposed. Both 10 mm nerves and arteries were excised in group A, and 20 mm nerves and arteries in group B. Whereas only 10 mm nerves were excised, and arteries and veins were preserved in group C, and only 20 mm nerves in group D. In all models any conduits were not used. Animals were sacrificed at 24 weeks after the operation. The lengths of regenerated nerves were macroscopically measured, and the retrieved specimens were histologically examined in transverse sections at 2 mm interval from proximal nerve stump. **RESULTS AND DISCUSSIONS:** In group A and C, macroscopically, regenerating nerves reached up to distal nerve stump. Histological examination demonstrated that a lot of nerves were regenerated on arteries, veins and fascia of muscles, and also observed in distal nerve stump in group A and C. In group B, only few myelinated axons regenerated up to 4-6 mm from proximal nerve stump. However, in group D, substantial number of axons regenerated along the arteries and some myelinated axons reached the distal stump over 20 mm gap. The result in 10 mm nerve gap model indicated that arteries, veins and fascia of muscles played as contact guidances in short gap. However, in 20 mm nerve gap model, only arteries could behave a good contact guidance.

THE NORMAL SEROLOGICAL RESPONSE TO AN ACUTE CAMPYLOBACTER INFECTION & THE ROLE OF SEROLOGY IN DIAGNOSING PRIOR CAMPYLOBACTER INFECTION IN NEUROLOGICAL DISEASE

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Positive campylobacter serology has been used as a marker of antecedent infection in the Guillain Barré Syndrome (GBS) and the Miller Fisher Syndrome (MFS). However, little is known of the normal serum antibody (Ab) response to acute campylobacter infection. We have assessed the serological responses using an ELISA method. 420 blood donors with no recent diarrheal illness were used as controls. Three groups of campylobacter patients were assessed: 98 patients with an acute infection, 68 of these patients 6 months later and 74 patients with infection at least 20 months prior. Serology was also performed on 59 patients with neurological disease, 15 with GBS/MFS, 10 with ALS, 7 with inflammatory neuropathy and 27 controls. Following an acute infection, IgG, IgM and IgA Abs tend to rise, but not uniformly in the majority (91%), and then all fall gradually. At greater than 20 months the mean Ab levels were all significantly elevated and 9% had antibody levels consistent with acute infection. No neurological control patient had elevated levels of Abs, however, mean Ab levels in all subclasses, particularly IgG, were elevated in the GBS/MFS patients. Despite only 1 patient having a documented campylobacter infection, high Ab levels were seen in 2 neuropathy patients but not in ALS patients. Therefore, using serology alone may overestimate the antecedent infection rate in GBS/MFS as infection many months prior may result in elevated titres. Additionally, patients with GBS/MFS may have elevated titres of Abs without evidence of recent symptomatic infection.

DOMINANCE OF AUTOREACTIVE T CELL MEDIATED DELAYED-TYPE HYPERSENSITIVITY OR ANTIBODY MEDIATED DEMYELINATION RESULTS IN DISTINCT FORMS OF EXPERIMENTAL AUTOIMMUNE NEURITIS IN THE LEWIS RAT

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The role of anti-myelin antibodies in the pathogenesis of EAN induced in the Lewis rat by immunization with peripheral nerve myelin has been assessed. Passive transfer with lymph node cells or purified serum immunoglobulin from rats with EAN was employed to directly measure the contribution of B cells and anti-myelin antibodies to demyelination and disease. Lewis rats with EAN transferred by lymph node cells or purified serum immunoglobulin from EAN donors in conjunction with a low dose of P2-specific CD4+ T cells demonstrated profound histopathological and neurophysiological evidence of demyelination during disease. In contrast, the classical adoptive transfer model of EAN in the Lewis rat induced by the injection of P2-specific CD4+ T cells was characterized by histopathological and neurophysiological evidence of axonal dysfunction and degeneration with limited demyelination. These findings demonstrate that the synergistic action of T cells and anti-myelin antibodies mediating demyelination or purely T cell mediated axonal dysfunction and degeneration are distinct pathways by which a specific autoimmune response in the peripheral nervous system can cause neurological disease.

EXPRESSION OF RHO-FAMILY GTPASES (RAC, CDC42, RHOA) AND THEIR ASSOCIATION WITH P-21 ACTIVATED KINASE IN ADULT RAT PERIPHERAL NERVE

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The Rho-family GTPases (Rac, cdc42, RhoA) are the major determinant towards neurite formation or retraction and the Rho-family GTPases-p21-activated kinase (pak) signaling pathway drives polarized outgrowth of the actin cytoskeleton in the developing neurite in neuronal cell lines. However, the expression patterns of the signaling pathway have not been clarified under *in vivo* conditions in the PNS. Thus, to clarify the presence of the signaling pathway in the PNS, we have examined their expression, the association between the small GTPases and pak, and the pak kinase activity in the PNS using immunoblot analysis, immunohistochemistry, co-immunoprecipitation study, and *in vitro* kinase assay. Immunoblot analysis showed the expression of Rac, cdc42, RhoA and pak in the dorsal root ganglion (DRG) and sciatic nerve. The localization of these proteins in the DRG neurons and axons and Schwann cells of the sciatic nerve was confirmed by immunohistochemistry. Co-immunoprecipitation studies indicated the *in vivo* associations of pak with Rac and cdc42, but not with RhoA, in both the DRG and sciatic nerve. The autophosphorylation of pak and phosphorylation of histone H4 by pak were also found in the DRG and sciatic nerve as well as in the CNS. These results suggest that the Rac/cdc42-pak signaling pathway exists and functions in the PNS and may mediate some intracellular signals.

MULTIFOCAL MOTOR NEUROPATHY AND CAMPYLOBACTER JEJUNI REACTIVITY

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Campylobacter jejuni (CJ) infection frequently precedes Guillain-Barré and Fisher syndromes where it correlates with reactivity with the gangliosides GM1, GD1a and GQ1b. Multifocal motor neuropathy (MMN) has been also associated with anti-ganglioside antibodies and a possible association with an antecedent CJ infection has been reported in 3 patients who developed MMN and anti-GM1 antibodies after CJ enteritis, while reactivity with the lipopolysaccharide (LPS) of CJ have been reported in patients with chronic motor neuropathies and high anti-ganglioside antibodies. To determine whether CJ may be involved in the pathogenesis of MMN, we examined 22 patients with MMN, including 6 with anti-ganglioside reactivity, for the presence of anti-CJ antibodies by Covalink ELISA and immunoblot, and correlated their presence with that of anti-ganglioside antibodies. As

controls, we examined 17 patients with chronic inflammatory demyelinating polyneuropathy (CIDP), 23 with amyotrophic lateral sclerosis (ALS), 43 with other neurological diseases (OND) and 23 normal subjects (NS). By ELISA we found high anti-CJ titers (1/640) in 8 MMN patients (36%), 2 CIDP (12%), 2 ALS (9%), one OND (2%), but no NS (MMN vs. neurological controls (NC), $p < 0.0005$; MMN vs. NS, $p < 0.005$). By immunoblot, 5 MMN patients (23%), one of whom also positive by ELISA, had an intense (1/32,000-1/100,000) reactivity with the 14 kD band of the CJ LPS, as compared to 2 ALS (9%), one CIDP (6%) and one NS (4%) (MMN vs. NC, $p < 0.01$). Overall, anti-CJ antibodies were detected by least one method in 12 MMN patients (55%), 3 CIDP (18%), 4 SLA (17%), 1 OND (2%) and 1 NS (4%) (MMN vs. NC, $p < 0.0005$; MMN vs. NS, $p < 0.0005$). Anti-ganglioside antibodies were similarly frequent in patients with (33%) or without (20%) anti-CJ reactivity. The high frequency of anti-CJ antibodies in MMN suggests an association between MMN and CJ, but it is unclear whether this reflects a concurrent or previous CJ infection possibly involved in the pathogenesis of MMN.

