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INTERFERON ALPHA MAY BENEFIT CIDP PATIENTS REFRACTORY TO STANDARD THERAPIES. DESCRIPTION OF LONG-TERM RESULTS IN 12 PATIENTS

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Although most patients affected by CIDP improve with Corticosteroids, Intravenous Immunoglobulins or Plasma-Exchange, about 20-30% show poor or no response and 7-10% die due to the disease. We describe 12 CIDP patients unresponsive to standard therapies who were treated with Interferon alpha at a dosage of 2-3 MIU/three times/week. Follow-up period ranged from 4 months to 8 years. Three patients recovered completely and three others improved of at least 2 grade in the Rankin scale. Beginning of improvement was observed within 2-4 days since starting therapy in most patients. In the remaining 6 patients no improvement was observed and two of them died as the result of progression of the disease to severe quadriplegia with respiratory failure. No significant adverse effects were observed. Over the last decade Interferons have been used in the treatment of CIDP with conflicting results. Selection criteria and the type of Interferon employed may explain these discrepancies. Our data show that 50% of patients treated with IFN alpha disclose a significant improvement thus suggesting that this therapy may represent an effective option in otherwise intractable CIDP patients.

METABOTROPIC P2Y RECEPTORS AND THEIR FUNCTIONAL ROLES IN RAT DORSAL ROOT GANGLION NEURONS

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Changes in intracellular free Ca²⁺ concentration ([Ca²⁺]_i) in small-sized dorsal root ganglion (DRG) neurons are closely implicated in the transmission of nociception. Although several types of connections of sympathetic nerves with sensory nerves have been reported, it remains unclear how sympathetic nerves transmit signals to sensory neurons. The study reported here was undertaken to examine the modulation of [Ca²⁺]_i by noradrenaline (NA) and ATP in isolated adult rat small-sized DRG neurons using the fluorescent Ca²⁺ indicator fura-2. Bath application of ATP (0.1 mM) evoked a rapid increase in [Ca²⁺]_i in small-sized DRG neurons, whereas NA failed to induce any change in [Ca²⁺]_i. Extracellular ATP induced substantial [Ca²⁺]_i increase even in the absence of extracellular Ca²⁺, suggesting an involvement of Ca²⁺ release from internal stores. UTP (0.1 mM) mimicked these stimulatory actions of ATP on [Ca²⁺]_i, indicating that [Ca²⁺]_i elevation during exposure to UTP is mediated through a metabotropic P2Y receptor. The RT-PCR analysis detected the expression of mRNAs encoding metabotropic P2Y1, P2Y2, P2Y4 and P2Y6 receptors in rat DRG neurons. The ELISA analysis also showed that an application of UTP significantly stimulated the release of CGRP, suggesting that P2Y receptor-mediated [Ca²⁺]_i increase is accompanied by CGRP release from DRG neurons. We conclude that rat small-sized DRG neurons express the metabotropic P2Y receptors, which would contribute, at least in part, to an elevation in [Ca²⁺]_i and to CGRP release in response to extracellular ATP.

CYCLOOXYGENASE-1 AND -2 IN NERVE INJURY AND TNF-INDUCED HYPERALGESIA IN THE RAT

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Prostaglandins have been suggested to contribute to hyperalgesia resulting from nerve injury. The inducible form of cyclooxygenase, COX-2, is upregulated in the spinal cord and thalamus after nerve lesion. We could previously show that COX-2 is also upregulated in the injured nerve itself, and that this upregulation is dependent on tumor-necrosis-factor-alpha (TNF). We therefore asked whether a COX-inhibitor would attenuate neuropathic pain in nerve injured rats with elevated endogenous TNF and in rats made allodynic by intraneural injection of recombinant TNF. Unilateral chronic constrictive sciatic nerve injury (CCI) was produced in 14 rats. Rats received vehicle, the COX-1 and -2 inhibitor ibuprofen (40 mg/kg), or the selective COX-2 inhibitor celecoxib (10 mg/kg). Drugs were given twice daily by tube feeding. Withdrawal latency to heat (Hargreaves' test) and mechanical thresholds (von-Frey-hairs) were regularly assessed. While vehicle treated rats developed thermal hyperalgesia (latency reduced from 17.3 ± 0.5 sec to 6.7 ± 0.8 sec), thermal hyperalgesia was significantly attenuated in both ibuprofen and celecoxib treated rats (latencies 8.6 ± 0.87 sec and 10 ± 1.6 sec on day 10, respectively). Similarly, mechanical allodynia was attenuated but not reversed by both treatments (19.7 ± 1.0 g at baseline, 3.1 ± 2.4 g in vehicle treated rats, and 4.9 ± 2.7 g and 5.6 ± 2.1 g in celecoxib- and ibuprofen-treated rats). In a pilot experiment, TNF (2.5 pg/ml) or 0.1% BSA (10 μ l) were injected into the sciatic nerve of 13 rats. Some rats additionally received ibuprofen (n=4) or celecoxib (n=5) as described above. TNF produced mechanical allodynia with reduction of von-Frey-hair thresholds to 50% of baseline. In ibuprofen- but not in celecoxib-treated rats, there was a significant reduction of allodynia at day 5. These findings indicate that nerve-injury- and TNF-induced pain-related behavior in rats is partly dependent on prostaglandins. Further studies are in progress to explore the possible role of COX-inhibition in TNF-induced allodynia.

