

NEUROPATHOLOGY AND NERVE OXYGEN SATURATION IN SUB-CLINICAL HUMAN DIABETIC NEUROPATHY

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Diabetic patients with established neuropathy demonstrate endoneurial and epineurial capillary disease with a reduction in endoneurial oxygen tension. These changes are considered to be important in the pathogenesis of nerve damage. However, the relevance of these changes in the causation of nerve damage is not clear as abnormalities which occur at the onset of neuropathy are not known. Type 1 diabetic patients underwent assessment of vibration perception threshold (VPT), sural nerve conduction velocity (SNCV) and peroneal motor nerve conduction velocity (PMNCV). 11 patients without neuropathy (VPT-6.3, SNCV-45.3, PMNCV-44.1), 6 patients with sub-clinical neuropathy (VPT-12.5, SNCV-38.9, PMNCV-38.4), and 7 patients with established neuropathy (VPT-36.4, SNCV-28.1, PMNCV-30.2) were compared to 8 non-diabetic control subjects. In-vivo sural nerve oxygen saturation (HbO₂%) was assessed by exposing the sural nerve and using micro-light guide spectrophotometry on the nerve surface. A significant reduction in nerve oxygen saturation was observed in diabetic patients with established diabetic neuropathy ($66.2 \pm 6.3\%$, $p=0.001$) compared to control subjects ($76.7 \pm 5.9\%$), and diabetic patients without neuropathy ($73.9 \pm 3.9\%$). However, in patients with sub-clinical neuropathy there was a paradoxical, increase in nerve oxygen saturation ($79.5 \pm 4.0\%$) compared to non-neuropathic diabetic patients ($p=0.01$). Fascicular sural nerve biopsy was performed in 4 diabetic patients without neuropathy and in 4 patients with sub-clinical neuropathy. An epineurial vasculitis in association with haemorrhage was observed in 3 patients with sub-clinical neuropathy and in 2 without neuropathy. Myelinated fibre density was not significantly reduced. However, there was evidence of endoneurial microangiopathy with significant basement membrane thickening, endothelial cell hyperplasia and hypertrophy in all diabetic patients. Nerve oxygen saturation is increased in patients with sub-clinical neuropathy and this may be related to epineurial vasculitis and haemorrhage with endoneurial microangiopathy. These observations lend further support to the key role of vascular abnormalities in the pathogenesis of human diabetic neuropathy.

DYNAMIC TRANSLOCATION OF PKC BETA I-EGFP IN RAT DRG NEURONS:
A SUB-CELLULAR EVENT CORRELATING WITH ACTIVATION,
DESENSITIZATION AND RECOVERY OF VR-1

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Desensitization of Ca²⁺ selective ion channels of vanilloid receptor subtype-1 (VR-1) in a subclass of sensory dorsal root ganglion (DRG) neurons plays a crucial role in blocking pain transmission by these neurons. [Ca²⁺]_i increase following VR-1 activation by capsaicin was proposed to cause dephosphorylation and desensitization of the receptor mediated by calcineurin [1]. A role for PKA in VR-1 desensitization was confirmed recently [2]. Direct phosphorylation and activation of VR-1 by PKC has also been shown [3]. This study presents evidence of a correlation between [Ca²⁺]_i transients and translocation of PKCβI-EGFP during activation, desensitization and recovery of VR-1 in capsaicin-sensitive neurons. Desensitization of VR-1 was induced by successive applications of 100 nM capsaicin, leading to progressively diminishing [Ca²⁺]_i responses in DRG neurons. These desensitized neurons recovered to a subsequent capsaicin stimulus on application of phorbol 12-myristate acetate (PMA), the recovery being complete at 20 μM PMA. 10 μM capsazepine blocked the re-sensitization to capsaicin by PMA. Inhibitory peptide PKC fragment 19-36 also inhibited re-sensitization to capsaicin by PMA. Desensitization to capsaicin correlated with reduced transient membrane association of cytosolic PKCβI-EGFP in transfected DRG neurons, as observed in real-time. Subsequent addition of 20 μM PMA led to the recovery of PKCβI-EGFP membrane association, which was sustained. Recovery of capsaicin response from desensitization following activation of PKC suggests a role for PKC in regulation of desensitized VR-1. The dynamic translocation of PKCβI-EGFP is suggested to be a sub-cellular signaling event correlating with recovery of VR-1 following desensitization in response to [Ca²⁺]_i. References: 1) Docherty RJ et al. (1996) Pflügers Arch 431, 828-37. 2) Bhave G et al. (2002) Neuron 35, 721-31. 3) Numazaki M et al. (2002) J Biol Chem 277, 13375-8.

CHANGES IN CARPAL TUNNEL WITH MANU HAND BRACE APPLICATION: A MAGNETIC RESONANCE(MR)IMAGES STUDY.

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Background: Manente and coll. have recently developed an innovative soft hand brace called Manu for conservative treatment of Carpal Tunnel Syndrome (CTS); a randomised controlled study showed that this hand brace, if worn at night for one month, is highly efficient in relieving symptoms and functional loss in CTS patients. Objective: This study aims at evaluating, through Magnetic Resonance Imaging (MRI), the changes in the carpal tunnel in CTS patients and controls wearing Manu, the innovative soft hand brace for CTS. Methods: 13 subjects (9 suffering from carpal tunnel syndrome and 4 controls) were enrolled in the study. T1 and GE STIR sequences were performed using a 0.2 T E-scan. Some morphologic parameters (tunnel diameters and area, carpus length and nerve signal intensity) were measured at three different scanning levels. The evaluation was carried out before and after the application of the brace. Results: Remarkable differences were recorded in the measurements performed before and after the application of the brace. Special attention should be paid to the constant increase of the front-rear diameter and the tunnel shape rounding. These data could explain the lighter symptoms as a result of the use of the brace. Conclusions: The Manu hand brace cause changes in the carpal tunnel, in particular increase the front-rear diameters and rounding the tunnel shape; hence the clinical efficacy in CTS. Moreover the MRI assures the best evaluation of the carpal tunnel morphologic changes.

PHENOTYPIC CHANGES OF GREEN FLUORESCENT PROTEIN-ENGINEERED, NEURITOGENIC T CELLS DURING EXPERIMENTAL ALLERGIC NEURITIS

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P0 glycoprotein is a member of the immunoglobulin supergene family that plays an important role in the maintenance of compact myelin in the peripheral nervous system (PNS). Auto-aggressive T cell responses to PNS myelin proteins, including P0, are implicated in the pathogenesis of GBS and CIDP. However, the functional interactions of P0-specific T cells in the PNS during inflammatory neuropathies are poorly understood. In order to investigate this further, T cells specific for the immunodominant epitope of P0 protein (P0₁₀₉₋₁₃₅) for the Dark Agouti (DA) rat were retrovirally transduced to express green fluorescent protein (GFP). Expression of GFP by these T cells influenced neither the proliferative response to the cognate antigen *in vitro* nor the pathogenic activity *in vivo*. The adoptive transfer of 10^7 T_{P0-GFP} cells induced clinical symptoms typical of EAN peaking in hind limb weakness in naïve syngeneic recipients at day 6 post-transfer. Expression of GFP has enabled us to track neuritogenic T cells *in vivo*, by confocal microscopy and FACS, as they migrated through the immune system and into the PNS. After their isolation from spleen, lymph nodes and cauda equine, FACS profiling of T_{P0-GFP} cells *ex vivo* using cell surface markers such as CD4, TCR, IL2-R, MHC class II and OX40 permits us to identify sites of T cell activation associated with disease induction. This model will be used to further address the migratory/homing patterns, functional status and site/mode of activation of antigen-specific T cells involved in the immunopathogenesis of inflammatory responses in the PNS.

LOCAL MANIPULATION OF ENDOGENOUS GROWTH-ALTERING SUBSTANCES PROMOTES EARLY PERIPHERAL NERVE REGENERATION

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Loss of function is a common result of peripheral nerve injury. Transection injuries are especially serious and recovery from such injuries is limited despite the use of modern microsurgical techniques. There is evidence that local molecular players in the regenerative microenvironment may promote or contrarily inhibit axon regrowth *in vivo*. We have developed a novel method of repeatedly introducing soluble factors directly into the microenvironment of regenerating peripheral nerve. The approach involves the implantation of a subcutaneous microinjection port, connected through a T-junction to a regeneration chamber encompassing proximal and distal stumps of a transected nerve. Soluble factors can be delivered fresh at multiple timepoints over an extended period. Using our model, we have confirmed that local IGF-1 delivery leads to increased numbers of regenerating myelinated fibers spanning a 3-mm gap between proximal and distal nerve stumps by 21 days. In contrast to IGF-1's growth promoting actions, we have also found that endogenous nitric oxide (NO) may deter regeneration. Inhibition of NO synthesis with the broad-spectrum NO synthase inhibitor L-NAME also increased the regeneration of myelinated fibers through our regeneration chamber. Quantitative electron micrography suggested that unmyelinated fiber regrowth similarly benefits from NO inhibition. Interestingly, NO inhibition would appear to enhance numbers of outgrowing fibers, rather than maturation since myelinated and unmyelinated axon caliber was not altered. Supported by CIHR, AHFMR.

MUTATIONS IN PERIPHERAL MYELIN GENES, PMP22, MPZ, AND CX32: ANALYSIS IN A LARGE COHORT OF DUTCH CMT PATIENTS

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Charcot-Marie-Tooth disease (CMT) is a clinically and genetically heterogeneous disorder of the peripheral nervous system. Alterations of several genes have been associated with the disease, among them sequence variations of PMP22, MPZ and Connexin 32 (Cx32) may cause a variety of distinct CMT phenotypes. To determine the frequency of mutations in these three genes in patients with CMT or a related peripheral neuropathy, we studied 2,205 CMT-affected patients, according to clinical and electro-physiological diagnosis. Our results show that: 511 (23.2%) of patients had a 17p12 duplication (CMT1A duplication) and 239 had a deletion of the 17p12 region (HNPP deletion) as assessed by Southern blot analysis. Among the remaining 1,455 no-duplicated cases, 400 patients were screened for mutations of PMP22, MPZ, and Cx32. Mutation detections were performed by SSCP and DHPLC analyses and confirmed by sequencing both DNA strands. Several mutations were identified: 39 patients had Cx32 mutations, 43 MPZ mutations (14 amino acid changes and 25 polymorphisms), and 172 had PMP22 mutations (151 polymorphisms). In the process of screening the above cohort of CMT patients we identified several unreported mutant alleles. For PMP22 we found 12 unreported mutations (1 missense, 1 frame shift, one 5 bp deletion, and 8 polymorphisms); 12 unreported mutations were identified in MPZ (5 missense and 7 polymorphisms), and 5 missense mutations were found in Cx32. These results highlight the genetic heterogeneity of hereditary neuropathies. Our investigation will be expanded for screening mutations in other CMT-causing genes. Association studies between the different polymorphisms and CMT disease will also be performed. This large number of genotyped patients will provide an opportunity to examine genotype-phenotype correlations in inherited and sporadic neuropathy.

DIFFERENTIAL GENE EXPRESSION IN FAMILIAL AMYLOID POLYNEUROPATHY: IMPLICATIONS IN PATHOGENESIS

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Familial amyloid polyneuropathy (FAP) is characterized by extracellular deposition of mutated transthyretin (TTR) amyloid fibrils, particularly in the peripheral nervous system (PNS). Due to the invasiveness of biopsying nerves, salivary glands (SG) with amyloid deposition were used to perform cDNA microarrays in order to approach mechanisms underlying degeneration in FAP. Genes found differentially expressed between FAP and normal SG biopsies with an average fold change $> \pm 2.5$ were validated by immunohistochemistry (IHC) and RT-PCR; to test if differential expression in SG was relevant in the PNS, control and FAP nerves in different stages of disease progression were compared by IHC. Two of the differentially expressed genes coded for extracellular matrix (ECM)-related proteins, biglycan and neutrophil gelatinase-associated lipocalin (NGAL) both of which were upregulated in FAP SG biopsies. In FAP nerves, biglycan overexpression started in a stage where TTR is deposited as non-fibrillar aggregates, before amyloid is detected (FAP 0) whereas NGAL upregulation occurred after TTR fibril formation. The matrix metalloproteinase-9, that is co-expressed with NGAL, was also increased in FAP in a similar pattern to the one observed for NGAL. Upregulation of ECM-related proteins is consistent with tissue remodeling occurring upon TTR deposition. Two other differentially expressed genes were involved in signaling through growth receptors: the mitogen-activated protein kinase phosphatase-1 and the insulin-like growth factor binding protein-4 (downregulated and upregulated in FAP tissues, respectively). The overall outcome of signaling pathways activated through binding of TTR aggregates to cellular membranes should be further addressed. Since we previously reported that FAP nerves show increased expression of pro-inflammatory cytokines, we extended our analysis to IL-10, and observed a balance between pro- and anti-inflammatory mechanisms during the course of the disease. Chemoattractant cytokines were unaltered in FAP nerves relative to controls, which may be related to the absence of cellular infiltrates in this disorder. The changes found in ECM-related proteins, signaling and inflammatory events may be relevant for future therapeutic approaches in FAP and other neurodegenerative disorders.

REGENERATION INTO PROTECTED AND CHRONICALLY DENERVATED PERIPHERAL NERVE STUMPS

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Delayed repair of peripheral nerve injuries often results in poor motor functional recovery. This may be due to the deterioration or loss of endoneurial pathways in the distal nerve stump before motor axons can regenerate into it. In this study we have developed and evaluated a rat hindlimb model to determine if regeneration of either motor or sensory nerve into a distal nerve stump will allow the successful regeneration of a cut motor nerve 2 months later, as compared to the regeneration of a cut motor nerve into a 2 month chronically denervated distal nerve pathway. Using the rat femoral nerve, we protected the distal endoneurial pathways of the saphenous nerve with either cross-suture of the quadriceps motor nerve (Group A) or re-suture of the saphenous nerve (Group B) to compare later motor regeneration into the "protected" saphenous nerve pathway to chronically denervated and "unprotected" saphenous nerve (Group C). A total of 45 rats, 15 per group, were operated on. After this protection (or lack of protection) for 8 weeks, the motor branch of the femoral nerve was re-severed and sutured to the distal saphenous to allow reinnervation of protected and unprotected saphenous nerves. The model was validated by histologic examination of the nerve stumps, showing myelinated axons in the protected as compared to the very few unmyelinated axons in the unprotected nerve stumps. Quantitative assessment of motor axonal regeneration was carried out after 6 weeks, 30 mm distal to the suture site by applying a 4% solution of Fluorogold (FG). Rats were perfusion fixed 6 days later, the T11 to L2 spinal cord collected and the number of FG labeled femoral motoneurons in all the spinal cord sections counted. In Group A, 39.1 (mean \pm 9.16 SEM) motoneurons regenerated axons into the distal stump, significantly more than the unprotected (10.2 ± 4.3), while 32.4 ± 10.6 motoneurons regenerated into the sensory protected pathway. Histomorphometric analysis of axonal regeneration into the distal pathway is ongoing. Even 2 months of denervation of the distal pathway is deleterious to regeneration, while protection of the pathway improves subsequent regeneration. Mechanistic issues will be discussed at the meeting.

FIBROSIS IN PERIPHERAL NERVES

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Fibrosis may be located in the gliding tissue around the nerve (paraneurium), the epifascicular epineurium, the interfascicular epineurium and the endoneurium. Fibrosis of the perineurium causes contracture in longitudinal, fibrosis of the epineurium contracture in transverse direction with compression of the endoneurial space. A sequence of surgical procedures are at hand from simple external neurolysis, paraneuriotomy, paraneuriectomy, epineuriotomy, epifascicular epineuriectomy to epineuriectomy which have to be carefully selected according to localisation and extension. If the endoneurium is fibrotic as well respectively the fascicular pattern is lost resection of the involved nerve segment and restoration of continuity by nerve grafting is the treatment of choice. In case of recurrent fibrosis enveloping the nerve by a gliding tissue flap is indicated. 247 cases are presented in whom these principles were adopted (Peripheral nerves: 86, Brachial plexus lesions: 81, Pain syndromes originating from peripheral nerves).

THERAPEUTIC EFFICACY OF ORAL HEDGEHOG AGONISTS IN EXPERIMENTAL DIABETIC NEUROPATHY

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Expression of hedgehog proteins and their receptor, patched-1 (Ptc), in adult peripheral nerve suggest a role for the hedgehog pathway beyond modulating the embryonic development of the nervous system. Our recent studies demonstrating impaired expression of desert hedgehog (dhh) in experimental diabetic neuropathy and the efficacy of sonic hedgehog (SHh) protein in ameliorating hyperglycemia-induced dysfunction of large motor and sensory fibers support this notion and imply expression deficits of these proteins may contribute to nerve disorders in this and other disease states. Here the therapeutic utility of two different orally available small molecule agonists of the Hh pathway at reversing functional nerve disorders resulting from experimental diabetes was investigated. Control and diabetic (50 mg/kg streptozotocin by intraperitoneal injection) female Sprague-Dawley rats were maintained for 4 weeks. After 4 weeks, diabetic rats were treated (5 mg/kg) thrice weekly by oral gavage with either of two agonists (A, B) or vehicle alone. After a further 4 weeks of diabetes and treatment, thermal response latency was assessed using a focused heat source directed onto the plantar surface of the foot. Motor and sensory nerve conduction velocity (MNCV and SNCV) were then measured using latencies of M and H waves recorded from interosseal muscles. Treatment with either hedgehog agonist did not prevent the diabetes-associated loss of body weight and had no impact on plasma glucose. Progression of diabetic rats from a state of transient thermal hyperalgesia toward one of protracted thermal hypoalgesia was prevented by both agonists, such that after 4 weeks of treatment agonist-treated groups remained significantly hyperalgesic (both $P < 0.05$). The MNCV and SNCV deficits seen after 8 weeks in vehicle-treated diabetic rats when compared to control rats ($P < 0.01$) were ameliorated by both agonists. Specifically, the MNCV deficit was significantly ameliorated by agonist A ($P < 0.01$), while there was a nonsignificant trend for an increase with agonist B. In contrast, agonist B significantly ameliorated the SNCV deficit ($P < 0.01$) whereas agonist A promoted a nonsignificant improvement. The efficacy of these orally delivered hedgehog agonists point to the therapeutic utility of these compounds in treating nerve dysfunction in experimental diabetes.

DETERMINATION OF NERVE GROWTH FACTOR (NGFmRNA) IN SKELETAL MUSCLE, THE TISSUE TARGETED BY NERVE REGENERATION

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Objective: Nerve growth factor that is synthesized in tissues targeted by peripheral nerves are transported through the axon retrogradely, and play an important role in the maintenance of nerve function and regeneration of damaged peripheral nerves. There have been numerous studies on the chronological changes in nerve growth factor in the stump of resected distal nerves, but it has been difficult to quantify minute amounts of nerve growth factor in skeletal muscle, which is the tissue targeted by motor nerves. We have developed a technique to quantify minute amounts of NGFmRNA in skeletal muscles by using RT-PCR/HPLC with a deletion mutant RNA as the internal standard. This time, to investigate chronological changes in nerve growth factor during peripheral nerve regeneration, the sciatic nerve was cut in one model and was cut and fused in another model, and the levels of NGFmRNA in the gastrocnemius muscle were measured. Methods: Thirty-five 5-week-old female ICR mice were used. Four cut sciatic nerve groups (sacrificed 0, 1, 2 or 4 weeks later) and three cut and fused sciatic nerve groups (sacrificed 1, 2 or 4 weeks later) were prepared (5 mice in each group). For the four cut sciatic nerve groups, the proximal end of the sciatic nerve was turned over to prevent regeneration. For the three cut and fused sciatic nerve groups, fibrin glue was used to fuse the cut sciatic nerve. Mice were sacrificed at 0, 1, 2 or 4 weeks after cutting the sciatic nerve, and then the left and right gastrocnemius muscles (about 50 mg) were collected, quickly frozen in liquid nitrogen, and stored at -80°C. To each sample, 3 fg/mg of the internal standard (dNGA) was added, and RNA was extracted by the AGPC method. The resulting total RNA was subjected to RT-PCR, and the reaction products were subjected to HPLC. Results: The amount of NGFmRNA in mouse skeletal muscle at 0, 1, 2 and 4 weeks after cutting the sciatic nerve increased with time: 8.8, 24.4, 20.9, and 36.6 fg/mg, respectively. The amount of NGFmRNA in mouse skeletal muscle at 1, 2 and 4 weeks after cutting and fusing the sciatic nerve also increased with time: 17.4, 24.0 and 29.5 fg/ml, respectively. Conclusions: The amount of NGFmRNA in the gastrocnemius muscle after cutting or cutting and fusing the sciatic nerve increased with time. We are planning to investigate these changes beyond 4 weeks.

CORRELATION BETWEEN CLINICAL MANIFESTATIONS AND IgG ANTIBODY ACTIVITY IN SERA TO A MIXTURE OF GQ1B AND A PHOSPHOLIPID IN MILLER FISHER SYNDROME

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We have reported that anti-GM1 IgG antibodies in GBS sera had greater antibody activity against a mixture of GM1 and phosphatidic acid (GM1/PA) than against GM1 alone. In this report we conducted a similar examination about the anti-GQ1b IgG antibody which was specifically seen in MFS. Furthermore, we analyzed the correlation between the antibody reactivities and clinical manifestations. We measured IgG antibody activities in sera from 54 MFS patients by ELISA. Wells of microtiter plates were coated with 200 ng of GQ1b, 100 ng of GQ1b mixed with 100 ng of phosphatidic acid (GQ1b/PA), or 200 ng of PA. For each patient, anti-GQ1b and anti-GQ1b/PA assays were performed on neighboring wells of the same microtiter plate. In all the 54 examples, anti-GQ1b IgG antibody was positive. As a whole, antibody activities against GQ1b/PA were not greater than those against GQ1b alone. Twenty four of those 54 sera had greater activity against GQ1b than GM1/PA (group A), whereas 30 had greater activity against GQ1b/PA than GQ1b (group B). None of the sera had antibody activities against PA alone. We investigated clinical features of those two groups; antecedent infections (upper respiratory infection, gastrointestinal infection), and presence of various neurological symptoms (ophthalmoplegia/cerebellar ataxia/bulbar palsy/disturbance of deep sense). The prevalence of bulbar palsy was significantly higher in group A (8, 33%) than in group B (1, 3.3%) ($p < 0.02$). Difference in the fine specificities of anti-GQ1b IgG antibodies may be associated with presence or absence of bulbar palsy in MFS.

THE ENDONEURIAL MACROPHAGE RESPONSE DURING EXPERIMENTAL SLOW PROGRESSIVE NEUROPATHIES IS MAINLY OF INTRINSIC ORIGIN

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Macrophages have a major impact on the pathogenesis of many forms of peripheral neuropathies. We recently could demonstrate that a remarkable proportion of the early endoneurial macrophage response in traumatic and inflammatory neuropathies is generated by resident endoneurial macrophages rather than hematogenous macrophages only. It is conceivable that in milder forms of neuropathy, the lesion-induced macrophage response may be created to an even greater extent by resident endoneurial macrophages. To prove this hypothesis we created bone marrow chimeric mice by transplanting green fluorescent protein transgenic (gfp+) bone marrow into wildtype irradiated recipients. These mice allow the differentiation between resident (gfp-) and hematogenous (gfp+) endoneurial macrophages. Two different models of neuropathy were examined: a length dependent neuropathy induced by acrylamide three months after bone marrow transplantation, and a hereditary neuropathy in heterozygous P0-deficient mice, examined four months after bone marrow transplantation performed at the age of two months. Fluorescence microscopy and immunohistochemistry was used to quantify and characterize endoneurial gfp- and gfp+ macrophages. We found that (i) resident endoneurial macrophages undergo physiological turnover in normal mice amounting to approximately 50% after three months, (ii) gfp- and gfp+ resident endoneurial macrophages become activated and phagocytose during the course of both neuropathies, (iii) both types of resident endoneurial macrophages proliferate and increase in number, and (iv) the ratio of gfp+ and gfp- macrophages changed only slightly in favour of gfp+ macrophages. Quantitative analyses of macrophage shape revealed that gfp+ endoneurial macrophages in acrylamide-induced neuropathy are more rounded than gfp- macrophages (v). Taken together, our results argue against a major influx of lesion-attracted hematogenous macrophages but rather point towards a mostly intrinsic generation of the macrophage response in two models of a slowly progressive neuropathy. While both types of macrophages share basic properties like phagocytosis and MHC class II expression, the difference in shape between gfp+ and gfp- macrophages may suggest a functional difference between long term resident and recently immigrated endoneurial macrophages. Our results further support the notion that resident endoneurial macrophages serve as an intrinsic defense system much like the microglial cells in the brain.