MCP-1 CHEMOKINE IMMUNOREACTIVITY IN SENSORY NEURON CELL BODIES FOLLOWING SCIATIC NERVE LIGATION

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Members of the chemokine family, a super family of chemoattractant cytokines responsible for migration and activation of specific leucocyte cell types, are rapidly expressed at the site of peripheral nerve injury. Monocyte Chemoattractant Protein-1 is expressed by Schwann cells in response to nerve damage and is thought to play a critical role in the process of Wallerian degeneration. Early expression of MCP-1 precedes the release of other factors (including other cytokines and neurotrophins) and is thought responsible for macrophage recruitment and activation. We have previously demonstrated the expression of MCP-1 in the dorsal root ganglion following unilateral sciatic nerve ligation in adult male Wistar rats. Immunocytochemical analysis (polyclonal anti MCP-1, R&D Systems, UK) demonstrated immunoreactive neuronal profiles within the dorsal root ganglion 1, 7 and 12 days following nerve ligation. Immunofluorescent co-localisation of MCP-1 with markers of DRG neuronal cell populations (antibodies to CRGP, P2X3 and N52) revealed a predominance of expression within the small purinergic (immunoreactive to P2X3) cell population at the 7 day time period although a lesser degree of co-localisation with both small peptidergic (immunoreactive to CGRP) and large non-peptidergic cell (N52) populations was observed. At present, the role for this novel expression of MCP-1 within sensory neuron cell bodies is unknown but may represent mechanisms related to neuronal survival and regeneration.

MASSION-VERNIORY SYNDROME DUE TO ALPHA-METHYL-CoA-RACEMASE DEFICIENCY

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Massion-Verniory et al. (Rev Neurol, 1946) reported two siblings with a combination of distal muscle weakness and wasting in the limbs, and a pigmentary retinopathy. Onset was in the third decade. Dyck et al. (Peripheral Neuropathy, PJ Dyck et al. (eds), WB Saunders, 1975) stated that they had seen several families with this condition and designated it type VII hereditary motor and sensory neuropathy. We report a woman now aged 52 years who developed bilateral visual loss at the age of 28 due to a pigmentary retinopathy. She had no other neurological symptoms at that stage but examination showed distal weakness in all four limbs, depressed tendon reflexes, impaired appreciation for all sensory modalities in the lower limbs and mild pes cavus. Her parents were normal and not consanguineous. Nerve conduction studies demonstrated a sensorimotor neuropathy with a

demyelinating component. Nerve biopsy showed a predominant loss of larger myelinated fibres and some demyelination/remyelination. Her visual symptoms and limb weakness have been progressive. Recent biochemical studies revealed mildly elevated serum phytanate and markedly raised pristanate levels. Cultured fibroblast studies showed a deficiency of the peroxisomal enzyme alpha-methyl-CoA-racemase. She is currently being treated with a low phytanic acid diet.

EFFECTS OF FK506 ON NERVE REGENERATION AFTER NERVE GRAFT OR TUBE REPAIR OF LONG NERVE GAPS

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We compared the effects of FK506 administration on regeneration after sciatic nerve resection and repair with an autologous graft or with a silicone tube leaving a 6 mm gap in the mouse. Functional reinnervation was assessed by noninvasive methods to determine recovery of motor, sensory and sweating functions in the hindpaw over 4 months after operation. Morphometric analysis of the regenerated nerves was performed at the end of follow-up. Nerve graft allowed for faster and higher levels of reinnervation in the four functions tested than silicone tube repair. Treatment with FK506 (for the first 9 weeks) resulted in a slight improvement of the onset of reinnervation and of the maximal degree of recovery achieved after autografting. The recovery of pain sensibility and of the compound nerve action potentials in the digital nerves, which are directly depending on axonal regeneration, showed better progression with FK506 than reinnervation of muscles and sweat glands, which require reestablishment of synaptic contacts with target cells. The myelinated fibers in the regenerated nerves showed a more mature appearance in the FK506 treated rats. However, FK506 showed a marginal effect on situations where regeneration is limited, as in a silicone tube bridging a 6 mm gap in the mouse sciatic nerve. In conclusion, treatment with FK506 improved the rate of functional recovery after nerve resection and autograft repair. Sponsor: FK506 was generously supplied by Fujisawa Pharmaceuticals, Inc.

A HIGH AFFINITY MONOCLONAL ANTI-GANGLIOSIDE ANTIBODY CAUSES DEGENERATION OF MOTOR NERVES IN ORGANOTYPIC SPINAL CORD CULTURES

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The effect of high affinity monoclonal antibody against GD1a and GT1b, generated by inoculating GD1a into immune-naïve transgenic mice lacking complex gangliosides, on motor nerves was studied in organotypic rat spinal cord cultures. Using this antibody, GD1a/GT1b was immuno-localized to motor nerve soma and axons in post-fixed cultures. Cultures incubated with 100 ug/ml of antibody and heterologous complement, showed a 70% ($p < 0.0001$) decrease in mean motor neuron count. A lower concentration of antibody (50 ug/ml) achieved an intermediate effect at 1 week, (ANOVA, $p = 0.0002$), the dose effect being linear ($p < 0.0001$). Axons exposed to antibody and complement showed dose and duration-dependent changes of Wallerian-like degeneration. The mean motor neuron count in cultures exposed to heterologous complement alone was similar to that of control cultures. The degeneration of motor nerves in organotypic spinal cord cultures exposed to high affinity antibody against GD1a/GT1b supports the pathogenic role of anti-ganglioside antibodies found in certain sub-types of the Guillain-Barré syndrome. Sponsor: 1) NIH grant NS31528, 2) Ministry of

EXPERIENCE ON METHOTREXATE (MTX) AS AN IMMUNOSUPPRESSIVE DRUG IN NEUROMUSCULAR AUTOIMMUNE DISORDERS

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INTRODUCTION: MTX is a folate antimetabolite inhibiting dihydrofolate reductase and DNA-synthesis. Besides its implementation as a chemotherapeutic agent, it is increasingly used in autoimmune and neuromuscular autoimmune diseases. The advantage is a relatively short onset of action (1-3 months) which may reduce additional steroid treatment. The average dose for this indication is 10-20 mg/week, which also causes only few haematologic side effects. Patients with restriction of pulmonary function have to be excluded. We report 9 patients with different autoimmune neuromuscular diseases, benefiting from MTX medication. **MATERIALS AND METHODS:** Nine patients with autoimmune neuromuscular syndromes (4 myasthenia gravis, 2 dermatomyositis, 2 polymyositis, and one patient with vasculitic neuropathy) were observed in our neuromuscular clinic. Diagnosis was established by electrophysiologic testing, antibody testing and biopsy when appropriate. Patients received MTX between 4 month and 43 months (median 24 months), either in combination therapy or as monotherapy. MTX was given orally once a week with doses between 7.5 and 15 mg. A complete laboratory screen and pulmonary function test was obligatory before inclusion. **RESULTS:** Three of four patients with myasthenia gravis responded to MTX therapy with a significant reduction of steroids. Patients with polymyositis did not respond and had to be switched to other immunosuppressive drugs. Both patients with dermatomyositis went into complete remission. The patient with vasculitic neuropathy is still under maintenance therapy and not completely symptom free. We observed no haematological side effects and no pulmonary dysfunction. However, dizziness, nausea and fatigue were reported by some patients after weekly ingestion of tablets. **CONCLUSION:** Our clinical observations suggest that MTX is either the treatment of choice or an interesting alternative for immunosuppression in autoimmune neuromuscular disease. From our small study, patients with myasthenia gravis and dermatomyositis seem to profit most, contrary to polymyositis patients.