TREATMENT AND PREVENTION OF EXPERIMENTAL AUTOIMMUNE NEURITIS WITH CD 28-SPECIFIC MONOCLONAL ANTIBODIES

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To investigate therapeutic effects of the "superagonistic" monoclonal anti-CD 28-specific antibody (mAb) JJ316 in experimental autoimmune neuritis (EAN). In contrast to the conventional anti-CD28 mAb JJ319, the mAb JJ316 activates T cells without T cell-receptor occupancy *in vitro* and induces lymphocytosis and a shift towards a Th2-cytokine pattern *in vivo*. Active EAN was induced in female Lewis rats with the P2 peptide aa 53-78 in complete Freund's adjuvant. Groups of 5 rats received 1 mg JJ316 i.p. or isotype controls either as prophylaxis on day -21 or day -7 before EAN induction, or as preventive therapy on days 0 and/or 4 or 12. In some experiments, JJ319 was also used. Electrophysiological measurements were performed before onset and at the peak of disease. In *ex-vivo* studies, lymph nodes were taken on day 12 for FACS analysis of intracellular IFN-gamma production in P2-specific T cells. Treatment with JJ316 during the induction phase of disease on days 0 and 12 reduced disease activity up to a complete prevention. Moreover, prophylaxis with JJ316 7 days before induction of EAN ameliorated disease (mean 3.5 vs. 6.0 in controls). The effect of JJ319 was less marked. Electrophysiological studies in JJ316 treated rats showed improved F-latency (7.8 ± 0.4 vs. 9.7 ± 0.3 ms in controls, in both groups 7.5 ms before immunization) and SEP-N1-response (3.2 ± 0.3 vs. 4.0 ± 0.2 in controls, 3.1/3.0 before immunization). Intracellular cytokine staining and FACS analysis of P2-specific T cells revealed a significant reduction of IFN-gamma positive cells after

JJ316 therapy (13%) compared to untreated control-EAN (33%) and healthy animals (19.8%). In this study we could show a preventive and a prophylactic effect of the anti-CD28 mAb JJ316, associated with a Th2-type cytokine shift. These results may have implications for future therapies of autoimmune diseases of the peripheral nervous system.

THE DEGREE OF AXONAL DAMAGE OR CONDUCTION FAILURE DETERMINES CLINICAL DISABILITY AND OUTCOME IN PATIENTS WITH CIDP

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OBJECTIVE: To elucidate the significance of axonal damage and demyelination for clinical disability in the course of CIDP. **METHODS:** The results of at least three consecutive nerve conduction studies from 15 patients meeting established diagnostic criteria of CIDP were evaluated. All patients fulfilled electrophysiological criteria for CIDP proposed by the Ad Hoc Subcommittee 1991. Electrophysiological examination included nerve conduction studies of the tibial and the median motor nerves and sensory nerve conduction studies of the median and the sural nerves. Clinical disability was rated according to a modified Rankin scale. For statistical evaluation, Spearman's correlations were used. **RESULTS:** A significant correlation of clinical disease severity as classified by the Rankin scale and the compound muscle action potential (CMAP) amplitude of the median and the tibial nerve was established. Furthermore, a significant correlation of the amplitude of the sural nerve sensory nerve action potential (SNAP) and disease severity could be found. F-wave latencies of the median and the tibial nerve did not correlate with the clinical score, but we found a positive correlation of F-wave persistence in the median and in the tibial nerves and disease severity. There was a good correlation of the electrophysiological parameters of the tibial nerve with those of the median nerve. Conduction velocities did not correlate with the clinical score. In 5 of the 15 patients there was a good intraindividual correlation of the CMAP amplitudes or distal motor latencies and the clinical score before and after cortisone pulse therapy. **CONCLUSION:** CMAP amplitudes of the motor tibial and median nerves and the sural nerve SNAP amplitude showed a significant correlation with disease severity indicating that the degree of axonal damage or conduction failure, rather than conduction slowing, are most relevant for clinical disability.

DISEASE SUSCEPTIBILITY OF NEWBORN LEWIS RATS TO AT-EAN IS SIGNIFICANTLY REDUCED COMPARED TO ADULT RATS DESPITE MAST CELL ACTIVATION, INFLAMMATORY INFILTRATES, AND INCREASED MHC CLASS II ANTIGEN EXPRESSION

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Experimental allergic neuritis induced by the adoptive transfer of P2-specific rat T-cell line (AT-EAN) is considered as an animal model of the Guillain-Barré syndrome (GBS) in man. It is not yet known whether AT-EAN is inducible at early stages in the development of the peripheral nervous system or whether disease activity is modified because of immaturity of either the nervous or the immune system. P2 antigen was already present in 4-day old Lewis rats. After tail vein injection of 10⁶ activated, P2-specific T-cells, MHC class II (Ia) antigen expression at 4, 7, and 10 days post partum was increased in sciatic nerves and spinal ganglia. Unlike in adults, segmental or paranodal demyelination was minimal, and nerve fiber degeneration did not occur despite activation of mast cells, the presence of inflammatory cells, and Ia antigen expression in Schwann cells and macrophages. It is concluded that newborn Lewis rats are less prone to develop AT-EAN than adult

rats.

BORRELIA NEUROPATHY: HISTOLOGIC AND IMMUNOHISTOCHEMICAL CHARACTERIZATION
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The pathologic features of borrelia neuropathy are not well defined. Here, we investigated 20 patients with borreliosis (5 in stage 2, 15 in stage 3) in comparison to 10 patients with vasculitic neuropathy (VN) and 14 patients with idiopathic axonal neuropathy (AN). Routine histology and immunohistochemistry with antibodies to CD4, CD8, CD68, 27E10 (activated macrophages) and 25F9 (late macrophages), C3, C5 and membrane attack complex C5b-9, IgM, IgG and IgA, VCAM, NCAM, ICAM-1, tumor necrosis factor-alpha (TNF), interleukin (IL)-1, IL-6, metalloproteinases MMP-2 and MMP-9 were performed. The characteristic histology was an axonal neuropathy (17/20) with perivascular infiltration of inflammatory cells (18/20), in 5 cases with definitive vasculitis. Inflammation was more pronounced in stage 2 than in stage 3. Most of the borrelia-neuropathies (BN, 18/20) showed late (25F9), but not early (27E10) endoneurial macrophages. In all patients with BN, but in only one third of VN and AN, perineurial thickening and vascularization was prominent. Perineurial immunoreactivity for IL-6 and TNF was prominent in BN, but not in the other neuropathies. Endoneurial cytokine expression was increased in VN and BN with vasculitis but not in BN with only perivascular infiltrations. In the endoneurium, the expression of HLA-DR3, ICAM and NCAM was markedly increased in BN compared to VN and AN. In conclusion, neuropathy in patients with borreliosis in our series of 20 patients is associated with perivascular and vasculitic inflammation, upregulation of adhesion molecules and pronounced perineurial pathology. We suggest, that borreliosis induces an autoimmune reaction attacking the perineurium.