ANTI-DRG NEURON SPECIFIC ANTIBODIES ASSOCIATED WITH NEUROLOGICAL MANIFESTATIONS IN SJOGREN'S SYNDROME

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We evaluated anti-neuronal antibodies in three patients who presented peripheral neuropathy as an initial symptom of primary Sjogren's syndrome. The sera from patient 1 and 2 showing subacute sensory ataxia immunostained rat large DRG neurons. The sera from patient 3 presenting small fiber neuropathy stained small DRG neurons. Western blotting showed that these sera contained anti-DRG neuron antibodies that recognize DRG protein of different molecular size. The sera from patient 2 reacted with protein expressed by both DRG and salivary gland. Autopsied DRG of patient1 showed neuronal loss and infiltration of mononuclear cells stained by CD8 (suppressor and killer) and CD16 (macrophage). These results suggest that anti-DRG specific antibodies could participate in the pathogenesis of ganglionitis in Sjogren's syndrome and elicit neurological symptoms.

INHIBITION OF p38 MAPK ACTIVITY ENHANCES AXONAL REGENERATION

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Tumor necrosis factor alpha (TNF)-induced cellular signaling through the p38 mitogen-activated protein kinase (p38 MAPK) pathway plays a critical role in Wallerian degeneration and subsequent regeneration. This link is particularly strong with respect to Schwann cell (SC) activity. TNF dose-dependently induces macrophage and Schwann cell activation *in vivo* and apoptosis in primary SC cultures *in vitro*, while inhibition of p38 MAPK is thought to block these cellular processes. We show with Western blots that after sciatic nerve crush injury phosphorylated p38 MAPK is significantly increased ($p < 0.01$) in distal nerve. p38 colocalized immunohistochemically with GFAP (activated Schwann cells) and to a lesser degree with ED-1 (macrophages). We hypothesized that systemic inhibition of p38 MAPK activity following crush injury of rat sciatic nerve would enhance axonal regeneration, and that inhibition of p38 MAPK activity would reduce activation and cell death of Schwann cells. In experiments approved by the local Animal Studies Committee, animals were gavaged with Scios SD-169 (10 mg/kg, $n=11$; 30 mg/kg, $n=10$) or excipient (PEG300, $n=10$) one day prior to and daily after crush injury to the sciatic nerve. SD-169 is a proprietary oral inhibitor of phosphorylated p38 MAPK activity. That is, p38 phosphorylation occurs as expected following TNF challenge, but its detrimental cellular consequences are inhibited. The rate of axonal regeneration was determined by the functional pinch test; the distal sciatic nerve was exposed at 4 and 8 days after crush and the length of functional regeneration was determined by pinching the nerve. Oral administration of SD-169 significantly increased axonal regeneration when measured 8 days ($p < 0.05$) after crush injury. Neuropathological examination and TNF immunofluorescence of distal nerve suggested that SD-169 reduced SC TNF activity. In support of these findings, SD-169 significantly reduced ($p < 0.05$) TNF-mediated primary SC death in culture experiments. These unique characteristics of SD-169 may have therapeutic value.

PHARYNGEAL-CERVICAL-BRACHIAL WEAKNESS AND THE CONTINUOUS SPECTRUM WITH ANTI-GT1A IGG ANTIBODIES

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Background: Patients with Fisher syndrome (FS) and Bickerstaff's brainstem encephalitis (BBE) have anti-GQ1b IgG antibodies which cross-react with GT1a, whereas those with the pharyngeal-cervical-brachial variant (PCB) of Guillain-Barré syndrome have anti-GT1a antibodies with or without anti-GQ1b reactivity. However, the nosological relationship between PCB and the other conditions has yet to be established. **Methods:** Information on neurological signs during the illness was reviewed in 140 patients with anti-GT1a IgG antibody. **Results:** Based on the diagnostic criteria, we diagnosed 64 (46%) patients as FS, 22 (16%) as GBS, 14 (10%) as BBE, and 6 (4%) as PCB. There were also some patients with overlapping diagnoses such as FS and GBS (5%), PCB and FS (5%), BBE and GBS (4%), or PCB and BBE (1%). The patients who had had bulbar palsy as the initial symptoms developed not only PCB but also FS and BBE. In the whole population of the anti-GT1a-positive patients, the frequent features were hypo- or areflexia, external ophthalmoparesis, and cerebellar-type ataxia, which are the triad of FS. In contrast, patients who had anti-GT1a IgG but did not have anti-GQ1b IgG antibody frequently had a history of diarrhea and experienced oropharyngeal, neck, and limb weakness. Immunological results of overlapping cases showed that FS, BBE, and PCB are closely related disorders. **Conclusions:** The patients with anti-GT1a IgG antibody had variety of clinical manifestations, and showed continuous clinical spectrum. A part of the clinical variation was caused by coexistence of anti-GQ1b IgG antibody. Our clinical and serological studies suggested that PCB is closely related not only with GBS, but also with FS and BBE.

CISPLATIN PROTECTS AGAINST PACLITAXEL-INDUCED APOPTOSIS IN HUMAN NEUROBLASTOMA CELL LINE

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Cisplatin (CDDP) and paclitaxel are often used in combined anti-cancer therapy. Both drugs induce peripheral neuropathy. We tested the effect of the combination or the sequence of administration of mildly to moderately toxic concentrations of CDDP and moderately to highly toxic concentrations of paclitaxel. Human neuroblastoma (HN) SH-SY5Y cells were cultured in DMEM and exposed to CDDP (4 or 8 μ M) alone or in combination with paclitaxel (0.1, 0.5, or 1 μ M). In the HN cells, CDDP and paclitaxel induce death by apoptosis. Percentage of cellular death was determined with the MTT test. In this paradigm, both CDDP 4 μ M and CDDP 8 μ M were able to significantly reduce the percentage of cellular death induced by exposure to paclitaxel. In another paradigm, we tested the effect of pre-treatment for 15 min or 1 hr with CDDP 4 μ M on the apoptosis induced with paclitaxel 0.1, 0.5, and 1 μ M. In this paradigm CDDP was able to significantly reduce the percentage of cellular death, independently from the time of CDDP pre-treatment. In a third paradigm, cultures were pre-treated with paclitaxel followed by different concentrations of CDDP. In this case, no changes in the percentage of cellular death were observed. We hypothesized that the protective action of CDDP on paclitaxel-induced apoptosis was due to a block in the cell cycle, thus preventing HN cells to reach the mitosis phase during which paclitaxel exerts its pro-apoptotic action by hindering the formation of the mitotic spindle. To test this hypothesis, in the paradigm of combined administration of CDDP 4 μ M and paclitaxel, we determined the mitotic index (% of the cells in mitosis) using the Giemsa staining. In cultures exposed to CDDP alone, the mitotic index was as low as in control cultures. In cultures exposed to paclitaxel and CDDP in combination, the mitotic index was significantly reduced compared to cultures with paclitaxel alone. These data suggest that CDDP is protective by blocking the cell cycle and preventing the HN cells to reach the mitosis phase.

AXONAL ABNORMALITIES EXPRESSED IN LONG TERM DORSAL ROOT GANGLIA CULTURES FROM A TRANSGENIC RAT MODEL OF CMT1A NEUROPATHY

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Secondary axonal atrophy has been clearly shown in sural nerves of CMT1A patients and accounts for clinical worsening in this disease. Recently, we developed an *in vitro* model of dys-demyelination in short-term (one month) dorsal root ganglia (DRG) cultures from a transgenic (tg) rat model of CMT1A. We used this model to determine whether it is possible to induce an axonopathy in long term (three months) tg DRG cultures. Axonal and myelin structure has been studied by light and electron microscopy (EM). Axonal diameter, periaxonal area and neurofilaments (NF) density were also measured by EM. Phosphorylation of NF was evaluated by Western blot analysis. Moreover, as myelin associated glycoprotein (MAG) is critically involved in the interaction of myelin-forming glial cells with axons, confocal microscopy has been used to analyze its distribution along myelinated fibers. Both control and tg cultures were rich in bundles of myelinated nerve fibers. However, in tg cultures, along with dysmyelinated internodes, we also found peculiar abnormalities of the myelin sheath, which showed a “bead-like” appearance, as it may be observed in axonal dystrophy. EM examination showed uncompacted myelin and smaller axons with increased density of NF, in all the tg cultures. Morphometric evaluation demonstrated a significant reduction of axon diameter in tg cultures compared to normal ones (0.9 ± 0.06 mm vs 1.18 ± 0.05 mm; $p < 0.001$); conversely, an increase of periaxonal area in tg cultures compared to the controls has been observed (0.13 ± 0.17 mm² vs 0.08 ± 0.12 mm²; $p = \text{NS}$), together with a higher density of NF (776.9 ± 516 per mm² vs 684.7 ± 108.9 per mm²; $p = \text{NS}$). Western blot analysis showed an increased percentage of non-phosphorylated neurofilaments compared to the phosphorylated ones in both homozygous and hemizygous tg cultures compared to the wild type (4.1 vs 1.8 vs 0.03). Finally, confocal microscopy showed that, in tg cultures, MAG no longer is expressed at the node of Ranvier, Schmidt-Lantermann incisures and the mesaxon but shows focal accumulation along the myelinated internodes. In conclusion, we present an *in vitro* model of axonal atrophy due to overexpression of PMP22. The abnormal distribution of MAG allows some speculations on the possible role of this protein in mediating axonal impairment following a primary myelin defect.

EPIDERMAL NERVE FIBER REGENERATION IN FRIEDREICH ATAXIA: A FUNCTIONAL AND MORPHOLOGICAL STUDY

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Recently, in Friedreich ataxia, an involvement of unmyelinated nerve fibers has been observed in addition to the well known large fiber neuropathy. To define the regenerative capability of epidermal nerve fibers in this disease we evaluated time-course and patterns of nerve fibers regeneration after a suction blister lesion in 8 patients affected by genetically determined Friedreich's ataxia (4F, 4M, age 19-34) and in 5 healthy volunteers. In each subject we induced 5 couples of 2 mm blisters on the forearm. At different time points (1-2-3-4 weeks after blister induction) quantitative sensory testing was performed on one blister from each couple, and then a specimen including it was removed by a 3 mm punch. The second blister was reblistered (3 mm blister) and the roof removed. The larger size of these biopsies and blisters allowed to include normal skin immediately adjacent to the 2 mm blister wound. Epidermal nerve fibers were visualised using antibodies to the pan-neuronal marker protein gene product 9.5 (PGP 9.5) on frozen sections from skin biopsies and on the whole blister roofs. Quantification of ENFs was performed on skin sections using digitized images acquired by confocal microscopy (CARV) and Neurolucida software. Blister allowed to have a panoramic and global view of the regenerative process especially of the collateral extension of axons from adjacent normal epidermis, while biopsy allowed to observe the sprouting from the subepidermal neural plexus. Both these phenomena resulted markedly evident in all FA subjects indicating a normal capability of nerve fibers to regenerate. Blister wound probably induces the release of nerve growth factors stimulating nerve regeneration as in normal subjects. At least for the short period of observation (four weeks) nerve fibers maintained this capability challenging the noxa pathogena that leads to their loss.

MORPHOLOGICAL SUSCEPTIBILITY TO ISCHAEMIA/REPERFUSION IN STZ-DIABETIC NERVE: CRITICAL ISCHAEMIC TIME AND ENHANCED INFLAMMATORY RESPONSE

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Rationale: i) STZ-diabetic nerve reveals increased morphological susceptibility to ischaemia/reperfusion injury [1-5]. ii) STZ rat exhibits intensified inflammatory responses to reperfusion injury [6]. iii) Reperfusion nerve injury causes an acute inflammatory response [7]. iv) Diabetic patients with macrovascular diseases often notice newly-developed sensory symptoms after vascular reconstructive surgery. Aims: 1) To determine a critical ischaemic time to cause morphological susceptibility to ischaemia/reperfusion in STZ-diabetic nerves. 2) To investigate the acute inflammatory response in reperfused diabetic nerves. Methods: Transient (1-2 h) near-complete ischaemia was induced in right hindlimb of 2-mo-STZ-diabetic rat (16-17 wks of age) by occluding major arteries [6]. Pathology in right sciatic and tibial nerves was examined after 6, 24, 48 h and 1 wk of reperfusion. Immunohistochemistry for neutrophils (HIS48) and monocytes/macrophages (ED1, ED2) was also stained. Results: 1) Morphology: Following 2 h or 1 h 30 min of ischaemia, there was no morphological alteration in control nerves, whereas diabetic nerve revealed severe axonal degeneration beginning at the mid-thigh level. After 1 h 15 min of ischaemia, three out of six diabetic nerves revealed multifocal or patchy lesions of axonal degeneration, though the rest showed normal morphology. Nerve pathology was normal after 1-h ischaemia in diabetic nerves. 2) Immunohistochemistry: After 1 - 2 h of ischaemia and 6 h of reperfusion, ED2+ve endoneurial macrophages were seen at the mid-thigh level of diabetic nerves, but not in control nerves. These ED2+ve macrophages were smaller than ED1+ve phagocytosed macrophages which appeared at the later stage (1 wk) of reperfusion. Conclusions: In diabetic nerves, 1 h 15 min of severe ischaemia may be a critical ischaemic time to induce morphological changes, which was preceded by intensified acute inflammatory responses. ED2+ve macrophages during an early phase of reperfusion may be activated and proliferated endoneurial resident macrophages [8,9]. References: 1. Diabetes 1986;35:1058, 2. Muscle Nerve 1992;15:1116, 3. J Neurol Sci 1993;119:162, 4 Brain Res 1999;838:11, 5. JPNS 2002;7:37, 6. Diabetologia 1999;42:1350, 7. Ann Neurol 2000;47:71-79, 8. Am J Physiol 2001, 9. Eur J Neurosci 2002.

CHURG STRAUSS SYNDROME DURING TREATMENT WITH A LEUCOTRIENE RECEPTOR ANTAGONIST PRESENTING WITH POLYNEUROPATHY

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A 79-year-old male patient with bronchial asthma for twenty years was admitted due to progressing gait disorder throughout the last two weeks. No other relevant medical history was known. Asthma was treated with a leucotriene receptor antagonist (Montelukast) for four years, as well as low doses of inhaled steroids and β_2 -agonists. On admission, neurological examination revealed a mild ataxia on both upper limbs and focal sensory disturbances without motor deficits. On the lower limbs he demonstrated a moderate distal paresis more pronounced on the right side. He had severe ataxia and asymmetrical sensory deficits on both legs. Deep tendon reflexes on the upper and lower limbs were intact. Vibration was distal symmetrically impaired. He was not able to walk without assistance. Nerve conduction velocity studies demonstrated a sensorymotor neuropathy of the lower limbs and normal results for the upper limbs. Electromyography exhibited no spontaneous activity in the right tibialis anterior and rectus femoris muscle. Laboratory examination showed leucocytosis and eosinophilia. Test for rheumatoid factor was positive. Mild peripheral pulmonary infiltrations were found in the CT of the chest. Nerve-muscle biopsy revealed an eosinophilic vasculitis. Thus, the diagnosis was compatible with Churg-Strauss syndrome (CSS). The patient received steroids and azathioprine. Neurological symptoms slightly improved consecutively. From the literature it is well known that bronchial asthma is associated with CSS. Peripheral neuropathy, even as the initial manifestation of CSS, is common. There is also some evidence that leucotriene receptor antagonists (LTAs) may trigger CSS in asthmatic patients especially when steroids were previously tapered. However, the pathogenesis is unclear and the association between CSS and LTAs remains a matter of discussion. Our patient received continuous low dose inhaled steroids, any other medication was ruled out with respect to their propensity in triggering CSS. Steroids were not tapered before. This case demonstrates for the first time a severe neuropathy in an asthmatic patient, during long lasting treatment with a LTA, as the initial clinical feature of CSS. However, an unmasking of a possible preexisting CSS due to a four year lasting treatment with LTAs seems unlikely, but can not be excluded.

CHRONIC SENSORY DEMYELINATING NEUROPATHY (CSDN): THE MOST COMMON TREATABLE SENSORY NEUROPATHY

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In 1992, we have reported a new entity of chronic sensory demyelinating neuropathy (CSDN) (J NNSP 1992; 55: 677-680). We concluded that CSDN is a sensory variant of CIDP. We are reporting here our experience of this disorder in 30 cases with emphasis on immunotherapy. Diagnostic criteria of CSDN were (1) progression of neuropathy longer than 4 weeks, (2) pure sensory neuropathy, and (3) demyelination in motor and sensory nerve conduction study in two or more nerves. The majority (90%) of patients were male. Age ranged from 28 to 74. All patients had pure sensory neuropathy without any motor weakness. Onset of disease is subacute and slowly progressive. Duration of disease to the maximum disability ranged from 4 weeks to 12 years. No antecedent events or infection was identified. Cranial nerve and respiratory muscle involvement was not observed in any of cases. Monoclonal gammopathy was seen only in three cases. Spinal fluid protein in 22 cases was high in 64% of cases with oligoclonal band in a few cases. Serum autoantibody tests in 20 cases were positive only in four cases. Nerve conduction showed evidence of demyelination at least in two nerves in all cases. In 77% of cases, motor nerve conduction showed evidence of demyelination. In 7 (23%) cases, the near-nerve needle nerve conduction study was necessary to document the evidence of demyelination. Nerve biopsy in 20 cases showed demyelination in 16 cases and inflammatory cells in 2 cases. Various immunotherapies were administered in 22 cases during the progressive phase. In 85% of cases, immunotherapy showed an improvement with complete recovery in 4 (13%) cases and with stable course without any medication in 8 (26%) cases. This included steroid, azathioprine, plasmapheresis, and IVIG treatment. Relapse was rarely observed with withdrawal of immunotherapy. Only one case developed motor weakness during the follow-ups. CSDN is an identifiable disease which can be treated with immunotherapy successfully in the majority of patients. According to our experience, this is the most common treatable sensory neuropathy.

A CASE OF POEMS (CROW-FUKASE) SYNDROME WITH A HIGH LEVEL OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

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Background: POEMS (Crow-Fukase) syndrome is a rare multisystem disorder. Unlike the polyneuropathies associated with immunoglobulin M gammopathy, no immune mechanism directed toward peripheral nerve components has been associated with this disease. Some recent studies reported that serum vascular endothelial growth factor (VEGF) levels in patients with Crow-Fukase syndrome were elevated and it is speculated that overproduction of VEGF is an important factor in the pathogenesis. We report a case of Crow-Fukase syndrome with a high level of serum VEGF. Case Report: A 41-year-old man developed polyneuropathy, organomegaly, endocrine disorder, M-protein and skin change. A computed tomographic scan of the bone showed that he had multiple tiny sclerotic bone lesions in the lumbar and thoracic spines. Serum level of VEGF was elevated (426 pg/ml). A femoral lymph node biopsy was performed and the specimen showed that there were histological changes like Castleman's disease. Oral corticosteroid was administered and his condition was dramatically improved and serum level of VEGF was normalized. Conclusion: We suggest that VEGF is a useful diagnostic marker for Crow-Fukase syndrome as well as a marker of clinical improvement.

PERIPHERAL AND CENTRAL NERVOUS SYSTEM INVOLVEMENT IN X-LINKED CHARCOT-MARIE-TOOTH DISEASE (CMTX)

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The X-linked form of Charcot-Marie-Tooth disease (CMTX) is associated with mutations in the Connexin 32 gene (Cx32), and is characterized by no male-to-male transmission, intermediate motor conduction velocities (MCV), and more severe disease in males. In our series of CMT patients, we found 9 different Cx32 mutations in 11 families (one novel mutation). Overall there were 26 patients (13 males), aged 11-76 yrs. Age at onset ranged considerably (1-60 yrs.), but symptoms began earlier in males (mean 15.4 yrs.) than females (25 yrs.). All patients were autonomous, but disease severity was greater in males, while 4 female carriers were asymptomatic. One patient had Babinski sign and another had rest tremor. Upper limb MCV ranged between 25 and 57 m/s, and were in the range of CMT1 (<38 m/s) in 10/13 males but only in 3/10 females. We found a certain degree of asymmetry in nerve conduction abnormalities. In some cases nerve conduction slowing was non-uniform within single nerves, and in both genders distal motor latency was significantly more prolonged in the median nerve than in the ulnar nerve. We also investigated the presence of subclinical abnormalities of the central nervous system (CNS) by multimodal evoked potentials (EPs), magnetic resonance imaging (MRI) and proton magnetic resonance spectroscopy (H-MRS). Abnormalities suggestive of CNS dysfunction were found in 10 out of 12 patients (7/8 males, 3/4 females). In detail, central component latencies or interpeak times were prolonged in 8/10 brainstem auditory EPs, 2/6 pattern-visual EPs, 3/5 flash-visual EPs, 3/9 somatosensory EPs, and 3/6 motor EPs. MRI showed cerebral atrophy in two young males and mild hyperintensity of the cortico-spinal tracts in a female patient. H-MRS evaluation of the peak amplitude for the dominant metabolites was performed in 5 patients. Expression of Cx32 in the brain is the likely explanation of these findings that confirm previous non-systematic observations. Subclinical evidence of CNS involvement is very common in CMTX, and may be of help in addressing molecular studies. Partially supported by a grant from the Italian Ministry of Health to F.T. and D.P.

INVESTIGATION OF MECHANICAL INTERACTIONS BETWEEN INTRANEURAL ELEMENTS OF THE RAT SCIATIC NERVE AFTER REPAIR

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The development of tissue engineered conduits for the surgical repair of peripheral nerves has raised important questions with regard to the mechanical aspects of neuroanatomy. Restoration of the ability of a repaired nerve to accommodate the movements required during limb movement is an important consideration in the design of repair strategies. A model has been developed for use alongside existing experimental assays of nerve function to explore the restoration of mechanical features in the regenerating rat sciatic nerve. This is based on the presence of a distinct core and sheath with an interface at the level of the innermost perineurial cell layer. The core and sheath can be separated experimentally using tensile testing equipment which allows quantification of the force required. This force has been shown to be consistent in control nerves from unoperated animals, providing a reference for the subsequent investigation of the interaction between these neural regions after repair. In this investigation, the left sciatic nerves in 18 Wistar rats were transected and immediately repaired using epineurial sutures. Animals were sacrificed 2, 4, 8 and 12 weeks post-repair and the nerves underwent mechanical testing to measure the force required for separation of the core and sheath. Controls were unoperated contralateral sciatic nerves from animals in the experimental group. The ability to separate the endoneurial core from the nerve sheath depends on the maximal force required being less than the break-strength of either region. 4 weeks after primary repair, separation of core and sheath was possible and required a greater force than controls. At the other time points the endoneurial core failed before separation from the sheath could take place, suggesting that the strength of the interface was greater than the strength of the endoneurial core. These data provide an insight into the mechanical changes that occur following primary repair in the rat sciatic nerve. Knowledge of such changes provides an additional means by which to compare alternative approaches to nerve regeneration in this animal model with a view to developing implantable devices which restore mechanical integrity along with conduction functionality.