STRETCH REFLEX RESTITUTION AND FACILITATION AFTER DIFFERENT TYPES OF PERIPHERAL NERVE INJURIES AND REPAIR

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We investigated the restitution and properties of the monosynaptic reflex after nerve injury and its role in the recovery of walking. Adult rats were submitted to sciatic nerve crush (CRH, n=9), complete section repaired by aligned (CS, n=11) or crossed (CCS, n=9) fascicular suture, or an 8 mm resection repaired by autograft (AG, n=9) or by tubulization (SIL, n=12). The sciatic nerve was stimulated proximal to the injury site and the M and H waves were recorded from gastrocnemius and plantar muscles at monthly intervals during 3 months postoperation. Walking track was also assessed and the sciatic functional index (SFI) calculated. The H wave was recorded in all the animals showing muscle reinnervation reaching final amplitude values of 40-80% for gastrocnemius and 70-80% for plantar muscles with respect to preoperative values. The H/M amplitude ratio increased after injury, indicating that the H reflex was facilitated, and tended to decrease to near control values as muscle reinnervation progressed. However, final values of the H/M ratio for the plantar muscles remained significantly higher in all groups except CRH. Only group CRH showed an appreciable recovery of the

walking track pattern. Final SFI values correlated positively with values of the M wave amplitude but negatively with the H/M ratio. Changes in the stretch reflex circuitry and excitability may be involved in the deficient recovery of walking pattern after severe nerve injuries.

MULTICENTRIC TRIAL (PHASE II) OF BETA-INTERFERON-1A IN CHRONIC INFLAMMATORY DEMYELINATING POLYRADICULONEURITIS (CIDP): 19 CASES

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The effectiveness of cortisone, plasma exchanges and IVIg for CIDP is recognized. Nevertheless, many patients do not respond to these treatments or relapse, which justifies trial of beta-interferon-1a (AVONEX). 19 patients were recruited from 4 neurological centers (Boston, Limoges, London-Canada, Paris) and were treated for 6 months, with the primary objective of evaluating the tolerance of beta-interferon-1a in patients with CIDP (criteria of the "ad hoc committee"). A secondary objective was to evaluate the effectiveness of this interferon on the NDS and Clinical Grade (CG) as well as on the electrophysiological data. Recruitment was difficult due to the rigid nature of the electrophysiological criteria. Nearly all the patients had failed to respond to other therapies and so had longstanding CIDP with accompanying axonal impairment. No deterioration in the clinical or electrophysiological results could be ascribed to interferon. Amelioration of NDS and/or CG were observed in 6 patients. There was also a tendency to an improvement in the areas of the motor potentials of certain nerves, especially in the upper limbs. The limitations of such a trial, notably with respect to its methodology, will also be discussed.

A STANDARDISED ELECTROPHYSIOLOGIC PROTOCOL FOR DIAGNOSIS OF MULTIFOCAL MOTOR NEUROPATHY

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Multifocal motor neuropathy (MMN) is a multifocal neuropathy characterised by asymmetric weakness and motor conduction block (CB) or other signs of demyelination. Previously, we have shown that, in MMN, CB is always found if many nerves are investigated in a standardised protocol. Our aim was to assess which nerves have to be selected to find CB. We investigated 41 patients with MMN before treatment in a standardised protocol. We defined definite CB as area decrement = 50% and probable CB as amplitude decrement = 30% in an arm nerve. Motor conduction was investigated up to Erb's point in the median (recording from thenar and forearm flexor), ulnar, radial and musculocutaneous and up to the popliteal fossa in the peroneal and tibial nerves. Muscle strength was assessed by MRC grading. Weakness and CB correlated weakly (Gamma = -0.60). The distribution of CB was statistically not different from uniform. Investigating only nerves innervating weak muscles revealed CB in 36 patients (25 definite CB). Investigating: 1) the median (recorded from thenar) and ulnar nerves on both sides revealed CB in 39 patients (24 definite CB); 2) all arm nerves revealed CB in all patients (29 definite CB); 3) all arm and leg nerves revealed CB in all patients (31 definite CB). In conclusion, a standard investigation of the longest arm nerves on both sides reveals most segments with CB, probably because CB is distributed randomly. Sponsor: Supported by a grant from the Prinses Beatrix Fonds.

MULTIFOCAL MOTOR NEUROPATHY: FOUR TO EIGHT YEAR FOLLOW-UP DURING MAIN-

TENANCE THERAPY WITH INTRAVENOUS IMMUNOGLOBULINS

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Patients with multifocal motor neuropathy (MMN) respond favourably to intravenous immunoglobulins (IVIg), but there may be disease progression during maintenance therapy with IVIg (mIVIg). However, this has not been documented for a follow-up of more than four years, which is important to justify expensive maintenance treatment strategies. To determine the long-term follow-up of MMN during mIVIg, we selected eleven patients with a mean follow-up of mIVIg of six years. Measurement of muscle strength and nerve conduction studies have been performed every year during follow-up and disability before and after follow-up. Muscle strength significantly improved after the first course of IVIg, but worsened during mIVIg, which was significant. Muscle strength after follow-up was still better compared with before IVIg was given. Disability of the upper limbs was significantly higher after follow-up than before. Electrophysiologically, we found evidence for an increase in axonal degeneration and demyelinating features during mIVIg. These results indicate that there is progression of disease despite mIVIg, implicating that MMN runs a progressive course. Nevertheless, mIVIg seems to preserve muscle strength on a higher level than before IVIg was started, which justifies mIVIg.

DISEASE PROGRESSION IN MULTIFOCAL MOTOR NEUROPATHY

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Disease progression in multifocal motor neuropathy (MMN) has not been well documented. MMN is a chronic disorder of the peripheral motor nervous system with a presumed auto-immune pathogenesis. Most patients with MMN respond to treatment with intravenous human immunoglobulins (IVIg). Whether and to what extent muscle weakness and atrophy progress without treatment has never been measured. We assessed disease progression by measuring muscle weakness, disability, conduction block (CB), and distal and proximal compound muscle action potential (CMAP) amplitudes in 38 patients who had never received immunosuppressive drugs or IVIg. Disease duration ranged from 6 months to 34 years. Between patients, weakness and disability correlated positively with disease duration. Distal and proximal CMAP amplitudes significantly decreased. Numbers of CBs were significantly higher in patients with disease duration longer than 10 years compared with patients with disease duration shorter than 10 years. Thirty of 34 patients responded to IVIg. Severe weakness was an independent prognostic variable associated with a better response to IVIg. Our results provide further evidence that MMN runs a slowly progressive course. The response to IVIg in patients with severe weakness and prolonged disease suggests an ongoing immune-mediated disease process. These findings implicate that early treatment may prevent future progression of weakness and disability in MMN.