EVALUATION OF DEMYELINATION AND AXONAL DEGENERATION WITH INTRAFASCICULAR NEUROGRAPHY

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We investigated typical patients with demyelinating and axonal neuropathies by means of intrafascicular neurography. A total of 30 patients with pure motor AIDP, multifocal motor neuropathy (MMN) and CIDP were classified as demyelinating neuropathy. Twelve patients with vincristin neuropathy and cisplatin neuropathy, ischemic neuropathy due to angiitis were included as axonal neuropathy. A tungsten microelectrode with a tip diameter of about 1 micron was inserted percutaneously into the median nerve trunk at the elbow without anesthesia. With supramaximal electric stimulation to the median nerve at the wrist, the largest compound nerve action potential (CNAP) was recorded. In healthy controls, the median nerve CNAP showed a large triphasic wave (positive-negative-positive, 300 μ V in average amplitude) followed by small multiphasic waves. The CNAP evoked by the supramaximal stimulus can be regarded as summation of single nerve action potentials around the electrode. We prepared a program to calculate the fiber diameter distribution from CNAP. The obtained histogram revealed two peaks of conduction velocity distribution as is already shown in the biopsy specimen. In demyelinating neuropathy, CNAP demonstrated irregular multiphasic waves. The maximal nerve conduction velocity (NCV) and amplitude (Amp) of CNAP were decreased. The histogram calculated by CNAP showed severe temporal dispersion. In axonal degeneration, amplitude of CNAP decreased without changes in NCV and histogram pattern. Comparing to the conventional nerve conduction studies, intrafascicular neurography is much more sensitive and accurate in the evaluation of peripheral neuropathies.

CIRCULATING MATRIX METALLOPROTEINASE 9 (MMP-9) CORRELATES WITH ELECTROPHYSIOLOGICAL ABNORMALITIES IN GUILLAIN-BARRÉ SYNDROME (GBS)

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OBJECTIVE: To investigate correlations between MMP-9 circulating levels and electrophysiological abnormalities of GBS. **BACKGROUND:** Circulating MMP-9 is increased in GBS, but whether it may underlie nerve dysfunction remains unknown. **DESIGN/METHODS:** Electrophysiological studies and MMP-9 circulating levels (ELISA) were simultaneously performed within the first 72 hours of admission in 21 consecutive patients with GBS. Electrophysiological data were classified as demyelinating (DM) or non-demyelinating (NDM). The severity of the polyradiculoneuropathy was evaluated by Indexes of Polyneuropathy (IPN) ranging from 0.00 (absence of neuropathy) to 1.00 (extremely severe neuropathy), taking into CMAP, distal latencies, NCV, and F waves. Global (IPNG), upper (IPNUL) and lower (IPNLL) limbs indexes were calculated. **RESULTS:** 14 patients (67%) were classified as DM and 7 (33%) as NDM. MMP-9 (ng/mL) was significantly higher in the DM group than in NDM group (47.7 ± 44.7 vs 157.8 ± 141.3 ; $p=0.02$). MMP-9 positively correlates with IPNG ($p=0.05$) and IPNUL ($p=0.02$), but not with IPNLL ($p=0.1$). There was a positive correlation between MMP-9 and CMAP decrease ($p=0.02$) and F-waves increase latencies ($p=0.05$). High MMP-9 tended to be associated with higher CSF protein levels ($p=0.06$). **CONCLUSION:** Increased MMP-9 circulating levels are associated with demyelinating GBS, and correlate with electrophysiological severity, F-waves and CMAP alterations. These findings suggest a role of circulating MMP-9 in the pathogenesis of peripheral nerve dysfunctions in demyelinating GBS. **Sponsor:** Contrat de Recherche et d'Investigation Clinique AP/HP (CRC 00006).

ANTI-GANGLIOSIDE ANTIBODY MEDIATED AXONAL DEGENERATION

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The AMAN variant of GBS is usually a post-infectious acute monophasic autoimmune neuropathy; *C. jejuni* is the most frequently recognized antecedent infection. Lipopolysaccharides of *C. jejuni* isolates from patients with AMAN carry ganglioside-like moieties. Our studies in Northern China indicate that high affinity IgG antibodies against the ganglioside GD1a are specific markers for AMAN. Pathological studies in this disorder indicate non-inflammatory, antibody mediated complement dependent injury of motor axons. Work in our laboratory has focused on modeling this disease by targeting GD1a. We have produced several clones of high affinity IgG anti-ganglioside (including anti-GD1a) antibodies in immunologically naive genetically engineered mice lacking complex gangliosides. In our initial studies we have used a complement-fixing clone for passive transfer. This clone was implanted i.p. in wild-type mice to obtain high titers of circulating antibodies. Animals were also given heterologous complement. These studies demonstrate that animals implanted with this clone develop axonal peripheral neuropathy. Scattered axonal degeneration was seen in peripheral nerves and spinal roots. Most animals had plaque-like areas of contiguous multiple myelinated fibers undergoing Wallerian-like degeneration, almost always in the spinal roots. Pathological changes were also seen in distal intramuscular nerves. Importantly, the pathology was patchy, non-inflammatory, and most severe in the spinal roots and terminal nerve regions of the peripheral nervous system, areas with a relatively permeable blood-nerve-barrier. These observations strongly support a pathogenic role for anti-ganglioside antibodies in AMAN.

