

ABSTRACTS (H-L) PNS 2001 Meeting

DISTURBED FIBRINOLYTIC AND ANTITHROMBOTIC MARKERS IN HUMAN DIABETIC PERIPHERAL NERVE MICROVESSELS

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Nerve ischemia is recognized as a final common pathological sequelae of diabetic neuropathy, but the precise underlying mechanisms remain controversial. In vitro, inflammatory and metabolic abnormalities associated with diabetes alter endothelial function producing a pro-thrombotic state. This biopsy-based case control study investigates the hypothesis that key vascular endothelial proteins regulating fibrinolysis and antithrombotic mechanisms are deficient in diabetic nerve microvasculature. Formalin fixed sural nerve sections from 7 diabetics and 9 controls with axonal neuropathy without vasculopathy were immunostained for fibrinolysis regulatory proteins (tissue plasminogen activator (tPA) and plasminogen activator inhibitor (PAI-1); thrombomodulin (TM), which inactivates thrombin and accelerates activated Protein C formation; and vonWillebrand factor (vWF), an endothelial cell marker). Data was expressed as the proportion of tPA, PAI-1, and TM immunoreactive vessels relative to total vessel number (determined by vWF staining on serial sections). Vascular endothelial tPA was present on $99.4 \pm 1.3\%$ of endoneurial vessels in controls, but only on $74.5 \pm 3.8\%$ in diabetics ($p < 0.01$). Yet, PAI-1 was uniformly present on the nerve microvasculature of diabetics and controls. Vascular TM expression was rarely ($< 0.1\%$) detected on the perineurium of diabetic or control nerves. The proportion of TM immunoreactive epineurial and endoneurial vessels was significantly lower in diabetics $4\% [2-10]$ versus controls $39\% [24-63]$ ($p < 0.0001$). We demonstrate that tPA is absent from a substantial proportion (26%) of endoneurial vessels in diabetics. TM is nearly absent at the blood nerve barrier in all cases and further reduced in diabetic endoneurial microvessels. These findings suggest the coordinate impairment of endogenous fibrinolysis and the TM-Protein C antithrombotic mechanism constitute a prothrombotic state that may contribute to microvascular ischemia in human diabetic neuropathy. Sponsor: 1R01 DK59758-01 and NIH 1K08NS01595; Baltimore Veterans Administration, Geriatrics Research, Education, and Clinical Center.

ULTRASTRUCTURAL IMMUNOLocalISATION OF ANTI-GQ1B ANTIBODY DEPOSITS AT MOUSE NEUROMUSCULAR JUNCTIONS IN AN EX VIVO MODEL OF MILLER FISHER SYNDROME

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Miller Fisher Syndrome (MFS) is associated with anti-GQ1b ganglioside antibodies that arise through molecular mimicry with microbial antigens including *Campylobacter jejuni* lipopolysaccharides. The motor manifestations of MFS are restricted to paralysis of craniobulbar muscles and current experimental evidence suggests that the motor nerve terminal may be one important site of injury. We have derived anti-GQ1b murine monoclonal antibodies (mAbs) from mice immunised with neuropathy-associated isolates of *C. jejuni* and used these in electrophysiological and ultrastructural studies to elucidate the mechanism(s) of nerve terminal paralysis in mouse phrenic nerve hemi-diaphragm and flexor digitorum brevis (FDB) preparations. Ex vivo incubation with the mAb CGM3 (GQ1b, GD3 and GT1a-reactive) and human complement has revealed IgM and C3c deposits at the nerve terminal using low resolution fluorescence microscopy. In order to determine the precise location of mAb deposits, we have now performed pre-embedding immuno-electron microscopy. Here we present high resolution, silver-enhanced 1 nM gold-labeled images of FDB which reveal extensive mAb deposits on both the presynaptic nerve terminal and on the capping Schwann cell, but not on any post-synaptic structures. These data indicate that anti-GQ1b Abs may have a direct action on the presynaptic nerve

terminal and, in addition, may induce nerve terminal damage mediated through the overlying Schwann cell. Sponsor: GBS Support Group UK.

AXON DAMAGE IN CMT DUE TO MUTATION IN MYELIN PROTEIN P0

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Clear distinction between CMT1 and CMT2 has become more difficult since female CMT1X patients have slightly reduced or normal NCVs in the range of CMT2. More recently, some myelin gene mutations, esp. Thr124Met mutation in the major myelin protein P0, were found in patients presenting as CMT2. Most of these patients showed evidence of axonal disease by electrophysiological and histopathological criteria, while some had also evidence of demyelination. We describe a family carrying the Thr148Met mutation in the P0 gene. Contrary to other neuropathies caused by myelin gene defects, no demyelination could be found in our biopsies. Based on follow-up examinations, extensive morphometry and immunohistochemical analysis, we suggest that in our family the ultrastructural intact but too thin myelin sheath indicates mild hypomyelination which secondarily causes axonal degeneration and axonal loss of large and small fibers which predominates the clinical picture.

GLIAL GROWTH FACTOR AND SCHWANN CELLS IMPROVED NERVE REGENERATION FOLLOWING DELAYED REPAIR

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Peripheral nerve injuries often require secondary repair, and outcome is worsened because of the impaired Schwann cell (SC) proliferation occurring when repair is delayed. Glial growth factor (GGF) regulates the proliferation of SCs and their interaction with regenerating axons. It was our aim to demonstrate whether exogenous GGF may promote SC proliferation and indirectly improve nerve regeneration, and to compare the benefit that may follow transplant of cultured autologous SCs. At 2 months after axotomy, rat sciatic nerve was repaired using 1 cm conduits containing either GGF (500 ng/ml) or cultured SCs (80 x 10⁶/mm). Conduits were harvested at 6 wks after repair and analysed for quantitative immunohistochemistry of nerve regeneration. Regeneration distance was increased 56% by addition of GGF, and 75% by SC transplant. However, the total area of regenerating axon immunostaining was increased 442% by GGF and 55% by SCs. Similarly, percentage area of axonal staining within the conduit was increased 337% with the addition of GGF and 25% with SCs. In conclusion, exogenous GGF and cultured SC transplant represent promising treatment for delayed repair of nerve injury, although GGF may promote a more abundant regeneration response.

COMPARISON AMONG ELECTROPHYSIOLOGIC INDICES OF DIABETIC NEUROPATHY

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In Rochester diabetic neuropathy research, Dyck et al. introduced an abnormal value (99% reliability) in two or more nerves as the nerve conduction criteria of diabetic neuropathy. We prepared the polyneuropathy index-revised (PNI-R), sensory neuropathy index (SNI) and sensory conduction

index (SCI) for the quantitative evaluation of diabetic neuropathy. PNI-R is calculated as the mean percentage of the normal of motor nerve conduction velocity and F-latency parameters on the median, ulnar, tibial and peroneal nerves. SNI or SCI was similarly calculated from amplitudes or conduction velocities of 4 sensory nerve action potentials of the median, ulnar, sural and superficial peroneal nerves. To compare these indices, first we obtained the normal limits of each parameter or index. Then, number of abnormal nerves, PNI-R, SNI and SCI were investigated in 78 patients with diabetes mellitus. Diabetic patients showed abnormal Dyck's criteria in 56, values of PNI-R, SNI and SCI as 83.8 ± 7.0 %, 52.1 ± 32.4 %, and 85.0 ± 7.6 %, respectively. These indices were well correlated with each other. Coefficient of correlation between number of abnormal nerves and PNI-R was as high as 0.85. In 18 patients they were both normal, and in 54 both abnormal. Only in 6 they did not coincide. When PNI-R was decreased to 70%, SNI value became almost 0. In diabetic neuropathy the main feature is axonal degeneration, therefore, 10% conduction slowing can correspond to one-third axon loss. Correlation with vibration threshold and Achilles tendon reflex was best in PNI-R. PNI-R corresponds well with Dyck's criteria, and is a suitable index for the quantitative evaluation of diabetic neuropathy.

OXIDATIVE STRESS AND RECEPTOR OF ADVANCED GLYCOSYLATED ENDPRODUCTS (RAGE) EXPRESSION IN DIABETIC AND VASCULITIC POLYNEUROPATHIES

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INTRODUCTION: Oxidative stress is suggested to play an important role in the pathogenesis of long term diabetic complications and in chronic inflammation. Binding of N-epsilon-Carboxymethyl-lysine (CML) - the main AGE in human tissues and a marker for chronic oxidative stress - to RAGE causes intracellular oxidative stress and enhances NF-kappaB activation. **METHODS:** We investigated the localization of CML and RAGE in sural nerve biopsies in diabetic (dPNP; n=10) and vasculitic polyneuropathy (vPNP; n=8) and controls (C; n=4) by immunohistochemistry with the APAAP method. **RESULTS:** RAGE was expressed by perineurial cells (in dPNP 9/10 and vPNP 3/8), vessels (dPNP 7/10, vPNP 4/8, C 1/4), Schwann cells (dPNP 10/10, C 2/4) and mononuclear cells (vPNP 7/8). CML modified proteins were localized in the perineurium (dPNP 10/10, vPNP 6/8, C 1/4), vessels (dPNP 7/10, vPNP 7/8, C 1/4) and mononuclear cells (vPNP 8/8). RAGE was expressed by CD4+, CD8+ and CD68+ mononuclear cells. **CONCLUSIONS:** CML binding to RAGE leads to NF-kappaB activation which promotes the production of proinflammatory cytokines, adhesion molecules, the upregulation of RAGE production itself and reduces apoptotic cell death. Therefore, RAGE activation in mononuclear cells could lead to a perpetuation of inflammation in vPNP. In dPNP, increased oxidative stress in the endothelium and the perineurium may alter the barrier function of these structures and, therefore, cause nerve dysfunction.