CORRELATION BETWEEN DIFFERENT TOXICITY SCALES FOR GRADING OF CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

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In the present study was performed a comparative study with the aim to establish whether differences occurred between different clinically-based neurotoxicity scales and a more extended composite scale (Total Neuropathy Scale) already validated in diabetic patients, in order to improve the grading of chemotherapy-induced peripheral neuropathy (CIPN) severity. The presence of CIPN was assessed by a team of neurologists and oncologists with specific expertise in this field in a series of women affected by locally advanced squamous cervical carcinoma treated with cisplatin and paclitaxel-based chemotherapy. Patients underwent clinical examination, neurophysiological assessment and quantitative sensory testing of vibration threshold (VDT); the ECOG, Ajani and NCIC-CTC grades were assessed by a neurologist after clinical examination and the TNS was calculated after additional instrumental examination. At the same time, the treating oncologist, who was blinded as regards to the results of the neurological examination, assessed the NCIC-CTC sensory neurotoxicity grade. The correlation existing between the different evaluations was evaluated with the Spearman test on a total of 120 visits. For all the neurotoxicity scales commonly used by oncologists and evaluated in this study a significant correlation was observed with TNS; however, the inter-examiner agreement comparison evaluation evidenced that in several cases the neurologist attributed a higher grade in the NCIC-CTC scale than the oncologist, and the analysis of the single items of TNS demonstrated that this discrepancy was mainly due to a different evaluation of the sensory impairment. We believe that for clinical trials TNS is superior than common toxicity scales in grading the real severity of CIPN and it is very important that the results of this evaluation can be reliably correlated with the oncological grading of sensory peripheral neurotoxicity. However, the time needed to assess the TNS score and the need for technical equipment still represent a limitation for the widespread use in the clinical setting.

ACETYL-L-CARNITINE: AN EFFECTIVE NEUROPROTECTANT AGAINST PACLITAXEL AND CISPLATIN-INDUCED NEUROTOXICITY

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Different attempts have been previously performed in order to reduce the neurotoxicity of antineoplastic compounds, but so far an effective neuroprotective treatment is not available. In this study we have tested in two rat models the hypothesis that Acetyl-L-carnitine (ALC), a naturally occurring compound with neuroprotective activity in several experimental paradigms and in clinical use for the treatment of painful neuropathies, may have a protective role on cisplatin- and paclitaxel-induced neuropathy. Preliminary *in vitro* and *in vivo* experiments in different tumor systems indicated the absence of interference of ALC in the antitumor effects of cisplatin and paclitaxel. ALC co-treatment reduced the neurotoxicity of both cisplatin and paclitaxel in well-established rat models and this effect was correlated with a modulation of the plasma levels of nerve growth factor (NGF) in the cisplatin model. The protective effect of ALC was confirmed also in *in vitro* studies on rat PC12 cells, where ALC treatment counteracted the paclitaxel and cisplatin-induced cell damage, as measured by reduction of neurite elongation. In a search for the mechanism(s) of ALC neuroprotection, the transcriptional profile of gene expression in PC12 cells determined by microarray and Northern blot analysis indicated that ALC, in the presence of NGF, was able to modulate various genes relevant in the tissue-specific toxicity, such as NGFI-A. Moreover, we observed that labeled acetyl groups derived from ALC were transferred into histones and that cells co-treatment with NGF and ALC increased the level of histones H4 acetylation. These findings suggest that up-regulation of genes implicated in the protection against specific damage induced by neurotoxic agents may be the result of modulation of histone acetylation mediated by transfer of ALC acetyl groups. Our results indicate that ALC has a neuroprotective action through a novel mechanism which may be important against cisplatin- and paclitaxel-induced neurotoxicity.

EXPERIMENTAL DENERVATION OF THE EPIDERMIS DEMONSTRATES REDUCED REGENERATION OF NOCICEPTIVE FIBERS IN PEOPLE WITH DIABETES

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Background: Loss of cutaneous nociceptive nerve fibers is a fundamental complication of diabetes mellitus that contributes to injuries of the extremities and amputations. The possibility that diabetes leads to a defect in repair and regeneration of nociceptive fibers has not been explored. To investigate this possibility we developed a means of experimental denervation of epidermis. **Methods:** 31 healthy volunteers and 19 people with diabetes mellitus (I and II) with or without neuropathy were enrolled. The presence of neuropathy among diabetic subjects was defined as a Michigan Diabetic Neuropathy Score (MDS) > 6. Topical application of capsaicin to a normal area on the distal thigh produced superficial denervation of the epidermis and dermis. Skin biopsies were obtained at baseline, following denervation and at multiple subsequent time points. Biopsies were processed to allow visualization and quantitation of epidermal nerve fibers (ENF) expressed as fibers per mm. **Results:** Healthy volunteers recovered their ENF density at a rate of 0.185 fibers/mm/day (95% CI: 0.15 - 0.21 fibers/mm/day). The rate of regeneration was decreased among those with diabetes (0.069 fibers/mm/day, 95% CI: 0.044 - 0.11 fibers/mm/day, $p < 0.001$), irrespective of the presence ($p < 0.0001$) or absence ($p < 0.04$) of neuropathy. Among diabetic subjects, those with peripheral neuropathy had a greater reduction in regenerative capacity (0.0384 fibers/mm/day, 95% CI: 0.0176 - 0.089) than diabetic subjects with no neuropathy (0.124 fibers/mm/day, 95% CI: 0.069 - 0.178, $p = 0.03$). **Conclusions:** Diabetic subjects regenerate ENF more slowly than healthy controls after chemical axotomy. Diabetic subjects with neuropathy had a greater reduction in regeneration rate. These results suggest that impaired nerve regeneration may play a role in the pathophysiology of diabetic neuropathy and precede any clinical evidence of abnormality. Measurement of ENF regeneration has potential to be an efficient outcome measure in future trials of agents designed to promote regeneration in peripheral neuropathy.

A PILOT RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED
EXPLORATORY SAFETY STUDY OF THE USE OF INTERFERON-BETA 1a IN
THE TREATMENT OF GUILLAIN-BARRÉ SYNDROME

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Objective: This investigator-led pilot study aimed to assess the safety and tolerability of Interferon beta-1a (IFN- β 1a) for Guillain-Barré Syndrome (GBS) when used with intravenous immunoglobulin (IVIg). **Background:** IFN- β has multiple effects upon the immune system and is widely used for multiple sclerosis. In vitro IFN- β reduces the migratory capacity of lymphocytes from GBS patients and reduces serum levels of matrix metalloproteinase 9 and TNF α , which are elevated in the acute phase of GBS. IFN- β ameliorates experimental autoimmune neuritis. Two published cases had favourable outcome after treatment with IFN- β and either plasma exchange or IVIg. **Methods:** Patients with GBS were recruited to this double blind, randomised, placebo-controlled trial supported by Serono after ethics committee approval and individual consent. Patients needed to be chair or bed bound and not improving. IFN- β 1a (Rebif®) 22 μ g was administered subcutaneously three times weekly for the first week and then 44 μ g administered three times weekly until they were able to walk 10 metres unaided, or for 24 weeks, whichever was sooner. **Results:** 19 patients were recruited between 1998 and 2002. 13 patients were allocated to IFN- β and six to placebo. In the IFN- β group one patient died and three others stopped receiving study drug due to serious adverse events. In the placebo group two patients experienced serious adverse events which did not lead to cessation of study drug. One patient in the placebo group stopped study drug due to deranged liver function tests. Disability grades at one month and six months did not differ significantly between those treated with IFN- β compared with placebo. **Conclusions:** There were no serious adverse events due to IFN- β . The IFN- β and placebo groups had almost identical proportions of patients with serious adverse events. We conclude that IFN- β is tolerated in GBS and does not appear to have any unexpected adverse interaction with IVIg. The study was not designed to detect even moderate improvements in disability outcome after IFN- β .

ABNORMALITIES OF DIGIT III FINGERTIP INNERVATION IN CARPAL TUNNEL SYNDROME BEFORE AND AFTER DECOMPRESSIVE SURGERY

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We studied the effect of chronic compression of median nerve at wrist on cutaneous innervation and the efficacy of decompressive surgery on functional and morphological recovery. We obtained two mm punch biopsy from third fingertip in 15 patients (11 female and 4 male, age range 33-61 years) with carpal tunnel syndrome before, and six and twelve months after surgical decompression of median nerve at wrist. Patients with diabetes and other metabolic or endocrinological disorders or with electrophysiological findings of a diffuse neuropathy were excluded. By means of immunohistochemical techniques and confocal microscopy we counted epidermal nerve fibers, Meissner corpuscles and myelinated papillary endings. All patients before surgery and three, six and twelve months after, underwent electrophysiological study and quantitative sensory testing using Semmes-Weinstein monofilaments for tactile threshold and Medoc TSA II Neurosensory analyzer for thermal threshold evaluation. Morphological and functional data were compared with age and sex matched controls. We observed abnormalities of epidermal nerve fibers, mechanoreceptors and their myelinated afferents in all patients. These abnormalities ranged from mild to marked and appeared more severe in patients with a longer disease duration and a slower conduction velocity along the median nerve. A relief of painful symptoms and a significant electrophysiological improvement was evident after three months and remained during the year of follow up. Morphological findings and quantitative sensory testing did not correlate with such course.

THE SECONDARY MANIFESTATIONS OF CHARCOT-MARIE-TOOTH DISEASE (CMT) AND THEIR EFFECT ON HEALTH-RELATED QUALITY OF LIFE

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The manifestations of CMT and its impacts on quality of life were rated in a survey of 324 adults and children. Demographic data were obtained, along with responses in the following areas: family history; initial diagnosis; effect of CMT on pregnancy; genito-urinary manifestations; peripheral sensory changes; balance; 'restless legs'; physical changes (back, arms/hands, legs/feet); use of assistive aids; footwear; and treatment (conservative, surgical and alternative therapies). We report the prevalence and inter-relationships of these features, and health-related quality of life scores measured by the MOS SF-36, a well-validated instrument scoring eight distinct dimensions of health. SF-36 scores for patients with CMT were significantly worse than age- and gender-matched Australian norms in all eight dimensions, and lay in the mid range for comparable chronic disorders (cardiovascular disease, stroke, arthritis, Parkinson's disease). Regression models were constructed to establish predictive markers. Health related quality of life was associated strongly in many dimensions with peripheral weakness. Other significant factors included age, presence of cramps, and previous surgical interventions. Lower limb impairment was highly prevalent, with leg weakness reported by 90%, and falls to the ground by one-quarter of the sample. 79% reported leg cramps. Stretching and strengthening regimes evaluated relatively strongly in milder cases, whereas splints and casts are not favored by the CMT community. Ankle-Foot orthoses improve function in a proportion of people with more profound weakness, but age interacted significantly with reported outcome. In-shoe orthoses were considered helpful. 'Alternative' therapies did not evaluate well. The choice of surgical intervention remains contentious, and was largely independent of any of the disease related factors included in the analysis. Muscle weakness was the strongest predictive marker for quality of life, and for patient-reported effectiveness of many therapies.

DELAYED NERVE IMPAIRMENT IN LEPROSY

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Objective: To study the clinical signs, symptoms and course of patients with leprosy who after treatment developed nerve impairment, not explained by relapse or reversal reactions. **Methods:** We searched the case-records of leprosy patients, seen at the Department of Dermatology at our Centre, between 1985-2002. Included in the study were patients who had developed nerve impairment after treatment of leprosy in the absence of relapses or reversal reactions and who were referred to a neurologist. In these patients we recorded age, onset of leprosy, type of leprosy, treatment of leprosy, signs and symptoms of delayed nerve impairment, results of electrophysiological studies, responses to treatment and course. **Results:** Included were 16 patients, 8 with a (sub)acute multiple mononeuropathy (group I); and 6 with a slowly progressive multiple mononeuropathy (group II). Patients in group I had limited improvement of nerve impairment after treatment with corticosteroids and recurrence of symptoms and signs (usually of the motor nerves) when corticosteroids were tapered off. Patients in group II had slowly progressive predominately sensory nerve impairment. Initially, they had only subjective symptoms, after at least 3 years objective signs became detectable. These patients were not treated with immuno-suppressants. **Conclusion:** Patients of group I responded insufficiently to corticosteroids. In these patients more aggressive immuno-suppressive treatment should be considered. The pathogenesis of nerve impairment in group II is unknown and therefore it is unclear how to treat these patients.

DIAGNOSTIC VALUE OF SKIN BIOPSIES IN PATIENTS SUSPECTED TO HAVE A SMALL FIBRE NEUROPATHY - META-ANALYSIS

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Objective: To review systematically the diagnostic value of skin biopsies in patients with painful, numb or tingling feet/hands and normal electrophysiological examination. **Methods:** We searched the literature for studies examining skin biopsies in patients with a suspected isolated small fibre neuropathy (ISFN) and did a meta-analysis of the results. The frequencies of positive skin biopsies in ISFN patients and in controls were extracted from each paper. Pooled weighted frequencies were calculated, using the study size as weights. Sensitivity and specificity were determined. For each possible prior probability the incremental ruling-in and ruling-out gain were calculated. Adding these gains to the prior probabilities resulted in post-test probabilities (i.e. positive respectively negative predictive values). **Results:** Of the 35 identified papers, 8 studies fulfilled the criteria for further analyses, including a total of 155 patients with ISFN and 116 controls. In patients with a prior probability of more than 40% for having ISFN, the incremental ruling-in gain is between 15-40%, resulting in a positive predictive value of 80-100% for having a ISFN. In patients with a prior probability of more than 85% for having ISFN and thus a prior probability of less than 15% of not having ISFN, the ruling-out gain is approximately 15%, resulting in a negative predictive value of 35% for not having ISFN. **Conclusion:** In patients with painful, numb or tingling feet/hands and normal electrophysiological examination, with a prior probability of 40-85% for having ISFN, the skin biopsy is a useful diagnostic test to confirm or to exclude IFSN.

WHY IS THERE PAIN WITH CARPAL TUNNEL SYNDROME? CHRONIC NERVE COMPRESSION INDUCES ABERRANT AXONAL SPROUTING WITHOUT WALLERIAN DEGENERATION

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Although carpal tunnel syndrome affects millions of individuals, there is no understanding as to why there is actually pain with this condition. The current dogma is that the ischemia of chronic nerve compression (CNC) leads to mild Wallerian degeneration which may be the source of the pain. To better understand CNC, we rigorously evaluated the axonal architecture of nerves in a model for carpal tunnel syndrome. A nerve compression model was developed with Sprague-Dawley rats as a sterile one-inch silastic tube (I.D. of 1.3 mm) was atraumatically placed around the sciatic nerve. EMG/NCV studies were performed at the time of specimen harvest. For EM evaluation, the specimens were post-fixed in osmium tetroxide, dehydrated in acetone and embedded in Eponate 12 resin. Design-based, unbiased stereologic counting was used to obtain unbiased number and size estimates. Fluorescent immunohistochemistry was performed to evaluate neurofilament protein expression. There were no significant electrophysiological changes at the one-month time point. By the eight month time point, the NCV consistently decreased to 65% of the normal value. At the one-month time point, EM did not show significant alteration in axonal integrity, but rather demonstrated maintenance of the normal axonal cytoskeleton including neurofilament architecture. There was no evidence of granular disintegration of the axoplasm. Surprisingly, at the one-month time point, there was a 58% increase in unmyelinated axons relative to normal nerve, which was not present at the eight-month time point. Fluorescent microscopy demonstrated that at one-month post surgery, there was evidence of axonal sprouting marked by the appearance of thinner neurofilament proteins emerging from thicker base proteins. Consistent with the EM data, such alterations are only observed at the periphery of the nerve section, while the center retained its normal architecture. By eight months, there was a return to a near normal neurofilament protein and axonal architecture. The temporal changes of unmyelinated axons in the CNC model follow the same time course as the expression of pain in patients with compression neuropathies. The data suggests a novel hypothesis that CNC may provide an early stimulus for re-innervation that aberrantly results in the pain associated with compression neuropathies.

THE EXPRESSION OF CYTOKINES IL-1 β , TNF- α , INF- γ IL-10 AND TGF- β IN SILICONE TUBE-TREATED PERIPHERAL NERVE INJURY

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In order to help axonal reinnervation after a peripheral nerve trauma, in which the loss of structural continuity occurs, silicone tubes have been used both clinically and experimentally. After peripheral nerve injury there is a marked activation of different cytokines, which induce inflammation but may also help axonal reinnervation. In this experimental study we investigated the expression of cytokines as well as the morphological changes after implantation of silicone tube after removal of a piece of sciatic nerve. The clinical improvement was studied by pinprick and toe-spread tests. The samples from the tube, distal and proximal stumps were taken 1, 3, 5, 7, 14, and 42 days postoperatively. The expressions of the following cytokines were studied: IL-1 β , TNF- α , INF- γ IL-10 and TGF- β . The number of reinnervating axons was studied by neurofilament antibody. The morphological results showed that sutures used to fix the silicone tube induced foreign body reaction. There was a high expression of the studied cytokines at day one but at day three the expression somewhat decreased. The exception was INF- γ which showed an increasing expression up to five days, especially in the tube and in the samples taken distally to the tube. After day three the expression in the tube increased up to day 42, except INF- γ which declined. Similar expression pattern was noted in all the studied cytokines in the distal area beside the tube. In the contralateral nerve, which was not operated on, also showed some early phase expression of the studied cytokines. The present study shows that the insertion of silicone tube induces marked changes in the cytokine expression. Thus it appears that these are involved in the building up the missing part of peripheral nerve. This may give us new ways to treat peripheral nerve injuries. The increased expressions of the studied cytokines in the contralateral nerve confirm our previous findings and indicate that healing in of the peripheral nerve is much more complicated than previously imagined.

THE EXPRESSION OF CYTOKINES IN DENERVATED SCIATIC NERVE

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The aim of the present study was to examine the cytokine changes in sutured peripheral nerve when axonal regrowth has been prevented. The samples for cytokine assays were collected from the right sciatic nerve distally and proximally to the point of transection. Furthermore, endoneurium was separated from surrounding epi- and perineurium to examine the expression of cytokines in both of these different compartments. We investigated the expression of, IL-1 β , INF- γ , TNF- α , IL-4 and IL-10 mRNA. The samples were collected from 1 day up to 5 weeks after the transection of the sciatic nerve. For the control purposes sciatic nerves from non-operated rats were used. Quantitative Real-time-PCR (Taq Man) was used to determine the expressions of the studied cytokines. The results showed that remarkable changes in the studied cytokine expressions were observed. The expression pattern of the studied cytokines was cyclic. The epi-/perineurium of the proximal samples showed strong simultaneous expressions of IL-1 β , TNF- α and IL-10 cytokines at 35 days. At 42 days the expressions of the studied cytokines was markedly decreased. In the samples taken distally to the site of transection an increased early expression of TNF- α , IL-10 and IL-1 β was noted but there were some peaks of expression of IL-10, IL-1 β and INF- γ later on. The present results show that cyclic expression of the studied cytokines can also be found also in the denervated nerves. The high epi-/perineurial expression of cytokines noted at 35 days in the proximal area indicate that cytokines may have a role in the formation of the epi-/perineurial fibrosis.

OXIDATIVE NEURONAL INJURY IS ASSOCIATED WITH PROGRAMMED CELL DEATH (PCD) AND NEUROPATHY IN AN ANIMAL MODEL OF TYPE II DIABETES AND IMPAIRED GLUCOSE TOLERANCE (IGT)

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The female Zucker Diabetic Fatty (ZDF) rat (GMI ZDF drt/fa) exhibits obesity-linked insulin resistance, impaired glucose tolerance (IGT) with a control diet, and frank diabetes with a high fat diet. The ZDF rat is thus a model of type II diabetes. This study defines the relationship between oxidative stress and neuropathy in an animal model of diabetes. Zucker rats were divided into three groups of 8 rats with: 1) diabetes, 2) IGT, 3) controls. After 10 weeks of diabetes, mean blood glucose was 600 mg/dl (diabetic) and 150 mg/dl in IGT rats (400 mg/dl at 1 h on glucose tolerance testing). End-products of oxidative stress such as malondialdehyde, and 4-hydroxynonenyl were measured in at least 100 dorsal root ganglion neurons (DRG) per animal and were significantly increased in diabetic>IGT>control DRG neurons at 10 weeks. Cryosectioned tissue was either stained for cleaved (active) caspase-3, or assayed for nuclear degradation using a TUNEL stain. Percent DRG caspase-3 cleavage was: ZDF diabetic 7%, IGT 5%, lean controls <2% (p<0.01). Schwann cells: ZDF diabetic 4%, IGT 3%, lean controls <0.5% (p<0.01). Percent DRG TUNEL staining: ZDF diabetic 18%, IGT 14%, lean controls 1% (p<0.001). Schwann cells: ZDF diabetic 8%, IGT 5%, lean controls <2% (p<0.01). Sensory nerve action potentials (SNAP) were reduced in the tail (75%) and digit (10%), and conduction velocities were slowed (distal>proximal) both in ZDF rats with frank diabetes and IGT (p<0.01). Motor conduction velocities were reduced in animals with diabetes and IGT, and hind limb motor distal latencies increased in diabetic animals (p<0.001). Measures of oxidative stress, PCD and NCS abnormalities were increased with impaired glucose regulation (diabetic>IGT animals). These results indicate: 1) an association between hyperglycemic oxidative stress and neuronal injury, 2) that neuronal injury and neuropathy occur with post-prandial IGT, in the presence of normal basal glucose levels. These results provide support for an association between IGT and peripheral neuropathy, and imply that even long-term transient increases in blood glucose may be associated with neuronal and axonal injury. NIH NS42056, VA Merit, and JDRF (JWR); NIH NS32843, JDRF and ADA (ELF).

PAIN SYNDROMES IN GUILLAIN-BARRÉ SYNDROME: INCIDENCE AND RESPONSE TO METHYLPREDNISOLONE

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Objective: Pain is a common and severe symptom in patients with Guillain-Barré Syndrome (GBS). A variety of pain syndromes may occur at different stages of the illness. Case reports suggest that corticosteroids may relieve severe pain in GBS. The objectives of this study were: 1) to evaluate the incidence, characteristics, severity and course of pain; 2) to assess the efficacy of methylprednisolone (MP) in treatment of pain. Methods: Patients were recruited from a randomised placebo-controlled study comparing IVIg + MP (500 mg for five days) versus IVIg + placebo. Presence and severity of pain were collected at randomisation and after four weeks. In addition, medical records of GBS patients treated in the EMC were screened retrospectively for different pain symptoms, course and severity: 1) pain needing paracetamol; 2) more than paracetamol; 3) extreme pain despite treatment. Pain was scored at different time intervals: 0-4 weeks before randomisation, 0-2 weeks, 2-4 weeks, 1-6 months and >6 months after randomisation. Patients were stratified for CMAP of the extensor digitorum brevis (EDB) ≤ 3.3 mV (median value). Efficacy of MP was evaluated using the endpoint: percentage of patients improving in level of pain-severity. Results: 55% of the 223 patients described pain at randomisation. Of the 39 retrospectively analysed patients, 67% described pain 0-4 weeks before randomisation. Painful par/dysaesthesiae (18%), backache (31%), radicular (18%), interscapular (28%) and muscle pain (23%) most frequently occurred. Most pain symptoms decreased within two weeks. However, painful par/dysaesthesiae and muscle pain remained rather constantly present during at least 6 months. The median CMAP of the EDB for patients without radicular pain was 4.6 (2.5-19.5), for patients with radicular pain 1.91 (2.5-4.9) ($p=0.075$). All patients with severe radicular pain (severity 2-3) had a CMAP < 3.3 mV ($p=0.01$). The efficacy endpoint showed similar trends for MP compared to placebo. Conclusions: Pain frequently occurs and causes severe complaints. Especially painful par/dysaesthesiae and muscle pain may persist for months. Severe radicular pain is related to a low CMAP, suggesting a relation with axonal damage. Methylprednisolone has no significant effect on the decrease of pain.