DEXAMETHASONE DECREASES NERVE BLOOD FLOW

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Dexamethasone is an anti-inflammatory glucocorticoid used to reduce edema in neurological tissue and to otherwise mitigate the consequences of neural inflammation. It has been reported to decrease upregulation of tumor necrosis factor alpha in subcutaneous tissue and to reduce transcapillary permeability. We studied the effect of dexamethasone on rat nerve blood flow (NBF) in normal animals to establish basic values for future studies on low back pain and radiculopathy. Blood flow was measured using a laser Doppler flow meter. Dexamethasone 0.4%, 0.1 ml or saline was applied topically to the nerve and NBF was measured continuously for 30 minutes. Some animals had repeat recordings at 4 hours (n=4). Application of saline to the exposed sciatic nerve did not significantly change nerve blood flow from the base-line values. Dexamethasone, however, significantly reduced NBF in both the 30-minute and 4-hour groups (72.3% ± 19.9% (SD) of normal at 30 min; 74.5% ± 13.5% (SD) at 4 hours; p=0.003 and p=0.03, respectively). Some animals showed an initial transient (10 minute) increase in blood flow before NBF began to steadily decline to the values given. Neuropathological changes were minimal and consisted only of edema in one animal and occasional subperineurial activation of Schwann cells in 2 animals. No demyelination or degeneration was seen at 2-6 days. While anti-inflammatory therapy is generally thought to be beneficial following acute injury to the nervous system, the pathophysiologic mechanisms of anti-inflammatory agents are largely unknown. Our study, in normal rat nerves, indicates that there is a significant effect of dexamethasone on reducing nerve blood flow. By itself, the approximately 25% reduction in NBF observed in this study is below the 50% reduction known to cause nerve fiber pathology.

PAINFUL PERIPHERAL NERVE INJURY INDUCES AXONAL TRANSPORT OF TNF α

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The outcome of peripheral nerve injury depends on the early events occurring at the injury site. Tumor necrosis factor- α (TNF) is a key determinant of both painful behavior and the early degenerative pathology in the chronic constriction injury (CCI) model of ischemic/inflammatory peripheral neuropathy. We determined injury-induced upregulation of TNF protein between the dorsal root ganglia (DRG) and distal nerve injury site at days 2 and 5 post-CCI using Western blot analysis. We further analyzed the ability of TNF to undergo axonal transport by using intraneural microinjection of biotin-labeled TNF into normal and injured sciatic nerve, as well as in the corresponding L5 DRG. Biotin tag was monitored in paraffin-embedded tissue using the avidin-biotin-peroxidase technique. The results indicate fast TNF transport in both the retrograde and anterograde directions. Neuronal neurobiotin tracer and vehicle served as controls. When injected at the injury site, TNF accumulated just distal to the L5 DRG at 6 and 24 hours post-injection, and reached the dorsal horn of the spinal cord by 48 hours post-injection. When injected into DRG, TNF migrated anterogradely towards the injury site and to the dorsal horn of the spinal cord. Biotinylated TNF co-localized with endogenous TNF ligand, suggesting that biotin tag did not dissociate from the TNF tracer, and with TNF receptors TNFRI and TNFRII, suggesting a receptor-mediated nature of TNF transport. We hypothesize that axonal transport is involved in a process of TNF protein and receptor regulation within the peripheral neural axis and spinal cord, and that this transport bears on the pathogenesis of painful neuropathies.

MUTATIONS IN MPZ CYTOPLASMIC DOMAIN ABOLISH ADHESION AND REVEAL ROLE

FOR PKC MEDIATED PHOSPHORYLATION IN PNS MYELINATION

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Mutations in the MPZ gene cause CMT1B. Analysis of the crystal structure of the MPZ extracellular domain indicates that adjacent MPZ molecules interact in cis to form tetramers, and that these tetramers further interact in trans to mediate homophilic adhesion. However, mutations in the MPZ cytoplasmic domain also disrupt adhesion and cause CMT1B by unknown mechanisms. To define regions of the cytoplasmic domain critical for adhesion and myelination, we stably transfected L-cells with cDNAs encoding wild type or mutated MPZ constructs and assayed the constructs for their abilities to form homophilic adhesions. Point mutations within the cytoplasmic domain that modify a PKC binding motif (RSTK) or an adjacent serine, a putative PKC target site, abolish adhesion, while mutations in other nearby serines or tyrosines do not. Inhibition of PKC activity also abolishes adhesion in a dose dependent manner. Mutations within the PKC binding motif, or in the adjacent serine, also cause CMT1B. These data suggest that regulation of PKC mediated phosphorylation of specific residues in MPZ are necessary for P0 mediated adhesion and that alteration of this process causes demyelinating neuropathy in humans. Sponsor: NIH, MDA.

EFFECTS OF NT-3 DELIVERY ON REINNERVATION OF FAST EDL AND SLOW SOLEUS MUSCLES

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We showed that delivery of NT-3 to proximal stumps of cut sciatic nerves resulted in specific normalisation in diameter and percentage of fast 2b muscle fibres in gastrocnemius muscle. Thus, we investigated if normalisation was related to greater improvement of reinnervation of fast (extensor digitorum longus: EDL, 75% 2b fibres) than slow motor units (soleus, 95% type 1 fibres). Then, we tried to clarify how NT-3 improves reinnervation by analysing markers in EDL and soleus motor pools. NT-3-impregnated (NT-3 group) or plain fibronectin (FN group) mats were inserted into a sciatic nerve gap. Neuromuscular junctions (NMJ) labeled with α -bungarotoxin and 4E2 antisera were imaged using confocal microscopy. 4E2 is a marker for structurally mature NMJ. After 120 days, 4E2-immunoreactive NMJs were more numerous in EDL of the NT-3 (40%) than of the FN group (7%), unlike in soleus (NT-3 \cong FN: 2%). Motoneurons (MN) were identified using retrograde tracers. NT-3 did not affect the number of reinnervating MN; axotomy-induced decrease in ChAT expression was selectively reversed by NT-3 in EDL MN; and in unoperated and NT-3 rats, trk C mRNA expression was higher in EDL than soleus motor pool. These results indicate that NT-3 preferentially improves reinnervation of fast muscle over slow muscle by enhancing neurotransmitter synthesis and maturation of NMJ rather than initial axonal regrowth, and this improvement is due to a selective effect of NT-3 on fast MN via trk C receptors.

