

## ABSTRACTS (A-G) PNS 2001 Meeting

### EXPRESSION AND DISTRIBUTION PATTERN OF TRANSCRIPTION FACTOR NF- $\kappa$ B AND ITS INHIBITOR I $\kappa$ B IN THE INFLAMED PERIPHERAL NERVOUS SYSTEM

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Members of the nuclear factor kappaB (NF- $\kappa$ B) family of transcription factors play important roles in immune, inflammatory and apoptotic responses through the induction of numerous cellular genes. In order to investigate the expression and distribution pattern of the transcription factor NF- $\kappa$ B and its inhibitor I $\kappa$ B in immune-mediated demyelinating polyneuropathies, sural nerve biopsies from patients with Guillain-Barré syndrome (GBS), chronic inflammatory polyradiculoneuropathy (CIDP), and, for comparison, various non-inflammatory neuropathies were studied. In inflammatory demyelinating as well as non-inflammatory neuropathies macrophages were identified as the primary cellular source expressing NF- $\kappa$ B, as determined by immunohistochemistry. Its inhibitor I $\kappa$ B, however, could be localized to macrophages as well as T cells in inflammatory demyelinating neuropathies, whereas in non-inflammatory controls Schwann cells were found to be the primary cell type expressing this inhibitor. Quantitation of immunoreactivity revealed a statistically significant increase of NF- $\kappa$ B expression in inflammatory demyelinating cases compared to controls. The present observations point to macrophages as the major cell type expressing NF- $\kappa$ B in acute and chronic inflammatory demyelination of the peripheral nerve, indicating a key position of this transcription factor in the signaling cascade of various proinflammatory mediators during ongoing disease. As such, NF- $\kappa$ B appears to be a suitable target for modulating the inflammatory process in the PNS.

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### ANTI-GANGLIOSIDE RESPONSES IN CHICKENS FOLLOWING ORAL INFECTION WITH C. JEJUNI

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Anti-ganglioside antibodies occur in C. jejuni-associated Guillain-Barré patients. Immunization studies in rabbits and mice have demonstrated that these anti-ganglioside antibodies can be induced using purified LPS from C. jejuni in a strong adjuvant. To investigate whether administration of ganglioside-bearing C. jejuni via the natural infection pathway also induces an anti-ganglioside response in laboratory animals, we infected five strains of chickens with different C. jejuni strains. Infection of chickens with a GM1-bearing C. jejuni strain from a GBS patient induced specific IgM and IgG anti-LPS and anti-GM1 antibodies. Infection of chickens with the Penner O:3 serostrain, without a GM1-like structure, induced anti-LPS but not anti-ganglioside antibodies. The anti-LPS and anti-ganglioside antibodies were cross-reactive indicating that they were induced by molecular mimicry between the LPS and GM1. The five strains of chickens showed a markedly different pattern of anti-LPS/ganglioside response, indicating the influence of host factors in the response to ganglioside-like structures. In one experiment, some of the infected chickens developed neurological symptoms but we could not demonstrate that this was due to immune-mediated damage of the peripheral nerves.

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### CLINICAL FEATURES OF CHRONIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY (CIDP) IN NORTHERN JAPAN

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We evaluated clinical profiles in 61 patients of CIDP from the Northern area of the main island of Japan. All patients met the electrophysiologic criteria of CIDP by AAN and had motor dominant neuropathy. With a prevalence day of October 1, 1996, we confirmed 33 patients in Aomori Prefecture with a population of 1,478,065. The crude prevalence of CIDP was estimated as 2.2 per 100,000 population. 89% of patients were able to walk without walking aids or other assistance. 60% showed distal dominant muscular weakness. In 12 patients with onset under 15, pes cavus deformity was seen in 5. 64% complained of numbness in the extremities during the progressive phase. One patient showed considerable loss of pain sense, which improved 12 months later after steroid therapy. Four showed ataxia with loss of deep sense. Twenty-five patients showed laterality of weakness more or less, while almost all cases showed electrophysiologic multifocality. The longest record of median nerve distal latency was 50 ms, and the slowest MCV was 2 m/s. 69% responded to steroids. In some, the effects were apparent within a few hours. Plasma cleaning done for steroid-resistant cases showed efficacy in 64%. IVIg was effective in 6 of 12 cases.

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#### AN EVOLUTIONARY STUDY OF 72 CHILDREN WITH GUILLAIN-BARRÉ SYNDROME

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A one Institution series of 72 patients aged less than 15 years (36 girls and 36 boys) followed up for 1 year was studied. There are few series of GBS in children (GBSC) with a similar or higher number of patients. A preceding event occurred in 47 (62%) patients. The first neurological symptom was leg weakness for 62 (86%). The nadir was reached during the first week by 75%. The first examination showed a flaccid paresia with 18 anatomical patterns of involvement. Besides patients with arreflexia there were 11 associations of tendon jerk alterations. The plateau duration was 9.2 days (range: 1 to 34 days). The facial nerve was committed in 27 (37.5%), autonomic dysfunction was detected in 21 (29%) and 5 (7%) needed mechanic ventilation. Pain occurred in 52 (72%). Nine patients (12.6%) had 10 to 50 cells in CFS without evidence of meningoradiculitis or AIDS. 84% had a demyelinating pattern, 8% an axonal pattern and 8% a not well defined pattern at ENMG. Average hospitalization time was 14 days. Most children walked independently before 3 months and all walked by 5 months of follow-up. SEQUELS: 3 children with dropped feet and 3 with limitation of foot dorsiflexion. One patient died. Treatment of 29 (40%) children with IVIg reduced the time to reach the plateau and its duration. Our data suggest that GBSC is more benign than in adults. A light hypercellularity and high levels of protein in CSF are not a rare finding in GBSC. The patterns of clinical involvement at the first neurological examination can reflect the anatomical multifocality of nerve lesions documented by different authors in necropsies or biopsies. Sponsor: Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP. Conselho Nacional de Pesquisa - Conselho Nacional de Pesquisa - CNPq. Coordenadoria de Aperfeiçoamento do Pessoal do Ensino Superior - CAPES. Fundação de Amparo ao Ensino e Assistência do Hospital das Clínicas de Ribeirão Preto - FAEPA.

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#### CHRONIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY IN DIABETICS (CIDPD) AND CHRONIC IDIOPATHIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY (CIDP)

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Clinical aspects, follow-up and responsiveness to treatment of 12 CIDPD patients and 28 CIDP

patients were compared. There are few series comparing CIDPD and CIDP. We used the clinical criteria of AAN and the electrophysiological criteria proposed by Albers et al. Motor strength was graded using the MRC scale and functional evaluation was based on the Winer et al. score. Both groups were followed up from 10 months to 13 years. CIDPD started mainly after 50 years of age and CIDP after 40 years and the difference was statistically significant. CIDPD predominated in men. For most of the CIDP patients the first symptoms were motor and for the diabetics they were sensorimotor. A predominantly distal motor deficit occurred in both groups. Sensory loss occurred in both the lower and upper limbs but some CIDPD patients only had motor deficits. CSF protein content was high in both groups. The majority of patients responded well to immunotherapy. Thickness of microvessels, as well as a possible higher axonal loss, was more prominent in the diabetic group biopsies. Other typical biopsy findings did not differ between groups. In summary, our diabetic patients seemed to have CIDP at an older age and the first symptoms were frequently sensorimotor. Both the CIDPD and CIDP patients responded to immunotherapy, but more diabetics needed a continuous treatment. It is recommended to investigate diabetes in all CIDP patients and to investigate CIDP in diabetic patients with any motor complaints. Sponsor: CAPES - Coordenadoria de aperfeiçoamento do pessoal do ensino superior. FAPESP - Fundação de Amparo à Pesquisa do Estado de São Paulo. FAPEPA - Fundação de Amparo à Assistência e à Pesquisa do Hospital das Clínicas de Ribeirão Preto.

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#### NEW METHOD FOR THE DIAGNOSIS OF FRIEDREICH'S ATAXIA IN LONG-TERM STORED TISSUE

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In Friedreich's ataxia (FRDA) the majority of patients are homozygous for an abnormal expansion of a polymorphic GAA-triplet repeat of the X25 (frataxin) gene. We investigated this triplet-repeat of 25 long-term stored, formalin fixed, paraffin-embedded sural nerve biopsies which had been clinically and microscopically conformable to FRDA. The DNA from long-term stored tissue is prone to degradation and disturbs amplification of fragments. The new method consists of two independent PCRs, which allow a fragmentation-independent detection of high number of GAA-repeats. The first PCR by the use of primers flanking the GAA-repeats gives products with a normal number of triplet repeats. The second PCR was used for the detection of abnormal repeat lengths in those cases in which no product was achieved by the first PCR. Here an abnormal number of GAA-repeats is seen by stutter peaks longer than 360 bp identified by primers, which anneal in the flanking and the GAA-repeat region. Five of the twenty-five cases showed an abnormal number of GAA-repeats including one which had been diagnosed as being definitely afflicted. Furthermore, the method has been tested by a comparison of results from a long range PCR of the GAA-repeat region using DNA from fresh frozen tissue with those obtained with the new method using DNA from paraffin sections of the same patients. This method allows for the first time the examination of GAA-repeats for the diagnosis of FRDA using DNA from long-term stored formalin fixed paraffin-embedded nerve biopsy sections.

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#### FOLLOW-UP STUDY IN PATIENTS WITH RELAPSED GERM CELL CANCER TREATED WITH PACLITAXEL, IFOSFAMIDE, AND CISPLATIN (TIP) PLUS HIGH-DOSE CARBOPLATIN, ETOPOSIDE AND THIOTEPA (CET)

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Eleven patients treated with 3 cycles of TIP followed by one cycle of high-dose chemotherapy (CET) were followed up for 2 to 3 years. Neurological and conduction studies of sensory and motor nerves were performed before, during and after treatment. All patients developed a neurotoxic

neuropathy with axonal degeneration, ranging from mild signs and symptoms with good recovery (4 pat.) to severe and remaining sensory deficit. Sensory potentials of the medial plantar nerves were lost in all patients and recovered in only 2 of them; sural nerve potentials were less affected; median and ulnar nerves recovered in all but 3 patients. Motor conduction velocity of leg nerves was normal or slightly slowed; compound muscle action potentials were diminished in all patients, although weakness of leg muscles was not obvious in most patients. High-dose chemotherapy (CET) was tolerated well by those patients with minor neurological deficit after TIP treatment.