HIGH INCIDENCE OF GASTRO-ENTERITIS-ASSOCIATED GUILLAIN-BARRÉ SYNDROME

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BACKGROUND: An increase in the number of Guillain-Barré syndrome (GBS) patients has

been observed in Curaçao, the Netherlands Antilles. **METHODS:** Patients fulfilling the NINCDS criteria for GBS and admitted between 1987 and 1999 were included. The results were compared with a large Dutch epidemiological study. **FINDINGS:** 49 patients were identified resulting in an incidence rate (IR) of 1.62 in the period 1987-1991 and 3.10 in the period 1992-1999, RR 5.22, $p=0.02$. There was a clear seasonal preponderance, $p=0.06$. Patients from Curaçao were characterized by a severe course (mortality rate 23%, in the Dutch group 3.4%, $p<0.01$), a higher % of preceding gastro-enteritis ($p<0.01$) and less involvement of the sensory system ($p<0.01$). Serologic evidence was found for a recent infection with *C. jejuni*. **INTERPRETATION:** This is the first study reporting a steady increase in incidence of GBS over the years in association with a pronounced seasonal preponderance and a severe course. The clinical characteristics suggest a role for *C. jejuni*. An isolated island population is an excellent base to study a multifactorial disease of unknown pathogenesis.

COMBINED THERAPY OF INTRAVENOUS IMMUNOGLOBULIN AND METHYLPREDNISOLONE IN PATIENTS WITH GUILLAIN-BARRÉ SYNDROME. THE RESULTS OF A MULTICENTRE DOUBLE-BLIND PLACEBO CONTROLLED RANDOMISED CLINICAL TRIAL

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OBJECTIVE: Based on the positive results of a pilot study, the additional treatment of high-dose methylprednisolon (MP) on standard treatment with intravenous immunoglobulin (IVIg) was studied in patients with Guillain-Barré syndrome (GBS).

METHODS: 232 GBS patients, fulfilling the inclusion criteria, were randomised for treatment with either IVIg and MP or IVIg and placebo. The predefined primary outcome was improvement at four weeks by at least one grade on the "Hughes disability score."

An interim-analysis was performed after the inclusion of 75 and 150 patients. To study the long-term effect of both treatments, the follow-up of the patients was 1 year.

RESULTS: 232 patients were included between 1994 and 2000. 225 patients could be analysed according to the intention-to-treat principle. The interim-analysis showed no significant difference at the primary endpoint for both groups. Further analysis is currently being performed. **CONCLUSION:** The final results including subgroup-analysis will be presented. **Sponsor:** An educational grand was supported by Hyland Immuno Europe (Baxter).

LONG-LASTING ϵ T CELL NON-RESPONSIVENESS IN PATIENTS WITH CAMPYLOBACTER JEJUNI ASSOCIATED GUILLAIN-BARRÉ SYNDROME

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Two thirds of the patients with the Guillain-Barré syndrome (GBS) report an antecedent infectious disease, most frequently with the enteric pathogen *Campylobacter jejuni*. Based on the observation that GBS patient's serum anti-GM1 antibodies cross-react with *C. jejuni* lipopolysaccharides, it has been hypothesized that 'molecular mimicry' plays a role in the induction of this disease. To seek further evidence for this hypothesis at the level of T lymphocytes, we studied *C. jejuni* reactive T cells in patients with GBS. Peripheral blood MNC of GBS patients and healthy donors were stimulated in vitro with crude sonicates of *C. jejuni*. The T lymphocyte proliferative response was measured at day 5 and phenotypical analysis of expanding T cells was performed on freshly isolated cells and after 12 days of culture. We found that in healthy individuals, peripheral blood T lymphocytes expressing

but not T cell receptors vigorously proliferate after exposure to crude sonicates of *C. jejuni*. In striking contrast, peripheral blood T cells in GBS patients with antecedent acute *C. jejuni* infections completely failed to respond. GBS patients without evidence for antecedent *C. jejuni* infections and a control group of patients with acute *C. jejuni*-related enteritis without GBS responded to *C. jejuni* stimulation as healthy individuals. In the majority of GBS patients with antecedent *C. jejuni* infection, T cell non-responsiveness to *C. jejuni* stimulation lasted for years after the patients had recovered from GBS. T cells may play a role in the pathogenesis of GBS.

INTRAVENOUS IMMUNOGLOBULIN (IVIg) FOR CHRONIC INFLAMMATORY DEMYELINATING POLYRADICULONEUROPATHY (CIDP): A COCHRANE SYSTEMATIC REVIEW

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The results of a systematic cochrane review will be presented. The methods of searching the literature, assessment of quality, data-extraction and statistical analysis can be found on the Cochrane library. All (quasi-) randomised studies investigating IVIg versus placebo or any other treatment and including patients with definite or probable CIDP were eligible. The primary outcome was defined as the proportion of patients with significant improvement in disability, as defined by original investigators, within one month after the onset of treatment, according to a disability scale used in the study. This outcome was expressed as a relative risk for each study. To assess overall efficacy, the pooled relative risk estimate was calculated. Secondary outcomes were change of mean disability score, change of mean MRC sum score, change of mean median nerve distal CMAP amplitude, change of mean median nerve MCV, change of mean median nerve distal DLT, change of mean disability score at 3 or more months, change in walking distance, and frequency of adverse effect. Secondary outcomes were expressed as effect size, apart from the change in walking distance and frequency of adverse effects. Pre-screening of 170 articles, a full text investigation of 14 studies resulted in 6 trials fulfilling all inclusion criteria. One study addressed the comparison between IVIg and PE and one between IVIg and prednisolone. Both treatments appeared equally effective compared to IVIg. Four studies addressed the comparison between IVIg and placebo. The chance to improve in disability within one month after IVIg treatment is 3.12 times higher than after placebo (95% CI: 1.72-5.67). This result should be interpreted with caution because of different disability scales used and the cross-over design of two included studies. Individual patient data analysis has to solve these problems.

A NATURAL HISTORY STUDY OF 51 PATIENTS WITH HMSN IA: BASELINE CLINICAL AND ELECTROPHYSIOLOGICAL FEATURES

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Hereditary motor and sensory neuropathy type Ia (HMSN 1a) is a primarily demyelinating neuropathy, but there is increasing evidence that axonal involvement plays a major role in disease progression. Previous studies, of which only a few were longitudinal, showed disease progression predominantly during childhood and to a lesser extent later in life. It is unknown what causes the differences in disease severity even within patients from the same family. To investigate the natural history of HMSN Ia and, in particular, to document axonal involvement, we started a longitudinal study of 5 years in April 2000 with 51 clinically affected patients with HMSN Ia, confirmed by the presence

of a DNA duplication in at least one member of the family. We included 27 males and 24 females from 28 families, ranging in age from 6 to 69 years (median 39 years). At baseline, strength was quantified by hand-held dynamometry and a standardized sensory evaluation was done. Physical performance tests were timed. Functional status was assessed by a modified Guy's neurological disability scale and a part of the Sickness Impact Profile. A standardized EMG was done to quantify nerve conduction and especially amplitude abnormalities, as an indirect measure for axonal involvement. Baseline data will be presented. The initial results confirm the presence of both a predominantly distal, symmetrical weakness and sensory disturbances, with the legs more affected than the arms. Disability was associated with distal weakness. Weakness correlated with CMAP amplitude in distal muscles. Clinical follow up is planned after 1, 2 and 5 years. By repeating the standardized EMG after 5 years, we hope to be able to better characterize the relationship between progression of disease and increasing axonal dysfunction. Sponsor: Grant from the Medical Research Council of the Netherlands Organisation for Scientific Research 940-33-024.