LONG TERM TREATMENT WITH PLASMA EXCHANGE LEADS TO A SUSTAINED IMPROVEMENT IN NEUROPATHY AND FUNCTION IN A PATIENT WITH REFSUM'S DISEASE

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Plasma exchange has been reported to be of benefit in short term 'rescue therapy' in the treatment of acute deterioration of ataxia or neuropathy in patients with Refsum's disease who have very high phytanic acid levels (typically >900mmol/l) unresponsive to dietary measures. We report the case of a 36-year-old female, who was symptomatic in her late teens with anosmia and poor night vision with Refsum's disease being finally diagnosed at age 25 when she presented with ataxia and progressive distal neuropathy. Despite being compliant with dietary measures (mean serum phytanic acid level 500mmol/l), by age 30 she required a walking stick and ankle splints for ambulation and consequently had to cease employment. The patient continued with a strict diet and underwent 3-6 monthly plasma exchanges for the next 5 years, enabling her to maintain a serum phytanic acid level of less than 300mmol/l. Over this time, not only was the deterioration in her condition halted, but there was also definite clinical and electrophysiological evidence of a sustained progressive improvement in her neuropathy. The mean conduction velocities in legs improved from 30 to 45m/s, correlating with the patient being able to walk without aid and in doing so returning to full time employment as a teacher. Thus plasma exchange may be of significant benefit in not just the acute setting but also when given as repeat courses, in the long-term management of patients with Refsum's disease who have only moderately elevated serum phytanic acid levels.

AMELIORATION OF RETARDED NEURITE OUTGROWTH OF DORSAL ROOT GANGLION NEURONS BY OVEREXPRESSION OF PKC δ IN DIABETIC RATS

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To examine which isoform in protein kinase C (PKC) may be attributable to impaired nerve regeneration in diabetes, we compared neurite outgrowth of isolated dorsal root ganglion (DRG) neurons between streptozocin (STZ)-induced diabetic and control rats with special reference to its causative isoform. The neurite outgrowth was significantly retarded in diabetic neurons. Rottlerin, a PKC δ specific inhibitor, significantly retracted neurite outgrowth, whereas Gö6976, an inhibitor specific for classical PKCs, did not have any effect on it, suggesting a significant role of PKC δ in neurite outgrowth of DRG neurons. The expression of phosphorylated PKC δ , but not total PKC δ in DRGs was decreased in diabetic rats. When this reduced expression was restored by overexpressing the PKC δ in isolated DRG neurons, retardation of neurite outgrowth was significantly reversed in diabetic rats. These results suggest that a decrease in phosphorylated PKC δ is at least in part responsible for impaired neurite outgrowth in diabetes, and that PKC δ plays a significant role in the pathogenesis of diabetic neuropathy. This observation provides a useful clue for the treatment of diabetic neuropathy.

IDENTIFICATION OF COBALAMIN DEFICIENCY IN A NEUROPATHY CLINIC

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Background: Diagnosing cobalamin (Cbl) deficiency as a cause of polyneuropathy (PN) is problematic as the frequency of both disorders increase with age and serum Cbl levels can be difficult to interpret. Objective: To identify unique clinical or laboratory features among PN patients (pts) with Cbl deficiency (Cbl-PN) and to examine the role of serum metabolite testing in identifying Cbl deficiency. Methods: Retrospective review of PN pts seen over a 2 year period at an academic neuromuscular clinic. Cbl-PN was diagnosed using low serum Cbl or elevated serum methylmalonic acid or homocysteine levels. Results: Twenty-seven of 324 PN pts were diagnosed with Cbl-PN. Twelve had normal Cbl levels but elevated serum metabolites. Compared to pts with cryptogenic sensory/sensorimotor PN (CSPN), Cbl-PN pts were more likely to have concomitant involvement of the upper and lower limbs, experience symptom onset in the hands, and manifest a sudden onset of symptoms ($p < 0.005$). Pernicious anemia was identified in 50% of Cbl-PN pts with normal serum Cbl levels. Nerve conduction studies (NCS) were abnormal in 70% of Cbl-PN pts compared with 92% of CSPN pts ($p = 0.008$). There were no significant differences between individual NCS or EMG parameters between the two groups. In all cases, when abnormal, NCS indicated axonal neuropathy. Cbl-PN pts with Cbl deficiency showed little objective improvement following parenteral replacement therapy, however, progression occurred less often compared to pts with CSPN ($p = 0.016$). Conclusions: Cbl-PN is not rare and has clinical features distinct from CSPN. Serum metabolite testing may identify Cbl deficiency in some cases with normal serum Cbl levels. The PN of Cbl deficiency is axonal.

PATHOMECHANISMS UNDERLYING CHARCOT-MARIE-TOOTH TYPE 1A (CMT1A) DISEASE

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CMT1A is an inherited demyelinating neuropathy due to an increased genetic dosage of the peripheral myelin protein 22 (PMP22). PMP22 plays a dual role in regulating Schwann cell (SC) growth and peripheral myelin compaction. However, it is still unclear how its altered dosage leads to dys-demyelination in CMT1A. We first evaluated expression and processing of PMP22, proliferation, migration, motility and shaping properties of SC from a transgenic rat model of CMT1A. Primary SC cultures in the presence or absence of forskolin and co-cultures of SC and sensory neurons were used. In basal conditions, both homozygous and hemizygous transgenic Schwann cells, although expressing higher PMP22 levels than control ones, showed normal motility and migration capacity. Exposure of affected SC to the axon, further stimulating PMP22 expression, determined a significant decrease in cell migration and motility, and an impairment of SC differentiation as judged by failure in increasing cell area and perimeter. Moreover, we used transmission electron microscopy morphometry and small-angle X-ray scattering with a synchrotron radiation microbeam (5 μ m diameter), to study the ultrastructure of myelinated fibers in experimental models of CMT1A and in sural nerves from CMT1A patients. Myelin periodicity was significantly increased in sciatic nerves of transgenic CMT1A rats, in organotypic dorsal root ganglia cultures established from this rat line and in human CMT1A nerves, compared to normal controls. In conclusion, we suggest that both the functions of PMP22, as a protein regulating SC differentiation and the compaction of peripheral myelin, may be affected by its overexpression thus leading to the development of the CMT1A phenotype.

FINE STRUCTURAL COMPARISON OF INTERMEDIATE TYPES OF NEUROPATHY CAUSED BY MUTATIONS IN GENES PRIMARILY AFFECTING EITHER MYELIN (PMP22, MPZ, CX32, PRX, GDAP1) OR AXONS (NEFL)

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Mutations affecting myelin genes have repeatedly been shown to result in a surprising, slowly progressive loss of axons. This is well documented in our series of biopsies from adult cases with CMT 1A, HNPP (PMP22); CMT 1B (MPZ); CMT X (Cx32); and CMT 4A (GDAP1). Nevertheless, in an infantile case of CMT 4F (periaxin, PRX) neuropathy, demyelination and hypomyelination was shown to predominate. Recently, we detected a new mutation in the NEFL gene (CMT2E) which supposedly should primarily affect axons; other genes were excluded as possible candidates. Diagnostic sural nerve biopsies had been performed in two cases at the age of 25 and 52 years from one family because of diagnostic purposes before the underlying mutation was found. These are, as far as we know, the first biopsies studied in CMT2E. In addition to degeneration and regeneration of axons, evidence of demyelination and remyelination with some degree of onion bulb formation was a prominent feature in this type of neuropathy. Thus 'intermediate' forms of neuropathy do occur not only in primarily demyelinating conditions (CMT1) but may also occur in a primarily axonal or neuronal type (CMT 2). Thus far our efforts in a large co-operative study have failed to identify pathogenic mutations in the KIF1B gene which is also supposed to cause a primarily axonal type of neuropathy (CMT2A). Therefore looking specifically for potential primary or secondary demyelinating changes in this or other genetically defined axonal types of neuropathy remains a task for future research.

MUSCLE HYPERTROPHY AND NEUROMYOTONIA IN MULTIFOCAL MOTOR NEUROPATHY

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Multifocal motor neuropathy (MMN) is a discrete clinical entity characterized by progressive predominantly distal, asymmetric limb weakness and minimal or no sensory involvement. Arms are affected earlier and more severely than legs. It is due to multifocal persistent partial conduction block in motor but not sensory nerves. Some patients have raised titres of IgM antibodies to ganglioside GM1 and usually respond to intravenous infusion of immunoglobulins but not with steroids. Typically the weakness and wasting is evident distally. In contrast we have observed 2 patients with neuromyotonia and hypertrophy of more proximal muscles in conjunction with typical distally located MMN. Patient 1 is a 55-year-old man with weakness of left grip for 12 years. He also noted cramps and flickering movements of right supraspinatus muscle. Clinical examination revealed hypertrophy of right supraspinatus muscle with myokymia. In addition he had weakness of left median, ulnar and radial innervated muscles, normal deep tendon reflexes and sensory examination. Patient 2 is a 37-year-old man who started to have weakness of right leg 5 years ago and subsequently of right hand. Examination showed hypertrophy of gastrocnemius and supraspinatus muscles with myokymia. He had weakness of right posterior tibial, median and ulnar innervated muscles with normal deep tendon reflexes and sensory examination. Neurophysiology showed conduction block in the proximal segments of median and ulnar nerves. EMG revealed evidence of neuromyotonia in the form of doublets, triplets, high frequency discharges and complex repetitive discharges. Serum immunoglobulins and immune electrophoresis were normal. Anti-GM1 antibodies and antibodies to voltage gated potassium and calcium channels were negative. Multifocal motor neuropathy responded very well to intravenous immunoglobulin infusion therapy but neuromyotonia responded only partially. Muscle hypertrophy is rare in peripheral neuropathies especially in multifocal motor neuropathy. Denuded axons in areas of focal demyelination may act as spontaneous impulse generators causing continuous motor unit activity and resulting in muscle hypertrophy. It is intriguing to find the contrasting abnormalities of conduction block and hyperexcitability coexisting.

PASSIVE TRANSFER STUDIES WITH ANTI-GANGLIOSIDE ANTIBODIES

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Anti-ganglioside antibodies are strongly implicated in the pathogenesis of the acute motor axonal neuropathy (AMAN) variant of Guillain-Barré syndrome. IgG antibodies against GM1, GD1a, and related gangliosides are frequently present in patients with post-Campylobacter AMAN. In a recently developed animal model of AMAN generated by immunization with gangliosides, the presence of serum IgG anti-GM1 antibodies correlated with clinicopathological disease. The pathophysiological role of anti-ganglioside antibodies in GBS continues to be debated because (a) a reproducible passive transfer model has not been established and (b) both *in vitro* and animal models have yielded divergent results. GBS sera or anti-ganglioside antibodies are sub-optimal for passive transfer studies in animals because of difficulties inherent in purifying large quantities of antibodies and the potential for serum sickness. We have recently raised several monoclonal IgG anti-ganglioside antibodies. Passive transfer of neuropathic disease was attempted by intraperitoneal (i.p.) hybridoma implantation and systemic administration of purified anti-ganglioside antibodies in mice. The majority of animals implanted with an i.p. clone of anti-ganglioside antibody secreting hybridoma developed scattered axonal degeneration in a small proportion of nerve fibres in spinal roots and or peripheral nerves. Animals implanted with an isotype matched control hybridoma did not develop neuropathy. In contrast to hybridoma implantation, passive transfer with systemic anti-ganglioside antibodies did not cause nerve fibre degeneration despite high titre circulating antibodies. Our findings raise the possibility that in addition to circulating antibodies, factors like antibody accessibility and nerve fibre resistance to antibody mediated injury play a role in the development of neuropathy.

CLINICAL OBSERVATIONS IN A LARGE PEDIGREE HIGHLY SUGGESTIVE OF X-LINKED CHARCOT-MARIE-TOOTH DISEASE (CMTX), NOT ASSOCIATED WITH A MUTATION IN THE CODING REGION OF CONNEXIN 32

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X-linked Charcot-Marie-Tooth (CMTX) disease is a hereditary motor and sensory neuropathy, associated typically with a more severe phenotype in affected males. CMTX is most commonly caused by mutations affecting the coding region of the connexin 32 gene (Cx32), which encodes a gap junction protein. To our knowledge, only 4 CMTX families have been reported where the mutations involved the non-coding region of Cx32. We report a large CMT family, with an X-linked dominant inheritance pattern, where a mutation in the coding region of Cx32 has been excluded. By history, there are 24 affected individuals within the pedigree, which spans 8 generations. 38 family members were interviewed and examined, including 6 affected females and 4 affected males. Early signs of disease are noted typically in the first or second decade with pes cavus and associated mild ankle instability. The disease progresses to atrophy and weakness of the hand intrinsic and anterolateral leg muscles, with absent ankle jerks. Sensory symptoms are prominent with glove and stocking loss to pain, temperature, and vibration. Foot pain of an aching burning quality is a common symptom in affected females and is frequently triggered by standing or walking. Contrary to classical descriptions of CMTX, weakness and sensory disturbances appeared to be of equal severity in affected males and females. In a pair of twins, the female was even more severely affected. Findings from electrophysiological studies are in keeping with a primary demyelinating polyneuropathy with secondary length-dependent axonal degeneration. Results of haplotype analysis suggest linkage to the Cx32 gene. Sequence analysis of the promoter region of this gene is currently underway.

NGF-INDUCED NERVE SPROUTING AND ELONGATION OF PC12 CELLS ARE DEPENDENT ON MATRIX METALLOPROTEINASE-9

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Matrix metalloproteinase-9 (MMP-9) is a basal-lamina degrading protease that we have recently shown is upregulated after nerve injury and expressed during nerve fiber regeneration. Since Schwann cell basal lamina is important in guiding peripheral nerve regeneration, we hypothesized that MMP-9 expression was essential to axonal sprouting and elongation. Using NGF-stimulated neuronal PC12 cell lines, we induced differentiation and sprouting by application of NGF, and analyzed the role of exogenous MMP-9 and its inhibitor on axonal sprouting, elongation and branching. Following treatments with MMP-9, anti-MMP-9 antibody or a broad-spectrum MMP inhibitor (Ro 31-3790) introduced into the media of differentiating PC12 cells, we analyzed the morphology of live cells with phase-contrast microscopy, fixed cells with confocal microscopy, and cell lysates with Western blotting and gelatin zymography. MMP-9 treatment increased levels of β -tubulin (neuron-specific cytoskeleton) while anti-MMP-9 treatment caused its decline. MMP-9 increased the extent of neuronal elongation by 62% ($p < 0.05$), branching by 37% ($p < 0.05$), but had no significant effect on the number and formation of new sprouts. Western blot analysis showed that treatment with MMP-9 or anti-MMP-9 antibody had no effect on the levels of GAP-43 (growth cone marker), however, the broad-spectrum MMP inhibitor reduced GAP-43 to levels of non-NGF-induced PC12 cells. These results indicate that MMP-9 plays an important role in neuronal elongation in-vitro and that another MMP isotype is involved in the membrane remodeling associated with formation of new axonal sprouts.

C-PEPTIDE CORRECTS DEFICITS IN NERVE FIBER REGENERATION IN TYPE 1 DIABETIC BB/W-RAT

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Nerve fiber regeneration is severely affected in insulin and C-peptide deficient type 1 BB/W-rats but not in normo-insulinemic and C-peptidemic type 2 BB/Z-rats. To study the role insulinomimetic proinsulin C-peptide may play in nerve regeneration, type 1 BB/W-rats were supplemented with rat C-peptide from onset of diabetes and compared with non C-peptide-replaced isohyperglycemic diabetic and non-diabetic BB/W-rats. Following sciatic nerve crush injury, early gene responses such as insulin-like growth factor, c-fos and nerve growth factor were examined longitudinally in sciatic nerve. Neurotrophic factors, their receptors and β -tubulin and neurofilament expression were examined in dorsal root ganglia. C-peptide replacement significantly normalized the early gene responses in injured sciatic nerve and partially corrected the expression of endogenous neurotrophic factors, their receptors as well as neuroskeletal protein expression in dorsal root ganglia. These effects translated into normalization of axonal radial growth and significantly improved axonal elongation of regenerating fibers in C-peptide-replaced hyperglycemic BB/W-rats. We conclude that impaired insulin action is probably more important than hyperglycemia per se as a mechanism underlying impaired nerve fiber regeneration in type 1 diabetic neuropathy.

GCPII (NAALADASE) INHIBITION PARTIALLY REVERSES NERVE CONDUCTION VELOCITY DEFICITS, HYPERALGESIA AND MORPHOLOGICAL ABNORMALITIES FOLLOWING CHRONIC DIABETES IN BB/WOR RATS

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Excitotoxic glutamate is involved in pain mechanisms and neuronal ischemia. One source is thought to derive from the neuropeptide N-acetyl-aspartyl-glutamate, via action of GCPII (NAALADase). We have reported GCPII inhibitors ameliorate peripheral neuropathies and pain (Brown et al., 1999; Zhang et al., 2000; 2002; Calvin et al., 2000). Here we report on two studies in BB/Wor rats. In the first, rats were treated for 8 mo with the GCPII inhibitor GPI 5693 (30 mg/kg daily) from onset of diabetes (PREVENTION). In the second study, rats with chronic untreated diabetes of 6 mo duration were treated with GPI 5693 (30 mg/kg daily) for 2 mo (REVERSAL). In the prevention study, GPI 5693 attenuated the nerve conduction deficit and hyperalgesia compared to age-matched diabetic controls (by 62% and 78%, respectively, $p < 0.05$). In the reversal study, 2 mo GPI 5693 treatment partially but effectively REVERSED both nerve conduction deficits and hyperalgesia (by 36% and 56%, respectively, $p < 0.05$). Additionally, pathological assessment of sural nerve teased fibers showed significant improvement manifest as: increased normalcy of fibers (by 20%), decreased paranodal swelling (by 62%) and demyelination (by 41%), less Wallerian degeneration (by 30%) and more regenerating fibers (increased by 90%) than in diabetic vehicle rats. Certain nerve morphology parameters were also significantly improved (total fiber number, axon area and circularity index). Findings support GCPII involvement in diabetic neuropathy and most notably, potential utility of GCPII (NAALADase) inhibitors in effective reversal of established diabetic neuropathy complications.

INFLAMMATORY SENSORY POLYRADICULOPATHY

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Background: We describe patients with sensory symptoms due to involvement of dorsal root segments proximal to the sensory ganglia. Peripheral nerves distal to and including the dorsal root ganglia were mainly unaffected.

Objective: We describe patients with sensory symptoms due to non-compressive involvement of proximal posterior roots and report on the histological findings of a fascicular sensory nerve root biopsy and response to immune modulating therapy.

Methods: We identified patients at Mayo Clinic, Rochester, MN that had: a predominant sensory syndrome, nerve conduction studies with no or minor abnormalities, somatosensory evoked potentials consistent with a proximal nerve root lesion, imaging studies that exclude spinal cord pathology or compressive nerve root lesions.

Results: Fifteen patients (41 to 77 years) were identified. All patients had significant sensory symptoms with only minimal motor findings. Four had subacute onset of paresthesias or ataxia. Eleven patients presented with lower limb ataxia progressing over a period of several months to thirteen years. Of those, four had upper extremity involvement. All patients had either normal or minimally abnormal nerve conduction studies. Thirteen patients had SSEP testing consistent with nerve root lesions. One of these patients and two others showed thickening of lumbar nerve roots on MRI. CSF examination on 8 patients showed elevated protein (57-117 mg/dL) with normal cell count. Sural nerve biopsies were normal in two patients. One patient's fascicular nerve root biopsy showed absence of large fibers and endoneurial inflammation. This patient and another with thickened lumbar nerve roots on MRI showed marked improvement of sensory ataxia after IVIg treatment.

Conclusion: We describe an entity of sensory ataxia due to predominant involvement of dorsal root segments proximal to the sensory ganglia. This syndrome is recognized by essentially normal nerve conduction studies with abnormalities localized to the root level on MR imaging or SSEP testing. An inflammatory immune mechanism is suggested by thickened nerve roots, elevated CSF protein, inflammatory infiltration of the nerve root and improvement of symptoms and findings with immune modulatory treatment. This entity is probably an inflammatory sensory polyradiculopathy and may represent an extreme proximal sensory variant of CIDP or Sjogren's Syndrome.

NEUROLOGIC COURSE TRIPLET REPEAT SIZE AND ENDOCRINE DYSFUNCTION IN KENNEDY'S DISEASE

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Background: Kennedy's disease [MIM 313200] is caused by expanded polyglutamines in the androgen receptor. Patients have varied involvement of lower motor and sensory neurons and endocrine systems, including gynecomastia and diabetes. Clinical variability remains unexplained. Recent animal models demonstrate testosterone modifies disease expression. Elevated testosterone levels occur in the gynecomastia of Kennedy's disease. Diabetic neuropathies range from distal symmetric sensory to proximal asymmetric motor, features which are found in Kennedy's. Objective: Determine in Kennedy's patients whether neurologic presentations or rates of progression correlate with the presence of diabetes, gynecomastia and triplet repeat size. Methods: Using a computerized record retrieval system all charts of patients diagnosed with Kennedy's at the Mayo Clinic, Rochester, MN, were reviewed. Only patients with confirmatory genetic testing were included (20). To quantify neurologic abnormalities Neuropathy Impairment Score (NIS) was utilized. Slopes of disease progression were calculated for eight patients with serial examinations. Relationships between CAG repeat length, age of onset weakness, presence of diabetes or gynecomastia and slope of disease progression were examined. Results: Patients were Caucasians and unrelated. Reported age of weakness onset ranged from 19 to 75 years and triplet repeat sizes from 36 to 55. Reported age of onset correlated with triplet repeat length ($r = -0.53$, $p = 0.01$). Slope of disease progression did not correlate with triplet repeat size ($p > 0.99$). Six patients had adult onset diabetes and three had impaired fasting glucose without association to age of onset or size of triplet repeats. Eleven patients had gynecomastia and six did not. Presence of gynecomastia was not associated with triplet repeat size or worse progression. Conclusion: Combined our data supports the importance of the size of polyglutamine expansions in neurotoxic gain of function for disease onset in Kennedy's. Modifiers of disease progression other than triplet repeat size, diabetes and gynecomastia must exist. Caution in the counseling of patients and their families based on triplet repeat size, gynecomastia, and diabetes regarding severity or course of disease is suggested. Design of clinical trials may be difficult given the varied and unpredictable rates of progression.

DIFFERENTIAL GENE EXPRESSION IN HUMAN PERIPHERAL NERVE AND NEUROMAS

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Introduction: The molecular pathways that lead to the formation of a neuroma in continuity after peripheral nerve trauma, as opposed to successful nerve regeneration are poorly characterised. We have used cDNA microarray technology to determine genes that are differentially expressed in human neuroma tissue as compared to normal healthy nerve. Such genes may include genes associated with Wallerian degeneration and inflammation, axonal guidance genes, neuronal repair genes, growth factors and their receptors and Schwann cell markers. The possible participation of identified genes in important processes such as axonal regeneration, neuroma formation and pain generation offers the potential to improve our understanding of nerve injury and repair.

Methods: Tissue samples were collected at the time of surgery and immediately flash frozen in liquid nitrogen. Samples were then stored at -80°C until the RNA was extracted. RNA from human peripheral nerve (n = 12) and neuroma tissue (n = 12) was extracted via the TRIzol method. The RNA was amplified and fluorescently labeled via RT-PCR. After overnight hybridization to a 19,000 cDNA microarray chip, confocal laser microscopy and computer analysis yielded a list of differentially expressed genes. **Results:** High quality mRNA was successfully extracted from 3 normal nerves and 7 neuroma samples, which were subsequently used for microarray analysis. Initially, 400 genes were identified whose expression was different in the neuroma and nerve samples as compared to a commercially available human universal control RNA. Further analysis of the data shows that 20 of these genes are differentially expressed between the normal nerve samples and the neuroma samples. **Next Steps:**

Immunohistochemistry and Western blotting will provide protein confirmation of the differentially expressed genes. Further analysis of differentially expressed genes and the function of their protein products may suggest molecular mechanisms of neuroma formation and maintenance.