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#### GADOLINIUM ENHANCED MRI OF DENERVATED MUSCLE IS A SENSITIVE METHOD FOR THE DIAGNOSIS OF PERIPHERAL NERVE LESIONS

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The purpose of the study was to use gadolinium enhanced MRI to visualize denervated muscles and to assess the value of this technique for the diagnosis of peripheral nerve lesions. In a first part of the study, MRI of the musculature of the lower leg was performed before and after application of gadolinium in 30 patients presenting with foot drop following peroneal nerve lesion or L5 radiculopathy. Both types of lesions produced a highly significant enhancement of denervated muscle by gadolinium and, based on contrast uptake of the tibialis posterior muscle (affected in L5 lesions, but not in peroneal nerve lesion), a differential diagnosis between both conditions was generally possible. MRI changes were closely correlated to signs of denervation assessed with EMG. In a second experiment changes in denervated muscle were studied in rats after transection of the sciatic nerve. Transection of the sciatic nerve in rats produced essentially identical changes in the denervated muscle as in human patients. Significant contrast enhancement was seen as early as 24 hours in some muscles and was maximal after 21 days. When the sciatic nerve was allowed to regenerate, changes in MRI signal were reversible. We conclude that gadolinium enhanced MRI is a sensitive, minimally invasive technique that can visualise denervated skeletal muscle. This technique may be particularly valuable for the diagnosis of denervated musculature that is otherwise difficult to investigate with standard EMG techniques.

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#### PERINEURIUM MEDIATES AXONAL DAMAGE IN ACUTE INFLAMMATORY DEMYELINATING POLYNEUROPATHY (AIDP)

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**OBJECTIVE:** To assess if axonal damage in severe AIDP correlates with the appearance of epiperineurium in nerve trunks. **BACKGROUND:** Increase of endoneurial fluid pressure in nerve trunks possessing epiperineurium may be an important mechanism of axonal damage in AIDP. **PATIENT AND METHODS:** A 79-year-old man had a 2-day history of acroparesthesias and ascending paralysis culminating in quadriplegia and mechanical ventilation. He died on day 60. Electrophysiologic studies (days 4, 17 and 50) initially showed normal nerve conduction velocities with further slowing, progressive attenuation of compound muscle action potentials and profuse denervation. We studied the preforaminal anterior and posterior L3 and L5 spinal roots, third and fifth lumbar nerves and their branches and femoral and sural nerves. **RESULTS:** Density of myelinated fibres was preserved in L5 ventral and dorsal roots and reduced in sural nerve. Mild de-remyelination was observed in lumbar roots. In both lumbar nerves and their branches, there were extensive de-remyelination and centrofascicular or wedge-shaped areas with marked loss of large myelinated fibres. Axonal degeneration was the predominant lesion in sural nerve. **CONCLUSION:** The presence

of epiperineurium correlates with a drastic change of pathology with superimposed ischemic lesions and distally accentuated axonal loss, suggesting that endoneurial fluid pressure increase could cause axonal damage in AIDP. Sponsor: Fondo de Investigacion Sanitaria (grant no. 99/0046-01) and Fundacion la Caixa (grant no. 98/107-00).

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#### ASSESSMENT OF NOCICEPTIVE C-FIBER FUNCTION IN RESPONSE TO HISTAMINE AND CAPSAICIN IN DIABETIC PATIENTS

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Diabetes is often accompanied by a small fiber sensory neuropathy, a complication that is difficult to evaluate clinically. C-fiber function in the skin may be assessed using models of experimental neurogenic inflammation induced by substances such as acetylcholine, histamine and capsaicin. In the present study, primary afferent C-fiber function was assessed by applying histamine and capsaicin into the forearm skin in 14 type 1 and 12 type 2 diabetic patients and compared to age-matched controls. The flare reaction and the areas of hyperalgesia and allodynia in response to topical application of capsaicin, and the flare reaction and the area of allodynia induced by histamine iontophoresis were evaluated. C-fiber (axon reflex) mediated changes in cutaneous blood flow in response to histamine were recorded by laser Doppler flowmetry (LDF) at a distance of 8 mm from the direct stimulation site. A visual analog scale (VAS) was used to quantify the itching and burning sensations evoked by histamine. Diabetic patients did not differ from controls in the flare reaction, nor did the areas of hyperalgesia, allodynia or allodynia differ. In contrast, the C-fiber mediated vasodilation was reduced in both type 1 and type 2 diabetic patients ( $p < 0.05$ ). There was a trend towards prolonged itching and burning sensations in the type 2 diabetics. Of all tests applied, only the combination of histamine iontophoresis with LDF was sensitive enough to detect C-fiber dysfunction in diabetic patients.

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#### NEW DATA ABOUT THE EVALUATION OF THE NEUROGENIC FLARE REACTION IN SMALL FIBER NEUROPATHY

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Because of high intra- and interindividual variability, the analysis of the neurogenic flare reaction is not established as a diagnostic tool in the evaluation of small fiber neuropathies. In this study we used a Laser Doppler Scanner (LDI, Moor-Instruments) to analyze the size of axon reflex flare and laser-doppler-flux intensity simultaneously at foot and thigh in patients with suspected small fiber neuropathy and age-matched controls. A neurogenic flare reaction was induced by histamine iontophoresis (10%, 0.7 mA, 20 s). Laser-doppler-flux, but not flare size was correlated to age in patients and controls. Flare size clearly differentiated between patients and controls at both test spots, while laser-doppler-fluxes were not different. CONCLUSIONS: The size of the neurogenic, axon reflex mediated flare is dependent on integrity, number and overlap of c-fiber nociceptors and can be used as a parameter in the evaluation of small fiber neuropathy. Measurement of the local laser-doppler-flux alone does not provide enough information, dependency on vascular reactivity and a ceiling effect may play a role. This could explain negative results in earlier studies, where only laser-doppler-flux was used for the analysis of the neurogenic flare reaction.

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## ELECTROPHYSIOLOGICAL PATTERNS IN SUBTYPES OF THE GUILLAIN-BARRÉ SYNDROME: A STUDY OF 119 CASES

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Guillain-Barré syndrome (GBS) is the most common cause of acute flaccid paralysis. Electrophysiologic examination is a useful tool for diagnosis confirmation, prognosis and categorization of GBS into recognised demyelinating (AIDP) and axonal (AMAN and AMSAN) subgroups. This is a study of 119 patients presenting to the laboratory of neurophysiology from January 1998 to October 2000 with a clinical diagnosis of GBS. The mean age was 25.3 (19 years, there were 45 children (38%) aged 16 months to 14 years and 74 adults (62%) aged 15 to 74 years. AIDP was diagnosed in 93 patients (79%) who fulfilled the published criteria of demyelination, the remaining 25 cases (21%) had the axonal form of GBS, most of which with the AMAN subtype. Only 1 patient had the Miller-Fisher syndrome. Recurrent GBS was diagnosed in 9 patients (8%) who experienced their second episode at an interval ranging from 1 to 16 years; they had either AIDP (7 cases) or axonal (2 cases) forms. Involvement of cranial nerves was the symptom of onset in 5 patients. The clinical severity with bulbar palsy leading to mechanical ventilation was significantly higher in the axonal subgroup when compared to the AIDP subgroup (70% vs. 30%,  $p=0.012$ ). In children, the axonal form was significantly more frequent than in adults (40% vs. 10%,  $p=0.0003$ ). The clinical characteristics and the evolution are compared between demyelinating and axonal subtypes in both adults and children subgroups. The recognition of GBS subtypes using the electrophysiological study is crucial as each may have an independent immunopathogenesis and, therefore, may require selective treatments.

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## CYTOTOXIC T LYMPHOCYTES CAN BE GENERATED AGAINST Hu ANTIGEN-DERIVED PEPTIDES: A NEW STEP IN DECIPHERING PATHOGENESIS OF ANTI-Hu SYNDROME

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**INTRODUCTION:** Anti-Hu syndrome is associated with CD8 T cell infiltrates in both lung cancer and the nervous tissue. The nucleoprotein HuD, expressed in both tumor cells and neurons, potentially constitutes a target antigen for auto-reactive T cells. **OBJECTIVES:** To generate and to evaluate cytotoxic T lymphocytes (CTL) directed against HuD in animals and humans. **METHODS:** Peptides were selected from the HuD sequence to match the anchoring sites in the peptide fold of HLA-A2.1 molecule. MHC binding assay was performed using TAP-deficient T2 cell line. Peptides shown to efficiently bind A2.1 were injected into humanized HHD mice, which are both A2.1 transgenic and murine MHC class 1-KO mice, to generate cytotoxic CD8 T lymphocytes (CTL). The studied peptides are present in both the murine and human HuD sequence. Each peptide was injected in 5 or 6 mice. Cytotoxicity was assessed against target cells loaded with HuD and irrelevant peptides. The HuD peptides were also used to generate CTLs in healthy A2.1 humans using an in vitro system stimulating peripheral blood CD8 T cells with peptide loaded on autologous dendritic cells. **RESULTS:** 14 peptides were initially selected, and 10 efficiently bound A2.1 on T2 cells. CTLs could be generated with 7/10 peptides in HHD mice, CTL clones being obtained for 5 peptides. In addition, CTLs were generated in vitro with 2/7 peptides in humans. **CONCLUSION:** We were able to generate CTLs directed against HuD peptides in vivo and in vitro. Since CTLs that are potentially harmful to self are usually eliminated or tolerated, the present results add to previous evidence that T-cell mediated responses are involved in anti-Hu syndrome.