DEMYELINATIVE AND AXONAL FEATURES OF CMT PATIENTS WITH PMP22, MPZ AND Cx32 MUTATIONS: ANALYSES OF 196 JAPANESE PATIENTS

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Charcot-Marie-Tooth disease (CMT) is caused by a wide variety of gene mutations, including PMP22, MPZ and Cx32. We evaluated the clinico-pathological and clinico-electrophysiological correlations in the CMT patients with PMP22 duplication, MPZ and Cx32 mutations. Clinical phenotypes of

distal and lower limb pronounced muscle weakness and sensory deficits were common in the patients with three mutations. Interestingly, about 30% of patients with MPZ and Cx32 mutations showed axonal features of well preserved NCV, abundant axonal sprouting and less frequent segmental pathology. These patients with axonal features were relatively late-onset and frequently associated with neural deafness and pupillary abnormality. The patients with PMP22 duplication, however, showed exclusively a segmental pathology. The CMAP amplitude was significantly correlated with the corresponding distal muscle strength, but not correlated with the proximal muscle strength in three mutations. On the contrary, slowing of MCV was not correlated with the distal muscle strength. These findings strongly support the view that neurological deficits are due to the reduction of axons rather than the segmental demyelination commonly in the patients with PMP22 duplication, MPZ and Cx32 mutations, irrespective of axonal or demyelinative features.

IMPAIRED LIMB BLOOD FLOW IN FABRY PATIENTS

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Fabry disease is associated with neuropathy, myocardial infarction, renal failure and stroke. To evaluate blood flow and vessel reactivity of Fabry patients, we performed venous occlusion plethysmography and post-ischemic flow measurements. In 14 Fabry patients and 15 controls, blood flow (% vol change/min) was averaged from 8 artifact-free venous occlusion measurements. Post-ischemic vasoreactivity was determined from the first venous occlusion peak following 3 min of ischemia. Skin blood flow (SBF) was monitored at the index finger using laser flowmetry. Fabry patients had lower baseline blood flow ($5.6 \pm 4.2\%/min$ vs. $6.1 \pm 1.4\%/min$) and post-ischemic hyperperfusion ($14.9 \pm 7.8\%/min$ vs. $16.9 \pm 2.9\%/min$) than controls ($p < 0.05$). Before ischemia, SBF did not differ between patients and controls. SBF decreased more in patients during ischemia (0.1 ± 0.4 PU vs. 3.2 ± 0.9 PU) and was significantly higher during reactive hyperemia (561.1 ± 315.2 PU vs. 266.1 ± 65.1 PU) than in controls ($p < 0.05$). Fabry patients show impaired forearm blood flow with deficient vessel reactivity and increased sensitivity of skin vessels towards ischemia. The discrepancy between reduced overall influx and increased SBF after ischemia suggests different impairment of arterioles and small skin vessels. Sponsor: The study was sponsored by Genzyme Corp., Cambridge, MA, USA.

DIFFERENTIAL EXPRESSION OF NEUROTROPHIN mRNA IN DENERVATED SCHWANN CELLS OF MOTOR AND SENSORY NERVE FIBERS

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The Schwann cells in the peripheral nervous system respond to denervation by changing the levels of expression of various neurotrophins. Often the levels of the neurotrophins are upregulated in order to support regenerating axons. We do not know which of these changes are specific to denervation due to loss of motor versus sensory axons. In order to study the changes in neurotrophin expression levels in denervated Schwann cells, we created a model of motor axonal injury by performing a ventral rhizotomy close to the spinal cord and a sensory axonal injury using a dorsal rhizotomy close to the dorsal root ganglion at the L5 level. After 1 week of denervation, L5 ventral and the dorsal roots were harvested for RT-PCR. Using standard techniques we reverse transcribed total RNA and then performed semi-quantitative multiplex PCR using primers for Brain-Derived Neurotrophic Factor (BDNF), Nerve Growth Factor (NGF), Glial cell-line Derived Neurotrophic Factor (GDNF) and

Neurotrophin-3 (NT-3). BDNF and NGF were upregulated in denervated Schwann cells after dorsal rhizotomy but not ventral rhizotomy. In contrast, GDNF was upregulated in denervated Schwann cells after either ventral or dorsal rhizotomy. The changes in the expression level of NT-3 was less pronounced but there was a small decrease in denervated Schwann cells after ventral rhizotomy. These results show that Schwann cells that are associated with motor neurons do not upregulate their levels of expression of BDNF or NGF in response to axonal injury, but the Schwann cells of sensory neurons do. This finding implies that these neurotrophins probably play an important role in sensory regeneration but not in motor regeneration. In contrast, the upregulation of GDNF by Schwann cells that are associated with motor and sensory axons suggests that this neurotrophin may play an important role in regeneration of both neuronal populations.

ACUTE GLUCOSE DEPRIVATION LEADS TO APOPTOSIS IN A CELL CULTURE MODEL OF ACUTE PAINFUL DIABETIC NEUROPATHY

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The pathogenesis of diabetic neuropathy is not fully understood. Hypoxia due to vascular insufficiency and the metabolic effects of chronic hyperglycemia are thought to be major factors in producing peripheral nerve damage. In about 1-2% of the diabetic patients, neuropathy presents with an acute or subacute course. Acute painful neuropathy or insulin neuritis following rapid improvement in glycemic control is well recognised but its pathogenesis is not fully understood. An understanding of these processes may give an insight into the early pathogenetic factors leading to diabetic neuropathy in general. We developed an in vitro model of acute diabetic neuropathy using rat dissociated dorsal root ganglion (DRG) neurons incubated in hyperglycemic medium (Glucose=700 mg%) and room air (PO₂=150 torr) conditions. After 5 days, DRG neurons were placed into hypoxic conditions (PO₂=7.6 torr) with normoglycemic medium (Glucose=100 mg%) or hyperglycemic medium (Glucose=700 mg%) containing 3 or 100 ng/ml NGF. Acute lowering of glucose levels under hypoxic conditions led to apoptosis of DRG neurons. Apoptosis was demonstrated by electron microscopy (EM), bis-benzimide staining for nuclear fragmentation, TUNEL staining, and DNA laddering. Inhibition of death by the caspase inhibitor z-VAD.fmk (100 μ M) confirmed that death was apoptotic. Hypoxia-induced death was reduced when DRG were maintained in hyperglycemic medium suggesting that high levels of substrate protected against hypoxia. Apoptosis was completely prevented by increasing NGF concentration from 3 to 100 ng/ml and partially prevented by addition of α -lipoic acid (1.25 mM). These results suggest a link between metabolic abnormalities induced by hyperglycemia and hypoxia. The demonstration that this involves apoptosis, which is preventable by NGF and by an antioxidant, provides a novel model for studying the pathogenesis and treatment of early stages of diabetic neuropathy. Sponsor: NS40471, St. Marianna University School of Medicine, and Mayo Foundation.

MUTATIONS IN THE 5' REGION OF THE MYOTUBULARIN-RELATED PROTEIN 2 (MTMR2) GENE IN AUTOSOMAL RECESSIVE HEREDITARY NEUROPATHY WITH FOCALLY FOLDED MYELIN

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Focally folded myelin has been recognized as a distinctive feature in some individuals with severe demyelinating neuropathy with an onset in childhood. These cases have been shown to be genetically heterogeneous. Alterations in the myotubularin-related protein 2 (MTMR2) on chromosome 11q22 have recently been shown to be associated with this phenotype in some patients. Mutations

have been described in the 3' region of the MTMR2 gene in four unrelated families, two of whom had previously been linked to chromosome 11q22. We have sequenced the entire coding region and flanking intronic regions of the MTMR2 gene in eight families with early onset autosomal recessive neuropathies. Two novel mutations were identified in exon 4 at the 5' end of the MTMR2 gene in an English and an Indian family, respectively. The clinical features including the sural nerve pathology in these two families differ in severity with the proband in the English family having an earlier onset and more severe neuropathy with prominent cranial nerve involvement. This is likely to be due to mutation type and involvement of small nucleotide polymorphisms (SNPs) in phenotype modulation. Detailed sural nerve pathology will be presented in both cases. Mutations in the MTMR2 gene are thus an important cause of autosomal recessive demyelinating neuropathy. Identifying further mutations and defining their phenotype will help to clarify the classification of the autosomal recessive demyelinating hereditary motor and sensory neuropathies.