FULMINANT IMMUNE NEUROPATHY ASSOCIATED WITH GLOMERULONEPHRITIS AND ADULT RESPIRATORY DISTRESS SYNDROME: RESPONSE TO SIMULTANEOUS INTRAVENOUS IMMUNOGLOBULIN AND INTRAVENOUS METHYLPREDNISOLONE

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BACKGROUND: Although the most common forms of immune-mediated acute flaccid paralysis constitute the Guillain-Barré syndrome (GBS), some cases of severe inflammatory autoimmune

polyradiculopathy are subacute in presentation and do not respond to traditional immunotherapeutic strategies. CASE HISTORY: Following rapid onset of peripheral edema, 10 g daily proteinuria, and biopsy-proven membranous glomerulonephritis which responded to cyclophosphamide and prednisone, a 27-year-old Caucasian man presented with progressive dyspnea, night sweats, and oral temperatures in excess of 40°C, diagnosed as adult respiratory distress syndrome with an unidentified infectious agent, requiring 7 days of artificial ventilation. One week after recovering from this respiratory illness, he noticed progressive tingling and weakness in the upper limbs. Two weeks later he was found to have mild to moderate distal>proximal upper limb weakness, normal sensation, and global areflexia (Neuropathy Impairment Score [NIS] 36). The CSF protein measured 20 mg/dL. A diagnosis of GBS was made and no immune treatment was given. Symptoms worsened and spread to the lower limbs. Despite treatment with IV immunoglobulin (IVIg) 0.4 g/kg for 5 days on three occasions, the NIS rose steadily to 118. Plasmapheresis qod x5 d was ineffective. Blood tests, repeat CSF, and nerve biopsy were nondiagnostic. At NIS 137, a decision was made to pursue simultaneous IV methylprednisolone 1 g/d x5 and IVIg 0.4 g/kg qd x5. Symptoms began to improve within two weeks. By three months the NIS was 0 and the patient was back to his baseline, working full time and playing up to 72 holes of golf on weekends. CONCLUSION: Although subacute inflammatory polyradiculopathy may prove refractory to commonly-used immune interventions, it may sometimes respond to combination therapy with IVMP and IVIg.

PROTECTION OF AXONS FROM DEGENERATION RESULTING FROM IMPULSE ACTIVITY IN THE PRESENCE OF NITRIC OXIDE

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Axonal degeneration is an important cause of permanent disability in neuroinflammatory disorders, including Guillain-Barré syndrome. The degeneration may result from exposure to the *mêlée* of inflammatory mediators, including nitric oxide (NO), and we previously reported to this society that the combination of impulse activity and NO can cause axonal degeneration. We now report that it is possible to protect axons from such degeneration. The dorsal roots of anaesthetised rats were exposed *in vivo* to NO (4 or 8 µM) while serially monitoring conduction. As before, persistent conduction block due to axonal degeneration occurred when exposure to NO was combined with sustained impulse activity within the physiological range (50 or 100 Hz for 2 hours). Axons were protected from persistent conduction block by treatment with the sodium channel blocking agents lignocaine or flecainide, even when these agents were administered at concentrations below those which blocked conduction. Parallel morphological experiments demonstrated that lignocaine or flecainide were effective in preventing axonal degeneration in roots incubated with NO. We conclude that partial blockade of sodium channels can protect axons from degeneration caused by the combination of impulse activity and NO exposure. These findings may indicate new avenues to prevent axonal degeneration and thereby disability in neuroinflammatory disorders. Supported by the Multiple Sclerosis Soc., and The Guy's & St. Thomas' Charitable Trust.

SPONTANEOUS AUTOIMMUNE DEMYELINATING NEUROPATHY IN B7-2 DEFICIENT NOD MICE: ELECTROPHYSIOLOGICAL FINDINGS

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The NOD mouse is a prototypic murine model of Type 1 diabetes mellitus. The elimination of

B7-2 expression in NOD mice prevents the development of hyperglycemia, but these mice develop a symmetrical hindlimb paresis at 20 weeks of age progressing to generalized weakness at 32 weeks (Salomon et al., submitted). We report here the electrophysiological findings from studies performed on sciatic nerves of these animals. CMAP amplitudes were 10.8 ± 1.5 mV in controls (n=8) and 3.0 ± 0.6 mV in B7-2 deficient NOD mice (n=12). Conduction velocities were 50.5 ± 3.8 m/s in controls and 17.9 ± 3.2 m/s in B7-2 deficient NOD mice. Other features noted in the CMAPs from the latter include prolonged distal latencies and temporal dispersion. These findings correlated with the histologic evidence of demyelination. Thus, the B7-2 deficient NOD mouse constitutes the first model of a spontaneous autoimmune demyelinating neuropathy that resembles the human disease, chronic inflammatory demyelinating polyneuropathy (CIDP). Sponsor: NIH PO1 DK49799 (J. Bluestone), Juvenile Diabetes Foundation fellowship (B.Salomon), NIH RO1 NS39346 and a gift from Mr. J. Miller (B. Soliven).