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## MYELIN ABNORMALITIES IN FELINE $\alpha$ -MANNOSIDOSIS

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$\alpha$ -mannosidosis is a disease caused by the deficient activity of  $\alpha$ -mannosidase, a lysosomal hydrolase involved in the degradation of glycoproteins. While myelin abnormalities of the CNS have been described in feline mannosidosis, a homologue of the disease in human patients, a detailed examination of peripheral nerves has not been reported. In the present study, we examined selected nerves in cats with a four base pair deletion in the gene encoding  $\alpha$ -mannosidase. Teased nerve fiber studies showed pronounced demyelination (involving 13 to 48% of teased fibers) in affected cats. Multiple endoneurial cell vacuolation was seen in semithin sections. Thin sections revealed almost universal attenuation of myelin sheaths, multiple vacuoles in macrophages, endothelial cells, pericytes, perineurial cells, and Schwann cells of myelinated and unmyelinated fibers. Thinly myelinated fibers were numerous, often with redundant basement membranes which sometimes formed 'necklaces' around remyelinating fibers. Naked axons and onion-bulbs were also seen. These changes, along with significantly increased G-ratios ( $p < 0.0001$ ), suggest both demyelination and hypomyelination in peripheral nerves of cats with  $\alpha$ -mannosidosis. These myelination abnormalities will serve as surrogate markers for the efficacy of gene transfer/bone marrow transplantation therapies for feline  $\alpha$ -mannosidosis.

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## CORRELATION OF QUANTITATIVE SENSORY TESTING, NERVE AND SKIN BIOPSIES IN PERIPHERAL NEUROPATHY

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In the present prospective study we investigated the correlation of epidermal nerve fiber (ENF) density to sensory thresholds and pain in neuropathies. 35 patients were studied. All patients underwent the following evaluation: 1) clinical and electrophysiological examination, 2) quantitative sensory testing, 3) sural nerve biopsy, and 4) skin biopsy. The average age was  $58.6 \pm 12$  years. In 10 patients the diagnosis was CIDP, in 9 vasculitic neuropathy, and the others had varying diagnoses. Pain was a prominent symptom in 11 patients. In patients with mild fiber loss in the sural nerve, cold thresholds were  $26.5 \pm 1.8$  °C and warm thresholds  $37.9 \pm 2.7$  °C. In patients with extensive fiber loss, cold thresholds were  $17.4 \pm 10.9$  °C and warm thresholds  $42.9 \pm 3.5$  °C. Decreased epidermal nerve fiber density as visualised by the panaxonal marker PGP 9.5 and morphometric evaluation using Image Pro Plus software was not always associated with pathologic temperature thresholds. Heat pain thresholds did not correlate with fiber loss in the sural nerve or the epidermis. Mean ENF densities were reduced in the biopsies to  $4.0 \pm 4.2$ /mm and did not correlate with myelinated fiber counts in the sural nerve. ENF counts could not differentiate between painful and painless neuropathies. These data do not support the notion that ENF is responsible for thermal thresholds and pain in peripheral neuropathies.

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## STUDIES OF INTRATHECAL INSULIN IN EXPERIMENTAL DIABETES

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Insulin has direct trophic actions on neurons by acting through insulin receptors or cross activating IGF-1 receptors. Previous work has suggested that insulin is capable of direct modulation

of neurofilament synthesis, an identified deficit in diabetic neurons and axons. In this work, we postulate that experimental diabetes is associated with defective direct signaling on neurons that supply peripheral nerve axons. We studied the influence of intrathecal low dose subhypoglycemic insulin in Sprague-Dawley rats with experimental STZ-induced diabetes and nondiabetic controls. After 2 months of hyperglycemia that was associated with slowing of motor and sensory conduction velocity, we placed a silicone (0.012" x 0.025") catheter into the lumbar intrathecal space between L6 and S1 connected to an AZLET infusion pump in the dorsal back subcutaneous space. Diabetics and nondiabetic controls received either 0.1 units of regular humulin or its saline carrier daily for 1 month. Insulin and saline had no influence on motor or sensory conduction in nondiabetics. In diabetics, insulin, but not saline, significantly improved motor, but not sensory conduction velocity. These findings have suggested a direct action of insulin on motor neurons and its axons. Work is ongoing to confirm these results, to examine dose dependency, to compare insulin's action with that of IGF-1 and to evaluate the impact of intrathecal insulin on neuropathic pain, expression of neurofilament protein and mRNA. If verified, these results provide support for the hypothesis that abnormalities in direct support of neurons by insulin contribute to experimental diabetic neuropathy. Sponsor: CIHR and AHFMR.

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#### THERAPEUTIC IMMUNOGLOBULIN NEUTRALIZES BLOCKING ANTIBODIES IN GUILLAIN-BARRÉ SYNDROME

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Treatment with high-dose intravenous immunoglobulin-G (IVIg) is accepted as an effective and convenient alternative to plasmapheresis for treating patients with Guillain-Barré syndrome (GBS), but its specific mode of action remains unknown. We here investigated the functional effect of IVIg on neuromuscular blocking antibodies in two adults and two children with GBS. Quantal endplate currents were recorded by means of the perfused macro-patch-clamp electrode in mouse hemidiaphragms. All GBS sera obtained during acute stage of disease before IVIg treatment blocked evoked quantal release by about 90%. Blocking activity was lost in sera obtained two days after intravenous immunoglobulin (IVIg) treatment. Coincubation of the active, blocking serum with the post-treatment serum or with the IVIg preparation used for treatment markedly reduced the blocking activity of the active serum. The extent of the reduction in blocking properties depended on the IVIg concentration. Fab-fragments prepared from the IVIg were equally effective. Our study provides evidence that IVIg is capable of neutralizing blocking antibodies directed at the motor nerve terminal in patients with GBS, possibly by an antiidiotypic mechanism.

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#### ERYTHROPOIETIN AND ERYTHROPOIETIN RECEPTORS IN THE PERIPHERAL NERVOUS SYSTEM: DRG APOPTOSIS AND JAK2 PHOSPHORYLATION IN SCHWANN CELLS

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Erythropoietin (Epo) is a hematopoietic factor that has recently been shown to function in the CNS. The presence or function of Epo or the erythropoietin receptor (EpoR) in the PNS is unknown. Our results demonstrate the presence of both Epo and EpoR in the rat sciatic nerve. Using two different models of nerve injury, chronic constriction injury (CCI) and crush injury adjacent to the DRG, we established functional properties of Epo and EpoR in peripheral nerve injuries. After CCI, Epo was present in axons and was upregulated in Schwann cells. EpoR was present in endothelial cells, axons and Schwann cells; it was not upregulated after injury. EpoR was also localized to both NF200 and IB4 positive DRG cell bodies. Morphometry of uninjured and adjacent crush injured DRG cell

bodies showed a significant reduction of EpoR in DRG cell bodies after injury that correlated with the induction of apoptosis. Crush injury, but not CCI, resulted in appearance of apoptotic cell bodies of DRG, as evidenced by TUNNEL labeling. Western blotting for EpoR of injured and uninjured DRGs showed a reduction of both a 70 and 90 kDa band. Greater phosphorylation of the 90 kDa band was observed after injury. Confocal microscopy demonstrated that EpoR and phosphorylated JAK2 colocalized after nerve injury. Epo induced phosphorylation of JAK2 and ERK1/ERK2 in primary and transformed Schwann cells within 5 minutes. Transient transfection of wild type JAK2 or kinase deficient JAK2KE into primary Schwann cells induced a morphological change and JAK2KE significantly inhibited Schwann cell proliferation; measured by BrdU incorporation. These findings demonstrate the presence of both Epo and EpoR in the PNS and the recruitment of phosphorylated JAK2 after painful nerve injury. These findings suggest that Epo-EpoR may be involved in both PNS degeneration and regeneration.

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#### ANTECEDENT INFECTIONS IN GUILLAIN-BARRÉ SYNDROME (GBS) IN NORTHERN ITALY: A CLINICAL AND SEROLOGICAL CASE-CONTROL STUDY

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Antecedent gastrointestinal (GI) or upper respiratory infections (URI) are frequently reported in GBS where they are often related to *Campylobacter* Jejuni (CJ), Cytomegalovirus (CMV) or Epstein-Barr virus (EBV). To determine whether these infections are risk factors for GBS in Northern Italy, we performed a prospective case-control study on 47 GBS patients, 45 neurological (NC) and 44 non-neurological (NNC) age-, sex- and residence-matched hospital controls. All patients and controls were interviewed for preceding events within 2 months before the onset of their disease. Patients' sera were tested for reactivity with the lipopolysaccharide of CJ by Western blot and with CMV, EBV, Herpes simplex virus (HSV), *Mycoplasma Pneumoniae*, Hepatitis A, B, C and HIV by ELISA. Nine GBS patients (19%) had GI compared to 3 NC and 3 NNC (7% each) (GBS vs. NC/NNC  $p < 0.05$ ) while an antecedent URI was reported by 32 GBS (68%), 16 NC (35%) and 16 NNC (36%) (GBS vs. NC+NNC  $p < 0.05$ ). IgG reactivity ( $>1/100,000$ ) with CJ was detected by Western blot in 7 GBS (15%) patients, including 5 with GI (71%), but in no NC or NNC, including 6 with GI ( $p < 0.005$ ). In the 3 positive GBS patients re-examined after 3 weeks, antibody levels were decreased supporting a recent CJ infection. Anti-CMV and anti-HSV IgM antibodies were found in 28% and 30% of GBS patients and in 4% and 11% of controls ( $p < 0.005$  and  $p < 0.01$ , respectively). Anti-CMV and anti-HSV IgM antibodies were similarly frequent in GBS patients with or without URI. In our population, URI and serological evidence of recent CMV and HSV infections were more frequent risk factors for GBS than GI and CJ infection. While, however, CJ was significantly associated with GI in GBS, the association of CMV and HSV with URI was elusive.

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#### ANTI-CD3 mAb AND IL-4 THERAPY AMELIORATES EXPERIMENTAL ALLERGIC NEURITIS (EAN)

**Carter N, Giang Tran G, He X, Killingsworth M, Hall B, Hodgkinson S.** Medicine, Liverpool Hospital, Liverpool, Australia.

