mechanisms for up regulation and down regulation are not fully understood but lack of activity—for example, after denervation or nerve conduction block—results in up regulation. Down regulation is seen in myasthenia gravis, in which the receptors are destroyed by autoimmune mechanisms. There is experimental evidence of down regulation of AChRs in the presence of agonists.13

Down regulation of AChRs in the presence of AChE inhibition would be expected to cause a different syndrome from myasthenia gravis. In myasthenia gravis the progressive weakness is explained by the smaller amounts of ACh released at the neuromuscular junction with each successive nerve impulse. The reduced number of ACh molecules are less likely to activate the few remaining AChRs before they are enzymatically destroyed. In the intermediate syndrome, however, any liberated ACh is likely to have time to activate one or more receptors once or even several times before it diffuses away. Receptor activation, however, fails to produce muscle contraction because there are insufficient simultaneously activated receptors. Even exposure to small amounts of organophosphorus can cause increase in jitter at three days.14 If the half life of AChR is 10 days, why should intermediate syndrome appear so rapidly 24–96 hours after poisoning? A reason may be that heavily activated receptors become desensitized, rendering them more readily endocytosed. The process may be related to the increased postjunctional non-contractile Ca2+.15 Recovery from intermediate syndrome in 5–18 days is explicable in terms of the AChR production.

With few AChR receptors an increase in single fibre jitter with blocking would be expected as it is more difficult to depolarise the fibre to firing threshold. However, even the reduced amounts of ACh released late in tetanic trains would be enough to activate all the receptors available. Repetitive stimulation would be expected to show neither increment nor decrement.

Although this sequence of events cannot be confirmed by clinical studies, supportive evidence could be obtained by estimating AChR numbers from end plate biopsies and more detailed single fibre EMG studies.

Correspondence to: Professor Sedgwick.


Raised antibody titre against conjugated S-nitrosocysteine in IgM paraproteinemic peripheral neuropathy: possible role of nitric oxide in pathogenesis

Peripheral neuropathies associated with IgM monoclonal gammopathies may represent a particular subgroup of the dysglobulinemic autoimmune neuropathies. Fifty to 70% of these patients have antibodies reacting with the 100-kDa myelin-associated glycoprotein (MAG). The clinical picture is that of a mixed sensorimotor polyneuropathy with ataxia and tremor. The pathological changes include ongoing segmental demyelination and remyelination, with a characteristic widening of myelin lamellae and often a pronounced loss of large fibres. The pathological process of anti-MAG IgM neuropathy is generally thought to stem from activation of the complement cascade secondary to binding of anti-MAG antibodies to the myelin sheath. However, several findings suggest that autoimmunity mechanisms may contribute to the peripheral nerve damage: (1) there is no obvious relation between the fall in antibody levels and the clinical effect of immunosuppressive treatment; (2) nerve infiltration associated with myelin lamellae by macrophages, indicative of an inflammatory process, have been found in some biopsy specimens; and (3) raised levels of soluble interleukin-2 receptors point to a role for T cell mediated immune response.12 Nitric oxide (NO) has been postulated to play a part in autoimmune disorders. Activated macrophages and lymphocytes produce high amounts of NO for long periods after transcription of the inducible NO synthase (iNOS) gene in response to cytokines. Furthermore, peripheral glial cells express iNOS mRNA in response to various stimuli. Sustained production of NO results in the nitrosation of cysteine residues of various proteins.16 This chemical modification of proteins carrying cysteine residues may give rise to immunogenic proteins which induce production of specific antibodies. We have previously developed an enzyme linked immunosorbent assay (ELISA) for the detection of antibodies against conjugated S-nitrosocysteine which represent an indirect indicator for sustained NO release.

In the present study we hypothesised that production of NO might be implicated in demyelinating anti-MAG IgM peripheral neuropathy.

We compared serum titres of antibodies directed against the NO-Cys-g-glycoprotein epitope from: (1) patients with neuropathy associated with non-malignant anti-MAG IgM monoclonal gammopathy (anti-MAG, n = 29), (2) patients with amyotrophic lateral sclerosis (ALS, n = 37), (3) anti-MAG sub-ectors (controls, n = 61), (4) patients with other autoimmune diseases including insulin dependent diabetes and systemic lupus erythematosus (OAD, n = 38), and (5) patients with benign IgG paraproteinemia (IgG, n = 21).

Antibodies to a chemically synthesised S-nitrosocysteine epitope carried by bovine serum albumin, NO-Cys-g-BSA, were measured by ELISA.17 Polystyrene well plates were coated with a solution containing either the NO-Cys-g-glycoprotein serum albumin (BSA) or BSA-g (10 μg/ml) in 0·05 mol/l carbonate buffer (pH 9·4) for 16 hours at 4°C. Free binding sites were saturated and the wells plates filled with 200 μl serum at a dilution of 1:500 in phosphate buffered saline (PBS)-Tween 1% (PBS-Tween) containing 0·1% BSA, and left for two hours at 37°C. The well plates were then incubated for one hour at 37°C with goat anti-human IgM secondary antibody labelled with horseradish peroxidase diluted 1:5000 in PBS-Tween containing 0·1% BSA. After subtraction of a blank value, immunological binding was expressed as the ratio (ODmax-ODmin)/ODmin where ODmax is the value of a positive control serum sample (for destructive measurement), and ODmin is the mean absorbance of serum samples from an independent group of controls. Differences between anti-MAG, ALS, OAD, IgG, and controls were evaluated by Mann-Whitney U test. Specificity of the IgM binding to NO-Cys-g-BSA was tested by inhibition experiments with NO-Cys-g-BSA and Cys-g-BSA in the liquid phase. Antibody binding was specifically displaced with NO-Cys-g-BSA, but not with Cys-g-BSA.