SUCCESSFUL AUTOLOGOUS STEM CELL TRANSPLANTATION IN A PATIENT WITH CIDP

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In 1988, a man of 38 years had his first signs and symptoms of CIDP. The diagnosis was made in 1991: CSF protein 0.67 g/l no cells, neurophysiological and sural nerve biopsy studies consistent with CIDP. After prednisone (1mg/kg) weakness (MRC 4, arms and legs) disappeared and, of the sensory symptoms, only mild impairment of the fingers remained, but tapering to less than 20 mg prednisone/day was followed by deterioration. Azathioprine 150 mg/day (2 years) and methotrexate 7.5 mg/week (4 months) had no effect. Finally, he was dependent on prednisone 20 mg/day and IVIg at regular intervals, but suffered from side effects of prednisone (mood disturbances after change in dose of prednisone and gastric symptoms). After IVIg infusion he had arthralgias for a few days. Because of these side effects, the relatively young age and chronicity of the disease, autologous stem cell transplantation (AST) was considered. In May 1998, he received induction chemotherapy, cyclophosphamide 8 g, followed by granulocyte-colony stimulating factor to mobilize stem cells. CD 34+ cells were collected by leukopheresis and cryopreserved. Reinfusion was withheld to discriminate the induction effect from the effect of AST. Prednisone was tapered to 8 mg/day. After 5 months he relapsed and IVIg was restarted. AST was carried out in April 1999 after a BEAM regimen: BCNU, Etoposide, Cytosine Arabinoside and Melphalan. Prednisone was tapered to 5 mg. At present, January 2001, he is 1 year and 8 months in remission. He has only mild sensory impairment of his fingers, works full-time and neurophysiological parameters have improved.

UNCOUPLING PROTEINS ARE ASSOCIATED WITH REGULATION OF HIGH GLUCOSE INDUCED OXIDATIVE STRESS AND NEURONAL DEATH

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Elevated glucose in animal models of diabetes is associated with induction of programmed cell death (PCD) and with loss of DRG neurons. Increased cellular glucose supplies additional electrons to the electron transport chain, leading to an excessively high proton gradient across the mitochondrial membrane (Mt) that can generate reactive oxygen species. Uncoupling proteins (UCPs) are inner Mt proteins that uncouple oxidative phosphorylation and decrease the Mt potential by operating as a proton carrier. Therefore, we investigated the potential for UCPs to modulate the induction of PCD in dorsal root ganglion (DRG) neurons following exposure to high glucose. The expression of UCP-1,

UCP-2 and UCP-3 in DRG neurons was determined using Western blot analysis. Glucose-induced PCD was assessed through cytochemical determination of caspase 3 cleavage in E15 rat DRG neurons in serum-free defined medium containing a range of glucose concentrations. Basal glucose (25 mM) activates caspase-3 in 10-20% of neurons using cytochemical techniques. Caspase-3 cleavage over 6 hours increases in a dose-dependent manner to 30 + 15% (35 mM glucose), 52 + 13% (40 mM glucose) and 68 + 17% (45 mM glucose). In contrast, overexpression of UCP-1 in DRG neurons, using an adenoviral transfection with Ad-GFP controls, completely prevented the activation of caspase 3 with high glucose as follows: 5 + 5% (35 mM), 7 + 13% (40 mM), and 17 + 5% (45 mM). UCP-3 is the most abundant UCP in DRG neurons both *in vitro* and *in vivo*, and is significantly decreased under high glucose conditions. The data demonstrate that UCPs can prevent glucose-induced neuronal PCD and that the loss of UCP-3 following exposure to high glucose leads to neuronal PCD. Preventing free radical generation through upregulation of UCPs may provide an effective strategy to prevent neuronal injury in diabetes. Sponsor: Supported in part by NIH NS01938, Juvenile Diabetes Research Foundation, and funds from the Ann Arbor Geriatric Research and Clinical Center (GRECC) (JWR).

MONOCLONAL CRYOGLOBULIN RELATED TO WALDENSTRÖM'S MACROGLOBULINEMIA: PERIPHERAL NERVE DAMAGE

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Peripheral neuropathy (PN) is a classical complication of Waldenström's macroglobulinemia (WM), but nerve fibers may be damaged by various mechanisms. We report two cases associated with monoclonal cryoglobulin, with special emphasis on morphological findings at peripheral nerve biopsy. These two male patients were respectively 82 and 73 when they were investigated for a severe PN occurring in the course of WM. A search for seric anti-MAG activity was positive only in the second case. Direct immunofluorescence study showed endoneurial IgM deposits in both patients, and a binding of IgM to several myelin sheaths was also noticed in the second. At electron microscopic examination, the endoneurial deposits had the tubular structure of cryoglobulin. There was marked axonal damage in both patients, but coexistence of a demyelinating process with typical widened myelin lamellae only in the second. These two cases and a few previously reported observations demonstrate that PN associated with monoclonal cryoglobulin has to be distinguished from those associated with mixed cryoglobulin. Epineurial vasculitis is frequently observed in mixed cryoglobulinemia, whereas this is not the case in monoclonal cryoglobulinemia. Both our cases had endoneurial cryoglobulin deposits responsible for compressive or ischemic axonal lesions. The second case differed by the coexistence of demyelinating lesions likely related to seric anti-MAG activity.

POST-VACCINAL INFLAMMATORY NEUROPATHY: PERIPHERAL NERVE BIOPSY IN THREE CASES

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Autoimmune inflammatory polyneuropathy (PN) can be triggered by vaccination. Case 1: a 36-year-old female nurse presented acute sensory disturbances in the lower limbs 15 days after hepatitis B vaccination (HBV). She had severe ataxia but no weakness. Cerebrospinal fluid (CSF) was 84 mg/100 mL, with 3 lymphocytes. Case 2: a 66-year-old man presented a severe sensory motor PN in all four limbs 21 days after HBV. Case 3: a 66-year-old man presented a progressive sensory-motor PN in all four limbs and bilateral facial paralysis 15 days after anti-yellow fever vaccination. His CSF was 300 mg/100 mL, with 5 lymphocytes. Six weeks later, a tracheostomy was performed. In the

three patients, the nerve deficits lasted for months. There were a few KP1-positive histiocytes but no T-lymphocytes in the endoneurium of the three nerve biopsies. At ultrastructural examination, there was axonal degeneration in the first two cases; in case 2, a few myelinated fibers exhibited an intra-axonal macrophage with an intact myelin sheath. There was only one example of macrophage-associated demyelination in case 2, but they were numerous in case 3. It is likely that in the first two cases, an autoimmune reaction against some axonal or neuronal component was triggered by HBV, and that it induced an acute sensory ataxic PN in case 1 and an acute motor-sensory axonal neuropathy (AMSAN) in case 2. The third patient had a chronic inflammatory demyelinating PN.