Experimental Allergic Neuritis (EAN) is the animal model for Guillain-Barré Syndrome (GBS), a T cell mediated demyelinating disease. Although there are many therapies currently in use for GBS, most are of limited effectiveness. We used G4.18 a non-mitogenic anti-rat CD3 mAb, alone and in combination with IL-4 to treat actively immunised Lewis rats at the onset of clinical symptoms. The mean clinical scores for G4.18 alone ( $1.1 \pm 0.4$ ), IL-4 alone ( $0.6 \pm 0.4$ ) and G4.18/IL-4 ( $0.4 \pm 0.4$ ) were

significantly lower than the untreated controls ( $1.7 \pm 0.5$ ,  $p < 0.001$ ). Weight loss was also significantly reduced in the treated rats as compared to untreated controls ( $p < 0.001$ ). Furthermore, the level of demyelination in treated rats was significantly lower than that in untreated controls. Although there was no difference in circulating lymphocyte numbers between the groups, eosinophils numbers were higher in G4.18/IL-4 treated animals. Our results demonstrated that a combination of non-mitogenic anti-CD3 with Th1 cytokines could successfully be used to treat immune-mediated demyelinating diseases.

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**ANTI-CD3 mAb AND IL-5 THERAPY AMELIORATES EXPERIMENTAL ALLERGIC NEURITIS (EAN)**  
**Carter N, Tran G, He X, Killingsworth M, Hodgkinson S, Hall B.** Medicine, Liverpool Hospital, Liverpool, Australia.

Experimental Allergic Neuritis (EAN), an animal model for Guillain-Barré Syndrome (GBS), is a T cell mediated demyelinating disease. In this study, we examined the effectiveness of non-activating anti-CD3 mAb and IL-5 to reverse established EAN in the Lewis rats. Rats were treated with G4.18, a mouse IgG3 non-mitogenic anti-rat CD3 mAb alone and in combination with IL-5, as well as IL-5 alone, at the onset of clinical symptoms. The mean clinical scores for untreated controls ( $1.5 \pm 0.8$ ,  $p < 0.001$ ), were significantly higher than for G4.18 alone ( $0.9 \pm 0.7$ ), IL-5 alone ( $1.0 \pm 0.5$ ) or G4.18/IL-5 ( $0.8 \pm 0.9$ ) treated rats. Furthermore, the level of lymphocyte infiltration and demyelination in treated rats was significantly lower than in untreated controls. Circulating lymphocyte numbers were reduced in the G4.18 treated rats and the CD3 receptor was blocked by G4.18 treatment. IL-5 treatment was associated with an increase in circulating eosinophils. Our results demonstrated that a combination of non-mitogenic anti-CD3 with the cytokine IL-5 could be used successfully as a therapy in immune-mediated demyelinating diseases.

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**ACETYL-L-CARNITINE REDUCES CISPLATIN NEUROTOXICITY IN THE WISTAR RAT**  
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Peripheral neurotoxicity is the dose-limiting side effect of the antineoplastic drug cisplatin (CDDP), despite the several attempts which have been performed in order to prevent or reduce this toxicity. Acetyl-L-carnitine (ALCAR) is an ester of the trimethylated amino acid L-carnitine which is produced in the human nervous system, liver and kidney. Several preclinical evidence suggested that the exogenous administration of ALCAR may enhance neuronal metabolism and act on different cellular proteins like tubulin, actin and histones by means of their acetylation. Female adult Wistar rats were treated with CDDP 2 mg/kg ip twice weekly x 9 times alone or in association with ALCAR (100 mg/kg/day po from day -7 to the end of the CDDP treatment). Nerve conduction velocity (NCV) in the tail nerve was assessed before the beginning of the treatment and after drug treatment withdrawal. Weight changes were recorded twice weekly. At baseline, no difference was observed between the 3 groups. At the end of the treatment period, a significant difference in bodyweight was observed between the 2 CDDP treated groups and the control group. Similarly, when the NCV results were compared, a significant difference was observed between controls (mean  $41.1 \text{ m/sec} \pm \text{SD } 3.06$ ) and CDDP alone or CDDP+ALCAR-treated rats. However, the difference between the results obtained in the CDDP group was significantly worse than those obtained in the group co-treated with ALCAR ( $27.8 \text{ m/sec} \pm 1.86$  vs.  $33.4 \text{ m/sec} \pm 2.77$ , respectively;  $p < 0.001$  by one-way ANOVA). These results indicate that ALCAR is able to modulate the peripheral neurotoxicity of CDDP. However, since the mechanism(s) of action of ALCAR in the reduction of CDDP-induced peripheral neurotoxicity is(are)

still unknown, further studies are underway in order to clarify this important aspect.

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**SUPPRESSION OF AXONAL GROWTH BY HIGH DOSE NGF INVOLVES TrkA INTERNALIZATION**  
**Conti A M, Brimijoin W S, Miller L J, Windebank A J.** Neurology, Mayo Clinic, Rochester, MN, USA.

We previously demonstrated that high doses of nerve growth factor (NGF) inhibit neurite outgrowth from embryonic rat dorsal root ganglion neurons within one hour. Candidate mechanisms include downregulation of NGF receptors at the cell surface or alterations in p75 function. To test the role of p75 we investigated: 1) rat dorsal root ganglion cultures treated with a specific blocking antibody directed against p75 and 2) dorsal root ganglion cultures from p75 knockout mice. Despite the functional blockade or absence of p75 in these systems, high doses of NGF inhibited neurite outgrowth. To test the role of TrkA, we used receptor binding techniques. After 1 h exposure to 200 ng/ml NGF, radioligand binding assays indicated a virtual disappearance of high affinity binding sites, with minimal effect on low affinity sites. The data thus suggest a relocation of TrkA into the cytoplasm. Direct evidence for receptor internalization was obtained from confocal images of neurons exposed to fluorescence labeled NGF. In conclusion, axonal growth suppression due to high concentrations of NGF is closely linked to decreased presence of TrkA receptors on the surface of neurons. A better understanding of the physiology of NGF receptors is a necessary step to design therapeutic trials in neurodegenerative diseases.

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**NITRIC OXIDE SYNTHASE EXPRESSION IN EXPERIMENTAL ALLERGIC NEURITIS (EAN)**

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Nitric oxide (NO), which is synthesised from L-arginine by the nitric oxide synthases (NOS), is produced by inflammatory cells during cell mediated demyelination in the central nervous system. NO may subserve different functions, from cytotoxicity to neuroprotection, and trigger either necrotic or apoptotic cell death. In this study we detected inducible form of NOS (iNOS) gene expression in experimental allergic neuritis (EAN), induced in Lewis rats by injection of "SP26," emulsified in complete Freund's adjuvant (CFA), which clinical, electrical, and pathological features resemble those of human idiopathic demyelinating polyradiculoneuritis. Northern blot and single nerve fiber immunostaining showed that both iNOS mRNA and protein were induced in the PNS of EAN rats by day 14 after immunization, at the beginning of EAN clinical signs, but became undetectable by day 18 with the clinical peak of the disease. Moreover, the single nerve fiber immunostaining showed that iNOS protein was mainly localized in inflammatory cells and concomitant with the observation of T-cell apoptosis, but preceding in time the evidence of Schwann cell death that was found beginning by day 18 after immunization. In conclusion, these results suggest that endoneurial expression of iNOS by infiltrating macrophages and NK cells participates in the immunopathogenesis of EAN with a possible active role in promoting T-cell clearance via apoptosis. Sponsor: "Dini Ferrari" Center, A.I.S.M. and Telethon.

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**ORAL GLUCOSE TOLERANCE TESTING IN PATIENTS WITH IDIOPATHIC DISTAL NEUROPATHY**  
**Cornblath D R, Sumner C J.** Neurology, Johns Hopkins University, Baltimore, MD, USA.

**OBJECTIVE:** To examine the diagnostic value of oral glucose tolerance test (OGTT) in

idiopathic neuropathies. **BACKGROUND:** Many patients with neuropathy have no defined etiology after extensive evaluation. While established diabetes mellitus (DM) causes neuropathy, controversy exists regarding the relationship of newly-discovered DM to neuropathy and the appropriateness of evaluation for DM in those presenting with neuropathy. OGTT is controversial as it also detects impaired fasting glucose (IFG) and impaired glucose tolerance (IGT), metabolic stages intermediate to normal glucose homeostasis and DM. **DESIGN/METHODS:** Twenty-six patients with idiopathic small fiber sensory or small and large fiber sensorimotor neuropathies were prospectively identified. All had idiopathic neuropathy based on history and routine lab studies including HbA1C (21), random glucose measurements (20), and skin biopsy (23). Patients underwent fasting glucose and OGTT and classified as normal, IFG, IGT, or DM by ADA/WHO criteria. **RESULTS:** Eleven women and 15 men, median age 62 years (range 47-87), noted neuropathy for a median of 5 years (range 0.5-15). Median NIS score was 12 (range 0-36). Nine had mainly small fiber neuropathies, and 17 had combined small and large fiber neuropathies. Of the 26, 24 had fasting glucose measurements, and all underwent OGTT. Ten (38%) were normal. Seven (27%) had DM; 3 (12%) both IFG and IGT; 5 (19%) IGT; and 1 (4%) IFG. No significant differences in age, duration, or type of neuropathy were observed between patients with normal and abnormal testing. **CONCLUSIONS:** In 26 patients with distal neuropathies, fasting glucose and OGTT identified 16 (62%) with impaired glucose metabolism, and these tests were abnormal in those with normal random glucose or HbA1C. The relationship of the newly-found DM, IGT, and IFG to the development of neuropathy is unknown but longitudinal studies assessing the effects on the neuropathy of treating these, especially IFG and IGT, are needed.