THE CURRENT STATUS OF PKC α INHIBITION AS A TARGET FOR THE TREATMENT OF DIABETIC PERIPHERAL NEUROPATHY

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The neuropathies associated with diabetes mellitus (DM) are thought to be a heterogeneous group of disorders. The pathogenesis of the most common variety, diabetic peripheral neuropathy (DPN), is an unsettled issue. It is not clear whether chronic hyperglycemic exposure injures nerve fibers or Schwann cells (or myelin) directly, or does so indirectly by damaging the microvascular endothelium and either altering the endoneurial blood flow or the blood-nerve barrier. It has been hypothesized that activation of protein kinase C (PKC) α by the metabolic changes associated with DM results in microvascular damage and impairment in blood flow. Chronically elevated glucose levels increase the activity of the glycolytic pathway leading to synthesis of diacylglycerol (DAG), which, in turn, activates PKC α in many in vitro systems. Activation of PKC α in endoneurial blood vessels results in their increased permeability, impairment of nitric oxide-dependent vasorelaxation, increased leukocyte adhesion and alterations in local blood flow resulting in nerve hypoxia which may play an important role in the development of DPN. In addition, activation of PKC α may be associated with the induction of nerve growth factor expression. In experiments on animals with DM, ruboxistaurin (RBX) mesylate, a PKC inhibitor with high selectivity for the α 1 and α 2 isoforms, prevented and reversed hemodynamic changes observed in diabetic neuropathy (as well as in diabetic retinopathy and diabetic nephropathy). The improvement in blood flow was paralleled by improvement in nerve function. A recently completed Phase 2 clinical trial of RBX for 1 year in 205 patients with DPN has shown improvements in the following outcome measures: neurological examination, positive neuropathic sensory symptoms, composite scores of nerve function and overall patient well-being. Phase 3 trials of RBX for the treatment of DPN are underway.

POSTERIOR ANTEBRACHIAL CUTANEOUS MONONEUROPATHY: CASE REPORT AND PRELIMINARY NORMAL VALUES

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Case Report: A 68-year-old woman was sent to the EMG laboratory for evaluation of bilateral wrist pain and possible carpal tunnel syndrome. The nerve conduction studies (NCS) and concentric needle examination were normal in all respects. That same day she underwent removal of a 1 cm lipoma from the posterior right forearm. Over the next few days she noticed pain and positive sensory symptoms over the posterior aspect of the right forearm without weakness. Repeat NCS one week later showed symmetric posterior antebrachial cutaneous sensory responses (8 uV bilaterally, distal latencies 2.8 and 2.5 ms on left and right, respectively). Five weeks later posterior antebrachial cutaneous sensory NCS demonstrated absence of the response on the right side (the response on the left side measuring 9 uV with a distal latency of 2.6 ms).
Controls: Posterior antebrachial cutaneous sensory NCS were performed on 21 nerves in 12 asymptomatic normal volunteers, gender ratio (M:F) 5:7, mean age 42 years (range 19-68), showing a mean amplitude of 11 uV (range 5-26) and a mean distal latency of 1.9 msec (range 1.4-2.8).
Conclusion: Isolated mononeuropathy of the posterior antebrachial cutaneous nerve has rarely been reported in association with trauma, surgical procedures, or injection. This forearm sensory nerve may be studied by a relatively simple NCS technique and may be useful to distinguish postganglionic disorders involving the cutaneous branch or the proximal radial nerve/plexus from mid cervical radiculopathy.

PAIN RELATED BEHAVIOR IN ENOS AND INOS-DEFICIENT MICE

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Nitric oxide (NO) generated by endothelial (eNOS) or inducible (iNOS) NO-synthase has repetitively been implied in the generation of neuropathic and inflammatory pain (Levy et al., 2000; 2001; Gühring et al., 2000). Here we investigated, whether mice genetically deficient of eNOS (eNOS $-/-$ mice) or of iNOS (iNOS $-/-$ mice) differed in their pain related behavior compared to wild type littermates. We used a neuropathic (chronic constrictive injury of the sciatic nerve, CCI) and an inflammatory pain model (intraplantar injection of complete Freund's adjuvant, CFA) and standardized behavioral tests assessing hypersensitivity to heat (Ugo Basile algometer), to tactile stimuli (von Frey hairs), and to cold (acetone). Baseline thresholds to all tests were not different between groups. In contrast to previous findings, iNOS $-/-$ mice developed reduced withdrawal thresholds to heat (thermal hyperalgesia) not distinguishable from WT-mice. In eNOS $-/-$ mice, there was a trend to reduced thermal hyperalgesia during the first week after CCI. Mechanical withdrawal thresholds in iNOS $-/-$ mice were reduced equally to those from those in WT mice after CCI (mechanical allodynia). In eNOS $-/-$ mice, there was a trend to an attenuation in mechanical allodynia. Exaggerated responses to acetone (cold allodynia) was present in all mice after CCI without differences between genotypes. Intraplantar CFA-injection induced paw swelling in WT and eNOS $-/-$ mice, but not in iNOS $-/-$ mice. Thermal hyperalgesia and mechanical allodynia were less pronounced in iNOS $-/-$ mice at 12 hours post injection ($p < 0.05$), and in eNOS $-/-$ mice at 12 ($p < 0.005$) and 16 hours post injection ($p < 0.05$). Mechanical withdrawal threshold were reduced in all animals irrespective of genotype, with no differences between groups. We conclude from these findings, that the iNOS and eNOS knock-out phenotype in mice plays a greater role concerning inflammatory thermal hyperalgesia than in other types of pain related behavior and in neuropathic pain.

MYCOPHENOLATE MOFETIL IS EFFECTIVE THERAPY FOR REFRACTORY CIDP

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Although many patients with chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) respond to safe immunomodulatory therapies such as intravenous immunoglobulin (IVIg) and plasmapheresis, long term immunosuppressive therapy is often required, with significant associated toxicity. Corticosteroids, azathioprine and cyclosporin are all widely used, but controlled trials have shown efficacy only for steroids. Occasional patients require even more aggressive immunosuppressive therapy such as cyclophosphamide. Mycophenolate mofetil is an antiproliferative agent most widely used for the prevention of solid tissue transplant rejection that is gaining popularity in the treatment of a variety of autoimmune disorders. Mycophenolate blocks *de novo* synthesis of purine by inhibition of IMPDH. Its mechanism of action is therefore similar to that of azathioprine but it has an improved toxicity profile. We have used mycophenolate in four patients with refractory CIDP with encouraging results. Case 1: A 68-year-old female with CIDP of greater than 10 years duration had previously been treated with plasmapheresis, corticosteroids and azathioprine with resultant osteoporosis and transient hepatitis. At review in mid 2001 she was quadraparetic, wheelchair bound and unable to feed herself despite regular plasmapheresis and cyclosporin. Mycophenolate was commenced in August 2001. By January 2002 she was able to feed herself and by April was ambulant with a pick-up frame. She has continued to improve. Case 2: A 70-year-old farmer was diagnosed with CIDP in December 2000. He had a good initial response to IVIg but in mid to late 2001 he deteriorated in the face of combination therapy with IVIg, corticosteroids and azathioprine. Mycophenolate was commenced in January 2002 with subsequent essentially full recovery. The two remaining patients had also failed to respond to IVIg and steroids (and in one case plasmapheresis, azathioprine and cyclosporin) and have either stabilised or markedly improved with mycophenolate. Mycophenolate is an effective, relatively safe form of immunosuppression that should be considered in cases of refractory CIDP

AUTOSOMAL DOMINANT HEREDITARY SENSORY NEUROPATHY WITH GASTRO-OESOPHAGEAL REFLUX AND COUGH IS LINKED TO CHROMOSOME 3P23-24 IN TWO FAMILIES

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Background: In this study we characterized two families with an autosomal dominant hereditary sensory neuropathy (HSN 1) with gastro-oesophageal reflux (GOR) and cough. Although HSN 1 with acral ulceration is commonly caused by mutations in the serine palmitoyltransferase (SPTLC1) gene on chromosome 9, there is clinical and genetic heterogeneity. Cough is a well-recognized manifestation of GOR, but in the few known GOR pedigrees, cough and neuropathy have not been reported. **Methods:** Twenty-seven individuals from a large Australian family and 11 members of a smaller family provided clinical information and blood for genetic analysis. Symptomatic individuals underwent neurological, gastrointestinal and respiratory investigations. To map the chromosomal location of the gene causing the disorder, a whole human genome screen with markers spaced at 10cM intervals was undertaken in the large family. **Results:** In the large family, 7 individuals had an adult onset of cough, reflux symptoms and distal sensory loss, with painless injuries in 4, and 5 others had cough or reflux without neuropathy. There were 2 definitely and 2 possibly affected individuals in the second family. Cough could be triggered by strong odours and could lead to syncope. Nerve conduction studies, sural nerve and skin biopsies in subjects with neuropathy demonstrated a sensory axonal neuropathy, with prominent small fibre loss. Autonomic function was either normal or showed mild adrenergic impairment, and sweat testing demonstrated distal hypohidrosis in some individuals. Audiometry revealed sensorineural hearing loss (5 of 12 subjects). Gastric emptying studies were normal, but some subjects had minor abnormalities of oesophageal peristalsis, with normal lower oesophageal sphincter pressures, on manometry. Twenty-four hour ambulatory oesophageal pH monitoring demonstrated multiple episodes of distal and proximal reflux, closely temporally associated with coughing. SPTLC1 DNA sequencing was normal. Linkage to hereditary neuropathy loci, CMT2A, 2B, 2D, 2E and 2F was excluded. Two-point linkage analysis between the disease phenotype and marker loci showed linkage to chromosome 3p23-24 in both families. **Conclusion:** These families represent a novel clinically and genetically distinct variant of HSN 1, associated with focal involvement of the upper aerodigestive tract and sensorineural deafness.

SMALL FIBRE NEUROPATHY STUDIES IN AN AUSTRALIAN POPULATION WITH PAINFUL BURNING FEET

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Background: Painful burning feet are a common clinical problem. In isolated small fibre neuropathy (SFN) routine nerve conduction studies (NCS) are often normal, making diagnosis difficult. Methods: We prospectively evaluated subjects with distal pain, burning or paraesthesia for evidence of a sensory neuropathy. Patients with diabetes or another definite cause of neuropathy were excluded. Patients and controls underwent clinical assessment, NCS, quantitative sensory testing (QST-CASE IV), autonomic function testing, skin biopsy for epidermal nerve fibre density (ENFD), antineuronal and anti-sulfatide antibody testing and routine blood tests to exclude an underlying cause for neuropathy. Results: Ninety-five patients were studied (age range 20-81 years, mean 55 years). The 26 controls were aged 24-79 years. The most frequent symptoms in the patients were: burning dysaesthesia (88%), paraesthesia (73%) and hyperaesthesia (66%). Twenty percent also had restless legs syndrome. The QST cold detection threshold (CT) was elevated (above 95th percentile) in 31%, and heat-as-pain threshold (HPT) was reduced (below 5th percentile) in 61% of patients. While significantly more patients than controls had an elevated CT ($p < 0.01$), there was no significant difference between the HPT of the control and affected subjects. The mean ENFD for patients (8.6 nerves per millimetre) was significantly lower than that of controls (14.5, $p < 0.0005$) and 49% of cases had an ENFD less than 8.5 (2 standard deviations below the control mean). Varicosities, or axonal swellings, were seen in 33% of patients. There was no correlation between ENFD and CT or HPT in affected subjects. Overall, the addition of a quantitative abnormality on CT or ENFD testing increased the diagnostic yield from 21% following NCS alone, to 66%. If varicosities were also included, this rate increased to 81%. Additional diagnoses in our cases included: subsequent diagnosis of cancer within the next 6 months (2 cases - 1 small cell lung carcinoma and 1 breast cancer), impaired glucose tolerance (10) and anti-sulfatide antibodies (4). Conclusion: These results are comparable to those described in other predominantly Caucasian populations. Skin biopsy for ENFD and QST (cold threshold) are minimally invasive tests that greatly improve the accuracy of diagnosis of SFN.

NON-INVASIVE MONITORING OF MACROPHAGE INFILTRATION DURING EXPERIMENTAL AUTOIMMUNE NEURITIS BY MAGNETIC RESONANCE IMAGING

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We report on a new method to assess macrophage infiltration in vivo and non-invasively by magnetic resonance imaging (MRI) during autoimmunity of the peripheral nervous system. Adoptive transfer experimental autoimmune neuritis (EAN) peaking at day 5 was induced in Lewis rats by intravenous injection of 12x 10⁶ P2-specific T-cells. Groups of rats received intracardial injections of superparamagnetic iron oxide particles (SPIO) (Resovist®) at days 2, 3, 4, 5, or 9 after cell transfer, and were scanned by MRI 24 hours later. All MR measurements were performed on a standard 1.5 T unit for human clinical investigations. The MR-protocol included a T2-w and a 3D CISS sequence in the axial plane with a slice thickness of 1 mm. SPIO accumulation in nerves leads to signal loss on T2-w images due to their paramagnetic effect. MRI revealed focal signal loss on T2-w images of the cauda equina at the preclinical stage (day 3) and at the disease onset at day 4, but signal loss already declined at the peak of clinical disease at day 5. No more signal loss was seen on days 6 and 10 after T-cell transfer. Perl's stain of nerve sections for iron detection showed focal accumulation of SPIO in cells at days 3,4, and 5 that could be identified as ED1+ monocytes/macrophages by immunocytochemistry. Spinal nerves at day 6 and 10 exhibited massive macrophage infiltrates, but no more SPIO deposition indicating lack of passive SPIO diffusion and uptake. Thus, signal loss on MRI and cellular iron deposition in AT-EAN indicate periods of active monocyte infiltration into nerves which have phagocytosed SPIO in the circulation. SPIO-enhanced MRI provides a sensitive new tool to visualize macrophage transmigration and infiltration in vivo even at a preclinical stage. This method may help to evaluate macrophage-targeted anti-inflammatory treatments in autoimmunity.

CHARACTERIZATION OF AUTONOMIC NEUROPATHY IN TRANSGENIC MICE OVEREXPRESSING HUMAN ALDOSE REDUCTASE

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Numerous studies identified the polyol pathway as an important pathogenetic factor for diabetic peripheral neuropathy. Little information is available for the role of the polyol pathway in the involvement of autonomic neuropathy in diabetes. We therefore examined the pathology of the autonomic nervous system in diabetic mice transgenic (Tg) for human aldose reductase and compared to littermate control mice (Lm). Diabetes was induced in mice at 8 weeks of age by streptozotocin and followed for 16 weeks. Celiac ganglion and mesenteric nerve were structurally examined with morphometric analysis. Effect of aldose reductase inhibitor (ARI, epalrestat; 40 mg/kg/day) was also examined. Most characteristic findings were reduction of cell size and an increase in small-sized mitochondria in celiac ganglion cells. Reduction of cell size is ascribed to the atrophy of cytoplasmic area and the nuclear size was preserved. Consistent with the changes in celiac ganglion, the mesenteric nerve was characterized by reduced mean axonal size, reduced population of axons, and increased frequency of axons with degenerative features in postganglionic fibers. Axonal pathology was exemplified by degenerative membranous profiles, ingrowth of Schwann axon networks, accumulation of organelles as well as frequent appearance of type 2 fibers. The changes in diabetic Lm were modest compared to diabetic Tg and the treatment with ARI for 12 weeks partially restored the changes in diabetic transgenic mice but not so in diabetic littermate. These findings confirmed that structural autonomic neuropathy with proximo-distal gradient in diabetes was accentuated with the activation of the polyol pathway with high expression of AR and ARI is effective for the prevention of such AR-induced changes.

PHENOTYPING OF NOVEL TRANSGENIC MODELS OF DIABETIC NEUROPATHY

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The Animal Models of Diabetic Complications Consortium (AMDCC) at the University of Michigan is developing models of diabetic neuropathy in mice via genetic engineering. Because animal models of diabetic neuropathy do not entirely duplicate human neuropathy, the AMDCC emphasizes cell-type specific knockout strategies that will predispose diabetic mice to this complication. To this end, we created a transgenic mouse with decreased superoxide dismutase expression (C57black6 SODhet). Our rationale is based on the fact that excess glucose increases oxidative stress which leads to cell damage and death resulting in diabetic complications. SOD is an intracellular enzyme that protects cells from oxidative damage. By “knocking out” this protective gene, we increase the damage caused by high glucose and should observe accelerated complications. As transgenic models are developed, neuropathy phenotyping will determine the degree of injury to neurons, axons and Schwann cells (SC). Phenotyping of neuropathy includes a) electrophysiological methods, b) morphometry of sciatic nerves and epidermal nerve fibers, and c) measurement of apoptosis. SODhet and their litter mate control mice (n = 24) were made diabetic by streptozotocin injection. Mice with a homozygous KO are not viable. Nerve conductions were assessed at 4, 12, 16 and 20 weeks following induction of diabetes. Four groups (wtC, wtD SODhetC, SODhetD) were analyzed. At 4 weeks, motor latencies in the tail were 1.9 ± 0.3 , 2.1 ± 0.2 , 1.9 ± 0.1 , 2.3 ± 0.3 . At 20 weeks, motor latencies in the tail were $2.1 \text{ ms} \pm 0.1$, $2.6 \text{ ms} \pm 0.3$, $2.1 \text{ ms} \pm 0.1$, 2.5 ± 0.4 . Results indicate changes in conduction latencies among diabetic mice; however, at these timepoints, mice lacking SOD did not display significantly greater neuropathy. Anatomical assays including fiber counts and immunohistochemical analyses of cell death and oxidative stress end products are currently being performed. This work was supported by the NIH DK60994.

FACIAL DIPLEGIA AND PARESTHESIAS: A RARE VARIANT OF GUILLAIN-BARRÉ SYNDROME

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Facial diplegia and paresthesias (FDP) is a rare variant of Guillain-Barré syndrome (Ropper, 1994). Only a few cases have been reported in the literature, and the clinical features of FDP are yet to be well-known. We conducted a retrospective observation of FDP patients. About 7,000 cases were referred to our neuroimmunological laboratory for serological tests during the past 7 years, and acute facial diplegia was a chief complaint in 40 patients. Clinical signs in 12 of the 40 patients were compatible with FDP described in Ropper's original report. Their clinical records were reviewed in order to clarify the history, neurological signs, laboratory findings, and electrophysiological data. They had progressive, relatively symmetrical bifacial weakness, distal dominant paresthesias in the limbs, and hypo- or areflexia. Other cranial nerve involvements, limb weakness, or ataxia were absent or only minimal. Clinical courses were monophasic and the nadir was reached within 4 weeks. Eleven patients had preceding infectious symptoms within 4 weeks before the onset of neurological illness. Antecedent pathogens were serologically confirmed in 3 patients: 2 with cytomegalovirus and 1 with *Mycoplasma pneumoniae*. Albuminocytologic dissociation was noted in cerebrospinal fluid (CSF) examination. Nerve conduction study showed demyelinating findings in the limbs. Sera obtained during the acute phase were tested by enzyme-linked immunosorbent assay for anti-ganglioside antibodies. Anti-GM2 IgM antibody was detected in one patient after cytomegalovirus infection. In the patients with acute facial diplegia, neurologists must take into account the possibility of FDP. The presence of antecedent illness, distal paresthesias, areflexia, CSF albuminocytologic dissociation, and demyelinating neuropathy help us to diagnose FDP.

ANIMAL MODEL OF AXONAL GUILLAIN-BARRÉ SYNDROME: DIFFERENT METHODS FOR IMMUNIZATION

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We had produced a disease model of axonal Guillain-Barré syndrome (GBS) by sensitization of male Japanese white rabbits (Yuki et al., 2001). Immunogens had been the emulsions of a bovine brain ganglioside (BBG) mixture or isolated GM1, keyhole limpet hemocyanin (KLH), and complete Freund's adjuvant (CFA). Repeated use of CFA, however, raised some concerns about the level of pain and distress inflicted on the rabbits. Whether a similar model can be induced in other species was another important issue. Therefore we tried alternative methods for the axonal GBS animal model. We investigated the following factors in rabbits: adjuvant, carrier protein, breed, and sex. A 5 mg portion of BBG was injected subcutaneously to the back of rabbits every 3 weeks, until the onset of clinical symptoms or maximum sensitization of 5 times. The endpoint was 15 weeks from beginning inoculation. All 3 rabbits developed limb weakness by the previous method. In 3 rabbits, CFA was used for the first immunization; thereafter, incomplete Freund's adjuvant (IFA) was used. All the rabbits developed limb weakness, but it took more time from the first sensitization to the onset and the paralysis tended to be mild, compared to the previous method. When methylated bovine serum albumin was used instead of KLH, only 1 of 3 rabbits showed limb weakness. Two of the 3 male New Zealand white rabbits developed tetraparesis. All 3 female Japanese white rabbits showed similar outcomes to the male rabbits. All the rabbits had plasma anti-GM1 IgG antibody, and axonal degeneration in the nerve roots, even in the rabbits without obvious symptoms. Ten male Lewis rats were immunized by 0.25 mg portion of BBG with KLH and CFA. No rats developed limb weakness or plasma anti-GM1 IgG antibody. The adjuvant, carrier protein, and rabbit breed in the previous method were more effective. Different methods, however, also could induce paralysis or subclinical disease in rabbits. Our axonal GBS rabbit model can be reproduced using IFA or New Zealand white rabbits.

ABSENCE OF PMP-22 MUTATIONS IN A FAMILY WITH HEREDITARY NEUROPATHY WITH LIABILITY TO PRESSURE PALSIES

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Introduction: Hereditary Neuropathy with Liability to Pressure Palsies (HNPP) is an autosomal dominantly inherited condition that typically presents with symptoms of recurrent peripheral nerve entrapment. 85% cases are due to a 1.5 Mb deletion of chromosome 17p11.2-p12 that results in loss of the PMP-22 gene. Ten different mutations of the PMP-22 gene that result in abnormal gene splicing or introduction of a premature stop codon have been described in cases lacking this deletion. Aim: To characterize the PMP-22 gene in individuals with HNPP lacking the 1.5 Mb deletion. Methods: Eight individuals from 5 families with the clinical and electrophysiological findings of HNPP were assessed.

Microsatellite analysis with polymorphic markers D17S122, D17S457, D17S61, D17S459, D17S2220 and D17S2230 was used to detect a 17p11.2-12 deletion and confirmed by detection of a 7.8 Kb junctional fragment following Southern blot of EcoRI/SacI digested DNA. In cases lacking the 7.8 Kb fragment the 4 PMP-22 exons were amplified by PCR and analysed by Single Strand Conformation Polymorphism (SSCP). Results: The 1.5 Mb deletion was identified in 5 individuals. Three individuals from one family all lacked the 1.5 Mb deletion, but no mutation could be identified within any of the 4 PMP-22 exons using SSCP. Neurophysiological findings could not distinguish those individuals without the PMP-22 deletion: motor studies revealed prolonged median and peroneal distal motor latencies and a reduction in ulnar nerve conduction velocity around the elbow. Sensory nerve action potentials were small/absent and sensory conduction velocities were decreased. Conclusion: In the absence of the 17p11.2-p12 1.5 Mb deletion it can be difficult to determine the molecular etiology of HNPP. We were unable to detect evidence of a PMP-22 mutation with SSCP in 3 affected individuals and we are currently directly sequencing all 4 PMP-22 exons in these patients. Haplotype analysis on other family members using microsatellite markers may determine linkage to the 17p11.2 locus.