PLASMAEXTRAVASATION AFTER RAT SCIATIC NERVE LESION

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Neurogenic inflammation and plasmaextravasation (PE) may play a role in neuropathic pain. Here we investigated the acute time course of PE in different tissues innervated by a damaged nerve. In rats with a chronic constriction injury (CCI) of the sciatic nerve, Evans blue was injected intravenously 5 min prior to or 24 h after the lesion. Controls received only Evans blue. 30 min after the injection, samples from the plantar skin (PS) and dorsal skin (DS) of the hindpaw, the sciatic nerve (SN) and the gastrocnemius muscle (GM) were excised. PE was assessed by the photometric measurement of the extravasated Evans blue after formamide extraction. 25 min after CCI, PE ipsilateral to the CCI was markedly increased in PS ($158 \pm 37\%$), DS ($200 \pm 48\%$), GM ($157 \pm 50\%$), and in SN ($362 \pm 81\%$). On the contralateral side PE was only increased in SN ($235 \pm 48\%$) but not in the other tissues. 24 h after CCI, PE was exclusively increased in ipsilateral PS ($177 \pm 24\%$). These results show that PE is acutely increased after a nerve lesion within minutes in all peripheral tissues innervated by the damaged nerve as well as in the contralateral nerve and after 24 h only in selective skin areas. We hypothesize that the early PE is induced by the injury discharge elicited by the nerve lesion. Activation of spinal neurons may be responsible for the contralateral PE. The delayed, selective PE is likely to be due to a different mechanism, possibly associated with immune mechanisms in the skin.

EPIDERMAL NERVE FIBER MALDISTRIBUTION ACCOMPANIES FIBER LOSS IN PAINFUL SENSORY NEUROPATHY

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A decrease in epidermal nerve fiber (ENF) density is sometimes the only confirmatory finding in patients with symptoms of painful sensory neuropathy. ENF density represents a valuable measure of pathology because it is quantifiable and can be easily compared to normal values. Various morphologic findings have also been proposed as possible signs of pathology in epidermal nerves. Three mm skin biopsies from the foot dorsum were immunostained to localize nerves (PGP 9.5) and basement membrane (Type IV collagen). ENF density was determined from confocal Z-series using Neurolucida software. In several patients with symptoms and signs of painful sensory neuropathy, we have noted an inhomogeneous distribution of epidermal nerves. In some cases, many of the epidermal nerves appear to cluster, often with long stretches of epidermis without nerve between these clusters. Such clusters often arise from a common origin in the subepidermal neural plexus, forming a tuft of nerves arborizing near the epidermal basement membrane. While the cause of this finding is unknown, it is reminiscent of the collateral sprouting known to occur in the setting of denervation and reinnervation of muscle. Irregular epidermal nerve fiber density with tufts of nerve arising from a common dermal nerve may represent useful markers of pathology in epidermal nerves.

Specimens with this finding, which is not seen in normals, might be interpreted as normal if the overall ENF density is used as the only criterion for abnormality. Sponsor: Minnesota Medical Foundation.

THE WLDS MUTATION IS NEUROPROTECTIVE IN MODELS OF PERIPHERAL NEUROPATHY

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The WLDS mutant demonstrates the unique phenotype of delayed Wallerian degeneration in both the PNS and CNS. The mutation has now been mapped to an 85 kb triplication on chromosome 4 that generates a novel gene and protein, whose function is yet unknown. We were interested in testing whether this mutation, in addition to delaying axonal degeneration after axotomy, would also provide for protection against axonal degeneration in models of slowly progressive neuropathy. Dorsal root ganglion (DRG) cultures from WLDS and C57BL/6 mice were allowed to extend neurites for 5 days and then were transiently exposed to 0.05 μ M vincristine for 24 hours. After vincristine withdrawal, cultures were observed for an additional 20 days, measuring axonal length and DRG area at multiple time points. Wild type DRGs showed progressive reduction in neurite length and DRG area with no evidence of recovery. They were essentially “dead” by 4 days after exposure. By comparison, WLDS neurites showed growth arrest that lasted about 10 days, and then resumed growth, showing significant increases in both neurite length and DRG area. These data provide the first demonstration of the WLDS mutation modifying the course of neuropathic disease, suggesting that the WLDS protein is truly neuroprotective. These findings further emphasize the importance of characterizing the WLDS protein; understanding its role in inhibiting axonal degeneration will potentially provide clues for new interventions in human neuropathic disorders. Sponsor: National Institutes of Neurological Diseases and Stroke.

ACTIVATION OF COMPLEMENT AND EXPRESSION OF CD59 IN GUILLEIN-BARRÉ SYNDROME

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Activation of complement is implicated in the process of myelin damage in the Guillain-Barré syndrome (GBS). Upregulation of complement inhibitory proteins on human Schwann cells might limit the inflammatory process. We studied archival autopsy tissues from 6 subjects fulfilling the criteria for GBS, who died between 1971 and 1999. Four patients had short survival times from the onset of neurological symptoms until death ranging from 1 to 14 days. Two patients succumbed 5 and 8 weeks after onset of the disease. Treatment modalities included steroid therapy in two patients, plasmapheresis in one, and intravenous immunoglobulins and plasmapheresis in another patient. Two patients received no immunomodulatory therapy. Immunocytochemistry was performed on spinal roots and ganglia to localize complement components, especially C3, the terminal complement complex C5b-9, including C9 neoantigen, and CD59, a complement regulatory protein. All cases displayed rather diffuse deposition of C3 on myelin sheaths. Labeling for C5b-9 on myelin sheaths and occasionally mononuclear cells was present in all but one case with 5 weeks duration. Whereas 1- to 14-day cases only faintly expressed CD59, cases with 5 and 8 weeks duration, respectively, showed moderate to strong staining for CD59 on Schwann cell surfaces. These preliminary data indicate upregulation of CD59 on human Schwann cells in cases of GBS with longer survival. Investigations will be extended on 11 cases.

CPI-1094, A POTENT ANTI-INFLAMMATORY AGENT WITH EFFICACY IN NEUROPATHIC PAIN
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Nitrones have been shown to be active as anti-inflammatory agents in animal models of arthritis and uveitis, but this is the first report of their efficacy in analgesic models. CPI-1094, a novel PBN analog, was evaluated in four analgesia models: mouse phenylquinone writhing (PQW) (Siegmund, 1957), rat hot plate (Carter, 1991), Hargreaves test in collagen arthritic rats (Hargreaves, 1988), and cold allodynia assay of chronic constriction injury (CCI) in rats (Hunter et al., 1995). In the PQW assay, mice dosed with 10 mg/kg CPI-1094, po, showed 85% inhibition ($p < 0.001$ vs. vehicle) of writhing activity. This agent was also active in collagen-induced arthritic rats (day 20) in the Hargreaves test and exhibited an ED_{50} of 6.25 mg/kg. By contrast, CPI-1094 was completely inactive in the rat hot plate (55°C) assay at 100 mg/kg, po. CPI-1094 showed significant, dose-dependent analgesic activity in CCI- neuropathic animals when tested in the cold allodynia assay. The mean latencies increased from 5.0 seconds in vehicle treated rats to 10.4 ($p > 0.05$), 12.6 ($p < 0.05$) and 16.2 ($p < 0.01$) seconds for the 10, 30, and 100 mg/kg CPI-1094 treated rats, respectively. This activity, in addition to the analgesic activity reported above, suggests the possible use of nitrones as analgesic agents to treat neuropathic pain.