RANDOMIZED CONTROLLED TRIAL OF IVIg VERSUS ORAL PREDNISOLONE IN CHRONIC INFLAMMATORY DEMYELINATING POLYRADICULONEUROPATHY (CIDP)

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Previous trials have shown that oral steroids and IVIg are effective in CIDP. To compare oral steroids and IVIg we did a multicenter randomized double blind crossover trial of six weeks of oral prednisolone starting with 60 mg daily with IVIg 2.0 g/kg given over one to two days in CIDP. Twenty-four of 32 patients completed both treatment periods. Both treatments produced significant improvements in the primary outcome measure, change in an 11-point disability scale two weeks after randomization. There was slightly but not significantly more improvement after IVIg than prednisolone, the mean difference between the groups in change in disability grade being 0.16 (95% CI - 0.35 to 0.66). There were also slightly greater improvements favoring IVIg in the secondary outcome measures, time to walk 10 m after 2 weeks and improvement in disability grade after six weeks. Results may have been biased against IVIg by the eight patients who did not complete the second treatment period. With the assumption of no change in the second treatment period for those patients, the mean (SD) improvement in disability grade during the prednisolone treatment period was 0.29 (1.18), which was not significant ($p = 0.18$), and with IVIg was 0.87 (1.46), which was significant (0.002). There was a marked trend towards more improvement with IVIg than with prednisolone, the difference being 0.56 (95% CI - 0.05 to 1.17) of a grade more improvement in the IVIg group ($p = 0.07$). A serious adverse event probably related to treatment (psychosis) occurred in one patient with prednisolone and in none with IVIg. This trial confirmed the efficacy of oral steroids and IVIg and suggested that IVIg works faster in CIDP. It did not examine the known long-term side effects of prolonged steroid treatment, which would favor treatment with IVIg. Sponsor: European Union BIOMED program 4 BMH-CT96-0324; INCAT Group; Novartis.

DETECTION AND PREVALENCE OF THE €LATROTOXIN-LIKE EFFECT OF SERA FROM PATIENTS WITH MILLER FISHER AND GUILLAIN-BARRÉ SYNDROME

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INTRODUCTION: Sera from patients with Miller Fisher syndrome (MFS), a variant of Guillain-Barré syndrome (GBS), contain anti-GQ1b antibodies. In mouse diaphragm *in vitro*, these sera induce muscle fibre twitching, a temporary increase of spontaneous quantal acetylcholine release at neuromuscular junctions (NMJs) and, eventually, blockade of neuromuscular transmission. These effects are similar to those of -latrotoxin (-LTx) and are complement-dependent. **AIM:** To develop a rapid assay based on muscle fibre twitching for detection of the -LTx-like effect of sera. **Screening** of 89 sera from controls and GBS/MFS patients, to determine the prevalence of the -LTX-like effect in relation to the presence of anti-ganglioside antibodies and clinical symptoms. **METHODS:** Mouse diaphragm strips were incubated with complement-inactivated serum samples and normal human serum as a complement source. Twitching was scored visually by stereomicroscopy. The assay was optimized and standardized using -LTx, mouse monoclonals and serum samples with anti-GQ1b activity. Sera of 41 patients were also tested with standard micro-electrode methods. Muscle strips were double-stained with fluorescent -bungarotoxin and anti-complement C3c to determine C3c-deposition at NMJs. **RESULTS:** Twitching was observed with 3/3 anti-GQ1b IgM monoclonals, 13/17 (76%) of MFS, 5/50 (10%) of GBS, and with none of 22 neurological, infection and normal controls. Twitching was highly associated with ophthalmoplegia, serum anti-GQ1b antibodies, increased MEPP frequency and C3c-deposition at mouse NMJs (all items, $p < 0.001$). **CONCLUSION:** This study strongly suggests that anti-GQ1b antibodies are responsible for the -LTx-like activity in serum from GBS and MFS patients. The muscle fibre twitching assay is an efficient test for detection of this effect.

SCHWANN CELL EXPRESSION OF OSTEOPONTIN AND ITS REGULATION DURING WALLERIAN DEGENERATION

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Osteopontin (OPN), an RGD-site-containing phosphoglycoprotein, exerts cytokine-like, chemotactic, and proadhesive effects via binding to specific integrin and CD44 cell surface receptors. During wound healing, OPN is expressed by infiltrating macrophages and has been implicated in tissue repair. To delineate a role in the regenerative response to axotomy we examined the expression of OPN in Wallerian degeneration of the sciatic nerve in rats. Unexpectedly, we found high constitutive expression of OPN by myelinating Schwann cells (SCs) in control nerves. Upon axotomy, SC-expressed OPN in the degenerating distal nerve stump transiently increased during the first two days after injury, but was continuously downregulated thereafter. Macrophages invading axotomized nerves were OPN-negative. During late stages after axotomy, SC-OPN was reexpressed in regenerating but not permanently transected nerves. We also found OPN expression by myelinating SCs in human sural nerves with a dramatic reduction in axonal polyneuropathies. Taken together, our study identifies OPN as a novel Schwann cell gene product that is regulated by axon-derived signals. The lack of OPN induction in infiltrating macrophages indicates fundamental differences in tissue repair between axonal injury in the peripheral nervous system and structural lesions in other organ systems. Sponsor: DFG, Schilling-Stiftung.

TWO FAMILIES WITH AUTOSOMAL RECESSIVE CMT TYPE II SHOWING EVIDENCE FOR LINKAGE TO "DEMYELINATING" AUTOSOMAL RECESSIVE CMT LOCI

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We present two families with a recessive form of CMT II showing evidence for linkage to “demyelinating” autosomal recessive CMT chromosomal loci. In each family, two siblings were affected. Family I was linked to chromosome 19q13.1-13.3; family II, to chromosome 11q23. There were some clinical differences between these two families. The common features, however, were axonal lesion with concomitant secondary demyelination revealed by electrophysiologic study and morphology of sural nerve biopsy. The differences concerned the clinical picture. The first symptoms in family I appeared at approximately 10 years of life, while in family II the symptoms were noted during infancy. Moreover, the course of the disease in family I was mild, whereas in family II, more severe, particularly in one of the siblings. In conclusion, these observations may serve as a new evidence for the lack of correlation between genotype and phenotype in HMSN.

LOCALIZATION OF A GANGLIOSIDE, N-ACETYLGALACTOSAMINYL GD1A, IN HUMAN PERIPHERAL NERVOUS TISSUE

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Previously, we reported that a ganglioside, N-acetylgalactosaminyl GD1a (GalNAc-GD1a), is a target molecule for serum antibody in some Guillain-Barré syndrome (GBS) patients, and that IgG anti-GalNAc-GD1a antibody is closely associated with the pure motor variant of GBS characterized by distal weakness and infrequent cranial nerve involvement. To investigate a pathogenetic role of anti-GalNAc-GD1a antibody in GBS, we studied localization of GalNAc-GD1a in human peripheral nervous tissue with double fluorescence labeling technique. IgG anti-GalNAc-GD1a monospecific antibody was purified through an affinity column from anti-GalNAc-GD1a antibody-positive rabbit sera. Anti-neurofilament-200 monoclonal and anti-HNK-1 monoclonal antibodies were used as markers for axon and myelin, respectively. The anti-GalNAc-GD1a antibody immunostained the outer surface of axolemma or Schwann cells adjacent to axons in ventral root (VR), in the small-diameter fibers in dorsal root (DR), in some nerve fibers around dorsal root ganglion (DRG) cells, and in intramuscular nerve fibers. The positive staining in the intramuscular nerve fibers was observed inside the axon as well. Anti-GalNAc-GD1a antibodies may bind to those regions in VR and in the intramuscular nerve fibers where GalNAc-GD1a is localized and play some role in the pathogenesis of pure motor GBS. Interpretation of the staining by anti-GalNAc-GD1a antibody in DR and DRG, however, required further investigation.

CHRONIC MOTOR AXONAL NEUROPATHY ASSOCIATED WITH ANTIBODIES AGAINST GANGLIOSIDES

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Although acute motor axonal neuropathy (AMAN) is known as an axonal motor-dominant form of Guillain-Barré syndrome (GBS), its counterpart in chronic inflammatory demyelinating polyneuropathy (CIDP) has been unrecognized. AMAN is mostly associated with elevated titers of anti-GM1 or GalNAc-GD1a IgG antibodies. We studied 11 patients with slowly progressive lower motor neuron

syndrome diagnosed using criteria set by Pestronk and others (Ann Neurol 17:316,1990). Anti-glycolipid antibodies were examined using enzyme-linked immunosorbent assay. Tested antigens included GM1, GM2, GM3, GD1a, GD1b, GD3, GalNAc-GD1a, GT1b, GQ1b, and galactocerebroside. Positive reactions were confirmed with high-performance thin-layer chromatogram immunostaining procedure. Six out of 11 patients had elevated titers of anti-ganglioside antibodies. Three of 6 had IgG antibodies against GalNAc-GD1a and normal titers of antibodies against other gangliosides. The other 3 showed those against GM1 (2 with IgG, 1 with IgM). Presenting with progressive muscular atrophy, fasciculations, and no sensory deficits, the patients had been diagnosed to have motor neuron disease. Electrodiagnostic features were predominantly axonal with minimal conduction delays and no evidence of conduction block. Five patients were treated with repeated intravenous immunoglobulin infusions, and 4 of them showed clinical improvement. These 6 cases represent a chronic motor axonal neuropathy with possible immunopathogenesis, which may constitute an axonal motor-dominant variant of CIDP. They could disguise as a motor neuron disease, yet responding to immunotherapy. Sponsor: Supported by Grants-in-Aid for neuroimmunological diseases from Japan Ministry of Health and Welfare.