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#### INTERFERON-BETA DECREASES ADHESION AND TRANSMIGRATION CAPACITIES OF LYMPHOCYTES FROM PATIENTS WITH GUILLAIN-BARRÉ SYNDROME

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To investigate the effect of interferon-beta (IFN- $\beta$ ) on adhesion and transmigration capacities of lymphocytes from patients with Guillain-Barré syndrome (GBS). GBS is characterized by infiltration of immune cells in nerve. IFN- $\beta$  decreases nerve tissue infiltration of mononuclear cells in experimental autoimmune neuritis (EAN). The effect of IFN- $\beta$  on leukodiapedesis was evaluated using two *in vitro* functional tests: (1) cell adhesion on plates coated with rVCAM-1 or rICAM-1; (2) cell transmigration with a Boyden chamber assay coated with fibronectin. Lymphocyte expression of VLA-4 and LFA-1 was evaluated by FACS analysis. Cells were incubated without and with IFN- $\beta$ 1a during 18 hours at increasing concentrations before cell adhesion assay, and during the transmigration assay. Very short incubation (5 minutes, 8,000 UI) was also performed for cell adhesion experiments. IFN- $\beta$  induced a dose dependent inhibition of lymphocyte adhesion to rVCAM-1 (adhesion index: 3.12 without IFN- $\beta$ ; 0.61 with IFN- $\beta$  8,000) ( $p < 0.0001$ ) and rICAM-1 (adhesion index: 13.9 without IFN- $\beta$ ; 10.1 with IFN- $\beta$  8,000) ( $p < 0.01$ ). At the highest dose, inhibition of adhesion to rICAM-1 was similar after 5 minutes or 18 hours of IFN- $\beta$  incubation (9.8 vs 10.1). Incubation with IFN- $\beta$  did not modulate the expression of VLA-4 and LFA-1. IFN- $\beta$  also induced a dose-dependent inhibition of lymphocyte transmigration across fibronectin (-32% with IFN- $\beta$  1000; -74% with IFN- $\beta$  8,000) ( $p < 0.0001$ ). IFN- $\beta$ 1a decreases, within minutes, adhesion capacities of lymphocytes from GBS patients to rVCAM-1 and rICAM-1, expressed by activated endothelial cells, and transmigration across fibronectin, a component of extracellular matrix. These results confirm the potential therapeutic interest of IFN- $\beta$  in acute inflammatory neuropathies. Sponsor: Contrat de Recherche et d'Investigation Clinique AP/HP (CRC 00006).

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## TWO INJECTIONS WITH STEROIDS CLOSE TO THE CARPAL TUNNEL ARE A GREATER HELP IN CTS THAN ONE INJECTION; 76.5% AND 50% SUCCESS

**Dammers H J, Veering M M.** Neurology, M.C. Alkmaar, Alkmaar, Netherlands.

After our study on the effect of 40 mg methylprednisolone with lignocaine compared to lignocaine alone in carpal tunnel syndrome (Dammers et al., BMJ 319:884,1999), we realized we had to confirm our findings. We planned a study, randomized and double blinded, with 3 different doses of methylprednisolone. We used 20, 40, and 60 mg. In this study, the first endpoint was the need for further treatment after the first injection. The second injection was stronger than the lowest dose. The second endpoint was the need for further (surgical) treatment after the second injection. 132 patients were included, 35 on 20 mg, 34 on 40 mg, and 33 on 60 mg. Groups were identical. A second injection was asked by 22, 22, and 18 patients (in total 62) in the 3 different groups, after 10.5, 10.5, and 12.4 months. There are no statistically significant differences between the groups. Not all patients wanted a second injection; 16 asked for the neurosurgeon after 1 injection because of different reasons. The patients who received 2 injections fared well; only 27 asked for the surgeon, which is only 23.5% (27 of 116 patients). This is much better than 50% asking for the surgeon after 1 injection. Follow-up averaged 21 months, longer than in our first study. We looked for correlations between facts, findings and endpoints. There is only one statistically significant correlation between the absence of sensory conduction over the distal median nerve and the need for neurosurgery. Of this part of the population, even 50% has good effect of the second injection; this still is satisfactory. The second injection gave an average delay in neurosurgery for another 7.1 months. Patients averaged more than 30 months of complaints. Actually, there is no contraindication to start treatment of CTS with 1 or, if necessary, 2 injections of at least 40 mg methylprednisolone with lignocaine proximal to the carpal tunnel. CONCLUSION: When patients do need a second injection for CTS, up to 76.5% long-term effect can be obtained. Injections must be given before operation is considered.

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## SERIAL ANALYSIS OF GENE EXPRESSION OF THE PERIPHERAL NERVOUS SYSTEM

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To get a better understanding of the molecular pathways of the physiological process of myelination, we generated gene expression profiles of human sciatic nerve and cultured human Schwann cells (SC) using SAGE. The analysis of sciatic nerve might give an insight into genes involved in myelin maintenance, whereas a comparison of the expression profile of SCs with other tissues will yield novel SC specific transcripts. We sequenced 20,000 tags per library, resulting in 9,422 different nerve transcripts and 7,480 SC transcripts. 29 of the high abundant transcripts (>5 per 10,000 tags) were nerve specific and 13 were SC specific. In the nerve library, myelin basic protein, apolipoprotein D and S100 calcium-binding proteins are found. A novel finding is the high expression of factors of the complement system and insulin-like growth factor-binding proteins. In the human SC library, high expression of genes involved in extracellular matrix formation is found. Both libraries show similar expression of house-keeping genes, such as Heat shock proteins, Ferritin and GAPDH. Northern blot analysis and RT-PCR were performed to confirm the expression levels found by SAGE for several genes. This study gives an overview of genes expressed in the peripheral nervous system.

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## MYELIN PROTEIN ZERO IN INHERITED NEUROPATHIES: A COMPARATIVE STUDY OF DIFFERENT MUTATIONS ASSOCIATED WITH DISTINCT PHENOTYPES

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Myelin protein zero (P0) is the major integral protein of the peripheral myelin. Its atomic structure for the extracellular domain was determined by x-ray crystallography. Mutations in the protein zero gene (MPZ) were found associated with different forms of inherited peripheral neuropathies, both of the demyelinating (Charcot-Marie-Tooth type 1, Dejerine-Sottas syndrome, congenital hypomyelination) and axonal (Charcot-Marie-Tooth type 2) forms. Aim of the present study is to discuss the relationships between the molecular defects and the variability in the clinical and neuropathological features associated with P0 mutations. We compared the clinical, neurophysiological and neuropathological findings associated with four different MPZ mutations. Two missense mutations are located in the extracellular domain of the protein: i) a novel variant at codon 61 (Asp61Asn) found in a patient with a severe neuropathy, characterised at sural nerve biopsy by severe demyelination and axonal loss; ii) a Thr124Met mutation, which segregated in a family with three affected subjects, diagnosed as CMT2. We also report two non-sense mutations affecting the cytoplasmic region of P0: a Ser233fs deletion, causing a frameshift and a stop at codon 250, detected in a severe form of CMT1, and a de novo Gln215stop which was found in a patient with CH. Molecular modeling of P0 extracellular domain allowed us to explore how different mutations, even at the same codon, affect the high-order structures of the protein. We then attempted to relate the molecular defect with the structural and functional abnormalities of myelin observed in patients' nerves. Sponsor: Partially granted by MURST and Ateneo to FA, Ministero della Sanità to PM. The laboratory is a member of the European CMT Consortium.

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#### NECROTIZING VASCULITIS OF BIOPSIED NERVES: HETEROGENEITY OF PATHOLOGIC ALTERATIONS AND OF CLINICAL SYNDROMES

**Dyck P J B, Engelstad J K, Dyck P J.** Neurology, Mayo Clinic, Rochester, MN, USA.

**BACKGROUND:** Classification of necrotizing vasculitis (NV) is based on size of blood vessels, nature of inflammation, associated clinical syndromes and putative mechanisms. Because the size of blood vessels of nerves biopsied are limited (arterioles, venules and microvessels), little variability of pathologic alterations and of clinical syndromes might be expected. **METHODS AND RESULTS:** Of the 8,301 nerve biopsies evaluated since 1964, 472 were interpreted as strongly suggestive and 264 as diagnostic of NV. A higher ratio of nerves with large arteriole involvement was diagnostic of NV, whereas for microvessels, the higher proportion were in the suggestive category of NV - implying that large arteriole NV is more obvious than microvessel NV. Since fibrinoid necrosis is readily recognized and characteristic of large arteriole NV but is seldom seen in microvasculitis, the differences in detection of large arteriole NV and microvessel NV is explained. The clinical syndromes associated with large arteriole involvement were polyarteritis nodosa, rheumatoid vasculitis, Churg-Strauss syndrome (CSS), Wegener's granulomatosis (WG) and others. The syndromes associated with microvessel NV were immune sensory neuropathy with sicca, non-systemic vasculitis, diabetic and non-diabetic lumbosacral and brachial radiculoplexus neuropathies and others. The nature of the inflammatory infiltrate was often informative (e.g. macrophages in WG and eosinophils in CSS). **CONCLUSION:** 1) The pathological features and criteria for the diagnosis of microvasculitis are difficult and not easily discriminated from other inflammatory lesions, whereas the features of large arteriole NV are more stereotypic and obvious. 2) Improved diagnosis of microvasculitis can be achieved from use of serial sections and immunohistochemistry of smooth muscle and inflammatory cells. 3) Stereotypic clinical syndromes exist for both large vessel NV (e.g. distinct multiple mononeuropathy) and for small vessel NV (e.g. lumbosacral radiculoplexus neuropathy). Sponsor: Supported in part by grants from the National Institute of Neurologic Diseases and Stroke (NS36797).