SARCOID NEUROPATHY WITH MULTIFOCAL DEMYELINATION

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Background: Sarcoid neuropathy is classically known as axonal, and rarely demyelinating electrophysiologically. We present two patients with chronic progressive weakness associated with sarcoidosis, showing electrophysiological features of multifocal demyelination of the peripheral nerve. Cases: Two patients, a 74-year-old woman (Case #1) and a 61-year-old woman (Case #2), were referred to us for progressive weakness and numbness of the limbs. Both patients had muscle weakness and sensory involvement of the distal limbs and recurrent cranial nerve palsies over a few months. Tendon reflexes were absent in the lower limbs. In both cases, the biopsied sural nerves and muscles revealed non-caseous granulomas containing multinuclear giant cells, consistent with sarcoidosis. Corticosteroid therapy dramatically improved their neurological symptoms. Electrophysiology: Motor nerve conduction studies revealed a fall in amplitude and changes in waveform of CMAPs after proximal stimulation. MCVs were less than 35 m/s in some nerves. CMAP P/D ratios were 0.23 and 0.36 in the median nerves of case #1, and 0.55 and 0.56 in the ulnar nerves in case #2; inching studies revealed lesions with CMAP changes outside the physiological entrapment sites. While distal CMAP of some nerves progressively decreased in amplitude within a couple of weeks, low P/D ratio in many nerves persisted several weeks. Most of the conduction failures rapidly reversed after corticosteroid therapy. Conclusion: The main electrophysiological findings in the present cases consisted of considerably-small P/D ratio of CMAP and slowing of MCV, similar to those seen in acquired demyelinating neuropathy as CIDP. Since the conduction failures were reversible during the motor recovery, demyelination could be a crucial process causing muscle weakness in these cases with sarcoid neuropathy. A mechanism of demyelination in sarcoidosis is still unknown. Reference: Said et al., Brain 125:264-275, 2002.

DEATH OF PERISYNAPTIC SCHWANN CELLS LEADS TO FAILURE OF BRIDGING OF SCHWANN CELL PROCESSES AND INHIBITION OF SPROUTING IN PARTIALLY DENERVATED NEONATAL MUSCLES

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Perisynaptic Schwann cells play an important role in axonal sprouting and reinnervation in adult rat muscles upon partial denervation. Perisynaptic Schwann cells extend processes from both innervated and denervated endplates and form bridges, therefore inducing and guiding axonal sprouting (Son and Thompson, 1995; Tam et al., 2001). In contrast to adult muscles, nerve regeneration and muscle reinnervation is relatively poor in neonates. In light of the important role of Schwann cells in axonal sprouting in adult muscles and the evidence that perisynaptic Schwann cells died as a result of apoptosis in denervated neonatal muscles (Trachtengerg and Thompson, 1996), we wish to investigate whether perisynaptic Schwann cells in partially denervated neonatal muscles fail to survive and produce processes. Hence bridge formation of Schwann cell processes and in turn, axonal sprouting is inhibited. In this study, tibialis anterior (TA) muscle of 3-day-old rats was partially denervated by cutting the L4 spinal root. At 3 day partial denervation, TA muscles were removed and fixed in a solution of 30% sucrose and 4% paraformaldehyde overnight. Fourteen micrometer cryostat sections were cut and processed for triple immunofluorescent labeling with S100 and neurofilament antibodies to visualize Schwann cells and axonal sprouts, and with alpha-bungarotoxin to locate motor endplates. We found that perisynaptic Schwann cells, which are normally found at the denervated endplates in adult muscles, were not found at denervated endplates in the neonatal TA muscles. Despite the production of Schwann cell processes at the innervated endplates, no bridge formation of processes between denervated and innervated endplates was evident and hence axonal sprouting was inhibited. Our findings show that in consistence with the findings in adult muscles, bridging of perisynaptic Schwann cell processes is essential for axonal sprouting and muscle reinnervation in neonates. Without perisynaptic Schwann cell processes at the denervated endplates, Schwann cell processes at innervated endplates are not sufficient to support axonal sprouting.

ATYPICAL GUILLAIN-BARRÉ SYNDROME WITH PRESERVED TENDON REFLEXES ASSOCIATED WITH ANTIGANGLIOSIDE ANTIBODIES

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Anti-GM1, anti-GM1b, anti-GD1a, and anti-GalNAc-GD1a antibodies are associated with axonal Guillain-Barré syndrome (GBS). Although hypo- or areflexia is the clinical hallmark required for the diagnosis of GBS, the presence of axonal GBS associated with preserved tendon reflexes has been proposed (Yuki & Hirata, *Ann Neurol*, 1998). We investigated clinical features in a large number of atypical patients who showed normal or brisk tendon reflexes during the course of illness. Information on antecedent illness and neurological signs during the illness was obtained from 559 patients who had antibody reactivity against gangliosides GM1, GM1b, GD1a, and/or GalNAc-GD1a. The following signs were assessed: ophthalmoplegia, facial weakness, bulbar palsy, limb weakness, deep tendon reflexes, and deep or superficial sense impairment. The diagnosis of GBS was made for 392 (70%) and atypical GBS with preserved tendon reflexes for 67 (12%). The proportion of antecedent illnesses did not differ significantly between GBS and the atypical GBS with preserved tendon reflexes (diarrhea, 58% vs. 61%; upper respiratory tract infection, 38% vs. 36%), and that of neurological signs did not differ between them (ophthalmoplegia, 7% vs. 6%; facial weakness, 13% vs. 15%; bulbar palsy, 15% vs. 4%; distal dominant limb weakness, 52% vs. 63%; deep or superficial sense impairment, 34% vs. 30%). The frequent antecedent illness was diarrhea in both GBS and the atypical GBS with preserved tendon reflexes. Both patients showed predominantly distal weakness, and cranial nerve involvement and sensory impairment were less common. These findings together with the common autoantibodies supported our hypothesis that both conditions form continuous spectrum.

PENTOSAN POLYSULFATE (ELMIRON) INHIBITION OF NEURONAL ANTIBODY REACTIVITY

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Pentosan polysulfate (PPS) is a semisynthetic heparinoid polysaccharide derived from beechwood, that has been used orally in the treatment of interstitial cystitis (IC). PPS action in IC is thought to be related to the recovery of the protective polyanionic surface of the bladder. Many of the targets of immunological attack of nerve, are very anionic antigens like MAG, sulfatide, and sialic acid containing lipids and glycoproteins. We studied the effects of PPS on the binding of human neural antibodies by looking at the inhibition of indirect immunofluorescence with normal human nerve as a substrate. A variety of sera were examined including those from patients with Guillain-Barré syndrome, paraprotein neuropathies, systemic lupus erythematosus, and diabetes mellitus. Serum from normal patients were also studied that contained low titer IgG anti-axonal antibodies, possibly to neurofilaments. Antibodies to axonal antigens in lupus were readily inhibited by PPS, and there was a corresponding decrease in the anti-nuclear antibody localization in the nerve adventitia and endoneurium. This may imply that some of the axonal antibodies may be anti-DNA antibodies that cross react with anionic phosphorylated neurofilaments. IgM anti-MAG antibody was difficult to inhibit, due to the high avidity of pentameric IgM. When the IgM was partially reduced and alkylated to the size and avidity of IgG, PPS was effective in blocking MAG anti-MAG binding *in vitro*. The effectiveness of PPS in blocking GB and diabetic anti-neural antibodies was more variable, with IgM generally more resistant to PPS inhibition than IgG. Many of normal sera containing IgG axonal antibodies were inhibited by PPS. We are in the process of affinity purifying some of these antibodies by using PPS, and will then characterize their reactivities by more specific elisa testing. Conclusion: Pentosan polysulfate can inhibit a variety of neuronal antibodies *in vitro*. This suggests that PPS may be effective orally in blocking some antibodies to anionic antigens *in vivo*.

MULTIFOCAL MOTOR NEUROPATHY: PATHOLOGIC ALTERATIONS AT THE SITE OF CONDUCTION BLOCK

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The pathologic changes of nerves in multifocal motor neuropathy (MMN), a rare neuropathy with selective focal conduction block of motor fibers in mixed nerves, remain essentially unstudied. Fascicular nerve biopsy of 8 forearm or arm nerves in 7 patients with typical MMN was undertaken at the site of the conduction block using intra operative nerve conduction studies. Abnormalities were seen in 7 of 8 nerves: a varying degree of multifocal fiber degeneration and loss and altered fiber size distribution with fewer large fibers and many regenerating fiber clusters. Small epineurial perivascular inflammatory infiltrates were observed in 2 nerves but no evident vasculitis, inflammatory demyelination, or onion bulb formation. We conclude that nerves at sites of conduction block in MMN do not demonstrate obvious demyelination or onion bulb formation. The unequivocal multifocal fiber degeneration and loss and regeneration at sites of conduction block does not explain the observed conduction block but explains the observed denervation, muscle weakness and atrophy. The occurrence of conduction block and multifocal fiber degeneration at the same sites suggests that the two processes are linked, with functional conduction block perhaps a precursor to motor fiber degeneration.

EXPRESSION PROFILING OF SCIATIC NERVE IN A CMT1A MOUSE MODEL

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The hereditary peripheral neuropathy CMT1A is often characterized by the duplication of the gene encoding peripheral myelin protein 22 (PMP22). The molecular mechanisms underlying the pathogenesis are unknown. We have used the CMT1A mouse model system C22 (with 7 copies of the human gene) for expression profiling of the sciatic nerve using SAGE (serial analysis of gene expression). SAGE libraries of roughly 10,000 tags were made from normal (wild-type) and the diseased (transgenic) nerves. Comparison of the expression patterns of these nerves shows that 263 tags are significantly differentially expressed ($p < 0.05$, tagcount at least 4 per 10K in one of the libraries). Of these, 103 tags are upregulated, of which 89 hit on a single gene. These are 41 known and 48 unclassified (ESTs or not found in database) genes. Downregulated are 160 tags, containing 141 single gene hits, with 99 known and 42 unclassified. The upregulated set contains genes implicated in signaling and apoptosis. However, the most significantly upregulated tags represent no-matches, that do not identify any gene in the databases. The downregulated set contains genes for myelin, mitochondrial, extracellular matrix and cell structure components. A similar comparison of SAGE libraries has been made from cultured Schwann cells derived from these mice (of roughly 35K tags each). This showed that 159 tags are differentially expressed with $p < 0.05$, with 75 tags up- and 84 tags downregulated. Apart from mitochondrial matches and ribosomal proteins these contain very diverse functions (ECM, cytokines, signaling). The follow-up of these profiling exercises is in progress, and focuses on the expression of some of the regulated genes in different genetic backgrounds in time. To this end the C22 mouse has been crossed back to genetic homogeneity into FVB and C57/B6 strains. The analysis of the SAGE data is done by Northern blot analysis and RT-PCR, as well as immunohistochemistry on cross-sections from paraffin-embedded sciatic nerves dissected on different postnatal time-points.

EFFECT OF IVIg ON CIRCULATING LEVELS OF CYTOKINES IN MULTIFOCAL MOTOR NEUROPATHY

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Intravenous immunoglobulins (IVIg) are successfully used as immunomodulatory therapy in patients with multifocal motor neuropathy (MMN) but their mechanism of action remains unknown. An anti-idiotypic block of pathogenic autoantibodies has been often postulated even if other possible mechanisms, including a modulation of the release of various cytokines, have been proposed. To evaluate the possible effect of IVIg on the expression of cytokines in MMN, we first determined circulating levels of TNF α , INF γ , IL2, IL4, IL10, IL12 by ELISA in the sera of 17 patients with MMN and compared them with 12 patients with amyotrophic lateral sclerosis (ALS), 12 with multiple sclerosis (MS), 6 with chronic inflammatory demyelinating polyneuropathy (CIDP), 5 with myasthenia gravis (MG) and 12 healthy controls (NS). Comparable levels of INF γ , IL2, IL4, IL10 and IL12 were detected in patients' sera and controls. Even if TNF α levels did not differ significantly among patients' groups, they were higher than in any healthy control (mean + SD 1.2 + 0.5 pg/ml, range 0.7-2.4 pg/ml), in 12 (70%) MMN patients (mean + SD 3.6 + 1.9 pg/ml; range 0.2-7.5 pg/ml), all ALS, 3 MS (25%), 2 CIDP (40%) and 2 MG (40%). We then measured the concentration of TNF α before and after IVIg therapy in 9 MMN and 2 ALS patients. In all but one MMN patients, circulating levels of TNF α slightly increased after treatment with IVIg (mean values 4.3 vs 7.2 pg/ml) and decreased 3 weeks after therapy while in both ALS patients they decreased or remained unchanged. No detectable level of TNF α was found in IVIg preparation. Similarly to what previously reported in other autoimmune neuropathies as GBS and CIDP, TNF α serum levels are slightly increased in MMN but, at odds with what reported in these disease, their concentration tend to increase parallel to clinical improvement after IVIg therapy. Further studies are necessary to clarify the pathogenetic implication of this finding and in particular whether a possible deviation from a presumed Th2 to a Th1 immune response may help explaining the effect of IVIg in MMN.

LONG-TERM DISABILITY IN MULTIFOCAL MOTOR NEUROPATHY AND ITS RELATION TO IVIg THERAPY

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Multifocal motor neuropathy (MMN) is characterized by a slowly progressive distal asymmetric limb weakness, mostly affecting upper limbs. The majority of MMN patients improves with high-dose intravenous immunoglobulins (IVIg) even if their effect on the long-term disability is still unclear. We report on the long-term outcome of 22 patients with MMN (mean age of onset 40 years; range 21-62) first examined by us between 1990 and 2000. In all patients the severity of neuropathy was assessed using a modified Rankin disability scale (score 0-5) and a functional impairment scale for upper (UL) and lower (LL) limbs (score 0-5, each). At entry, after a mean duration of symptoms of 9.3 years (range 0.25-25 years) the mean Rankin and UL+LL scores were 2.2 and 3.7, respectively. Fourteen patients (63%) were unable to perform some manual activities (UL score >2) including 7 (31%) with a Rankin score >2 (symptoms significantly interfering with lifestyle). The mean duration of symptoms was higher in disabled (12.2 years) than in non-disabled patients (5.3 years). None had LL score >2. Of the 22 patients initially examined, 19 (86%) were followed until January 2003. All but one patient were treated during follow-up with periodic IVIg infusion for a mean of 5.6 years (range 3-12). By the end of follow-up, after a mean duration of symptoms of 15.2 years (range 4-35, at least 5 years in 21 patients and 10 years in 17), the mean Rankin and UL+LL scores were 1.8 and 2.7, respectively. Six patients (27%) had an UL score >2 and 4 (18%) a Rankin score >2. The disability rates at 5, 10, 15 and 20 years from neuropathy onset were 5, 18, 33 and 42%, respectively, with a considerable difference between treated and untreated patients at each interval (0 vs. 6%, 0 vs. 25%, 14 vs. 60% and 25 vs. 66%). Our findings indicate that MMN induce a progressive disability in the majority of patients after several years and that IVIg therapy is effective in preventing the long-term disability in most of them.

TWO PATIENTS WITH HEREDITARY MOTOR AND SENSORY NEUROPATHY TYPE 1A AND INITIAL PRESENTATION OF CLINICAL AND ELECTROPHYSIOLOGICAL FASCICULATIONS

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Muscle fasciculations, either clinical or electrophysiological, have been associated with endocrinological, metabolic disorders and motor neuropathies as well as the benign fasciculation syndrome. The presence of fasciculations in predominantly demyelinating disorders has not been reported in adults with hereditary causes of neuropathy. We report two patients with Hereditary Motor and Sensory Neuropathy type IA, confirmed by genetic testing, whose initial clinical presentations consisting of prominent spontaneous fasciculations of long duration. Evidence of fasciculations was found both clinically and electrophysiologically. We propose that motor neuron instability secondary to the primary demyelinating pathology in these patients led to the prominent fasciculations in this unique presentation of a common disorder.

DRIVING PERIPHERAL SENSORY AXON REGENERATION WITH LOW DOSE INTRATHECAL INSULIN

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Background: In the two-compartment culture model *in vivo*, Mearow and colleagues suggest that growth factor support of regenerating neurons may depend upon its delivery site. We have found that low dose systemic insulin accelerates axonal regeneration. Intrathecally-delivered insulin is taken up by dorsal root ganglia, with insulin receptors expressed on perikarya and on regrowing axons. Methods: We studied the impact of intrathecal low dose insulin on regeneration of CGRP expressing sural axons distal to a crush in Sprague-Dawley rats. Chronic delivery (0.1 IU regular insulin daily or saline carrier) was provided using an ALZET mini-osmotic pump with a catheter inserted at the L6/S1 level and longitudinal sections through and distal to the crush zone underwent counting of CGRP labeled profiles. Results: Intrathecal insulin delivery was associated with greater numbers of regenerating profiles distal to the crush zone compared to rats given saline carrier. Insulin similarly increased CGRP expression in sensory neuron perikarya but had no apparent impact on ipsilateral intact central branch dorsal horn CGRP expression. The benefits of intrathecal delivery appeared to exceed that of its action when given near nerve, but awaits further analysis. Conclusions: Central intrathecal delivery of a growth factor *in vivo* is capable of driving peripheral regeneration of sensory fibers. In this case, benefits from insulin may accrue from shared actions with IGF-1 signaling and may either reflect rescue of sensory neurons from retrograde loss, or more rapid sprouting from surviving neurons. Supported by CIHR, CDA and AHFMR.

LONG TERM EXPERIMENTAL STREPTOZOTOCIN-INDUCED DIABETES IN MICE: ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL FEATURES

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Background: In previous work, we have suggested that murine streptozotocin (STZ)-induced diabetes may offer significant advantages over the more common rat model because of accelerated neuropathy. The model may offer a starting point for understanding how superimposed diabetes may interact with transgenic models. Methods: We studied serial electrophysiological measures of sciatic-tibial motor conduction velocity (MCV), sciatic-tibial interosseous compound muscle action potential amplitudes (CMAPs), caudal sensory conduction velocity (SCV) and mixed caudal nerve action potential amplitudes (NAPs) in STZ diabetic (glucose >16.0 mmol/L) male Swiss mice and littermate controls given the STZ citrate carrier alone. These findings were associated with quantitative morphological assessment of axon numbers, caliber and myelin thickness. Results: By four weeks, there were declines in SCV and NAPs. MCV declined by 6 weeks and CMAP amplitudes fell by 22 weeks of diabetes. Accompanying the electrophysiological changes were early macrophage infiltration, myelin thinning, axon atrophy and loss of distal axons. Conclusions: Murine STZ-induced diabetes is associated with robust features of experimental neuropathy that can be addressed with electrophysiological and morphometric approaches. These features render a more useful accelerated model than similar work using rats and may more closely resemble human polyneuropathy. Supported by CIHR and AHFMR.

CLOSE OBSERVATION OF CHRONOLOGICAL CHANGES OF ANTI-GQ1b IgG ANTIBODY TITER IN MILLER FISHER SYNDROME AND GUILLAIN-BARRÉ SYNDROME WITH OPHTHALMOPLEGIA, WITH SPECIAL REFERENCE TO ITS PATHOGENIC ROLE TO OPHTHALMOPLEGIA

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Objective: To investigate the pathogenic role of anti-GQ1b IgG antibody in ophthalmoplegia in MFS and GBS based on the close chronological observation of the antibody titers. **Materials and Methods:** In 8 patients with MFS and GBS with ophthalmoplegia, the first and follow-up serum samples were obtained within 6 and 14 days, respectively, after the appearance of ophthalmoplegia. In 4 of them, serum samples were taken during progressive phase of ophthalmoplegia, and the relationship between chronological change of the antibody titers and clinical symptoms was studied. Anti-GQ1b IgG antibody was measured by ELISA. **Results:** In 7 of the 8 patients in whom the first serum was taken after the appearance of ophthalmoplegia, the highest antibody titer was seen in the first sample, which decreased over time. Further, 3 of the 4 patients who had serum samples taken during progressive phase of ophthalmoplegia showed that the antibody titer was in the decreasing phase while the symptoms were worsening. In the patient whose first sample was taken prior to the appearance of ophthalmoplegia, the antibody titer had already elevated before ophthalmoplegia occurred, and then decreased gradually as ophthalmoplegia appeared and the symptoms progressed. **Discussion:** Our results found that the serum anti-GQ1b IgG antibody is elevated prior to the appearance of ophthalmoplegia and has already entered a decreasing phase while the symptoms continue to worsen. These findings suggest that the antibody is not produced secondarily as a result of tissue damage, but rather in relation to a preceding infection. Further, it may assume a pathogenic role as a trigger during the early phase of the process of ophthalmoplegia in patients with MFS and GBS.

FK506 ENHANCES TARGET ORGAN REINNERVATION BY REGENERATION AND BY COLLATERAL SPROUTING OF PERIPHERAL NERVE FIBERS

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We examined the effects of FK506 administration on the degree of target reinnervation by regenerating axons (following sciatic nerve crush) and by collateral sprouts of the intact saphenous nerve (after sciatic nerve resection) in the mouse. FK506-treated animals received either 0.2 or 5 mg/kg/day, dosages previously found to maximally increase the rate of axonal regeneration in the mouse. Functional reinnervation of motor, sensory and sweating activities was assessed by noninvasive methods in the hindpaw over a one-month period following lesion. Morphometric analysis of the regenerated nerves and immunohistochemical labeling of the paw pads were performed at the end of follow-up. In the sciatic nerve crush model, FK506 administration shortened the time until target reinnervation and increased the degree of functional and morphological reinnervation achieved. The recovery achieved by regeneration was greater overall with the 5 mg/kg dose than with the dose of 0.2 mg/kg of FK506. In the collateral sprouting model, reinnervation by nociceptive and sudomotor axons was enhanced by FK506. Here, the field expansion followed a faster course between 4 and 14 days in FK506-treated animals. In regard to dose, while collateral sprouting of nociceptive axons was similarly increased at both dosages (0.2 and 5 mg/kg), sprouting of sympathetic axons was more extensive at the high dose. This suggests that the efficacy of FK506 varies between subtypes of neurons. Taken together, our findings indicate that, in addition to an effect on rate of axonal elongation, FK506 improves functional recovery of denervated targets by increasing both regenerative and collateral reinnervation.

ALTERED EXPRESSION OF ION CHANNEL ISOFORMS AT THE NODE OF RANVIER IN P0 DEFICIENT MYELIN MUTANTS

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The node of Ranvier is the critical functional compartment for the generation and saltatory propagation of action potentials along myelinated nerve fibers. In order to study the impact of myelinating Schwann cells on the molecular architecture of the node of Ranvier in the peripheral nervous system, we investigated the nodal expression of distinct voltage gated sodium channel (VGSC) isoforms and the localization of paranodal and juxtaparanodal cell membrane proteins in a severely affected Schwann cell mutant. We chose the mouse deficient in the major peripheral myelin adhesion molecule P0, which displays abnormal myelin formation and impaired myelin compaction. Using immunohistochemistry on single fiber preparations, we found that in these mutants, nodal VGSC clusters reminiscent of developmentally retarded stages were abundantly present. In addition, mutant motor nerves displayed an ectopic expression of the tetrodotoxin (TTX)-resistant Nav1.8 isoform at almost all nodes, where it is coexpressed with the nearly ubiquitous TTX-sensitive Nav1.6 isoform. Furthermore, asymmetry or even absence of the paranodal Caspr protein was a characteristic feature of the myelinated nerve fibers in this mutant. Voltage gated potassium channels and Caspr2 were not confined to juxtaparanodes as typical for normal axons, but often showed an overlap with paranodal sites, reminiscent of an arrested development. Thus, intact myelin formation is an important prerequisite for the expression of the proper VGSC isoforms at the node, of Caspr at the paranodal junction and of K⁺-channels/Caspr2 at the juxtaparanodal region.

A PATIENT WITH POEMS SYNDROME WHO RESPONDED TO PLASMA EXCHANGE AND CORICOSTEROIDS

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A 51-year-old lady with POEMS syndrome is described. She presented with progressive cachexia, generalised weakness to the point of being bed-ridden, persistent fever, massive hepatosplenomegaly, severe ascites, peripheral edema, hyperpigmentation, scleroderma-like skin changes, clubbing, optic disc swelling, hypothyroidism, hypocortisolism, leucocytosis and thrombocytosis. Upper limb nerve conduction velocity was in the range of 25 m/s. Compound muscle action potentials (CMAPs) were severely diminished. Lower limb nerves were inexcitable. Sensory potentials were absent. IgA lambda chain was detected on immunofixation. Bone marrow examination revealed 3-5% plasma cells, immunostains confirming light chain restriction. Skeletal survey was normal. Liver biopsy showed nodular hyperplasia. Histology of the spleen was consistent with Castleman's syndrome. Vasoactive endothelial growth factor (VEGF) level was grossly elevated. The patient was treated with five plasma exchanges followed by high dose steroids (1 mg/kg). Over the next few weeks she made dramatic recovery. The ascites and edema settled. She regained her strength and started walking unaided. There was reduction in the VEGF levels. The paraprotein has remained stable. CMAPs, sensory potentials and conduction velocities, however, remained the same on repeat nerve conduction tests. The dose of prednisolone is being gradually reduced. Treatment options, their putative mechanism; and the role of VEGF in the manifestations of the disease will be discussed.