CLINICAL AND PATHOLOGICAL ASPECTS IN INTRAVENOUS IMMUNOGLOBULIN (IVIg) RESPONSIVE CHRONIC INFLAMMATORY AXONAL NEUROPATHY (CIAP)

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Chronic inflammatory predominantly motor-sensory neuropathy resembles motor neuron disease or hereditary motor sensory neuropathy (HMSN). It is particularly difficult to distinguish if persistent conduction block is absent. However, distinction of CIAP is very important, since inflammatory neuropathy is treatable. We describe the clinical, electrophysiological and pathological findings in 6 patients with a CIAP. Four of six patients had successful effect to IVIg. All patients showed slowly progressive severe symmetrical lower limb weakness and muscle atrophy with/without minor sensory deficits. CSF protein content was increased in 3 patients and there was no anti-GM1 antibody. All immunoelectrophoresis was normal. Motor conduction and EMG were consistent with axonal involvement. Although sural nerve conduction velocity was normal, SNAP was small. Peroneal F responses were delayed in 4 cases and absent in 2 cases. Sural nerve biopsy showed moderate loss of myelinated fibers (Fiber Density: $4,800 \pm 1,800/\text{mm}^2$; Mean \pm SD) and scattered axonal degeneration. Demyelination and remyelination were not remarkable, however, there some lymphocytes and macrophages infiltrated into the endoneurial and the perineurial areas. EM showed endothelial cell proliferation of the endoneurial vessels. These electrophysiological and pathological findings suggest that CIAP is polyradiculo-neuronopathy resulting from an inflammatory origin.

CLINICAL, ELECTROPHYSIOLOGICAL AND GENETIC STUDIES IN A COSTA RICAN FAMILY WITH HEREDITARY MOTOR AND SENSORY NEUROPATHY

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The clinical, electrophysiological and genetic findings are described in a Costa Rican family diagnosed with autosomal recessive hereditary motor and sensory neuropathy. We investigated this large family and here we present the data from six affected patients belonging to one of three branches of this consanguineous family with predominant Spanish ancestry. In the presented cases, genetic linkage analysis mapped to 19q13.3. The analyzed family originates from a single town in the province of Alajuela, Republic of Costa Rica in Central America. The age at onset of chronic symmetric sensory-motor polyneuropathy was 28 to 42 years. All patients presented with symmetric weakness of plantar extensor and flexor muscles in the lower extremities, distal weakness in the upper extremities were present in four patients. Sensory deficit in a stocking-glove pattern was present in all patients. Deep tendon reflexes were reduced or absent. The electrophysiologic data reflect an axonal degenerative process: Motor conduction velocities in the upper extremities were normal or slightly reduced, and in the lower extremities were absent or reduced. Sensory nerve conduction velocities could only be detected in one patient in the upper extremities. Amplitudes were decreased in affected nerves; motor and sensory nerve potentials did not show temporal dispersion. EMG showed an increased number of polyphasic motor unit potentials (MUP) and increased amplitudes of the MUPs.

SENSORY REINNERVATION IN DIABETIC NEUROPATHY

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The pattern of epidermal nerve fiber (ENF) loss in diabetic neuropathy suggests a cycle of denervation-reinnervation. This implies that persons with diabetic neuropathy have potential to reinnervate, given appropriate treatment. We investigated the efficiency of reinnervation of a model blister wound in normal and diabetic subjects. Pairs of 2 mm suction skin blisters were raised on the forearm of human subjects. At selected times one blister was removed by punch biopsy (3 mm), the pair member was reblistered (3 mm). Specimens were immunostained to localize nerves (PGP 9.5) and basement membrane (type IV collagen). ENF density was determined from confocal z-series using Neurolucida software. The blister roof presents a bird's eye view of all nerves in the sample. Biopsy sections give a view 90 degrees to the blister to show the ENF pathway into epidermis. Blister reinnervation in diabetic neuropathy followed a temporal course similar to that of normal subjects: 1) Reepithelialization; 2) Collateral extension of axons from adjacent normal epidermis; 3) Sprouting of fibers from the subepidermal neural plexus. Diabetic subjects with reduced density prior to blistering reinnervated to a similar reduced density. Sprouting often occurred in tufts of several fibers. Diabetic subjects are capable of regeneration of sensory nerves into nascent epidermis. The density of the regenerated nerves is similar to that before blistering. The skin blister is a minimally invasive model wound that may be useful for studying the potential for nerve regeneration of patients with neuropathy who are being considered for therapy or for a pilot study of local therapy. Sponsor: NIH (DK56708), Juvenile Diabetes Foundation (1-2000-318).

NITRIC OXIDE SYNTHASE ACTIVITY IN THE REGENERATIVE MILIEU OF INJURED DIABETIC PERIPHERAL NERVES

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Nitric oxide (NO) exhibits varied roles in the peripheral nerve regenerative microenvironment. For instance, liberation of NO by macrophage and Schwann cell derived immunologic nitric oxide synthase (iNOS) may serve as an important effector in the clearance of myelin/axonal debris, while simultaneously enhancing local nerve blood flow. Despite the aforementioned benefits, an enhanced

and prolonged expression of NO may also be neurotoxic. Previously, it has been demonstrated by ourselves, as well as others, that regeneration of a peripheral nerve is impaired in diabetes mellitus, perhaps related to delays in macrophage invasion. The current study was carried out to evaluate NOS activity and iNOS expression of the injured nerve in a rat model of diabetes mellitus. Briefly, Sprague-Dawley rats were made diabetic by streptozotocin (in citrate buffer) injection while controls received the buffer alone. Nerve conduction was carried out prior to nerve injury to verify neuropathy. Left sciatic nerves were transected and harvested 14 days post-injury for NOS activity. Shams consisted of nerve exposure, but no injury. NOS activity was assessed using an assay for conversion of radiolabeled arginine to citrulline, while expression will be quantified by reverse transcriptase-polymerase chain reaction (RT-PCR). As expected, noninjured diabetic sciatic nerves developed slowing of conduction velocity following 8 months of hyperglycemia. Intact nerves of diabetic animals exhibited higher baseline NOS activity than nondiabetics ($p < 0.001$). Transection induced a dramatic rise in proximal stump NOS activity in both groups, but diabetics displayed only a 52% increase compared to 225% in nondiabetics. Nondiabetic distal stumps had NOS activity comparable to intact shams, but diabetic NOS activity dropped by nearly 400% ($p < 0.05$). Diabetic sciatic nerves demonstrate abnormal NOS activity following peripheral nerve injury consistent with an impaired macrophage participation in nerve repair. Sponsor: CIHR & AHFMR.

URIDINE AND PYRUVATE AMELIORATE IN VITRO NEUROTOXICITY BY 2',3'- DIDEOXYCYTIDINE (ddC)

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BACKGROUND: Nucleoside Analogue Reverse Transcriptase Inhibitors (NRTIs) are an essential component of HAART (highly active antiretroviral therapy), substantially reducing the morbidity and mortality of HIV. However, the use of many of these drugs, including ddC, ddI and d4T, has been associated with a peripheral neuropathy that often necessitates drug discontinuation by the patient. We have recently developed an *in vitro* model of NRTI toxicity on primary dorsal root ganglion (DRG) neuronal cultures, and have been using this as a screening tool in the search for agents that prevent NRTI neurotoxicity. A study by Keilbaugh et al. (Mol Pharmacol 1993; 44:702-6) demonstrated amelioration of ddC toxicity on undifferentiated PC-12 cultures by uridine and pyruvate. We sought to investigate the therapeutic efficacy of uridine and pyruvate in our model. **METHODS:** Dissociated neurons from E15 rat embryo dorsal root ganglia were plated on a Schwann cell monolayer in the presence of NB1/GDNF medium. Various concentrations of uridine and pyruvate (0, 0.1, 10, 1,000 micromolar) were added to the culture wells at the time of plating, and three hours later, 10 micromolar ddC or no drug (controls) was added. After 15 hours of incubation, the cells were fixed and immunostained using anti-Beta III Tubulin as the primary antibody. Using this technique, neuronal cell bodies as well as their neuritic outgrowths could be clearly seen. The cell was scored positive for a neuritic outgrowth if at least one process was observed at a length of at least double the diameter of the cell body. **RESULTS/CONCLUSION:** The percentages of neurons bearing neuritic processes in the control and ddC cultures were 84% and 38%, respectively. In the presence of uridine and pyruvate, the ddC neurotoxicity was prevented. The percentages of neurite-bearing neurons were 68%, 87% and 69% at 0.1, 10, and 1,000 micromolar concentrations of uridine and pyruvate, respectively. In this model, uridine and pyruvate have been demonstrated to ameliorate NRTI neurotoxicity.