SCHWANN CELL APOPTOSIS IN EXPERIMENTAL AUTOIMMUNE NEURITIS AND THE
FUNCTIONAL ROLE OF TNF-ALPHA

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Schwann cell (SC) apoptosis may be a critical factor challenging nerve remyelination and regeneration in experimental autoimmune neuritis (EAN) in the Lewis rat. We, therefore, investigated possible ways to reduce SC apoptosis in EAN using two modes of immunotherapy. First we analyzed the fate of SC during high-dose antigen therapy of adoptive transfer EAN using rhP2 protein. P2 antigen therapy was associated with an increase of T-cell apoptosis mediated by TNF- and led to a useful treatment of this autoimmune disorder as shown in previous studies. We found that antigen specific therapy had no clear effect on SC apoptosis. Secondly we neutralized TNF-, an important proinflammatory mediator released in high amounts in abundance by antigen or released only in small concentrations during EAN, while the addition of a TNF-neutralizing antiserum resulted in a significant decrease in the rate of SC apoptosis in vivo compared to animals treated with control antigen rhP0 or with rhP2 only. Our results indicate that TNF- may be a critical mediator of SC apoptosis in EAN and could be highly active in rather small concentrations.

SENSORY REINNERVATION OF A SKIN BLISTER WOUND

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Epidermal nerve fibers (ENFs) are reduced in number in sensory neuropathies. The morphology of some ENFs in neuropathies suggests their involvement in a denervation-reinnervation cycle. We studied the regenerative capacity of ENFs using a skin blister wound model that separates epidermis from dermis above the basement membrane. Pairs of 2 mm suction skin blisters were raised on the forearm of normal human subjects. At selected times, one of the pair was reblistered

(3 mm); the other was removed in a 3 mm punch biopsy. Specimens were fixed and immunostained for localization of nerves (PGP 9.5) and basement membrane (type IV collagen). Fiber density was determined from confocal z series using Neurolucida Software. After reepithelization, ENFs in normal epidermis around the wound began to extend collaterals into the nascent epidermis. Soon after, about day 9, proximal ENF stumps in the base of the wound developed nerve sprouts. Some sprouts lengthened, crossed the basement membrane and entered the nascent epidermis. Eventually the collateral ENFs from adjacent normal epidermis disappeared, whereas the regenerating nerves from the proximal stumps of ENFs in the blister base achieved nearly normal reinnervation approximately four weeks after wounding. We conclude that the minimally invasive blister model is useful to study the efficiency of epidermal nerve regeneration. Sponsor: NIH (DK56708), Juvenile Diabetes Foundation (1-2000-318), The Salvadore Maugeri Foundation, Capoli MT, Italy.

THE CLINICAL AND LABORATORY FEATURES OF CANOMAD: CHRONIC ATAXIC NEUROPATHY, OPTHALMOPLEGIA, M-PROTEIN, AGGLUTINATION AND DISIALOSYL ANTIBODIES

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The CANOMAD Study Group gathered data through a questionnaire-based survey on 18 patients with a paraproteinaemic neuropathy syndrome herein termed CANOMAD (chronic ataxic neuropathy, ophthalmoplegia, monoclonal IgM protein, cold agglutinins and disialosyl antibodies). All cases have serum IgM antibodies occurring as benign paraproteins reactive with NeuAc(a2-8)NeuAc(a2-3)Gal disialosyl epitopes on gangliosides, including GD1b, GD3, GT1b, GT1a and GQ1b. The paraproteins are also cold agglutinins with anti-Pr specificity in ~50% cases. Isoelectric focusing showed the IgM paraproteins to be multiple in several cases, which was not always identified by standard agarose electrophoresis. The clinical picture comprises a chronic neuropathy dominated by sensory ataxia and areflexia with relatively preserved motor function in the limbs. 50% of cases have motor weakness affecting oculomotor and bulbar nerves as fixed and/or as relapsing-remitting features. This pattern of clinical distribution bears a resemblance to Miller Fisher syndrome, in which similar, but acute phase, anti-ganglioside antibodies are found. Clinical electrophysiology and nerve biopsy show both demyelinating and axonal features. A partial response to intravenous immunoglobulin and other treatments is reported in some cases.

COMPARISON OF ELECTROPHYSIOLOGICAL, MORPHOLOGICAL, AND FUNCTIONAL RECOVERY FOLLOWING SCIATIC NERVE LESIONS IN RATS

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The relationship between clinical and physiological outcome measures during peripheral nerve regeneration is poorly documented. We have, therefore, in rat after crush (n=10) or section/suture (n=15) of the sciatic nerve at mid-thigh, compared electrophysiological studies of the sciatic/tibial nerves and sciatic functional indices (SFI; 0=normal, -100=complete nerve lesion; Bain et al., 1989)

during weekly studies for up to 100-150 days. At termination, sections of tibial nerve were taken for histology. Electrophysiological studies showed earlier reinnervation of plantar muscles after crush (28 ± 1 days, mean \pm SEM) than after section (36 ± 1 days) and, at these times, the SFI's were -27 ± 4 and -63 ± 5 , respectively. At 100 days, conduction velocities were similar after crush (34 ± 1 m/s) and section (34 ± 1 m/s), whereas the amplitudes of the CMAP were three times larger after crush (7.2 ± 0.7 mV) than after section (2.5 ± 0.2 mV). Similarly the SFI's were close to normal at -4 ± 2 after crush whereas sections remained at -45 ± 5 . The total number of tibial nerve fibers at 35 mm distal to the lesion averaged about 6,000 after section and 3,600 after crush in comparison to only 2,600 in controls. The clinical, morphological and physiological parameters show different aspects of the recovery process after nerve lesions and should all be included in therapeutic studies. We are, at the moment, conducting such studies during treatment with FK506 (Fujisawa). (1) J.R. Bain et al. *Plast Reconstr Surg* 1989;83:129-138.

ALTERED PROTEIN KINASE C ACTIVITIES IN PERIPHERAL NERVE OF DIABETIC MICE TRANSGENIC FOR HUMAN ALDOSE REDUCTASE

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Changes in protein kinase C (PKC) activity are now considered to have a major role in pathogenesis of diabetic complications. However, alterations of PKC activity in diabetic nerve are controversial. We examined the relationship between polyol pathway hyperactivity and PKC activity using transgenic mice (Tg) that overexpress human aldose reductase (hAR). PKC activities were separately measured on the fractions of endoneurium and perineurium, the latter of which is rich in peri- and transperineurial vessels. Mice were made diabetic by STZ (200 mg/kg; ip). Non-diabetic Tg and littermate control mice (Lm) were used for comparison. After 12 weeks of diabetes, body weight and levels of hyperglycemia were comparable between Tg and Lm. Diabetes caused 5 fold increase in nerve sorbitol levels in Lm and diabetic Tg showed further 1.5 fold increase compared with diabetic Lm. Diabetic Tg showed 20% decrease in motor nerve conduction velocity (MNCV) compared with nondiabetic TG ($p < 0.01$), while the decrease in MNCV in diabetic Lm was only 5% ($p > 0.1$). Endoneurial PKC activity in membrane fraction in diabetic Tg was reduced by 28% ($p < 0.05$ vs Tg and diabetic Lm), and that in diabetic Lm was 13% less than Lm ($p < 0.05$). By contrast, diabetes caused increased perineurial PKC activity of membrane fraction in both Tg and Lm, but there was no difference between Tg and Lm. Na^+, K^+ -ATPase activity was significantly reduced in both diabetic Tg and Lm and the average value was the lowest in diabetic Tg. It was thus shown that the enhanced PKC activity occurred in tissues rich in microvessels, independent of polyol pathway, but increased flux of polyol pathway in endoneurial tissues was associated with reduced PKC activity. We conclude that the neuropathic changes in diabetic Tg may be accounted for by polyol-dependent impairment of PKC- Na^+, K^+ -ATPase activities superimposed by polyol-independent microvessel alterations exemplified by increased PKC activity. Sponsor: Supported by the Japanese Ministry of Education, Science & Culture, and JDFI.