EFFECTS OF THE P75 NEUROTROPHIN RECEPTOR ON AXONAL TRANSPORT AND REGENERATION AFTER AXOTOMY

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The role of the low affinity neurotrophin receptor (p75) in axonal regeneration and neurotrophic support was studied after unilateral sciatic nerve crush in p75(-/-) mice using an ELISA immunoassay, electron microscopy, and stereological counting techniques. The retrograde transport of NGF in intact nerves was 36.9 ± 8.2 pg/h and 3.2 ± 2.8 pg/h in control and p75(-/-) mice, respectively. In p75(-/-) mice the number of unmyelinated axons increased from $2,700 \pm 900$ to $5,800 \pm 1,500$ 14 days after crush. In control mice the number of unmyelinated axons increased from $6,200 \pm 2,400$ to $10,700 \pm 3,900$ 14 days after crush. No further increase was observed after 42 days. The relative number of regenerating unmyelinated axons was significantly increased in p75(-/-) mice ($2p < 0.05$). The number of myelinated axons in intact nerves was $2,600 \pm 800$ and $4,800 \pm 800$ in p75(-/-) and control mice, respectively, the number being unchanged 14 days after crush. The number of myelinated axons was increased to $3,300 \pm 700$ (NS) and $5,900 \pm 400$ ($2p < 0.05$) after 42 days in p75(-/-) and control mice, respectively. Myelination was reduced 14 days after crush in p75(-/-) mice as compared to controls. At 42 days after crush, myelination was similar in the two groups. In conclusion, the p75 receptor promotes the retrograde transport of NGF and inhibits axonal sprouting. A new observation is that knockout of the p75 receptor inhibits remyelination after axotomy.

INSIGHTS INTO NEURODEGENERATION IN FAMILIAL AMYLOIDOTIC POLYNEUROPATHY (FAP)

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FAP is related to the extracellular deposition of mutated transthyretin (TTR) amyloid fibrils in several organs, particularly in the peripheral nervous system. We investigated how TTR fibrils modulate peripheral neuronal death. We present evidence that the receptor for advanced glycation end products (RAGE), which is associated with the induction of cell stress, binds TTR and has increased expression in FAP tissues, in sites of TTR deposition. RAGE-dependent activation of NF- κ B, a pleiotropic activator that induces inflammation-associated molecules, by TTR fibrils was shown in cellular systems and in FAP nerves. Immunohistochemical semiquantitative analysis of FAP tissues showed increased expression of inflammatory markers such as TNF, M-CSF and IL1 in sites of TTR deposition. In cell culture, TTR fibrils induced the transcription of TNF, M-CSF and IL1 in neuronal,

endothelial and Schwann cells in a RAGE-dependent fashion. By similar approaches we identified activation of caspase-3 in FAP tissues and in cells exposed to TTR fibrils. These results show that cell stress is mediated by RAGE in FAP. Further insights into the molecular mechanisms triggered by the TTR-RAGE interaction may provide a better understanding of neurodegeneration associated with FAP. Sponsor: Praxis XXI (Portugal) and NIH (USA).

OCCURRENCE AND CHARACTERISATION OF PERIPHERAL NEUROPATHY IN NEUROFIBROMATOSIS TYPE 2

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Neurofibromatosis type 2 (NF2) is a rare autosomal dominant disorder with nearly full penetrance and a high rate of sporadic occurrence. NF2 is caused by a mutation in a gene called Schwannomin/Merlin. The clinical feature of NF2 is characterised by the occurrence of bilateral vestibular Schwannomas, additional various brain and spinal tumours and in lower frequencies peripheral nerve tumours, cutaneous tumours and juvenile posterior lenticular opacity. Few case reports demonstrate patients suffering from NF2 and peripheral nerve lesion unrelated to tumour masses. In large clinical studies, occurrence of peripheral neuropathy as a rare unexplained clinical feature were observed in 6% of patients. Sural biopsies in selected cases showed an identical histological appearance of an onion-bulb-like formation which seemed to have originated from Schwann cells. These single case reports of the peripheral nerve involvement and the observation that many patients with NF2 have areflexia and sensoric disturbances, which cannot completely explained by tumour burden, raise the possibility that neuropathy is under-recognized. Thus we conducted a systematic investigation to determine the occurrence of peripheral neuropathy excluding other courses of neuropathy. We investigated 15 patients suffering from definite NF2 to examine the frequency and type of peripheral neuropathy in Neurofibromatosis Type 2. We found in 11 patients (73.33%), electrophysiological evidence of neuropathy. In this study we present type of neuropathy, histological findings of NF2 neuropathy and correlation to clinical findings. As a result, we conclude that peripheral neuropathy, mostly of axonal type, is a common clinical finding in Neurofibromatosis type 2. Further details will be presented.

REPAIR OF THE ADULT RAT SCIATIC NERVE WITH A COLLAGEN-BASED CONDUIT

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In clinical nerve repair, the situation is often encountered when the gap between the nerve stumps is too large to permit repair by direct tensionless suture. Grafting with a segment of autogenous nerve is effective in such situations but has drawbacks such as donor site morbidity and incomplete recovery of function. Recent approaches have been developed in which a nerve conduit may be used to replace the need for nerve grafting. Previous studies have demonstrated that collagen-based nerve conduits are capable of promoting nerve fiber regeneration and partial functional recovery in the rat sciatic nerve (Chamberlain et al., *Exp Neurol*, 154:315, 1998) and the median nerve of the nonhuman primate (Archibald et al., *J Neurosci*, 15:4109, 1995). The present study investigated the ability of a collagen-based conduit consisting of a collagen tube filled with a highly porous collagen-glycosaminoglycan matrix to support nerve fiber regeneration across gap lengths up to 22 mm in the rat sciatic nerve. The experimental design consisted of bridging nerve gaps ranging between 12 and 22 mm (up to 6 animals per group) with collagen-based conduits, followed by

histological quantification of the number of regenerated nerve fibers at the gap midpoint after 9 and 12 weeks. Our data indicate that a substantial number of nerve fibers had regenerated across 12-mm gaps at 9 weeks ($3,123 \pm 890$, mean \pm SEM), while few or no fibers had regenerated across longer 20-mm nerve gaps at 9 weeks (79 ± 39). After 12 weeks, regenerated nerve fibers were identified at the gap midpoint in the 22-mm gap group (356 ± 110). Regeneration across smaller gap lengths at 12 weeks was more robust (16-mm gap, $3,184 \pm 773$). These data demonstrate the potential for a collagen-based nerve conduit to repair large gaps in transected rat peripheral nerves. Future studies will address the maximum gap length that can be repaired and the extent of functional recovery.