GREEN MARROW IN BLACK MICE: A POWERFUL TOOL TO DISSECT LESION RESPONSES OF LOCAL AND INFILTRATING MACROPHAGES DURING EXPERIMENTAL NEUROPATHY

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In a companion paper, we report on the identification and rapid response of resident endoneurial macrophages to traumatic and inflammatory nerve lesions using a rat transgenic bone marrow chimera model. Here we describe chimeric mice created by transplanting bone marrow from green fluorescent protein (GFP) transgenic mice into irradiated C57Bl6 wildtypes. Such animals exhibit green fluorescence in all bone marrow derived cells and allow easy differentiation between local and infiltrating macrophages. In normal peripheral nerve, approximately one half of all resident endoneurial macrophages were green after three months, indicating a 50% physiological turnover from the blood. Following a sciatic nerve crush, GFP-negative resident endoneurial macrophages became rapidly activated in the distal nerve segment from day two. A strong influx of GFP-positive haematogenous macrophages was not observed before day four. Although the number of GFP-positive infiltrating macrophages further increased with time, GFP-negative, long-term resident endoneurial macrophages with a highly activated morphology were strikingly frequent throughout all time points examined. Preliminary estimations suggest that their quantitative contribution to the total macrophage pool during Wallerian degeneration may be considerable. We then wanted to know about possible functional differences between GFP-negative and GFP-positive macrophage populations in normal nerves. In sciatic nerve organ cultures from chimeric mice, we found early and progressive morphological activation, proliferation and myelin phagocytosis by resident endoneurial macrophages, which was similar in GFP-positive resident macrophages of recent hematogenous origin and GFP-negative, long term resident macrophages. Our data suggest an early and active role for resident endoneurial macrophages following nerve lesions, but argue against the existence of functionally distinct populations of resident macrophages in the peripheral nerve. Sponsor: Deutsche Forschungsgemeinschaft.

TUMOR NECROSIS FACTOR--CONVERTING ENZYME IS EXPRESSED IN INFLAMMATORY DEMYELINATING DISEASES OF THE PERIPHERAL NERVOUS SYSTEM

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Tumor necrosis factor- (TNF-) is a major proinflammatory cytokine implicated in the pathogenesis of inflammatory demyelinating diseases, such as the Guillain-Barré syndrome. Soluble TNF- is released from its membrane-bound precursor by shedding through a proteinase, identified as TNF- α converting enzyme (TACE; ADAM-17), a member of the ADAM (A Disintegrin And Metalloproteinase) domain family of proteins. To investigate the expression pattern of TACE in inflammatory demyelinating disorders of the peripheral nervous system, sural nerve biopsies from patients with Guillain-Barré syndrome (GBS), chronic inflammatory polyradiculoneuropathy (CIDP), and, for comparison, various non-inflammatory neuropathies, were studied. Expression and distribution patterns of ADAM-17 were investigated immunohistochemically using an avidin-biotin detection system. Immunoreactivity for ADAM-17 was observed in GBS and CIDP cases, localized to small, round cells, primarily observed in perivascular cuffs. On serial sections, these cells, morphologically consistent with lymphocytes, were similar in appearance and distribution to cells detected with the T-cell marker anti-CD3. In the cerebrospinal fluid of GBS and CIDP patients, increased levels of TNF-RII, indicative of proteolytic activity of ADAM-17, could be found. Based on our present observations, we conclude that ADAM-17 is expressed by invading T lymphocytes in immune-mediated demyelinating diseases of the peripheral nervous system and might play a critical role in the pathogenesis of these disorders.

TWO CASES OF VARIANT TYPE GUILLAIN-BARRÉ SYNDROME WHOSE SERUM HAD LATROTOXIN-LIKE ACTIVITY

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We had two patients of variant Guillain-Barré Syndrome (GBS) whose clinical course, titers of anti-ganglioside antibodies and the effect of minimal endplate potentials (mepp) release (latrotoxin-like activity) were investigated. Patient 1: She had acute weakness, numbness of four limbs, ophthalmoplegia and cerebellar ataxia after diarrhea as a premonitory symptom. Her serum had only weak anti-GD1b IgG reactivity. There was no anti-GQ1b reactivity. Patient 2: She had acute weakness and numbness of four limbs after common cold during 2 weeks. She also had cranial nerves palsy (III, IV, VI, VII, IX, X, XI, XII). She required the respirator after admission. Her serum had strong anti-GQ1b, GT1a, GD1b IgG and IgM reactivity. For measurement of latrotoxin-like activity, we did conventional intracellular recording of mepp. We prepared the normal mouse phrenic-hemidiaphragm preparations bathed in the patient's sera, then measured mepp using glass microelectrodes filled with 3 M KCl. We could detect latrotoxin-like activity of both patients' sera when their symptoms were at the peak. Their sera facilitated mepp release then blocked neuromuscular conduction. However, when their symptoms improved, their serum facilitated mepp without neuromuscular blocking. Clinically, there were no neuromuscular disturbances by their electrophysiological study. The compound muscle action potentials of two patients, when the median and ulnar nerves were stimulated at the wrist, were completely normal. CONCLUSIONS: This latrotoxin-like activity may have a more specific correlation with the symptoms of Fisher's syndrome than anti-GQ1b antibody. However, this action may not influence the muscle weakness.

DORSAL ROOT GANGLION PATHOLOGY IN CHRONIC EXPERIMENTAL DIABETIC NEUROPATHY

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Putative mechanisms by which hyperglycemia causes diabetic neuropathy include nerve ischemia, auto-oxidation/glycation, overactivity of polyol pathway, protein kinase C, excessive lipolysis, and deficiencies of α -linolenic acid deficiency, or growth factor. All these mechanisms result in oxidative stress. Recent data suggests that dorsal root and sympathetic ganglia are specific targets of oxidative stress. Experimental diabetic neuropathy with a duration in excess of 6 months results in myelinopathy of dorsal and ventral roots and ischemia and a vacuolar neuronopathy of dorsal root ganglion (DRG). DRG mitochondria are especially susceptible to damage by reactive oxygen species (ROS). The vacuoles are mitochondrial, whose function appears to be defective. L5 DRG is ischemic, is under oxidative stress and shows defective respiratory enzyme activity and caspase-3 immunostaining, invoking an apoptotic mechanism. We undertook morphometric analysis of L5 DRG neurons in 7 diabetic rats and 6 age- and gender-matched littermates. Diabetes was induced with streptozotocin, and duration of diabetes was 12 months. DRG count for controls was $15,304 \pm 991$ neurons. Diabetic group mean count at $14,847 \pm 1524$ was not significantly reduced (although two values fell below the control range) when compared with controls. The number of small neurons (Type B neurons) considerably exceeded those of large neurons (Type A) with a ratio of 71:29. The percent of large cells was, however, significantly reduced for diabetics when compared with controls. As expected, the ratio of large: small cells was significantly reduced in diabetics ($p=0.01$). The large diameter population can be subdivided into two groups. With this subdivision, the intermediate size neurons ($<50 \mu\text{m}$) were not different in diabetics, but the largest size neurons ($\geq 50 \mu\text{m}$) were significantly reduced (by 41%). This selective reduction in large DRG neurons could explain the sensory conduction abnormalities. Sponsor: Supported in part by the Juvenile Diabetes Research Foundation and Mayo funds.

ASSOCIATION OF DIABETIC DERMOPATHY WITH NEUROPATHY

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Diabetic dermopathy (DD) is a common cutaneous finding in diabetes mellitus (DM). The objective of this study is to investigate whether there is an association between diabetic neuropathy (DNP) and DD. A total of 196 diabetic patients, 38 female (mean age: 60.4 ± 11.4), and 158 male (mean age: 59.1 ± 11.3), with clinical evidence of DNP and DD, underwent neurological examination, electromyography and nerve conduction studies. Mean duration of DM was 19 ± 10.6 and 13.8 ± 8.16 years in female and male patients, respectively. Negative sensory symptoms were found 84.2% and 63.9%, motor dysfunction was noted 13.1% and 23.4% in female and male patients, respectively. Almost half of the patients showed complete loss of sensory function. Motor nerve demyelination (7.8% and 15.8%) and severe axonal loss (26.2% and 32.2%) were found in female and male patients, respectively. Sural nerve was inexcitable in 71% of females and in 75.9% of males. Elderly patients with longer duration of diabetes had neuropathies characterised by severe sensory loss, whereas demyelinating and/or axonal neuropathy secondary to demyelination and motor neuropathy were more prominent in younger male patients. When compared to diabetic patients without DD, it seems that existence of DD correlates with the severity of DNP. Thus, DD as a clinical sign may indicate the presence of severe disabling DNP.