The results (figure) indicate that serum samples from patients with anti-MAG contained the most anti-NO-Cys-g-BSA antibodies, suggesting a sustained production of endogenous NO in these patients. Levels of circulating antibodies to NO-Cys-g-BSA were significantly higher in serum samples of patients with anti-MAG than in the samples from patients with ALS, OAD, or IgG, or controls (P < 0.0001, anti-MAG v controls; P = 0.002, anti-MAG v OAD, P < 0.0001, anti-MAG v IgG, Mann-Whitney U test). There was no significant difference in serum levels of anti-NO-Cys-g-BSA antibodies between the healthy subjects and the other pathological controls (P = 0.85) and OAD (P = 0.96). No correlation was found between the level of antibodies to NO-Cys-g-BSA and either the levels of total IgM (P = 0·47) or the antiglycolipid sulphoglycolipid paragloboside IgM level (P = 0·39) in the serum samples of the patients with anti-MAG.

To rule out any cross reactivity between the anti-MAG antibodies and S-nitrosocysteine antibodies, ELISA tests were done with a preparation of human myelin from healthy brain. A raised level of antibodies directed against the myelin preparation was found in serum samples of 21 patients with neuropathy associated with non-malignant anti-MAG IgM monoclonal gammopathy.
ELISA of IgM antibodies (mean (SEM)) directed against NO-Cys-g-BSA in the serum samples of patients with neuropathy associated with non-malignant anti-MAG IgM monoclonal gammopathy (anti-MAG, n = 29), patients with amyotrophic lateral scrosis (ALS, n = 37), healthy subjects (controls, n = 61), patients with other autoimmune diseases (OAD, n = 38), and patients with IgG benign hyperglobulinaemia (IgG, n = 21). The line depicts the cut off value, which is the maximal control value.

belonging to the group tested against NO-Cys-g-BSA. When compared with 30 controls previously tested against NO-Cys-g-BSA, this raised level approached significance (P = 0.056). However, no difference was found between the two groups on chemically nitrosylated myelin (P = 0.97) (data not shown).

Sustained NO production may participate in the pathogenesis of autoimmune CNS demyelinating diseases. Expression of iNOS has been reported in multiple sclerosis lesions and increased levels of circulating antibodies directed against NO-Cys-g-BSA have been found in the serum samples of patients with multiple sclerosis. A pathogenic role for NO in autoimmune demyelinating peripheral neuropathies has also been evidenced using NO synthase inhibitors, which partially suppress the lesions of T cell line mediated experimental allergic neuritis.

Our findings raise the question of the origin of the sustained NO production during the demyelinating process. In peripheral nerves, Schwann cells may function as accessory cells interacting with the immune system because they can express MHC class II molecules and secrete interleukin-1. Interestingly, Schwann cells produce NO in vitro when stimulated with tumour necrosis factor-α (TNF-α) and interferon-γ (Zielasek et al, personal communication). Moreover, NO might be produced by macrophages or by T cells. Indeed, changes in the distribution of T cell subtypes (an increase of the T helper/T inducer and T killer/T effector ratio), also point to a participation of cell mediated responses in the pathogenesis of the neuropathy.

Activation of the cytokine cascade and the cell mediated component of the immune response required for NO production may well occur in anti-MAG IgM peripheral neuropathy, although to date there is little direct evidence for the involvement of cytokines in the disease. The role of TNF-α in the pathogenesis of inflammatory demyelinating polyneuropathy, in which macrophage infiltration is always present, is better documented. TNF-α is an effector for the induction of iNOS expression by macrophages, microglia, astrocytes, or Schwann cells, which can result in a central or peripheral demyelination. Sustained NO release may participate in the peripheral nerve lesions characterising the anti-MAG IgM neuropathy by lipid peroxidation. Peroxynitrite resulting from NO production may damage myelin directly.

This work was supported by grants from INSERM poste d'accueil (EE), ARMA, Institut Fédératif de Recherche Biomédicales en Neurosciences Cliniques et Expérimentales, Ligue Française contre la Sclérose en Plaques, and Association pour la Recherche sur la Sclérose en Plaques (KGP).

EMANUEL ELLIE
JACQUES DEMOTES-MAINARD
KLAUSS G PETRY
INSERM U394 "Neurobiologie Inflammatoire",
Bordeaux, France.

Correspondence to: Dr Klaus G Petry, INSERM U394, Rue Camille Saint-Saëns, F 33077 Bordeaux Cedex, France.
Raised antibody titre against conjugated S-nitrosocysteine in IgM paraproteinaemic peripheral neuropathy: possible role of nitric oxide in pathogenesis.

A I Bouillerne, E Ellie, J Demotes-Mainard and K G Petry

*J Neurol Neurosurg Psychiatry* 1997 62: 202-203
doi: 10.1136/jnnp.62.2.202

Updated information and services can be found at:
http://jnnp.bmj.com/content/62/2/202.citation

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/