CAN STUDYING SMALL FIBER FUNCTION IDENTIFY A PRE-CLINICAL STAGE OF DIABETIC POLYNEUROPATHY?

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Analogous to microalbuminuria, that predicts development of diabetic nephropathy, the identification of a pre-clinical phase of diabetic polyneuropathy (DPN) may have important therapeutic implications as any treatment that is started after axonal degeneration is well-advanced is unlikely to be effective. We undertook to study small fiber function by intraepidermal nerve fiber (IENF) quantitation and autonomic function tests in asymptomatic and minimally symptomatic diabetic patients, namely stage 0 of the Michigan Diabetic Neuropathy Class (MDNC). In addition, we wanted to establish a method of ascertaining subtle but definite early neuropathy that could be used as an outcome measure in therapeutic trials. IENF quantitation of skin biopsies (stained for PGP 9.5) at the ankle and upper thigh was compared with controls. Resting heart rate, heart rate variation with standing, deep respiration and during the Valsalva maneuver were recorded. Blood pressure change on standing, isometric exercise and cold pressor stimulus was measured. Sympathetic skin response was recorded at the palm and sole. These values were compared with age-matched controls published in the literature. To date, we have found autonomic function tests to be normal in MDNC 0 patients. Average IENF quantity at the upper thigh is similar to the controls (59.7 ± 18 and 68.9 ± 17 , respectively, $p=0.37$). However, IENF at the ankle, although still within normal limits, is appreciably lower in MDNC 0 patients compared to controls (31.4 ± 13 and 49.7 ± 17 , respectively, $p=0.05$). The ratios of IENF numbers at upper thigh to ankle are 2.03 for patients and 1.44 for controls ($p=0.04$). It appears that even in asymptomatic and minimally symptomatic diabetic patients, cutaneous small fiber function may be subtly disordered. The ratio of IENF quantity at the upper thigh/ankle may be a sensitive marker of early diabetic neuropathy and conceivably could be used to track progression especially in a therapeutic context.

DIAGNOSTIC VALUE OF NERVE BIOPSY FOR ATYPICAL CHRONIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY: EVALUATION OF 8 CASES

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The objective of the study was to define how a nerve biopsy could be helpful in identification of atypical cases of chronic inflammatory demyelinating polyneuropathy (CIDP). An ad hoc committee in 1991 defined the clinical, electrophysiological and pathological criteria for diagnosis of CIDP. In common with other authors, we regard the rather specific electrophysiological criteria as being too restrictive, and we think that a significant number of patients may therefore not benefit from effective treatment or be excluded from therapeutic trials. The Inflammatory Neuropathy Cause and Treatment (INCAT) group (2001) has proposed new electrophysiological criteria of CIDP, which are more sensitive and do not lose any specificity. Over a period of three years (January 1999 to December 2001), we classified 44 patients into two categories: those presenting the strict criteria of the ad hoc committee and those who we regarded as cases of CIDP who did not meet these strict criteria. All these patients benefited from one or more clinical and electrophysiological examinations; extensive biological workup and genetic study when appropriate excluded other causes of neuropathy. Nerve biopsies were taken from all patients and samples were included in paraffin and epon for systematic light and electron microscopic examination. Out of 44 patients, 24 fulfilled the INCAT electrophysiological criteria with only 12 of these cases fulfilling the criteria of the ad hoc committee. Eight patients did not fulfill any of the widely accepted electrophysiological criteria of CIDP. However, study of nerve biopsies of these eight patients revealed histological features characteristic of CIDP according to histological criteria (AAN - 1991). Among these patients, six have been treated and five responded favorably to conventional treatments for CIDP. Without information from the nerve biopsy, these patients would not have been treated effectively because their electrophysiological profile was indicative of axonal impairment interpreted erroneously as primary.

ANTI-GANGLIOSIDE IgG IN SERA FROM GUILLAIN-BARRÉ PATIENTS INDUCES POTENT LEUKOCYTE EFFECTOR FUNCTIONS

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IgG antibodies specific for glycolipid constituents of peripheral nerves (gangliosides) are detected in sera from a substantial percentage of Guillain-Barré patients. The presence of these antibodies has been shown to be associated with severity of disease, and antibodies of different specificities may be related with specific subtypes of Guillain Barré syndrome (GBS). The role of ganglioside-specific antibodies in GBS pathogenesis has not been elucidated completely, but they may contribute to nerve damage in addition to leukocyte infiltrates and complement deposits that have been detected previously. IgG with sufficient affinity for the antigen is capable of inducing complement activation and activation of leukocytes via leukocyte IgG receptors (Fc γ R). Therefore, we studied the capacity of anti-ganglioside IgG with different specificities (GM1, GD1a, GM2, GQ1b) to induce leukocyte inflammatory functions. Control sera did not induce leukocyte activity, whereas GM1 and GD1a specific IgG from 50-70% of seropositive cases readily induced leukocyte effector functions such as degranulation and phagocytosis. There was no clear relation between antibody titers and the extent of activation detected. Blocking of Fc γ R with specific monoclonal antibodies abrogated leukocyte function completely, indicating that Fc γ R are crucial effector molecules in this process. The presence of 5-10 mg/ml pooled non-specific IgG ('IVIg') significantly attenuated degranulation and phagocytosis, which may suggest an immune modulatory role of IVIg via leukocyte Fc γ R during IVIg treatment of GBS. GM2 and GQ1b specific IgG induced leukocyte effector functions less efficiently than GM1 specific IgG despite high titers. These findings suggest functional heterogeneity of GBS-associated ganglioside-specific IgG and suggest that leukocyte Fc γ R constitute effector molecules in GBS pathogenesis as well as targets for immunotherapy.

LONGTERM IV IMMUNOGLOBULIN TREATMENT IN CIDP

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Objective: Chronic inflammatory demyelinating polyneuropathy (CIDP) can improve after intravenous immunoglobulin (IVIg) treatment. Most patients need intermittent IVIg to maintain improvement. Limited information is available on factors related to improvement. This study aims to analyse these factors especially in relation to longterm treatment and prognosis. Methods: Data were collected from all CIDP patients known at the Erasmus Medical Centre in Rotterdam, treated with IVIg and followed for at least 2 months. 50 clinical and laboratory parameters were analysed for a possible relation with improvement (Rankin scale). Patients presently needing longterm treatment received a questionnaire about their experiences with IVIg treatment. Results: 64 males and 31 females were followed for a period ranging from 2.5 months - 19.5 years (median 4.0 years). 15 patients received additional treatment to reduce the amount of IVIg. 77/95 (81%) patients showed improvement after start of IVIg. 66/77 (86%) patients needed intermittent IVIg treatment for at least 2 months, suggesting that improvement was not due to a spontaneous remission. Improvement was related with a relapse in the past (all 12 patients improved), progressive weakness until treatment and no discrepancy in weakness between arms and legs. Mean time on IVIg treatment until remission was 3.5 years (median 2.1 years). Patients with sensory-motor disturbances ($p=0.002$; HR 3.2) and a relative short duration of weakness ($p=0.008$; HR 2.6) had a higher chance to reach remission after discontinuation of IVIg. 10% of patients needed IVIg for a period over 8.7 years (maximum 19.5 years). Severe side-effects were not seen. Most patients needing longterm IVIg treatment (once every 2-6 weeks) were satisfied and not eager to switch to another treatment. Conclusion: Most patients need IVIg for a long period to maintain good clinical condition. Especially due to high costs, it may be justified to switch or add another immunomodulatory drug in a rather early stage of disease. Since most patients were treated with IVIg only, this study may serve as a national history survey of CIDP during longterm IVIg treatment.

DIFFERENCES BETWEEN RELAPSING GUILLAIN-BARRÉ SYNDROME AND ACUTE CIDP

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Introduction: The course of disease in inflammatory demyelinating polyneuropathies seems to be a continuum. Patients with Guillain-Barré syndrome (GBS) can have consecutive episodes of progressive weakness. It can be difficult to distinguish treatment related clinical fluctuations/relapsing GBS (rGBS) from chronic inflammatory demyelinating polyneuropathy with acute onset (ACIDP). Objective: Prognosis and treatment of rGBS and ACIDP differ considerably. The aim of this study was to identify characteristics that help to differentiate between rGBS and ACIDP. Patients and Methods: Records of all GBS and CIDP patients, fulfilling the international criteria and admitted to or evaluated at the Erasmus MC between 1987 and 2000 were screened. A case was defined as rGBS when deterioration (at least one f-score) occurred after initial improvement (at least one f-score compared to the f-score at nadir during the first episode). A case was defined as ACIDP when the nadir of the first episode was within 8 weeks from onset, and the consecutive course was like CIDP. We scored the number of fluctuations/relapses, time to reach nadir, severity of the relapse, time between relapses, duration of improvement and duration of decline. We took account of sex, age and therapy as possible confounders. Results: 13/190 (7%) GBS patients suffered from a treatment related clinical fluctuation or a relapse. 8/90 (9%) CIDP patients had acute onset of disease. The course of ACIDP was characterized by more relapses in the first year and longer duration between the relapses compared to rGBS. GBS patients with a subsequent relapse after a period of 10 weeks from onset, generally turned out to have ACIDP. An increase in severity during subsequent relapses was found in ACIDP, but not in rGBS. There was no difference between age and sex distribution between the two groups. Conclusions: The suspicion of ACIDP in a patient previously considered as GBS should rise when 1) a subsequent relapse occurs after ten weeks from onset; 2) after a third relapse; 3) when a subsequent relapse has a more severe course.

TREATMENT APPROACHES AND LONG-TERM OUTCOME IN A CANADIAN COHORT (1972-2002) WITH CHRONIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY (CIDP)

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The treatment of CIDP has evolved with the addition of intravenous immunoglobulin (IVIg) and plasmapheresis (PE) to corticosteroids as therapeutic options. To determine treatment outcome based on initial therapy, we conducted a retrospective review of individuals diagnosed with CIDP (n=90; 55 males) followed at the LHSC Neuromuscular Clinic for at least 6 months. Forty-two patients with CIDP-MGUS were excluded from the analysis. Mean age at onset of CIDP was 42.9 years (range 3-75) with a mean follow-up of 69.3 months (range 6-336); 43 patients were reassessed within 12 months prior to analysis. Associated conditions included diabetes mellitus (10%) and other autoimmune disorders (22%). For the cohort, the initial treatments were IVIg (n=43), prednisone (n=19), plasmapheresis (n=19) or combined therapy (n=5). Four patients improved spontaneously. Of the 43 patients initially treated with IVIg monotherapy, 29 (67%) improved, while 14 (33%) failed to respond. 10/29 responders (34%) achieved remission with IVIg pulse treatment alone, the remaining 19 patients (66%) were treated with additional immunomodulation, including prednisone, PE, azathioprine, cyclophosphamide or cyclosporine. In comparison, 14/19 (74%) patients improved with PE alone, while 5/19 (26%) failed to respond. Of the responders, 2/14 (14%) went into remission with PE alone, and 12/14 (86%) required an adjuvant agent, most commonly prednisone. In patients initially treated with prednisone, 9/19 (47%) improved with prednisone alone, whereas 10/19 (53%) required additional therapies. Overall, 62/73 (85%) patients treated with prednisone at any time responded favorably. At last follow-up, 56% of the IVIg group, 73% of the PE group and 47% of the prednisone group were in remission and off all therapies or on low maintenance dose prednisone. Overall, the response rate to initial treatment was 66% as documented by improvement in the Neurological Impairment Score and Clinical Grade [transformed to a modified Rankin score (mRS)]. At last follow-up 77/90 (86%) CIDP patients had a mRS score of 0 or 1 (no functional disability), with 68% improved by at least one mRS grade. With a unified approach to therapy, the long-term prognosis for patients with CIDP is excellent, however the majority require individualized therapy with more than one agent.

CLINICAL DISEASE SEVERITY, ABNORMAL MYELINATION AND AXONAL DYSFUNCTION IN A MOUSE MODEL FOR HMSN IA

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BACKGROUND: HMSN Ia is known as a primarily demyelinating neuropathy, most often caused by a duplication of the PMP22 gene. From our own baseline results in a cohort of HMSN Ia patients and from the literature, evidence is accumulating that (i) abnormal myelin status in adulthood is the result of initial dysmyelination, with afterwards de- and remyelination, (ii) clinical signs and symptoms develop due to axonal dysfunction and degeneration, and (iii) the extent of axonal involvement is dependent on the extent of initial dysmyelination. Genetic modifiers may be involved in disease severity. To further substantiate these findings, we used a mouse model (C22) with seven copies of the human PMP22 gene. **METHODS:** After backcrossing the C22 line to genetic homogeneity in the C57BL/6 and FVB strains, impairment and behavior, electrophysiology and pathological anatomy were assessed in mice overexpressing PMP22 and wildtype littermates (WT) in a longitudinal study with timepoints from 3 to 48 weeks after birth. **RESULTS:** With a modified behavioral assessment score (SHIRPA) significant differences were detected between PMP22 and WT mice at all timepoints. Disease progression was found in the PMP22 mice. In these mice nerve conduction velocities were markedly reduced at 3 weeks after birth and remained unchanged. Compound muscle action potential amplitudes were reduced at all timepoints in PMP22 as compared to WT mice. There were no strain differences between FVB and C57BL/6. In the C57BL/6 PMP22 mice electron microscopy showed a severe hypomyelination at 3 weeks with some increase in myelin thickness during life and a smaller axonal caliber at 3 weeks, which did not increase as in the WT mice at later timepoints. Immunofluorescence in the C57BL/6 PMP22 mice indicated changes in phosphorylated and non-phosphorylated neurofilaments. Further substantiation of axonal involvement during disease progression is currently under investigation. **CONCLUSIONS:** In a mouse model for HMSN Ia, clinical disease is detectable early with progression later in life. Hypomyelination is the initial feature, which seems to render the axon more vulnerable. As there were no major phenotypic differences between PMP22 FVB and C57BL/6, a further search for genetic modifiers in these strains is not justified.

RAPID ONSET OF OXIDATIVE STRESS FOLLOWING HYPERGLYCEMIA MEDIATES PERIPHERAL NEURONAL DEGENERATION

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Peripheral neuropathy, the most common complication of diabetes, has been linked to hyperglycemia-induced oxidative stress. Complete characterization of the development of oxidative stress and the neuronal innate defense systems will allow better understanding of the disease pathogenesis. This study takes a multifaceted approach to defining the progression of oxidative stress in animal and cell culture models of diabetic neuropathy. Cultured dorsal root ganglia (DRG) neurons provide a model for hyperglycemia-susceptible peripheral neurons that undergo programmed cell death, demonstrated by caspase 3 cleavage and TUNEL staining, following the application of as little as 5-20 mM glucose above baseline. Fluorimetric real-time analysis of reactive oxygen species (ROS) in cultured DRG demonstrated that hydrogen peroxide (using DCFDA), superoxide (using DHE) and nitric oxide (using DAF-2DA) all rapidly increased by 100% approximately 5 h following the application of 20 mM extra glucose. The end-products of oxidative stress malondialdehyde, 8-isoprostane F₂, 4-hydroxynonenyl, and nitrotyrosine significantly increased in cultured DRG exposed to hyperglycemia. Cellular antioxidant systems such as reduced glutathione, catalase and superoxide dismutase initially increased in cultured DRG within 3 h but longer-term studies demonstrated a significant decrease in these molecules that can avert oxidative stress. Most interestingly, a two-hour exposure to hyperglycemia produced a similar degree of injury as 6 h or longer periods, demonstrating that a post-prandial peak in plasma glucose is sufficient to produce neuronal degeneration. Taken together the studies provide strong evidence that oxidative stress is an early and robust response to hyperglycemia in peripheral neurons. Similar to other complications-prone tissues, therapies to promote the cellular antioxidant status may provide clinical benefit for diabetic patients.

TOPIRAMATE IMPROVES *IN VITRO* AND *IN VIVO* MEASURES OF NERVE FIBER LOSS IN PATIENTS WITH DIABETIC NEUROPATHY

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The degree of *in vitro* neuronal cell death in response to pro-apoptotic factors in sera from patients with diabetic neuropathy correlates with specific fiber losses *in vivo*. In addition to pain and impaired pain/thermal perception, C-fiber damage adversely affects skin blood flow, with severely impaired skin perfusion in type 2 diabetes (T2DM) co-segregating with features of the dysmetabolic syndrome. Topiramate (TPM) is a neurotherapeutic that appears to prevent neuronal apoptosis and stimulate neuronal growth. We conducted *in vitro* and *in vivo* evaluations of TPM's effects on nerve growth and function in patients with diabetic neuropathy. To assess the effects of TPM on pro-apoptotic factors in diabetic sera, N1E-115 murine adrenergic neuroblastoma cells were cultured in 10% serum, either PHS or serum from diabetic neuropathy patients (N=18). 100 ng/mL TPM (dose giving maximal antiapoptotic effect in dose-response study) reduced diabetic sera-induced apoptosis (from 16.5% to 4.1%); apoptosis was significantly reduced in 8 of 8 patient sera-treated cultures ($14.2 \pm 4.7\%$ reduction). 100 ng/mL TPM significantly increased cell growth: Day 1, $176 \pm 19\%$ without TPM vs. $217 \pm 23\%$ with TPM ($p < 0.01$); Day 2, $226 \pm 24\%$ vs. $275 \pm 31\%$ ($p < 0.03$). In a separate 8-week open-label trial in 11 patients with T2DM (60 ± 2 yrs; BMI 32 ± 2 ; C-peptide 2.35 ± 0.5 mg/mL), C-fiber neuropathy was diagnosed by total neuropathy scores, nerve symptom scores, and quantitative sensory tests. Intra-epidermal nerve fiber density (IENF) was determined by immunohistochemical staining. TPM was titrated to 400 mg/day or maximum tolerated dose. After 8 weeks' treatment, TPM significantly increased IENF ($p < 0.04$), dendrite length ($p < 0.04$), and conduction amplitude ($p < 0.04$), and improved symptoms of C-fiber dysfunction ($p = 0.04$). Metabolic parameters (HbA1c, $p < 0.04$; total cholesterol, $p = 0.002$) were also significantly improved. Side effects were consistent with clinical experience, with no serious side effects reported. These findings suggest that TPM may reverse cytotoxic effects of sera and improve/restore neuronal function in patients with diabetic neuropathy. Supported by a grant from Johnson & Johnson Pharmaceutical Research & Development.

DISRUPTION OF ENDONEURIAL MICROVESSELS IN AGE-INDUCED NEUROPATHY IN RATS

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To explore the role of advanced glycation end-products (AGE) in the pathogenesis of diabetic neuropathy, we examined the effects of AGE exogenously administered on the peripheral nerve function and structure in normal rats. In this study, purified AGE prepared by incubation of glucose with bovine serum albumin (BSA) at 200 mg/kg (high-AGE) were intra-peritoneally injected for 12 weeks to normal rats. Control group received BSA. Effects of aminoguanidine (AG) (50 mg/kg/day) were also examined. At end, serum AGE levels were 3.2 fold higher in AGE-treated group accompanied by significant delay of motor nerve conduction velocity (MNCV) by 42% compared to BSA group ($p < 0.001$). AGE-treated group showed 40% reduction in nerve Na,K-ATPase activity and increased plasma levels of thrombomodulin, a marker of endothelial injury. Immunohistochemistry revealed an intensified expression of 8-hydroxydeoxyguanosine (8OHdG) and NF- κ B in the nuclei of endothelial cells and Schwann cells in AGE-treated group and these reactions were significantly inhibited by AG treatment. Submicroscopic analysis demonstrated vacuolated endothelial changes in AGE-treated animals. While AG treatment significantly improved MNCV delay and Na,K-ATPase levels as well as 8OHdG expression, serum AGE and thrombomodulin levels were not influenced by AG. We conclude that AGE have a pathogenetic role in the development of diabetic neuropathy by disrupting endoneurial microvasculature and enhancement of oxidative stress.

DISTINCT TIME PATTERN OF COMPLEMENT ACTIVATION AND CYTOTOXIC T-CELL RESPONSE IN GUILLAIN-BARRÉ SYNDROME

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Humoral and cellular immune mechanisms contribute to the pathogenesis of Guillain-Barré syndrome (GBS). Activation of complement has been implicated in the initiation of myelin damage. We here provide data on the role of cellular cytotoxicity in the maintenance of nerve destruction in subacute GBS. Archival autopsy tissues including spinal roots, dorsal root ganglia, and peripheral nerve were examined from eleven subjects with GBS exhibiting a primary demyelinating pathology, who died one day to eight weeks after onset of symptoms. In order to study the extent of humoral and cellular immune processes with regard to disease duration a broad panel of antibodies to immunological and cellular markers was used to visualize the stage of demyelination, the deposition of complement components, expression of CD59, and to characterize cell infiltrates. Active myelin breakdown, as defined by the antigenic profile of myelin degradation products within macrophages, occurred at acute and subacute stages of GBS. Deposits of C9neo antigen on degenerating myelin sheaths were predominantly detected in acute cases. Expression of CD59 was upregulated on demyelinating fibers, but did not correlate with the presence of C9neo antigen or duration of disease. Quantitative analysis of endoneurial T-cells showed a correlation between the density of CD3+ T-cells per square unit and the degree of demyelination, but not with the duration of disease. The ratio of CD8+/CD3+ T-cells, however, was significantly increased in cases of GBS with a subacute course. Granzyme B positive lymphocytes and upregulation of MHC class I molecules on Schwann cells and myelin sheaths were detected in cases with longer than four weeks disease duration. These findings implicate an important role of cytotoxic T-cell responses for myelin damage in subacute stages of GBS.

DEVELOPMENT OF THE ENDONEURIAL VASCULATURE IN RAT SCIATIC NERVES

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Harlan Sprague-Dawley rats at ages of 2, 4, 10 and 26 weeks (n = 4 or 5) were sacrificed, mono-fascicular portion of sciatic nerves were harvested, and immersion-fixed in 2.5% buffered glutaraldehyde. The nerves were osmicated, embedded in araldite, sectioned at 1 to 2 μ m, and stained with toluidine blue. The cross sections were imaged in brightfield and photographed at a resolution of 2048 x 1536 pixels with a digital camera attached to the microscope. The digitized images were analyzed with NIH Image software to calculate cross sectional area of nerves. Each cross section was systematically scanned to identify and count microvessels within the endoneurium. Classification of microvessels was not undertaken in this study. Cross sectional areas in mm² (mean \pm SE) at the different ages were 2 weeks 0.14 \pm 0.02; 4 weeks 0.20 \pm 0.01; 10 weeks 0.39 \pm 0.04; 26 weeks 0.44 \pm 0.03. The largest observed vessel diameter was 55 μ m. Number of microvessels per fascicle at the different ages were 2 weeks 12 \pm 2; 4 weeks 42 \pm 3; 10 weeks 37 \pm 3; 26 weeks 34 \pm 2. Calculated microvessel densities per mm² were 2 weeks 96 \pm 26; 4 weeks 206 \pm 8; 10 weeks 98 \pm 10; 26 weeks 73 \pm 5. The number of microvessels per fascicle increases almost fourfold during the third and fourth week and remained relatively unchanged up to 26 weeks of age. This angiogenic spurt also gives rise to the highest vessel density at four weeks. On the other hand, the largest increase (\sim x2) in cross sectional area occurred between the fourth and tenth weeks. Thus, microvessel proliferation in the endoneurium precedes increased metabolic activity, and the dramatic growth of endoneurial content is reflected in the increased fascicular cross sectional area.