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AXONAL ATROPHY IN THE AORTIC DEPRESSOR NERVE CAN BE THE HISTOLOGICAL CORRE-

LATE OF AN ALTERED BAROREFLEX AFFERENCE IN SHORT TERM EXPERIMENTAL DIABETES  
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The aortic depressor nerve (ADN) is an important afference of the baroreflex in rats. Most of the reports showing impairment of baroreflex sensitivity attribute it to the autonomic efferent neuropathy. The afferent arm of the baroreflex, i.e. the carotid and aortic depressor nerves, has received less attention in diabetes. Previous results from our laboratory [1] have shown that rats with streptozotocin (STZ)-induced diabetes present baroreflex dysfunction as early as five days after the administration of STZ. To evaluate the morphology of the ADN myelinated fibers in short term experimental diabetes, Wistar rats were injected with STZ (40 mg/kg, iv, n=9) 15 days before the experiments. Control rats received a vehicle (n=7). Under pentobarbital anesthesia, the ADN was isolated and its spontaneous activity was recorded. After the recordings, distal segments of the nerves were prepared for light microscopy studies as described [2]. The ADN of diabetic rats showed a smaller diameter of the myelinated fibers ( $1.94 \pm 0.06 \mu\text{m}$  vs.  $2.11 \pm 0.07 \mu\text{m}$ ) and their axons ( $1.07 \pm 0.04 \mu\text{m}$  vs.  $1.23 \pm 0.07 \mu\text{m}$ ) and the G-ratio histograms were shifted to the left in 5 animals when compared to the controls. The fascicular area of the nerves was  $804 \pm 106 \mu\text{m}^2$  for the diabetic rats and  $1,114 \pm 118 \mu\text{m}^2$  for the controls, with a significant difference. The number of myelinated fibers was  $60 \pm 7$  for diabetic rats and  $72 \pm 4$  for controls, with no difference between groups. These data provide morphological support for previous reports of an altered baroreceptor afference in this model of diabetic neuropathy. [1] Fazan Jr R et al. *The Physiologist* 43 (2000) 261. [2] Fazan VPS et al. *J Auton Nerv Syst* 77 (1999) 133-139. Sponsor: PRONEX 1, FAPESP, CNPq, FAEPA, FUNEPU, FAPEMIG.

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## RECURRENT CUBITAL TUNNEL SYNDROME: ETIOLOGY AND TREATMENT

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Controversy surrounds the treatment of recurrent cubital tunnel syndrome after previous surgery. Irrespective of the surgical technique, namely pure decompression in the ulnar groove and the cubital tunnel distal of the medial epicondyle and the different methods of volar transposition (subcutaneous, intramuscular, and submuscular), the results of surgical therapy of cubital tunnel syndrome are often not favorable, especially in cases of long-standing symptoms and severe deficits. 25 patients who had previously undergone surgical treatment for ulnar nerve entrapment at the elbow were evaluated because of persistent or recurrent pain, paresthesia, numbness, and motor weakness. 5 patients had undergone a simple decompression of ulnar nerve and 20 patients had undergone a nerve transposition. 2 patients underwent surgery at our hospital, whereas 23 patients underwent their primary surgery at other institutions. Various surgical techniques were used during the subsequent surgery, such as external neurolysis, farther subcutaneous anterior transposition, and subsequent transfer of the nerve back into the sulcus. The causes of continued or recurrent symptoms after initial surgery included retention of the medial intermuscular septum, dense perineurial fibrosis of the nerve after subcutaneous transposition and adhesions of the nerve to the medial epicondyle. The average follow-up after the last procedure was 7 months (2-20 months). All 7 patients with subsequent transfer of the ulnar nerve back into the sulcus became pain-free, whereas only 14 of 18 patients who had external neurolysis or secondary subcutaneous transposition became free of pain. The recovery of motor function and return of sensibility were variable and unpredictable. In summary, reoperation after primary surgery of cubital tunnel syndrome gave satisfactory results in 21 of 25 cases. Subsequent transfer of the ulnar nerve back into the sulcus promises to be useful in cases in which subcutaneous

transposition had not been successful.

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#### FIBER LENGTH DEPENDENCY IN POLYNEUROPATHY ASSOCIATED WITH IGM

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Polyneuropathy associated with IgM monoclonal gammopathy (PNP-IgM) is characterized by symmetrically distributed sensorimotor deficits. Nerve conduction studies may show predominantly distal conduction slowing consistent with demyelination. This led to the suggestion of fiber length dependency despite the occurrence of this mechanism in axonopathies. Our aim was to assess if fiber length dependency also exists in demyelination. In 23 patients with demyelinating PNP-IgM, we performed electrophysiologic studies of nerves with short (motor: median to forearm flexor, sensory: lateral cutaneous to forearm), medium (motor: median to thenar, sensory: median to 3<sup>rd</sup> digit), and long (motor: tibial, sensory: sural ) fiber lengths. For nerves with short, medium, and long fiber lengths we found respectively: (1) distal motor latency consistent with demyelination in 9, 19, and 8 patients; (2) motor conduction velocity consistent with demyelination in 5, 8, and 4 patients; (3) disproportionate distal slowing of motor conduction in 1, 12, and 6 patients; (4) decreased distal compound muscle action potential (CMAP) amplitude in 1, 7, and 22 patients (below 0.5 mV in 0, 0, and 18); (5) decreased distal sensory nerve action potential amplitude in 12, 21, and 22 patients; (6) spontaneous muscle fiber activity on electromyography in 0, 2, and 16 patients. Due to very low CMAPs, items 1, 2, and 3 could not be determined reliably for the tibial nerve. We conclude that, in PNP-IgM, demyelination as well as axonopathy are fiber length dependent.

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#### THE SEQUENTIAL ANALYSIS OF CX3C CHEMOKINE AND CX3CR mRNA EXPRESSION IN THE CAUDA EQUINA OF RATS WITH EXPERIMENTAL ALLERGIC NEURITIS (EAN)

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Chemokine is implicated in the pathogenesis of EAN, an animal model of human Guillain-Barré syndrome. CX3C chemokine fractalkine (flk) is expressed on many cell types including neurons as well as immune cells, acts on leukocytes and microglia, and plays an important role for the development of the central nervous system (CNS) lesions, although its role in the pathogenesis of peripheral nervous system (PNS) lesions remains unclear. To investigate whether flk and its specific receptor (CX3CR1) are expressed in the PNS and, if any, their kinetics during PNS inflammation, mRNA was isolated from the cauda equina (CE) of EAN rats at days 0, 4, 7, 10, 14, 18, 21, 28, and 32 post-immunization (p.i.). We quantitated flk and CX3CR1 messages using competitive RT-PCR method. Each message was compared with GAPDH message in the same sample to compensate variations. All rats developed limp tail at d 11 p.i. Then paralysis advanced up to moderate to severe paraplegia, and peaked at d 17 p.i. Paralysis improved spontaneously thereafter; only mild tail weakness remained. Flk was weakly expressed in normal CE, started increasing at d 7 p.i., and peaked at d 18 p.i. in concert with the active stage. Flk message reduced at d 21 p.i., and stayed at low levels thereafter. CX3CR1 mRNA was scarcely found in the normal CE. Its expression remained at low level until d 7 p.i. then increased. The peak expression was observed during the active stage of EAN (d10~18 p.i.), followed by gradual decline. By d 32 p.i., CX3CR1 message recovered to normal levels. This is the first report that flk message was detected in the PNS as well as in the CNS. That flk and CX3CR1 messages were upregulated in a slightly different way during the active stage of EAN indicates not only their proinflammatory roles, but also the multiple pathways regulating these molecules under inflammatory conditions of PNS. The cell source and the regulation mechanism of flk and CX3CR1 need to be further investigated to gain insights into the inflammation of the PNS.

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## NOVEL CLASSES OF PLP MUTATIONS ARE ASSOCIATED WITH PNS AND CNS DISEASE

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PLP and its alternatively spliced isoform, DM20, are the main intrinsic membrane proteins of compact myelin in the CNS, but are also expressed by Schwann cells. Patients who do not express either PLP or DM20 protein, due either to gene deletion or protein truncation, have both Pelizaeus Merzbacher Disease (PMD) and a demyelinating peripheral neuropathy. Several individuals with non-conservative amino acid substitutions that alter both PLP and DM20 but are not predicted to abolish protein expression do not have a peripheral neuropathy, despite having severe CNS disease, while other individuals with missense mutations have neuropathy. To investigate the molecular mechanisms causing peripheral neuropathy, and to elucidate the function of PLP/DM20 in the PNS, we have evaluated more than 30 PMD patients for the presence of neuropathy. We find several novel classes of PLP mutations. Type I mutations lead to the absence of both PLP and DM20 expression and are associated with mild CNS disease and a demyelinating peripheral neuropathy. Mutations of this type include deletions of the entire PLP gene, mutation of the PLP start codon, and deletion of the fourth G of the PLP coding region. Type II mutations produce misfolded PLP and DM20 which accumulate in the ER, are associated with severe CNS disease, but do not cause peripheral neuropathy. Type III mutations are PLP gene duplications which produce increased amounts of PLP/DM20, cause moderate to severe CNS disease, but also do not cause neuropathy. Type IV mutations, noncoding mutations in the PLP gene, are associated with variable disease severity, and may or may not produce peripheral neuropathy. These data clearly demonstrate that there are functional domains of PLP and DM20 in Schwann cells and oligodendrocytes and that delineating how these domains cause PNS or CNS disease will be important in understanding the pathogenesis of PMD.

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## A DOUBLE-BLIND RANDOMISED, PLACEBO CONTROLLED CROSS-OVER TRIAL OF AMANTADINE FOR TREATMENT OF FATIGUE IN GUILLAIN-BARRÉ SYNDROME

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**BACKGROUND:** Severe fatigue and endurance intolerance are a major complaint in about 80% of patients after the acute phase of immune-mediated polyneuropathies. Since there are similarities in immunological mechanisms between multiple sclerosis (ms) and immune-mediated polyneuropathies, and amantadine is an effective drug in treatment of fatigue in ms-patients, we started a double-blind randomised and placebo controlled cross-over trial to study the effect of amantadine for treatment of fatigue in the Guillain-Barré Syndrome (GBS). **METHODS:** 80 GBS-patients fulfilling the inclusion criteria will be randomised. The primary end point is improvement of at least 1 point on the fatigue severity scale (FSS), a 9-item and 7-points scale, priorly evaluated in immune-mediated neuropathies. Follow-up of the patients will be 4 months; 2 weeks introduction and baseline measurements, 6 weeks amantadine or placebo, 2 weeks wash-out and 6 weeks the reverse, followed by another 2 weeks wash-out. Besides the FSS, the fatigue impact scale, hospital anxiety and depression scale, SF-36 health survey, social support scale and St. Mary's Hospital sleep questionnaire will be examined. **FINDINGS:** 40 patients are already included. This summer the study will be finished. Results from our pilot study showed that patients with immune-mediated polyneuropathies tend to respond to amantadine. 4 of 7 tested patients with GBS showed a fast (within a few days) and significant improve-

ment on the FSS. One patient had a subjective improvement of fatigue without a significant change on the FSS. The 2 other patients did not respond. INTERPRETATION: This is, to our knowledge, the first controlled trial in which treatment of severe fatigue in GBS-patients will be examined. The final results will be presented.