P0 IS A TARGET ANTIGEN IN CIDP

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The aims of this study were to investigate: I) the presence of antimyelin antibody in CIDP; II) to determine the pathogenicity of such antibodies; III) to define possible target antigens. Sera from 22 CIDP patients were tested by means of ELISA, immunofluorescence and Western blot techniques for binding to myelin or myelin antigens. Sera and purified IgG were injected intraneurally and systemically into Lewis rats which were assessed clinically, electrophysiologically and morphologically

before and after administration of CIDP serum or IgG. In this patient group one or more antibodies to whole peripheral nerve myelin, myelin lipids (GM1, GQ1b, GD1b, GM2 and Gal-S), myelin protein (P0) and complement (C3) activities were found in 17 (77.3%) of 22 CIDP sera. Six sera had anti-P0 IgG antibody, four of which also showed C3 activity. Five contained anti-myelin IgM antibody, of which four were directed to gangliosides and two of these showed C3 activity. One showed C3 activity only. Five sera reacted with neither whole myelin, P0, the gangliosides nor C3. No sera had detectable antibodies to myelin proteins, MAG, PMP22 or P2, nor to the glycolipids, Gal-C or GD1a. Pathogenicity to peripheral nerve was demonstrated only in four sera which contained IgG anti-P0 antibody and C3 activity. Conduction block and demyelination were induced following intraneural injection or passive transfer with these four sera and their IgG. The specificity of the response was shown by the binding of F(ab)₂ from these IgG antibodies to the P0 protein which was identified by sequencing and loss of pathogenicity following absorption by P0 but not by P2. The conclusions of the study are that CIDP is a heterogenous disease and that P0 is a target antigen in some patients.

ACUTE HYPOGLYCEMIA WITHOUT SEVERE HYPERINSULINEMIA CAN INDUCE PERIPHERAL NEUROPATHY IN RATS

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We investigated the effect of acute hypoglycemia without severe hyperinsulinemia on rat peripheral nerve. Glibenclamide (20 mg/kg body weight) was given orally via intraesophageal catheter in 8-week-old rats to induce continuous hypoglycemia (n=5). Then sciatic nerve motor nerve conduction velocity (MCV), nerve blood flow (NBF) and nerve blood velocity (NBV) were measured for 180 minutes after given glibenclamide. NBF and NBV were measured by laser doppler. In hypoglycemic rats given glibenclamide, MCV, NBF and NBV significantly decreased as compared to normal controls. There was no significant pathohistological change in the sciatic nerves of hypoglycemic rats on light microscopic and electron microscopic observation. In hypoglycemic rats, the serum levels of insulin were only fivefold as compared to normal level and no severe hyperinsulinemia was shown. These results indicate the possibility that acute hypoglycemia causes nerve dysfunction without severe hyperinsulinemia in rats.

RAPIDLY PROGRESSIVE POLYNEUROPATHY ASSOCIATED WITH A NOVEL VARIANT TRANSTHYRETIN

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Mutations in transthyretin (TTR) are a major cause of inherited neuropathy. Over 70 different TTR mutations have been reported and the majority of these cause amyloid deposition in peripheral nerve, although restrictive cardiomyopathy is the most common cause of death. The usual clinical picture is a slowly evolving peripheral neuropathy. A novel TTR mutation was found in the DNA of a 52-year-old woman with an acute onset neuropathy within a few days of an immunization, which then progressed to a severe degree over 18 months. The initial diagnosis was chronic inflammatory neuropathy, and the condition appeared to be somewhat responsive to an extended course of plasma exchange and immunosuppression. Sural nerve biopsy done 20 months after onset showed amyloid deposits within the endoneurium. Sequencing of genomic DNA revealed heterozygosity of a G to T transversion at the first position of codon 25 of the transthyretin gene, resulting in a serine for alanine substitution. While there was no obvious family history of neuropathy or amyloidosis, DNA analysis of family members revealed the same mutation in a 4 year older sibling who had very mild sensory

complaints since limb frostbite as a teenager. Haplotype analysis suggested this novel mutation originated in the germline of the father, who was free of neurologic symptoms at the time of his death at age 70 and did not have the mutation on RFLP analysis of his genomic DNA.

ANIMAL MODEL OF AXONAL GUILLAIN-BARRÉ SYNDROME INDUCED BY SENSITIZATION WITH GM1 GANGLIOSIDE

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Some humans develop the axonal form of Guillain-Barré syndrome after receiving bovine brain ganglioside. On sensitization with the ganglioside mixture, all the rabbits injected developed high anti-GM1 IgG antibody titers, flaccid limb weakness of acute onset, and had a monophasic illness course. Pathological findings for the peripheral nerves showed predominant Wallerian-like degeneration with neither lymphocytic infiltration nor demyelination. IgG was deposited on the axons of the anterior roots, and GM1 was proved to be present on the axons of peripheral nerves. Sensitization with purified GM1 also induced axonal neuropathy, indicating that GM1 was the immunogen in the mixture. A model of human axonal Guillain-Barré syndrome has been established that uses inoculation with a bovine brain ganglioside mixture or isolated GM1. This model may help to clarify the molecular pathogenesis of the syndrome and to develop new treatments for it.

DOES DIABETES TARGET GANGLION NEURONS? PROGRESSIVE SENSORY NEURON INVOLVEMENT IN LONG TERM EXPERIMENTAL DIABETES

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Direct involvement of dorsal root ganglia neurons could account for selective early sensory abnormalities in human diabetic polyneuropathy. In this work, we addressed lumbar dorsal root ganglia sensory neuron survival, phenotype and gene expression in a long term model of experimental STZ diabetes in rats. While there was slowing of motor and sensory conduction velocity by 2 months of diabetes compared to nondiabetic controls, this was not associated with alterations in sensory neuron caliber or gene expression using in situ hybridization for markers that included alpha and beta CGRP, SP, talpha1-tubulin, GAP-43, NfM, p75, and Trk A,B,C mRNAs. By 12 months, however, diabetics had developed neuron perikaryal and distal sural axon atrophy, accompanied by a generalized downregulation of alpha and beta CGRP, PACAP, SP, NfM, p75, TrkA, and TrkC mRNAs. With the exception of HSP-27, no elevation in mRNAs that increase after injury such as VIP, galanin, CCK, PACAP, GAP-43 or talpha1-tubulin was observed and constitutive levels, when detectable, trended toward lower rather than increased levels. There was relative preservation of neuron numbers at 12 months in diabetes with only an insignificant trend toward fewer diabetic neurons when a rigorous systematic physical dissector counting approach was applied to the entire L5 ganglion. There was no shift in the relative populations of CGRP and SP neurons compared to controls. Morphological features such as vacuolation or lipofuscin accumulation were not specific for diabetics. Our findings indicate that long term experimental diabetes is associated with atrophy and altered gene expression of sensory neurons with only a borderline reduction of the neuron pool. (Supported by CIHR, AHFMR).