ADAPTIVE REGULATION OF NERVE BLOOD FLOW

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In a vast majority of severe peripheral vascular diseases, nerves become ischemic. Consequently, these primarily vascular diseases manifest a peripheral nerve component. Additionally, in metabolic and other peripheral neuropathies ischemia is a significant complication directly contributing to nerve pathology. Thus, microcirculatory failure and the resulting ischemia play a significant role in the etiology of peripheral nerve disorders. Hence a comprehensive description of the mechanisms regulating nerve blood flow (NBF) is essential to understand the etiology of many peripheral neuropathies. The objective of this study was to investigate the reactive change of NBF in rat sciatic nerve when one or more of the nerve's major nutrient arteries (hypogastric-HG and superior gluteal-SG) are occluded. The hypothesis underlying this proposal is that homeostatic mechanism residing within the endoneurial microenvironment restores NBF when the nerve is rendered ischemic. NBF was measured with a Laser Doppler flowmeter connected to a fiber optic probe (250 μm in \varnothing) that was chronically implanted in the mid-thigh portion of the sciatic nerve (~ 1.2 mm in \varnothing). After four to five baseline measurements of NBF and indirect arterial blood pressure were obtained within 7 to 10 days of a fiber optic being implanted, one or both of the nutrient arteries was occluded (n=4 for all groups). Immediately after occlusion, NBF was monitored for four hours and then periodically for the next seven days. Cumulative changes in normalized NBF (nNBF) during 4 hours after occlusion of SG were not significantly different from that observed after sham occlusion. On the other hand, occlusion of only the HG or both vessels produced a severe drop in nNBF during the 4-hour post-occlusion period and the next 7 days. Measured in units of a 10% decrease for 1 hour, nNBF was decreased by 16.5 ± 4.1 (S.E.) during 4 hours of HG occlusion and by 20.1 ± 2.4 after SG-HG occlusion. After 4 hours of HG occlusion, nNBF was $73 \pm 14\%$ of pre-occlusion levels, and was $78 \pm 11\%$ on 7th day. After SG-HG occlusion it was $56 \pm 9\%$ at 4 hours and $59 \pm 6\%$ on 7th day. If HG was ligated 7 days after SG was occluded, then the reduction in nNBF was less than that after combined occlusion.

THICKENED EPIDERMAL BASEMENT MEMBRANE IN DIABETIC NEUROPATHY

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A specialized basement membrane (BM) has the distinguishing characteristic of forming a firm attachment between the epidermis and dermis. Epidermal Nerve Fibers (ENFs) must pass through this BM to reach their target endpoints within the epidermis. The density of ENFs is reduced in correlation to the severity of diabetic neuropathy. The interaction of ENFs with BM may be altered in patients with diabetic neuropathy as evidenced by the following morphological irregularities found in skin biopsies. Some nerve fibers follow a course along the dermal side of the BM but do not penetrate, frequently ending in a bulb-like swelling. In patient biopsies with low nerve fiber density, nerves entering the epidermis often branch at the point of penetration through the BM and form a tuft of nerve endings that extend into epidermis. Similarly, clustering of ENFs is frequently seen in disease states, wherein multiple nerve fiber penetrations are interspersed with relatively long segments of nerve-free epidermis. These findings led us to investigate whether the diabetes-related BM thickening seen in muscle, retinal, and renal glomerular capillaries also occurs in the dermal-epidermal BM; and if the thickness of the dermal-epidermal BM is related to the degree of neuropathy based on ENF density and neuropathy score. Skin biopsies from lateral calf were acquired from types 1 and 2 diabetic subjects and age and sex matched normal control subjects for electron microscopic measurement of skin BM thickness and double immunofluorescent localization of nerve and BM for confocal microscopic imaging and computer-assisted quantification of ENF density. The dermal-epidermal BM of diabetic subjects was significantly thicker than that of normal subjects. ENF density decreases and clinical neuropathy score assessment increases as BM thickness increases. Morphological manifestations of neuropathy were associated with increased BM thickness. Changes occurring in the dermal-epidermal BM that lead to its thickening in diabetic patients may prevent nerve fibers from entering epidermis. Repeating cycles of denervation and reinnervation with concomitant thickening of BM result in denervated epidermis with consequent dysesthesia, allodynia and loss of sensation.

ACETYL-L-CARNITINE ELIMINATES SENSORY NEURON LOSS AFTER PERIPHERAL NERVE INJURY: DOSE-RESPONSE RELATIONSHIP AND EFFECT OF DELAY IN ADMINISTRATION

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Inadequate sensory recovery in the majority of peripheral nerve injuries is largely due to the death of up to ~40% of the sensory neuronal cell population. As quality of sensation regained depends on innervation density, the preservation of this cell population is of crucial clinical importance. Acetyl-L-carnitine (ALCAR), the predominant acylcarnitine in human tissues, has essential roles in facilitating high-energy substrate oxidative metabolism within mitochondria, and may therefore be neuroprotective. The effect of systemic administration of various doses of ALCAR on sensory neuron loss, and subsequently, the effect of delay in its systemic administration are examined. Six groups of adult male Sprague-Dawley rats (n=5) underwent sciatic nerve axotomy. Each received either sham treatment, or one of five doses of ALCAR between 50 and 0.5 mg/kg/day. Five further groups (n=5) underwent unilateral sciatic nerve axotomy followed by the optimal ALCAR dose (50 mg/kg/day). Delay in initial ALCAR administration from time of axotomy in these five groups varied between zero and 1 week. In all groups the L4 and L5 dorsal root ganglia (DRG) were harvested bilaterally after 2 weeks and primary sensory neuron counts were obtained by the optical dissector technique. ALCAR eliminated neuronal loss at higher doses (50 and 10 mg/kg/day), while a dose-response effect was evident at lower doses: 12% loss at 5 mg/kg ($p < 0.05$), 19% at 1 mg/kg ($p < 0.001$), 23% at 0.5 mg/kg ($p < 0.001$), compared to contralateral control. Neuronal cell loss was eliminated at delays in ALCAR administration of 0 ($p = 0.559$), 6 ($p = 0.649$), and 24 hrs ($p = 0.725$) from axotomy, while delay of 1 week resulted in significant loss (18%, $p < 0.0001$) compared to contralateral control. Sham treatment from time of axotomy resulted in a loss of 25% ($p < 0.001$) demonstrating no protective effect. In conclusion, systemic administration of ALCAR eliminates axotomy-induced loss of primary sensory neurons in a dose-dependent fashion and this protective effect persists when administered up to 24 hrs after axotomy. ALCAR treatment may represent a new pharmacotherapy for improved outcome following peripheral nerve trauma.

MECHANISM OF CISPLATIN NEUROTOXICITY

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We have previously demonstrated in tissue culture and in animals that apoptosis of dorsal neuron ganglion (DRG) underlies cisplatin sensory neurotoxicity. Neuron death was preceded by aberrant re-entry into the cell cycle. Markers for this included upregulation of cyclin D1 expression, activation of the cyclin D1/cdk4 kinase and increased phosphorylation of the retinoblastoma gene product (J Clin Invest 101:2842-2850, 1998; Neurobiol Dis 8:1027-1035, 2001). We are now investigating whether neurons progress beyond G1-phase of the cell cycle, whether this pathway is necessary for death, and downstream signaling events. Using incorporation of BrdU into cisplatin treated E15 rat DRG neuron cultures, there was no evidence of new DNA synthesis, indicating that neurons did not progress into S-phase of the cell cycle. There was no increased expression of cyclin B or E as judged by Western blotting. Absence of these later markers of cell cycle progression suggested that neurons were not progressing beyond G1. To determine whether upregulation of cyclin D1 was necessary for death, E15 rat DRG neurons were transfected with an adenoviral vector expressing a full length antisense construct for cyclin D1 (AS-cyclin D1). Control neurons were treated with a similar vector expressing β -galactosidase. Expression of AS-cyclin D1 decreased the cisplatin-induced up-regulation of cyclin D1 and significantly protected neurons against death. This demonstrated that cyclin D1 upregulation is a necessary component of the death process and confirmed earlier findings with pharmacological inhibitors of cyclin D1. We have demonstrated that cisplatin binds at very high levels to DRG DNA (Neurology 58:A378-A379, 2002). Since DNA damage is thought to underlie cisplatin toxicity, we investigated whether this was a p53 dependent process. Using DRG neurons from p53^{-/-} mice, we found that p53 was not necessary for cisplatin-induced death. However neurons from Bax^{-/-} mice were significantly less susceptible to cisplatin-induced apoptosis confirming that activation of Bax is a necessary component of the death pathway (Neurobiol Dis 9:220-233, 2002). Current studies are directed towards determining whether other members of the p53 family (such as p73), which are known to be important mediators of DNA damage response in neurons, are involved in cisplatin-induced death. In conclusion, we have demonstrated that aberrant entry into G1 of the cell cycle, but not progression into S-phase, underlies cisplatin neurotoxicity. Downstream events include non-p53 induced activation of Bax. By understanding the specific mechanisms leading to DRG death, we hope to develop therapeutic strategies directed towards preventing the major, dose-limiting toxicity of this important class of chemotherapeutic agents.

MORPHOLOGY OF PERIPHERAL AXON REGENERATION

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A century ago, Santiago Ramon y Cajal described the morphology of regenerating peripheral axons. Our experiments reevaluate his findings, using modern techniques to quantify axon morphology and characterize the regeneration environment. Our goal is to predict function through analysis of form. Sciatic nerve grafts from C57BL6 mice were used to bridge defects in the sciatic nerves of mice expressing yellow fluorescent protein (YFP) in a subset of DRG and motoneurons (Feng et al., *Neuron* 28;2000). Five, seven, or ten days later the nerves were harvested, fixed in paraformaldehyde, sectioned at 100 microns, and reacted with antibodies to laminin (Sigma) labeled with Alexa Fluor 568 (Molecular Probes) to define the architecture of Schwann cell tubes. Sections were viewed on a Zeiss confocal laser scanning microscope and photographed at 40x or 100x. Among the designations used to describe regenerating axons were: 1) crossing the gap as a single sprout ("direct"), 2) arborizing to reinnervate multiple Schwann cell tubes of the distal stump ("arborizing"), 3) ending in a terminal retraction bulb within the gap ("bulb"). "Direct" projections often traveled laterally in the interstump gap before entering a distal Schwann cell tube, suggesting either ability to discriminate amongst distal targets or totally random behavior. "Arborizing" projections, in contrast, sampled 5-10 distal tubes from among the more than 100 within their 50-100 micron spread, consistent with random pathway entry followed by positive interactions with "correct" pathways and pruning of collaterals from "incorrect" pathways. Single axons traveling within the ideal environment of a distal Schwann cell tube continued to sprout collaterals, suggesting that the process of sprouting is a natural concomitant of regeneration. Retraction bulbs, far from being static collections of degenerating axoplasm, often sent out minute sprouts with multiple growth cones. Occasionally, these sprouts successfully reinnervated the distal stump. These observations suggest that axons interact with distal Schwann cell tubes in at least two different ways, and that collateral sprouting is such a fundamental property of regenerating axons that it occurs on a regular basis in spite of environment.

THE ROLE OF MMPS IN BNB BREAKDOWN

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Matrix metalloproteinases (MMPs) are a large group of endoproteinases that are produced by a wide range of cell types. MMPs appear to be involved in the pathogenesis of the inflammatory demyelinating disorders – multiple sclerosis (MS), Guillain-Barré syndrome (GBS) and chronic inflammatory demyelinating neuropathy (CIDP) since they are released from activated leukocytes in their transmigration from the vascular to neural compartments. However there is no direct evidence of their capacity to induce blood nerve barrier (BNB) breakdown. To investigate effect of MMP-9, one of the MMPs, we introduced MMP-9 into the sciatic nerve of rats and detected BNB breakdown. Intraneural injection of MMP-9 caused BNB leakage as shown by horseradish peroxidase (HRP) and Evan's blue accumulation following systemic administration. MMP-9 intraneural injection alone did not produce demyelination and only caused minor axonal degeneration in a higher dose. Electrophysiological study showed no significant change before and after intraneural injection. The ratios of amplitudes of hip and ankle (H/A) were 0.89 ± 0.031 on day 0 and 0.82 ± 0.055 on day 7. MMP-9 intraneural injection and experimental autoimmune neuritis (EAN) serum systemic injections resulted in demyelination around the endoneuronal vessels. The ratios of H/A were reduced from 0.86 ± 0.023 on day 0 to 0.54 ± 0.15 on day 7. More extensive demyelination was found after intraneural injection with MMP-9 and TNF- α . Electrophysiological changes detected in this experiment showed the H/A ratio was reduced from 0.88 ± 0.02 on day 0 to 0.40 ± 0.18 on day 7 and then recovered gradually. In conclusion, MMP9 breaches the local BNB after intraneural injection. Demyelination was caused when anti-myelin antibody existed in the circulation. MMP and TNF- α have a synergistic effect in breakdown of the BNB. Further studies will focus on preventing BNB leakage by MMP inhibitors.

SURAL NERVE GRAFTING AFTER RADICAL PROSTATECTOMY

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Erectile dysfunction is a common side effect of radical prostatectomy, which is believed to be due to intraoperative disruption of the autonomic cavernous nerves mediating penile bloodflow. Wide resection of the tissue and nerves surrounding the prostate is often necessary for cancer control. We report our 18-month results of a sural nerve grafting technique to assist cavernous nerve regeneration, for recovery of erectile function. Methods: Twenty-six sexually potent men underwent radical prostatectomy, with resection of one or both cavernous nerves. A sural nerve graft was harvested from the lower leg. After resection of the cavernous nerve and prostate, the graft was placed between the cut ends of the neurovascular bundle(s). The graft was secured using sutures and fibrin glue. Pre- and post-operative erectile function was measured using a validated self-report questionnaire, and nocturnal penile monitoring. Results: Of 26 men, 3 were determined to have locally extensive or metastatic disease immediately following surgery, requiring further treatment with radiation and/or hormone ablation. Twenty three men were appropriate for follow up. Six men had bilateral nerve grafts, and 5 were completely dependent on sexual aids for erections or were not sexually active. One man with bilateral grafts used sexual aids occasionally, but otherwise was able to get erections. Of the 17 men with unilateral grafts, 8 (47%) reported spontaneous erectile function of adequate duration and rigidity for satisfactory sexual intercourse, and 3 men required sildenafil (Viagra) to achieve satisfactory erectile function (18%). The majority of these men recovered function by 15 months following the operation. Two men (12%) had some recovery of erectile function, but still required sexual aids occasionally (less than 25% of the time). Thus, 77% of men with unilateral grafts recovered some erectile function. Four men (24%) were completely dependent on sexual aids for erections, or were sexually inactive. Conclusions: Unilateral sural nerve grafting appears to improve recovery of erectile function following radical prostatectomy, compared to historical data from men with unilateral nerve sparing prostatectomy without grafting. The time to recovery supports the concept that nerve regeneration is occurring. Bilateral nerve graft outcomes are less encouraging, and further refinement of the grafting technique is necessary.

EFFECT OF ACUTE HYPOGLYCEMIA ON OXYGEN PARTIAL PRESSURE OF SCIATIC NERVES IN EXPERIMENTAL ACUTE HYPOGLYCEMIC RATS

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Background: Acute severe hypoglycemia causes peripheral neuropathy experimentally and clinically. Previously, we reported the decreased nerve blood flow, increased plasma adrenaline level and derangement of nitric oxide in nerves of experimental acute hypoglycemic rats. Ischemic effect of acute hypoglycemia on peripheral nerves may be considered, but the condition of oxygen saturation in the tissues of peripheral nerves has not been studied directly. Objective: To clarify the ischemic effect of acute hypoglycemia, we studied the tissue oxygen partial pressure (PO_2) in the sciatic nerves of experimental acute hypoglycemic rats directly. Methods: Acute hypoglycemia was induced by insulin in rats. The tissue PO_2 was measured by inserting an electric catheter of PO_2 sensor in the sciatic nerve of the rat and the PO_2 was analyzed by digital PO_2 monitor. Motor nerve conduction velocities (MCV) were also measured in rat sciatic nerves. Age matched rats were used for controls. Results: The tissue PO_2 was significantly reduced in hypoglycemic rat sciatic nerves. MCV was also decreased in these rats. Conclusion: Acute hypoglycemia may induce ischemia which causes neuropathy in the peripheral nerves in rats.

GANGLIOSIDE MIMICRY OF CAMPYLOBACTER LIPOOLIGOSACCHARIDE INDUCES THE DEVELOPMENT OF GUILLAIN-BARRE SYNDROME MODEL

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We have shown that GM1 is one of the autoantigens for IgG antibodies in some patients with axonal Guillain-Barré syndrome (GBS) (Yuki et al., Neurology 1990), the IgG class of the autoantibody was produced against GM1 in experimental animals, and pathological changes of the peripheral nerves were similar to those seen in human axonal GBS (Yuki et al., Ann Neurol 2001). Some patients develop axonal GBS subsequent to Campylobacter jejuni enteritis, and the C. jejuni strains isolated from these patients have lipooligosaccharide (LOS) bearing GM1-like structure: The terminal structure [Gal beta1-3 GalNAc beta1-4 (NeuAc alpha2-3) Gal beta1-] is identical to the terminal tetrasaccharide of GM1 ganglioside (Yuki et al., J Exp Med 1993). To prove molecular mimicry theory between an environmental agent and the peripheral nervous system, we sensitized Japanese white rabbits with the GM1-like LOS of C. jejuni (CF 90-26) isolated from a patient with axonal GBS. The injected rabbits developed high anti-GM1 IgG antibody titers and flaccid limb weakness. Paralyzed rabbits had similar pathological changes of the peripheral nerves to patients with GBS subsequent to C. jejuni enteritis. IgG was deposited on the axons of the anterior roots. By immunizing the GM1-like LOS to genetically engineered mice lacking complex gangliosides, monoclonal antibody (mAb) that reacted with both the LOS and GM1 was cloned. Immunohistological study demonstrated the anti-GM1 mAb binding to human peripheral nerves. The anti-GM1 mAb blocked neuromuscular transmission in a rat muscle-spinal cord co-culture. These data demonstrate the importance of GM1 mimicry of the C. jejuni LOS as a cause of axonal GBS subsequent to C. jejuni infection.

DEVELOPMENT AND CHARACTERIZATION OF AN *IN VITRO* ANTI-GANGLIOSIDE ANTIBODY MEDIATED NEURONAL CELL LYSIS ASSAY

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Anti-ganglioside antibodies are strongly associated with certain variants of Guillain-Barré syndrome; for example, antibodies against GD1a, GM1, and related gangliosides are frequently present in patients with AMAN variant of GBS. The pathologic role of these antibodies in AMAN variant of GBS is now widely accepted. However, two basic issues related to anti-ganglioside antibody mediated neural injury remain unresolved: (a) some anti-ganglioside antibodies can cross react with glycoproteins and therefore the nature of antigens targeted by these antibodies is not well established and (b) although pathological studies suggest that complement activation is involved in the pathogenesis of GBS, experimental data for the role of complement continues to be inconclusive. To address these issues we developed an anti-ganglioside antibody mediated lysis assay using neural cell lines NG108-15, that expresses gangliosides GM1 and GD1a, and NG-CR-72, a mutant line derived from NG108-15 that does not express these gangliosides. The ganglioside expression/content of these cell lines was also manipulated by reconstitution and pharmacological inhibitors of ganglioside biosynthesis. Heat inactivation and complement inhibitors were used to determine the role of complement. Our results demonstrate that both GBS patient sera, containing anti-ganglioside antibodies, and monoclonal anti-ganglioside antibodies cause neuronal cell lysis by targeting specific cell surface gangliosides and this cell lysis is complement dependent. Further, IVIg, now the standard treatment for GBS, significantly decreased cell lysis in this assay. These findings support the concept that gangliosides are targets of immune attack in some variants of GBS and complement plays a role in anti-ganglioside antibody mediated nerve fiber injury. Studies with IVIg indicate the presence of factors capable of blocking anti-ganglioside antibody-mediated cytotoxicity in the commercially available IVIg used for the treatment of GBS. We propose the use of this simple lysis assay for screening of (a) pathogenic anti-ganglioside antibodies in patients with immune mediated neuropathies and (b) new/experimental therapies to prevent anti-ganglioside antibody mediated neural injury.

NEUROREHABILITATION IS EFFECTIVE IN PATIENTS WITH NEUROPATHIES – RESULTS OF A RANDOMIZED, PROSPECTIVE STUDY

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Objective: To describe the effect of neurorehabilitation in patients with neuropathies (NPs) on physical parameters, pain, functional scores and well-being. **Design:** Randomized prospective study. **Setting:** Inpatient neurorehabilitation in the Neurological Rehabilitation Centre Pirawarth. **Patients:** 29 patients (13 female, 16 male; mean age 63.3 years; range 26 to 88 years) with a NP as the primary diagnosis. **Interventions:** Physiotherapy, sports-therapy (strength and endurance training: isometric and isokinetic exercises, treadmill and bicycle training), occupational therapy, and passive physical therapies. **Main Outcome Measure:** Scores and parameters for endurance (bicycle exercise testing: Resistance, Total Cycling Time (TCT), percentage of the expected individual result), pain (VAS), functional scores (Barthel Score), and well-being (Zerssens Befindlichkeitsskala, BF-S) were assessed at the beginning and at the end of rehabilitation. **Results:** The overall mean scores for bicycle exercise testing were 102.1 W (pre) and 117.2 W (post) ($P=0.002$) for resistance, 551.5 s (pre) and 642.3 s (post) ($P=0.001$) for the Total Cycling Time (TCT), and 68.3% (pre) and 78.3% (post) ($P=0.001$) for percentage of the expected individual result. The overall mean pain score was 4.9 (pre) and 3.6 (post) on the VAS scale ($P=0.005$). The overall mean Barthel score was 76.1 (pre) and 80.3 (post) ($P=0.036$). The overall mean BF-S score was 11.6 (pre) and 6.9 (post) ($P=0.003$). **Conclusions:** Neurorehabilitation is effective in patients with NPs. The results of this study emphasize the importance of a full residential neurorehabilitation for patients with NPs.

THE NOVEL NEUROFILAMENT LIGHT (NEFL) MUTATION GLU397LYS IS ASSOCIATED WITH A CLINICALLY AND MORPHOLOGICALLY HETEROGENEOUS TYPE OF CHARCOT-MARIE-TOOTH NEUROPATHY

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Charcot-Marie-Tooth (CMT) disease comprises a heterogeneous group of hereditary neuropathies. CMT falls into two main groups: demyelinating CMT1 with reduced nerve conduction velocity (NCV) and axonal CMT2 with normal NCV. The neuropathological features correspond in most cases to this classification. Three genes were recently identified to cause autosomal dominant CMT2, neurofilament light (NEFL), kinesin superfamily motor protein 1B-beta (KIF1B-beta), and RAS-associated protein (RAB7). Thus far, six families with NEFL mutations have been reported. We identified a novel mutation, Glu397Lys, in a conserved motive signaling the end of the rod domain. The affected family members from three generations showed clinically strikingly different phenotypes ranging from minimal signs to weakness of the lower extremities, foot deformities, and deafness. The mutation was associated with NCVs ranging from 27 m/s in a 25-year-old female to 43 m/s in a 82-year-old male. Sural nerve biopsies of two affected subjects were analyzed by light and electron microscopy. The pathological changes consisted of a reduction of predominantly large myelinated nerve fibers and various stages of onion bulb formation as typically seen in CMT1. These findings represent the first correlative study between the clinically as well as the histopathological phenotype and a NEFL mutation in man indicating a wider clinical spectrum of NEFL mutations than previously assumed. Therefore, future diagnostic efforts should consider the possibility of NEFL mutations not only in pure axonal CMT neuropathies.