POST-INFECTIOUS SENSORY NEUROPATHY RESPONDING TO INTRAVENOUS IMMUNOGLOBULIN

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Idiopathic small fibre neuropathies are usually chronic disorders characterized by burning dysaesthesia in the extremities and reduced epidermal nerve fibre density (ENFD). The most common post-infectious polyneuropathy, the Guillain-Barré syndrome, typically involves demyelination or axonal damage with prominent large fibre involvement. Here we describe an unusual case of a post-infectious neuropathy with distal sensory loss but no evidence of large fibre neuropathy, and a complete clinical response to intravenous immunoglobulin (IVIg). A 62-year-old woman presented with a 6 week history of progressive paraesthesia and burning in the hands and feet developing 2 weeks after an upper respiratory tract infection. She was neurologically intact apart from reduced pin prick sensation in the fingers and toes. Nerve conduction studies and a lumbar puncture were normal. Inflammatory markers, anti-neuronal and anti-ganglioside antibodies were normal apart from a mildly elevated ESR of 26. Quantitative sensory testing demonstrated hyperaesthesia to heat as a painful stimulus, and an abnormal cold detection threshold. A distal leg skin biopsy revealed an ENFD in the normal range but some fibres were shortened and nodular. After responding well to 3 days of IVIg (0.4 g/kg/day) and relapsing one month later, the patient recovered fully within 7 days of another single IVIg dose. We propose that this case may represent a predominantly small fibre form of acute post-infectious inflammatory neuropathy. Sponsor: The Nerve Research Foundation and the National Health and Medical Research Council (NH&MRC) of Australia.

AN EVALUATION OF A MONOCLONAL ANTI-CD20 ANTIBODY (RITUXIMAB) IN THE TREATMENT OF THE ANTI-MAG ASSOCIATED POLYNEUROPATHY

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BACKGROUND: In polyneuropathy associated with immunoglobulin M (IgM monoclonal gammopathy), antibodies to myelin associated glycoprotein (MAG) have been associated with specific clinical and electrophysiological features. So far, results of trials with immunosuppressive drugs have been disappointing. **OBJECTIVE:** To study whether Rituximab is safe and effective in the treatment of anti-MAG associated polyneuropathy. In an ongoing open study, 7 patients have received Rituximab intravenously 375 mg/m² once weekly for 4 weeks. None of the patients had received immunosuppressive treatment during the previous 3 months. Clinical follow-up consisted in neurological examination with a Neurological Disability Score (NDS) and Neurological Symptoms Score (NSS) at baseline, month 1, 3, 6, 9 and 12. Electroneurography was done at baseline, month 6 and month 12. In addition, regular white and red blood cell counts, blood chemistry and measurement of IgM and IgG levels and anti-MAG-antibodies were performed. **RESULTS:** Rituximab was well tolerated with few side effects.

In all patients, B-cells were depleted at month 1, and this effect persisted until month 6 - 9. The IgM level decreased in all patients, but IgG level remained stable. In 3 patients a decrease in the anti-MAG antibody titer was observed. Clinical improvement was present in 3 patients, 2 progressed on the NDS and 2 patients remained stable. CONCLUSION: These results indicate that Rituximab can effectively and safely eliminate B-cells and lower IgM level in patients with anti-MAG associated polyneuropathy with associated clinical improvement in some patients. Sponsor: Clinical trial sponsored by Roche.

PHRENIC NERVE PALSY AS A FEATURE OF CHRONIC INFLAMMATORY DEMYELINATING POLYRADICULONEUROPATHY

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Phrenic nerve involvement is exceptionally described in chronic inflammatory demyelinating polyradiculoneuropathy (CIDP). We report 4 CIDP patients presenting phrenic nerve palsy. All patients underwent clinical examination, electrodiagnostic studies, cerebrospinal fluid analysis, spirometry, serum immunoelectrophoresis, serology for neurotropic viruses, Borrelia Burgdorferi, campylobacter jejuni, and antinuclear antibodies. Patients were treated with intravenous immunoglobulin (IVIg) or steroids. Two men and 2 women fulfilled the diagnostic criteria for CIDP. Age of onset ranged from 56 to 66 years. Two patients had severe distal and proximal sensorimotor deficit and 2 had distal sensorimotor deficit. Phrenic nerve palsy was respectively unilateral in 2 and bilateral in the 2 others patients, which required mechanical ventilation. Vital capacity ranged from 30% to 51% of normal. IgG and IgA monoclonal gammopathy was present in one patient. After treatment either with IVIg or steroids, sensorimotor deficit and respiratory parameters improved in all patients but one. Phrenic nerve may be involved in CIDP and can successfully respond to either IVIg or steroids.

INTERLEUKIN-18 IS INDUCED IN ACUTE INFLAMMATORY DEMYELINATING POLYNEUROPATHY

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Th1 cells producing the proinflammatory cytokine IFN-gamma have been implicated in the pathogenesis of immune-mediated PNS diseases. IL-18 is a potent IFN-gamma-inducing cytokine that is cleaved by caspase-1 into its active form. We analyzed the expression of IL-18 and caspase-1 mRNA in the nerve roots of rats with experimental autoimmune neuritis (EAN) using RT-PCR and immunocytochemistry. Both IL-18 and caspase-1 mRNA levels increased in nerve roots during the stage of active disease progression. Immunocytochemically, IL-18 induction was mainly associated with ED1+ macrophages. Using an ELISA, we furthermore determined IL-18 levels in serum and CSF samples from GBS patients and controls. IL-18 serum levels were significantly higher in GBS patients than in controls. Our data implicate the Th1-inducing cytokine IL-18 in the pathogenesis of acute immune-mediated PNS demyelination. Sponsor: Hermann- und Lilly-Schilling-Stiftung.