PERIPHERAL NEUROPATHY ASSOCIATED WITH HEPATITIS C WITHOUT CRYOGLOBULINEMIA

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There are an estimated 170 million persons worldwide infected with the hepatitis C virus (HCV). Peripheral neuropathy is a known complication of HCV, usually associated with cryoglobulinemia. However, little is known about peripheral nerve disease in cases where hepatitis C occurs in the absence of cryoglobulinemia. To investigate this, we reviewed the medical records of 211 patients seen at Mayo Clinic-Rochester between 1980-2000 who had been diagnosed with peripheral neuropathy and hepatitis C (or non-A, non-B hepatitis). These patients were identified by a computerized diagnostic coding system. Patients were excluded if they had a history or laboratory evidence of cryoglobulins, or if another cause of their neuropathy could be identified. Out of 100 patients analyzed thus far, a total of 25 patients (M=14, F=11; mean age at diagnosis: 54.7 y) were included in this study, all of whom were diagnosed between 1991-1999. Of these, 8 patients had clinical features of small fiber neuropathy and 6 had features of vasculitic neuropathy. Other clinical/EMG diagnoses included 5 patients with primarily distal, axonal sensorimotor peripheral neuropathy, and 2 patients with sensorimotor polyradiculoneuropathy. In addition, 2 patients had chronic inflammatory demyelinating polyradiculoneuropathy, 1 had acute inflammatory demyelinating polyradiculoneuropathy and 1 had a slowly progressive neurogenic process affecting lower motor neurons only. Six patients underwent sural nerve biopsy, 4 of which were diagnostic or suggestive of necrotizing vasculitis. Twenty-three patients were HCV RIBA positive, 1 was RIBA indeterminate but PCR RNA positive and 1 was antibody positive only. Five patients with elevated rheumatoid factor tested negative for cryoglobulins. These findings suggest a possible independent association between HCV and peripheral neuropathy, which is likely immune-mediated. Further analysis, outcomes and correlation with disease markers will be presented.

MECHANISTIC INSIGHTS AND BIOPSY EVIDENCE FOR AN IMMUNE-INFLAMMATORY COMPONENT IN HEREDITARY BRACHIAL NEUROPATHY

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Hereditary brachial plexus neuropathy (HBPN) is separable from the sporadic form by precipitating factors, history of recurrence and autosomal dominant inheritance. The symptomatology and time course of attacks is probably not different. Nerve biopsy data has provided evidence for an inflammatory process (autoimmune) in the sporadic form. The basis for the attacks in the familial form is unknown despite chromosomal localization at 17q25. Based on historical information, the putative mechanisms considered for neuropathic attacks are mechanical, hormonal, immunity and other. RESULTS: One patient with HBPN had 13 other affected members and prior postpartum attacks. She was examined serially during recent pregnancy for impairments (Neuropathy Impairment Score [NIS]), symptoms (Neuropathy Symptoms and Change [NSC]), and nerve conduction and quantitative sensation abnormalities. No neuropathic worsening occurred during the pregnancy. Elective cesarean section (37th week) was performed to eliminate mechanical and certain hormonal influences. On the first postpartum day, she had a typical attack. The second patient from a separate kindred, but with 7 affected members, had recurrent episodes and a new onset attack without precipitating event. Radial sensory nerve biopsy in both patients showed prominent axonal degeneration and epineurial perivascular inflammatory cells. Multifocal fiber loss was found in one nerve. In open trials with high dosage IV methylprednisolone, relief of symptoms occurred in both patients. CONCLUSIONS: 1) Elimination of the act of labor with parturition did not prevent an attack of HBPN, evidence against the role of mechanical and certain hormonal factors; 2) Prominent mononuclear cell infiltrates in biopsied nerve implies an inflammatory process, perhaps related to altered immunity and the mutant gene; 3) The pathologic process involves distal nerve; and 4) IV methylprednisolone may have ameliorated the symptoms of HBPN. Sponsor: Supported in part by grants from the National Institute of Neurological Diseases and Stroke (NS36797).

ANTI-GT1A ANTIBODY IN GUILLAIN-BARRÉ SYNDROME

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Several patients with Guillain-Barré syndrome (GBS) who show bulbar palsy have been reported to have serum anti-GT1a IgG antibody. Most of them, however, had also anti-GQ1b IgG antibody. Previous study has failed to detect GT1a in human cranial nerves, whereas GQ1b is abundant in human oculomotor nerves. Whether anti-GT1a IgG itself determines the clinical manifestations remains to be elucidated. In this retrospective study, anti-GT1a and anti-GQ1b IgG antibodies were positive, respectively, in 10% and 9% of 220 consecutive GBS patients. Patients with anti-GT1a IgG often had cranial nerve palsy (ophthalmoparesis, 57%; facial palsy, 57%; bulbar palsy, 70%) and 39% needed artificial ventilation. These features were also seen in those with anti-GQ1b IgG and there was no significant difference of clinical findings between the 2 groups. Anti-GT1a IgG in 5 patients did not have cross-reactivity with GQ1b. All of them had bulbar palsy, neck weakness, absence of sensory disturbance, and positive *C. jejuni* serology. Immunochemical study showed that GT1a is present in human oculomotor and lower cranial nerves. Anti-GT1a monoclonal antibody reacted with lipopolysaccharides from some *C. jejuni* strains. These studies provide further evidence that anti-GT1a IgG itself could determine the clinical manifestations. The distinctive clinical features of the patients with anti-GT1a IgG antibody without anti-GQ1b activity represent a specific subgroup within GBS.

CREATION OF BIOABSORBABLE TUBE COATED WITH SCHWANN CELLS FOR PERIPHERAL NERVE REPAIR THROUGH TISSUE ENGINEERING

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INTRODUCTION: Repair of severe traumatic peripheral nerve defect requires autografts. It sacrifices other peripheral nerves. This study assesses the feasibility of a tissue engineering approach to constructing bioabsorbable tube coated by Schwann cells. **MATERIALS AND METHODS:** We have synthesized copolymers with 5 types of compositions from L-lactide, D,L-lactide and caprolactone that are 50% L-lactide and 50% ϵ -caprolactone without collagen (A), 75% L-lactide and 25% ϵ -caprolactone without collagen (B), 50% L-lactide and 50% ϵ -caprolactone coated with Type I collagen (C), 50% L-lactide and 50% ϵ -caprolactone coated with Type IV collagen (D), and 50% L-lactide and 50% ϵ -caprolactone coated with Type I+IV collagen (E). We isolated Schwann cells from DRG in rats, and incubated on these copolymers. **RESULTS:** Two weeks after incubation, Schwann cells were identified histologically on all copolymers except (B). **CONCLUSION:** Schwann cells are clarified to be adhesive to these copolymers with collagen, and these results could be useful for making bioabsorbable tube coated by Schwann cells.

THE CMT NORTH AMERICAN DATABASE

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To date, six genes and many mutations have been identified as the cause of many cases of CMT. Linkage and family studies indicate that at least 8-10 other CMT-causative genes exist. The large amount of genetic heterogeneity in CMT makes phenotype-genotype correlations challenging. Extrapolation of data from published phenotype-genotype studies is difficult given that different parameters for measuring and interpreting electrophysiologic studies and clinical disability are used. A national database of clinical, family history, electrophysiologic and pathologic information on patients with all types of Charcot-Marie-Tooth disease (CMT) has been created so that large-scale natural history studies are feasible. The information in the database will be available to scientists performing quality CMT research. This comprehensive, computerized database has been developed at Indiana University School of Medicine to store, update and maintain clinical, family history, neurophysiologic and pathologic data on patients with all types of CMT. A centralized database will insure that all necessary forms are completed by the subjects and entered into the database in a timely, error-free fashion. Any physician in North America can submit data for entry into the database. Researchers interested in using this information will submit a proposal that will be reviewed by a panel of experts in the field of CMT. There has been significant interest in this project and it is supported by the CMT Association, CMT clinical laboratories and CMT experts across the country. A standard packet of information has been developed and is available to physicians from the CMT Association, CMT clinical laboratories, CMT research sites and our CMT clinic at Wayne State University. This packet contains information about the database, consent forms to participate, and standardized questionnaires. The creation of this database will greatly enhance CMT research and our understanding of the natural history of this condition. Sponsor: CMTA.

STRUCTURAL DIFFERENCES BETWEEN PAINFUL AND NON-PAINFUL NEUROMA - NEW EXPERIMENTAL ASPECTS FROM HUMAN NEUROMAS

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Overexpression of specific sodium channels after an injury to a peripheral nerve may underlie

the hyperexcitability associated with pain. It is hypothesized that either PN1 or PN3 sodium channels are involved in this phenomenon. Ankyrin G, a transmembrane protein of the axolemma, is involved in clustering of sodium channels at axon tips of unmyelinated sprouting fibers. Human neuromas were immunocytochemically examined for the localization of PN1/ PN3 sodium channels and ankyrin G. Ankyrin G content of painful and non-painful neuroma, as well as from normal nerve, was further evaluated by western blot and band intensities were quantitated by densitometry. PN1, PN3, and ankyrin G were detected and found to be upregulated in neuroma tissue. In two patients, samples of painful neuroma and normal nerve from the same patient were available for intraindividual comparison. Painful neuromas exhibited considerably higher levels of ankyrin G. A common (repair-) mechanism for clustering sodium channels at a high density is assumed. A dysregulation in this mechanism might be an initial step in a cascade that ends in a painful, rather than a non-painful, neuroma. Sponsor: Dr. T. Kretschmer's stay at LSUHSC/New Orleans was supported by a study grant from the DFG (Deutsche Forschungsgemeinschaft).