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## SOLUBLE CD27 IN RELATION TO CLINICAL AND IMMUNOLOGICAL SUBGROUPS IN GUILLAIN-BARRÉ SYNDROME

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**INTRODUCTION:** The Guillain-Barré syndrome (GBS) is a heterogeneous immune-mediated polyneuropathy which can be divided into clinical and immunological subgroups. Soluble CD27 (sCD27), a marker of T cell activation and member of the TNF-R family, has been shown to be associated with disease activity in a variety of immune-mediated disorders. **AIM:** To assess whether levels of sCD27 in serum and cerebrospinal fluid (CSF) are increased and reflect intrathecal or systemic T cell activation in clinical and immunological subgroups of GBS. **METHODS:** Pretreatment paired serum and CSF samples from 77 GBS patients were tested in sandwich ELISA for sCD27 (CLB, Amsterdam, The Netherlands). We used serum control samples from patients with other neurological diseases (OND) (n=40), CMV infection (n=21) and healthy controls (HC) (n=38). CSF control samples from patients with non-inflammatory (NI-OND) (n=45) and inflammatory (I-OND) OND (n=10) were tested. **RESULTS:** Serum sCD27 in GBS patients was higher compared to OND patients and HC (p<0.001) and lower compared to CMV controls (p<0.001). In CSF of GBS patients, sCD27 was higher than in NI-OND (p<0.001) and lower than in I-OND patients (p<0.01). CSF sCD27 was not related with serum sCD27 or traumatic puncture, indicating that it was produced intrathecally. The subgroup of patients with high CSF sCD27 levels more frequently had relatively slow progression, a global distribution of weakness and cranial nerve involvement. **CONCLUSION:** sCD27 levels in serum and CSF from GBS patients are increased compared to non-inflammatory controls indicating T cell activation. High levels of sCD27 in CSF in a subgroup of GBS patients was related to a clinical pattern compatible with proximal root and cranial nerve lesions.

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## ENDOGENOUS INTERLEUKIN-10 PROTEIN IN RODENT SCIATIC AND HUMAN SURAL NERVE IN EXPERIMENTAL AND HUMAN NEUROPATHY

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The anti-inflammatory cytokine interleukin (IL)-10 is involved in inflammatory as well as injury-induced peripheral nerve disease and has been suggested to be of therapeutic value in experimental settings. Here, endoneurial IL-10 was investigated in experimental and human neuropathy. The time course and cellular localization of IL-10 protein was characterized after crush (day 0, 1, 3, 7, 14, 28) in mouse and rat sciatic nerve and in experimental autoimmune neuritis (EAN; day 0, 7, 11, 14, 28) in rats using ELISA (n=12) and immunohistochemistry (IHC; n=3). Sural nerve biopsies from patients suffering from GBS, CIDP, hereditary, vasculitic or metabolic polyneuropathies (PNP) were analyzed for IL-10 by IHC on frozen sections. IL-10-immunoreactivity (IR) was rapidly depleted endoneurially after crush and EAN, recovered slowly afterwards and reached baseline levels again around day 14 after crush. Many invading epineurial macrophages were positive for IL-10 on day 1-3 after crush. Rarely detectable or even absent in GBS and CIDP, IL-10-IR was occasionally found endoneurially in hereditary and vasculitic PNP in Schwann cell-like structures and epineurially in vasculitic PNP. These results suggest that nerve lesion is associated with a relative deficiency of IL-10 protein locally.

Our findings support the hypothesis that treatment strategies increasing endogenous IL-10 levels may be beneficial.

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#### TEMPORAL DISPERSION IN MULTIFOCAL MOTOR NEUROPATHY

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Multifocal motor neuropathy with conduction block (MMN) is a disorder treatable with intravenous immunoglobulin (IVIg) that can be mistaken for motor neuron disease or other lower motor neuron syndromes. Existing electrophysiological criteria for MMN are restrictive, as the diagnosis of conduction block is often considered untenable in the presence of temporal dispersion (TD). To look into this further, we compared pre- and post-treatment electrophysiology in 9 patients who satisfied clinical and electrophysiological criteria for MMN and who responded to IVIg. Although some improvement in conduction block was seen after treatment, the most significant changes occurred in TD, which improved in 13/17 segments studied and by at least 20% in 7 of these segments. Improvements in TD were most marked in segments with conduction block but there was no correlation between improvement of TD and change in the magnitude of conduction block. We propose that TD should be considered an inherent feature of MMN. Changes in TD could be a sensitive marker of response to IVIg in these patients.

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#### CASPASES ARE PRESENT IN PERIPHERAL NERVES AND ARE ACTIVE DURING WALLERIAN DEGENERATION

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Axonal degeneration following nerve injury involves calcium entry and activation of calpains. Calpain protein levels, however, are reduced in peripheral nerves long before axonal degeneration occurs (JPNS 1999;4:182), leading to the hypothesis that proteases other than calpain may be involved. Caspase-3 is a key executioner of cellular apoptosis, and is a candidate enzyme in all models of cellular degradation. The presence of caspase 3 in axons has been demonstrated previously in the CNS. We investigated the presence and activity of caspase-3 in peripheral nerves and in the process of Wallerian degeneration. We found caspase-3 specific breakdown products of alpha-fodrin (120 kD) in normal peripheral nerves, and demonstrated that the caspase inhibitor DEVD-fmk inhibits Wallerian degeneration in cultured sensory neurons. Interactions between calpain and caspase-3 were also indicated by in vitro cleavage assays. These data suggest that caspase, and specifically caspase 3, is present in peripheral nerve and is important in the process of axonal degeneration. We propose that a proteolytic pathway involving both calpain and caspase is involved in the process of axonal degeneration after injury, and possibly in other models of axonal degeneration. Sponsor: National Institutes of Neurological Diseases and Stroke.

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#### THE CLINICAL SYNDROME OF LUMBAR SPINAL STENOSIS

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Lumbar spinal stenosis is well defined in patho-anatomical terms but its clinical features may be more heterogeneous than previously thought. 75 patients were reviewed with lumbar spinal stenosis presenting to a neurology and a neurosurgery clinic. Diagnosis was based on imaging studies to document the clinical features, radiological changes and outcome of this disorder. Patients presented

with complaints of weakness numbness/tingling, radicular pain and neurogenic claudication in equal proportions. Neurogenic claudication occurred in only 61.3%. Although there were abnormalities on neurological examination in 93.3% of patients, these were generally mild with reduced ankle jerks being most common. Imaging of the lumbar spine showed that moderate to severe central spinal stenosis correlated with complaints of weakness and abnormal motor power on clinical examination. Patients were reviewed at a mean of 4 years after diagnosis; 65.3% had undergone surgical decompression. A third of patients felt that their symptoms had improved while a quarter felt that they had worsened. More than half had good neurological function when reviewed. Outcome did not differ whether the patient was treated surgically or not. However, a poorer functional status correlated with motor weakness and associated co-morbid disease. Degenerative lumbar stenosis is a clinically heterogeneous neurological disorder of the lower limbs in the elderly. Some patients had been diagnosed with polyneuropathy initially. A high index of suspicion is required and neuroimaging confirms the diagnosis. Long-term outcome is mixed and in some patients non-surgical management remains a viable option.

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#### VACCINATION, PREVENTION AND TREATMENT OF EXPERIMENTAL AUTOIMMUNE NEURITIS (EAN) WITH AN OLIGOMERIZED T-CELL EPITOPE

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Using a polypeptide oligomer harboring 16 repeats (“16mer”) of the neuritogenic epitope (amino acids 58–73) of myelin P2-protein as basal element, we attempted to modulate the immune response to the P2-protein, which is an important neuritogenic autoantigen in EAN. We show that treatment of Lewis rats with P2-16mer had a tolerizing and vaccinating effect against the induction of EAN by immunization. Animals vaccinated or i.v. treated with 200 µg of the P2-16mer were almost completely protected against clinical disease, whereas control rats suffered from paraplegia. The beneficial clinical effect of 16mer therapy could be correlated with a reduced proliferative response of P2-53-78 specific lymph node cells. The rate of apoptotic T-cells in sciatic nerve or in lymph node cells was not increased by the 16mer-treatment, suggesting that induction of energy could be responsible for the effect. Our data suggest that oligomerized antigens are highly effective in prevention and suppression of a specific autoreactive T-cell mediated immune response.

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#### LOCALIZATION OF MAJOR GANGLIOSIDES IN THE PNS: IMPLICATIONS FOR IMMUNE NEUROPATHIES

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Anti-ganglioside antibodies (Abs) are associated with some immune mediated neuropathies, e.g. GD1a with predominant motor axon injury in GBS (AMAN). One conflict is that Abs recognizing widely distributed gangliosides are associated with clinical symptoms that imply specific cellular injury, for example motor axon injury in AMAN. Since, in humans, all major gangliosides are expressed in axons and myelin of both ventral and dorsal roots, the association of particular anti-ganglioside antibodies with primary axonal injury or predominant motor or sensory fiber damage remains to be explained. We have raised a series of high affinity monoclonal IgG class (IgG1, IgG2a or IgG2b) Abs

against the major nervous system gangliosides GM1, GD1a, GD1b, and GT1b. Immunohistochemical studies were done on peripheral nerves and spinal cord using these Abs. The following observations were made: 1) One anti-GD1a mAb (IgG1) showed preferential binding to motor axons; 2) Two anti-GD1a mAbs bound preferentially to unmyelinated fibers; 3) When the three anti-GD1a mAbs were used at the same concentration, different staining patterns were detected in the spinal cord due to different affinities; 4) Myelin was poorly stained in peripheral nerves. Our findings suggest that factors such as Ab specificity and affinity, differences in ganglioside accessibility, and differential expression of gangliosides may each influence preferential injury to different fiber systems and cell types.