TGF- β REVERSES THE ADVERSE EFFECT OF CHRONIC SCHWANN CELL DENERVATION ON MOTOR AXONAL REGENERATION

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Long-term denervation of Schwann cells (SCs) in the distal nerve stump severely compromises axonal regeneration and functional recovery (Sulaiman & Gordon, Glia 32:234-6, 2000). The recession of the inflammatory response associated with Wallerian degeneration after nerve injury coincides

with the failing capacity of SCs to support axonal regeneration. This suggests that macrophages and the cytokines that they secrete play a key role in maintaining the growth-promoting non-myelinating SC phenotype. Specifically, transforming growth factor, TGF- β , has been implicated in promoting the growth-permissive SC phenotype (Guenard et al., *Glia* 13:309-18, 1995). Experiments were conducted to test the hypothesis that exposure of chronically denervated SCs to exogenous TGF- β can reactivate atrophic non-growth supporting SCs to promote axonal regeneration *in vivo*. Six month chronically denervated rat sciatic nerve explants (3x3 mm²) were incubated *in vitro* for 48 hrs either with TGF- β and forskolin (experimental) or DMEM (control). The explants were then placed into a 10 mm silastic tube that bridged the proximal and distal nerve stumps of a freshly cut tibial nerve (n=10). The number of tibial motoneurons which regenerated axons through the explants and into the distal nerve stump after 6 months was determined by application of the retrograde tracers, fluororuby or fluorogold, to the cut tibial nerve. The mean number (\pm SE) of motoneurons that regenerated through the explants of chronically denervated SCs was significantly higher through the nerve explants treated with forskolin and TGF- β

(442 \pm 22) as compared with DMEM (258 \pm 13). These findings demonstrate that acute treatment of atrophic SCs with TGF- β can reactivate the growth-permissive SC phenotype and support axonal regeneration after chronic denervation. Sponsor: Paralyzed Veterans of America/Spinal Cord Research Foundation and CIHR/APF.

ANIMAL MODEL OF AXONAL GUILLAIN-BARRÉ SYNDROME: ELECTROPHYSIOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

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We performed further evaluations of electrophysiological or immunohistochemical study and appropriate amount of antigen in a model of axonal Guillain-Barré syndrome (GBS). We also compared this model to experimental demyelinating neuropathy. Male Japanese white rabbits were immunized by 0.5, 1, 2.5, and 5 mg portions of bovine brain ganglioside (BBG) mixture (GM1 21%, GD1a 40%, GD1b 16%, GT1b 19%), 0.5 mg portions of GM1 ganglioside, and 1 mg portions of galactocerebroside. On sensitization with the 0.5, 1, and 2.5 mg portions of BBG, 0 of 3, 3 of 6, and 6 of 6 rabbits, respectively, developed severe flaccid limb weakness of acute onset. Three of 8 rabbits immunized by GM1 showed severe limb weakness. The appropriate immunization protocol was thought to be inoculation with a 2.5 mg portion of BBG. Seven of 8 rabbits immunized by galactocerebroside showed obvious paralysis, in which motor nerve conduction study revealed typical evidence of demyelination. In contrast, motor nerve conduction study and needle electromyography on the acute phase of the axonal GBS model showed almost normal, in spite of severe paralysis. The absence of late components of F waves, however, was noted in some rabbits. Decreased amplitude of compound muscle action potential and denervation potential was shown on the recovery phase of axonal GBS model in one rabbit. Immunohistochemical studies using peroxidase-conjugated protein G demonstrated that IgG was deposited on some axons of the nerve roots and cauda equina, whereas similar findings were observed in few fibers of sciatic or tibial nerves. These findings suggest that the lesion during the acute phase of axonal GBS model are predominantly localized in nerve roots which were thought to be vulnerable due to relative deficiency of blood-nerve barrier.

CD8+ T CELL LINES ISOLATED FROM SURAL NERVE IN PATIENTS WITH ANTI-HU ANTIBODIES AND SENSORY NEUROPATHY

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INTRODUCTION: Paraneoplastic subacute sensory neuropathy (SSN) in patients with anti-Hu antibodies arises as a result of an inflammatory process within the dorsal root ganglia (DRG), although inflammatory infiltrates are also present in sural nerve biopsies. Previous studies suggest that neuronal destruction is mediated by oligo-clonal CD8⁺ cytotoxic T lymphocytes (CTLs) of uncertain antigen specificity. **OBJECTIVE:** To culture DRG and sural nerve biopsies from patients with anti-Hu antibodies/SSN to derive and characterise T cell lines that can be investigated for antigen specificity. **METHODS:** Post mortem DRG and two sural nerve biopsies from 3 patients with anti-Hu antibodies/SSN were cultured in T-cell medium containing 20 U/ml IL-2. Nerve-derived T lymphocytes were expanded with 1 g/ml PHA+50 U/ml IL-2, using irradiated allogeneic PBMCs as antigen presenting cells and phenotypes were determined using FACS. **RESULTS:** Immunohistochemistry of sural nerve sections revealed 14 0.68 CD8⁺ cells/mm² (Patient 1) and 2.24 0.64 CD8⁺ cells/mm² (Patient 2). CD8⁺ + T cell lines were obtained from both sural nerve biopsies, but not the DRG. **CONCLUSION:** CD8⁺ T cell lines can be isolated from sural nerve biopsies taken from patients with SSN and anti-Hu antibodies. Further work is being undertaken to determine whether these CTLs react with epitopes derived from the Hu antigen.