ABSORPTION OF ANTIGALACTOCEREBROSIDE ANTIBODIES IN SERA FROM PATIENTS WITH GUILLAIN-BARRÉ SYNDROME BY *MYCOPLASMA PNEUMONIAE*

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Elevated antibody titers against various glycolipids have been found in about 60% of acute phase sera samples from patients with Guillain-Barré syndrome (GBS). Galactocerebroside (Gal-C) is a major glycolipid antigen in the myelin of both the central and peripheral nervous systems. Anti-Gal-C antibody is known to be a demyelinating factor. We previously reported the presence of anti-Gal-C antibody in sera from patients with GBS subsequent to *Mycoplasma pneumoniae* (M. pneumoniae) infection. The mechanism of anti-Gal-C antibody production is not yet known. There are several reports of molecular mimicry between glycolipids and glycoconjugates of the microorganisms of the antecedent infections. We, therefore, investigated whether there is molecular mimicry between Gal-C and M. pneumoniae. M. pneumoniae CF antigen (Denka Seiken, Tokyo, Japan) was used as a M. pneumoniae reagent. The CF control antigen (Denka Seiken) was used as a control. Sera from 7 patients with GBS subsequent to mycoplasma infection with anti-Gal-C antibodies and those from 9 other GBS patients with antibodies against other glycolipids were investigated. Each serum sample, diluted 1:50, was incubated first with an equal volume of the CF antigen or the CF control antigen. Antibody activities of the serum samples incubated with the reagents were determined by the ODs obtained in the ELISA. The M. pneumoniae reagent specifically inhibited anti-Gal-C antibody activities in 7 GBS patients, whereas the control antigen did not. Antibody activities against such other glycolipids as GD1b, GQ1b and GalNAc-GD1a were not inhibited. This shows that a Gal-C-like structure is present in M. pneumoniae, indicative of molecular mimicry between Gal-C and M. pneumoniae. The Gal-C-like structure in M. pneumoniae may stimulate the immune system to produce anti-Gal-C antibody, which may act as a demyelinating factor in the pathogenesis.

AUTO-IMMUNOREACTIVITY TO SCHWANN CELLS IN PATIENTS WITH INFLAMMATORY NEUROPATHIES

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We studied the involvement of Schwann cells in patients with Guillain-Barré Syndrome (GBS), chronic inflammatory demyelinating polyneuropathy (CIDP) and multifocal motor neuropathy (MMN) by serological characterization using immunofluorescence microscopy. We found 21% of the GBS

(51/238), 22% of the CIDP (11/50) and 25% of the MMN (3/12) patients to have circulating IgG autoantibodies against proliferating non-myelinating human Schwann cells. In contrast, healthy donors showed positive staining in only 2 out of 34 sera. No reaction was found with sera from patients with non-inflammatory neurological disorders (HMSN type 1 0/47, Alzheimer's disease 0/4). Cell lines derived from non-neural origin (tumors) did not show this staining. Immunofluorescence was localized strongly at the distal tips (leading lamella) of the Schwann cell processes. Distal tips of neurites (nerve-growth-cones) of in vitro differentiated non-myelinated human neurons (hNT2 cells) were stained strongly as well. These data suggest that, at least part of the immune reactivity is not directed against myelin, but towards non-myelin proteins and epitopes possibly involved in Schwann cell-axon interaction. Several candidate epitopes were characterized by co-localization experiments. The correlation between clinical manifestation, intravenous immunoglobulin treatment and anti-Schwann cell serum antibodies will be discussed. Sponsor: Work was supported by the Prinses Beatrix Fonds under grant number 96-0108.

ANTIBODY-MEDIATED DAMAGE TO SENSORY NERVE FIBERS IN IgM MGUS NEUROPATHY

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Monoclonal gammopathy of undetermined significance (MGUS) is a hematologic condition characterized by an excess of serum M-protein (monoclonal antibody). Previous studies suggest that up to 50% of patients with MGUS also develop a debilitating peripheral neuropathy of unknown etiology. In this study, we present evidence that partially purified serum antibodies from a patient with IgM MGUS and sensory neuropathy are capable of causing complement-dependent damage to sensory nerve fibers. Antibody binding to dorsal root ganglion neurites was observed by immunocytochemistry using MGUS patient antibodies. This antibody sample also elicited complement-dependent increases in neuritic Ca⁺⁺ as measured by digital fluorescence microscopy using Fura-2 AM dye. Intraneural injection of complement-supplemented serum antibodies produced degeneration of some myelinated fibers and a marked attenuation of the sensory response (H reflex) while preserving evoked motor responses (M wave). Antibodies drawn from age-matched control patients without MGUS or neuropathy did not damage peripheral nerve fibers *in vitro* or *in vivo*. These data suggest that anti-neural antibodies present in some IgM MGUS patients produce sensory deficits through the recognition of antigens unique to sensory fibers. Supported by grants from Falk Charitable Foundation (Loyola University Chicago) and the Department of Veterans Affairs.

LONG-TERM TREATMENT OF MULTIFOCAL MOTOR NEUROPATHY WITH PERSISTENT CONDUCTION BLOCKS: A RETROSPECTIVE STUDY OF 43 CASES

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Patients with multifocal motor neuropathy (MMN) are known to respond to IVIg in 70-80% of cases, but the long-term treatment has been the subject of few studies (Van den Berg-Vos et al. 2000, Taylor et al. 2000). We retrospectively studied 43 patients (28 M and 15 F, mean age 52.2 years) with MMN followed from 4 to 7 years. All patients had 6 monthly courses of IVIg at 2 g/kg, then some of them had IVIg courses depending on their motor deterioration. Immunosuppressors were introduced in patients needing repeated IVIg courses after at least 18 months. Patients were divided in 4 groups. Group 1: 14 patients (32.5%) had prolonged remission (>12 months) following 6 (7 cases) or 6-18

(7 cases) monthly IVIg courses. Group 2: 10 patients (23.2%) had stabilization or slight deterioration depending on only IVIg courses repeated 2 to 6 times a year. Group 3: 10 patients (23.2%) had stabilization or slight deterioration depending on repeated IVIg courses and immunosuppressors (cyclophosphamide: 7 cases, azathioprine: 3 cases). Group 4: 3 patients (16.2%) did not respond to a first course of IVIg and 4 other patients secondly deteriorated despite repeated IVIg infusions and immunosuppressors. The last 2 patients dropped out of the study because of loss of follow-up. In this series, nearly 1/3 of patients with MMN had prolonged remission with 6-18 months IVIg courses. Half of them needed repeated IVIg courses, of whom 50% needed immunosuppressors. Lastly, 16.2% of patients failed to respond to both treatments. In conclusion, both treatments did not seem to bring a long-lasting remission of the disease in nearly 2/3 of cases.

RELIABILITY OF MORPHOLOGICAL METHODS IN VASCULITIC NEUROPATHY

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OBJECTIVE: To investigate the reliability of morphological investigations in vasculitic neuropathy. **METHODS:** In 104 cases of proven vasculitic neuropathy, biopsies of peripheral nerve and skeletal muscle were reevaluated by using available specimen: HE stain including serial sections, Trichrome stain, plastic embedded semithin sections and immunohistochemistry (CD4, CD8). **RESULTS:** There would be false negative results in 13.6% if isolated peripheral nerve and in 34.8% if isolated skeletal muscle would have been biopsied. Without performing serial sections of the peripheral nerve, 3.7%, without immunohistochemistry, even 12.2% and, disclaiming both methods, 20.7% of vasculitides of the nerve would not have been detected. If HE and Trichrome stain is negative in about 30%, vasculitis of the nerve was identified on the basis of serial sections, immunohistochemistry and/or semithin sections of the peripheral nerve. Performing additional muscle biopsy but not serial sections and immunohistochemistry of the peripheral nerve would result in 5.8% false negative diagnosis of vasculitis. **CONCLUSIONS:** We suppose that in case of suspected vasculitic neuropathy, not only combined biopsy of peripheral nerve and skeletal muscle should be performed routinely. Additionally, serial sections and immunohistochemistry of the peripheral nerve should be integrated in routine diagnostic investigation.

SPIKE TRIGGERED AVERAGING DEMONSTRATES MOTOR UNIT LOSS IN BOTH PROXIMAL AND DISTAL MUSCLES IN CHARCOT-MARIE-TOOTH DISEASE

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OBJECTIVE: We have utilized the spike triggered averaging (STA) technique of motor unit number estimation (MUNE) to determine the extent of motor unit loss in the biceps brachii (BB) and abductor digiti minimi (ADM) in patients with CMT. STA may correlate better with strength than compound motor potential amplitudes (CMAP) and may demonstrate differences between the various forms of CMT. **Background:** There is increasing evidence that the major determinant of weakness in all forms of CMT is axonal loss causing a reduction in the number of motor units. While reductions in CMAP of distal muscles have been found to develop over time, less is known about proximal muscles. **DESIGN:** 61 patients with CMT (CMT1A=27; CMTX=7; CMT2=27) and 7 normal controls had STA of the BB and ADM. The averaged motor unit potential amplitude (AMUP) and MUNE were determined and correlations were made to CMAP and clinical strength. **RESULTS:** Preliminary analysis of the first 25 patients reveals marked reduction of ADM MUNE in all patients (24; norm>100), but mild reduction in most but not all patients of BB MUNE (108; norm>150). ADM AMUP was markedly increased

(670 uv; norm < 100), but BB AMUP was only mildly increased (120 uv; norm < 70). MUNE correlated with CMAP for both muscles but better for BB. In some cases, MUNE correlated better than CMAP with clinical strength of the ADM. The patients with CMTX had more severe reduction in MUNE and larger AMUP of the ADM than other forms of CMT. CONCLUSIONS: STA demonstrates that proximal muscles have similar but less severe motor unit loss than distal muscles. Collateral sprouting is more extensive in the ADM than BB. There is a suggestion that MUNE correlates better with strength than CMAP, most likely related to the potential excessive influence of increased AMUP on CMAP. STA may allow one to follow the progression of motor unit loss in patients with CMT. An analysis of the 61 patients will be reported. This may show further differences between the various forms of CMT. Sponsor: Muscular Dystrophy Association.