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#### IONOTROPIC ATP RECEPTORS IN THE AXONAL MEMBRANE OF UNMYELINATED PERIPHERAL NERVE FIBRES

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It is now known that receptors for ATP on distal and central nerve endings contribute to the transduction of sensory stimuli. However, it is a common assumption that the axonal membrane in the mid-axonal nerve trunk does not have this kind of chemosensitivity. In the present study, we have tested possible ATP-induced changes in the excitability of peripheral axons by threshold tracking, using QTRAC (Institute of Neurology, London), a stimulus-response data acquisition program. The experiments were performed on isolated rat cervical vagus nerves, rat sural nerves, and mouse dorsal spinal roots. In all these preparations, changes in compound C fibre action potentials were seen during bath application of ATP and structural ATP analogues. These changes in electrophysiological parameters indicate the presence of ionotropic, depolarising receptors for ATP. Possible sources of ATP for activation of axonal receptors include Schwann cells (membrane stretch), endothelial, and tumor cells. Taken together, our data demonstrate ionotropic ATP receptors in the axonal membrane of unmyelinated fibres in various peripheral nerves. These receptors might contribute to the transduction of sensory, including nociceptive, stimuli in the trunk of a peripheral nerve. Sponsor: Supported by the DFG.

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#### EARLY ELECTROPHYSIOLOGICAL CHANGES IN A TRANSGENIC RAT MODEL OF CHARCOT-MARIE-TOOTH

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Recently, a reliable transgenic rat model of human CMT 1 A has been developed. So far, neurophysiological studies have been performed only in advanced stages of rat disease. Affected rats show overexpression of Peripheral Myelin Protein (PMP-22) and a peripheral demyelinating neuropathy. We performed a neurophysiological study in two heterozygous PMP-22 transgenic rats and in one normal control, matched for age (3 weeks) and weight (average: 60 g). Recordings were performed in vivo by stimulating the sciatic nerve at both sciatic notch and ankle sites and recording the H-reflex and direct muscle responses (CMAP). The H-reflex related SNCV and MNCV were calculated by measuring the distance between the sciatic notch and the ankle sites and the respective latencies. The two transgenic rats showed different levels of PMP-22 overexpression, as judged by quantitative PCR. The rat with a lower PMP-22 gene level showed a 30% reduction of MNCV compared to the normal control, while SNCV was not reduced. The CMAP was sized approximately 45% of the normal rat while the ratio between H wave amplitude and CMAP was 30% of the normal, the H wave amplitude being more affected than the CMAP. The action potentials in the

rat with a higher transgene level were not recordable. Our data demonstrate that slowing of MNCV is an early finding in CMT1A rat model. The marked reduction of H wave amplitude in front of a normal SNCV suggests a possible early axonal damage of sensory fibers. The entity of electrophysiological compromise positively correlated with the number of copies for PMP 22 gene. All together these considerations prove the sensitivity of this method, however, further studies are needed to confirm these results and to prove that this model may be suitable to investigate the effects of therapeutic approaches.

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**S-PBN, SPIN TRAP NITRONE, ATTENUATES REPERFUSION INJURY IN RAT SCIATIC NERVES**  
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Oxidative stress plays a key role in the development of reperfusion nerve injury [1-4]. We examined the effects of S-PBN (sodium 4-[(tert-butylimino) methyl]-benzene-3-sulfonate N-oxide) on reperfusion injury in rat sciatic nerves. S-PBN, closely related to NXY-059 (disodium 4-[(tert-butylimino) methyl]-benzene-1,3-disulfonate N-oxide), is more stable than previously available free radical scavengers. Immediately after the onset of 4-h ischaemia in rat right hindlimb [5-7], S-PBN (1.2 M) or 0.9% saline (control group) was administered via mini-osmotic pumps inserted subcutaneously. S-PBN was also injected by a single injection (50 mg/kg, ip). Morphology in sciatic, tibial and peroneal nerves was assessed after 72 h of reperfusion. In S-PBN-treated rats, plasma concentrations of S-PBN were 70.4, 88.3 and 96.5  $\mu$ M at 24, 48 and 72 h post-reperfusion, respectively, whereas there was no quantifiable concentration of S-PBN ( $<10 \mu$ m) in control plasma samples. Pump and dosing solution analysis indicated that the rats received between 80-100% of the target S-PBN concentration. After 72 h of reperfusion, S-PBN-treated nerves revealed normal-appearance myelinated fibres and vessels, or minimal pathological changes. By contrast, saline-treated control nerves showed severe changes in nerve and vessels, e.g. axonal degeneration and vascular swelling. The frequency of abnormal myelinated fibres was significantly less in S-PBN-treated nerves than in controls ( $6.6 \pm 2.7$  and  $42.2 \pm 3.9\%$ ,  $p < 0.001$ , respectively, at the upper-calf level of tibial nerves). In conclusion, S-PBN exhibits neuroprotective properties in our rat model of ischaemic/reperfusion nerve injury. REFERENCES: 1) Muscle Nerve 1996;19:37, 2) Brain Res 1997;772:156, 3) Neuroscience 1999;94:909, 4) Brain Res 1999;844:192, 5) J Anat 1994;185:259, 6) JPNS 1997;2:60, 7) Ann Neurol 2000;47:71.

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#### PEPTIDE SURROGATE ANTIGENS FOR THE CHARACTERISATION OF PARAPROTEINS ASSOCIATED WITH POLYNEUROPATHY

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In polyneuropathies associated with paraproteinaemia, the monoclonal antibody is suspected to be pathogenic. In some cases, a cognate antigen/hapten has been recognised. These are all anti-carbohydrate IgM antibodies, e.g. anti-MAG. For the remainder, including the IgG and IgA paraproteins, cognate antigens are unknown and it is not even known if these are autoantibodies. We are attempting to derive surrogate peptide antigens using a phage-displayed random peptide library for the characterisation of these antibodies. In trial experiments we have used sera with anti-MAG IgM and serum with an IgM anti-GM1. The murine monoclonal Leu-7 was used as the type anti-MAG antibody. Using a disulphide constrained heptameric random peptide library we have isolated the peptide -C N/P APH H/K HWM C- using Leu-7. This peptide also bound to the IgM of anti-MAG patients. The human anti-MAG sera selected peptides with homology to the above peptide. It is

suggested that these peptides are mimotopes for the carbohydrate hapten. A peptide, CATAPTTQSC, was isolated using the patient's serum with anti-ganglioside activity which did not bind antibodies from serum with anti-MAG activity. This technique will be used to obtain surrogate antigens for IgG paraproteins from patients and these used to determine the antibodies' properties.

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#### WALLERIAN DEGENERATION OF NEIGHBORING FIBERS INDUCES C FIBER CHANGES THAT CONTRIBUTE TO HYPERALGESIA

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Partial nerve injury in experimental animals has provided a useful group of model systems for studying mechanical and thermal hyperalgesia and, by extension, neuropathic pain. In most models it has been assumed that hyperalgesia depends on central sensitization at the level of the spinal cord, and that spontaneous activity in the cut end of the proximal stump of interrupted fibers helps drive central sensitization. However, data from Ramer and Bisby, Sorkin and Myers, and Sommer have suggested events in the distal nerve may play a role. Our recent data indicate: 1) After transection of the L5 mixed spinal root fibers, the L4 dorsal root fibers are sufficient to establish and maintain hyperalgesia (Li, Pain, 2000); 2) The uninjured C fibers in the L4 dorsal root develop spontaneous activity (Wu, J. Neuroscience, in press); 3) L5 dorsal root ganglionectomy produces hyperalgesia; 4) Degeneration of myelinated motor fibers in the sciatic as a result of L5 ventral rhizotomy is sufficient to establish persistent hyperalgesia; 5) In this model there is prompt entry of the Schwann cells of intact uninjured Remak bundles into the cell cycle; 6) In many of these models there is prominent remodeling of the Remak bundles, with fewer axons/Remak Schwann cell. Taken together, these data indicate that Wallerian degeneration of neighboring fibers induces a series of physiologic and structural changes in uninjured Remak bundles and their C fibers, and suggests that these changes may be sufficient to drive central sensitization and hyperalgesia.

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#### EFFECTS OF AXONOTMESIS UPON ADULT RAT DORSAL ROOT GANGLION NEURONS

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**INTRODUCTION:** The magnitude, mechanism and time course of sensory neuron loss following axonotmesis of the sciatic nerve at mid thigh level is unclear, as is the question of whether particular types of sensory neurons are more affected than others. **METHODS:** 15 adult rats had their right sciatic nerve crushed at mid thigh level. Animals were perfused at 1, 2 and 3 months. A stereological counting technique was used to estimate the total number of neurons, the incidence of neuronal apoptosis and the number of lectin reactive neurons from serially sectioned left and right L4 and L5 dorsal root ganglia. Expression of the neural differentiation markers nestin and N-myc was investigated at 1 month. **RESULTS:** Neuron loss in the ipsilateral ganglia at 1, 2 and 3 months was 7.2%, 0.3% and 0%, and the incidence of neuronal apoptosis was 0.05%, 0.01% and 0%, respectively. Approximately 1% of ipsilateral sensory neurons at 1 month expressed nestin and occasional neurons contained cytoplasmic N-myc immunoreactivity. **CONCLUSION:** Neurons are lost in significant numbers by apoptosis after axonotmesis. Functional recovery is complete by 2 months, when neuronal apoptosis is still occurring. Neuronal loss has stabilised or declined by 2-3 months indicating neuronal replacement, and this is associated with nestin and N-myc immunoreactive neurons that may represent ongoing neurogenesis. **Sponsor:** Supported by the Brain Research Trust.

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