THE DISTAL SLOWING IN HNPP IS NOT SOLELY DUE TO A DISTAL MYELINOPATHY

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OBJECTIVES/BACKGROUND: Previous electrophysiological studies of patients with Hereditary Neuropathy with Liability to Pressure Palsies (HNPP) have shown accentuated distal slowing superimposed on a generalized polyneuropathy with mildly reduced conduction velocity. Some authors have suggested that there is a distal myelinopathy as an underlying pathophysiologic mechanism in HNPP. We have evaluated patients with HNPP to determine whether more extensive studies are consistent with this concept. METHOD: We studied an HNPP family with not only standard nerve conduction studies but also studies of more proximal muscles in the arm and leg. Other families with HNPP will also be studied and reported. RESULTS: Both the proband, a 9-year-old girl with symptomatic onset at age 6, and her minimally symptomatic 43-year-old father, had abnormal conduction studies. Median, ulnar and peroneal distal motor latencies (DML) to the Abductor Pollicis Brevis, Abductor Digiti Minimi and Extensor Digitorum Brevis muscles, respectively, were significantly prolonged, but the DML to the anterior tibialis, and forearm flexor muscles (median and ulnar) were normal. The tibial DML to abductor hallucis and musculocutaneous DML to biceps brachii were also normal. CONCLUSION: Accentuated distal slowing is found to most, but not all, distal muscles. More proximal muscles do not have the same distal latency slowing. These findings do not support the concept of a distal myelinopathy as the sole or major determinant of the conduction abnormalities. Other factors, including distal compression, may be playing a more significant role than previously considered. Other possible physiologic explanations of these observations will be discussed.

RAPID RESPONSE TO COMBINED PLASMA EXCHANGE FOLLOWED IMMEDIATELY BY INTRAVENOUS IMMUNOGLOBULIN IN REFRACTORY CIDP

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Chronic inflammatory demyelinating polyneuropathy (CIDP) can progress rapidly and become refractory to available treatments. Response to intravenous immunoglobulin (IVIg) can wane and is, at times, improved by a course of plasma exchange. We report here 2 patients with rapidly progressive CIDP who had become refractory to immunosuppression with IVIg or plasmapheresis separately, as well as to a course of plasmapheresis followed by a course of IVIg. Both patients improved dramatically, from being bedridden and worsening to being ambulatory without assistance in two to three weeks, on a course of plasmapheresis followed immediately by IVIg on the same day. Nerve conduction studies were conducted in one patient and demonstrated dramatic improvement as well. These combined treatments were administered on alternate days for one to two weeks and then tapered. Though quite expensive, this regimen may be helpful in selected patients with severe, progressive CIDP that has become refractory to serial courses of either treatment alone. More

experience with this approach and a better understanding of its mechanism are necessary.

POLYCYTHEMIA VERA AND PERIPHERAL NEUROPATHY: A DESCRIPTION OF 3 PATIENTS

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Although paresthesias have been often described among neurologic manifestations of polycythemia vera (primary polycythemia), the involvement of peripheral nervous system is uncommon, and descriptions of electrophysiological and morphological aspects of this rare neuropathy are scanty. We report on 3 patients affected by polycythemia vera who showed clinical signs of peripheral neuropathy. Extensive neurophysiological examination and sural nerve biopsy were performed in all cases. Clinical features were heterogeneous among our patients. Two patients had a chronic indolent neuropathy, with predominant sensitive involvement in one and sensory-motor impairment in the other. The third patient showed two episodes of subacute-onset sensory-motor neuropathy without evidence of improvement over a long-term follow-up. In all patients, sural nerve biopsy was consistent with an axonal neuropathy with variable degree of fiber loss. Active fiber degeneration was obvious only in one patient. No features of demyelination were found. We didn't find any relationship between treatment of underlying myeloproliferative disorder and improvement of symptoms, as previously described by other authors.

CAMPTODACTYLY - A NON-NEUROGENIC CAUSE OF CLAWING OF THE FINGERS

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Camptodactyly (Greek; "kamplein" - bent and "dactulos" - finger) is a flexion deformity of the proximal interphalangeal joint, which usually appears during adolescence (Type 2), but may occur in infancy (Type 1). The contracture mainly affects the little finger and is bilateral in 50% of cases, but nearly always asymmetric. We report 9 cases (male:female = 2:7; age range 16-40 years) referred with clawing of the little finger, in whom a diagnosis of ulnar neuropathy or more proximal C8/T1 root lesion had been considered. All patients gave a history of painless clawing of the little finger with some involvement of the ring finger. The clawing interfered with fine manual skills such as playing a musical instrument or typing. In two cases, the mothers had a similar deformity. The flexion deformity was reversible in all cases with no motor or sensory deficit found on examination. Nerve conduction and electromyographic studies were normal. Two patients were referred for hand surgery, with restoration of normal function of the little finger.

POSTURAL TACHYCARDIA SYNDROME (POTS): A LIMITED AUTONOMIC NEUROPATHY

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POTS is manifested by orthostatic tachycardia without OH. Most patients are women between the ages of 20 to 50 years. Orthostatic symptoms consist of a combination of symptoms of cerebral hypoperfusion (such as lightheadedness, weakness, blurred vision) and sympathetic activation (such as tachycardia, nausea, tremulousness). POTS is heterogeneous; some patients have a limited, presumably immune-mediated autonomic neuropathy. An antecedent viral infection and evidence

of peripheral denervation occurs in approximately 50% of patients. On thermoregulatory sweat test or QSART, anhidrosis of the legs is commonly found. Peripheral adrenergic denervation is present. Evidence includes loss of phase II_L of the Valsalva maneuver and reduced lower extremity secretion of norepinephrine. Perivascular round cell infiltration is sometimes seen on nerve biopsy, and ganglionic antibody may be positive, especially in the more severely affected patients. Loss of epidermal fibers is sometimes seen on skin biopsy. As a result of peripheral denervation, capillaries are excessively leaky, and hypovolemia develops with continued standing. We recently demonstrated excessive splanchnic pooling, presumably due to partial denervation of splanchnic-mesenteric venous capacitance bed. In addition to peripheral denervation, pathophysiologic considerations include hypovolemia, beta-receptor supersensitivity, hyperadrenergic state from different mechanisms (hypovolemia, venous pooling, norepinephrine transporter defect), venous pooling (legs and splanchnic mesenteric bed) and brain stem dysfunction. Cerebral hypoperfusion is due to paradoxical vasoconstriction of cerebral arterioles in response to standing up. Brain stem dysregulation is suggested to occur. In some patients, it may be the dominant mechanism. Sponsor: Supported in part by funds from NIH PO1 NS32352, Mayo GCRC (M01 RR00585), and Mayo funds.

ANTI-MAG ANTIBODIES ALTER NEUROFILAMENT SPACING: A CONTRIBUTION TO THE PATHOGENESIS OF IgM ANTI-MAG PARAPROTEINAEMIC NEUROPATHY?

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BACKGROUND: Anti-myelin associated glycoprotein (MAG) IgM paraprotein associated neuropathy is a demyelinating neuropathy with prominent distal slowing of conduction. Anti-MAG antibodies are pathogenic but the mechanism of that pathogenesis remains unexplained. Regional variations in axon calibre depend upon the phosphorylation status of neurofilament sidearms. Animal studies indicate that MAG is one of the signaling molecules controlling neurofilament phosphorylation, neurofilament spacing and, hence, axonal calibre. We postulated that if MAG is involved in the control of neurofilament spacing, then nearest neighbour neurofilament distance (NNND) would be altered in patients with anti-MAG neuropathy. **METHODS:** In an EM morphometric study, we measured NNNDs in the axons of sural nerves from patients with anti-MAG paraproteinaemic neuropathies and compared these to normal human sural nerves and those from patients with Guillain-Barré Syndrome or CIDP. **RESULTS:** Axon calibre was similar in all groups. In normal human sural nerves axonal NNND was correlated with axonal diameter ($r=0.56$). In diseased axons, this correlation did not exist. NNND was significantly reduced in demyelinated axons (30.5 ± 3.2 nm) and axons with widely spaced myelin (28.9 ± 1.3 nm) from patients with anti-MAG antibodies compared to normal axons from normal patients (39.8 ± 3.2 nm) or those with demyelinating neuropathy (35.8 ± 4.6 nm) ($p=0.001$). **CONCLUSION:** This study supports the previous observations that MAG is involved in the control of neurofilament spacing and extends these findings to human anti-MAG paraproteinaemic neuropathies. A reduction in NNND may impair neural transport and conduction contributing to the pathogenesis of the neuropathy and the prominent distal slowing of conduction. Sponsor: MPL is supported by the Patrick Berthoud Charitable Trust.
