

*Gd-DTPA enhancing cranial MRI lesions (white columns) and serum Il-1ra concentrations (black dots) measured longitudinally during one year in patient 1 with active rr-MS. There was a clinical relapse at time point 9, which was treated with intravenous methylprednisolone. Changes in MRI activity tended to precede changes in serum Il-1ra concentrations by three to four weeks (one time point,  $P < 0.05$ ).*

sepsis) was published recently.<sup>4</sup> Its agonist, Il-1 $\beta$ —the predominant Il-1 isoform in humans—is consistently expressed in acute multiple sclerosis plaques.<sup>1</sup> It is conceivable, but so far neither confirmed nor disproved, that the natural specific antagonist Il-1ra is involved in the counterregulation of inflammatory activity in multiple sclerosis. If this were the case, Il-1ra would be a novel candidate for immunomodulatory therapy in multiple sclerosis.

We conducted a pilot study of nine patients with definite multiple sclerosis. The patients were followed up for one year by clinical examination (EDSS) and MRI every three to four weeks (resulting in 14 time points with code numbers 1 to 14), in parallel to measuring Il-1 $\beta$  and Il-1ra concentrations in serum and CSF. Five patients with a relapsing-remitting multiple sclerosis (two men, three women; ages 27–34; duration of disease one to three years), and four patients with chronic progressive multiple sclerosis (one man, three women; ages 40–59; duration of disease five to 28 years) were studied. Cranial MRI (Siemens Magnetom 1.0 Tesla, München, Germany) images were obtained according to standard guidelines. They were T2 and T1 weighted, with and without 0.1 mmol/kg Gd-DTPA enhancement. Serum ( $n = 118$ ) and CSF samples ( $n = 33$ ) were frozen within two hours of collection and stored at  $-80^{\circ}\text{C}$ . Il-1 $\beta$  and Il-1ra were measured by commercially available enzyme linked immunosorbent assay (ELISA) kits (R and D Systems, Minneapolis, MN; sensitivity 1 pg/ml for Il-1 $\beta$  and 20 pg/ml for Il-1ra; intra-assay precision  $< 8.5\%$ ). The assays were performed in a blinded fashion, one assay for each patient. A clinical relapse was defined as an increase by  $> 1.0$  EDSS point. A relative MRI maximum was defined as a time point with  $> 1$  Gd enhancing lesion if preceded and followed by less MRI activity. Serum Il-1ra concentrations were individually defined as extreme ( $> 3$  box lengths from upper boundary) using individual box plots for each patient (box length between 25th and 75th percentile) according to the SPSS procedure "Examine". The association between changes in Il-1ra concentrations and MRI activity was tested with the sign test (increase or decrease of Il-1ra concentration or number of active MRI lesions between two time points).

Relative MRI maxima and clinical relapses were only seen in the group of

patients with relapsing-remitting multiple sclerosis, not in the one with chronic-progressive multiple sclerosis. Il-1 $\beta$  was not detectable in serum ( $n = 118$  samples) or CSF ( $n = 33$  samples). Il-1ra could be detected in all serum samples, but not in CSF. Hence serum Il-1ra concentrations were subject to further analysis. The median serum Il-1ra concentrations varied interindividually (range 45–422 pg/ml), but were all within the normal published range.<sup>5</sup> In the patient with the highest total number (64) of Gd enhancing lesions (patient 1), an increase or decrease of MRI activity was followed by an increase or decrease of Il-1ra concentrations by three to four weeks (one time point,  $P < 0.05$  in sign test, figure). Individually defined extreme Il-1ra concentrations were only seen in patients with relapsing-remitting multiple sclerosis. There were four such extreme concentrations in four different patients. Two of those coincided with relative maxima of MRI activity, and one with a clinical (spinal) relapse.

Smaller than extreme fluctuations of Il-1ra were not specific for multiple sclerosis activity, as they were also found in the group of patients with chronic-progressive multiple sclerosis without clinical or MRI activity in the observation period. This is probably due to the fact that Il-1 and Il-1ra are involved in a wide range of inflammatory activities.<sup>3</sup> The longitudinal design including monthly visits proved to be essential for defining individual extreme Il-1ra peaks. Because of the few patients, a significant association could be shown in only one patient. In her, the very high disease load (demonstrated by MRI activity) probably allowed us to detect this association. We could not detect Il-1ra in CSF, or Il-1 $\beta$  in CSF or serum, probably because the concentrations were below the detection limit of our ELISA assays. Because the biologically active concentration of Il-1ra is at least one magnitude higher than that of Il-1,<sup>3</sup> Il-1ra may be more easily detectable than Il-1.

In conclusion, we found that fluctuations of Il-1ra may be associated with multiple sclerosis activity. The role of Il-1ra in multiple sclerosis therefore warrants further study.

RAYMOND VOLTZ  
MATHIAS HARTMANN  
SIMONE SPULER  
ANGELA SCHELLER  
NORBERT MAI  
REINHARD HOHLFELD  
*Department of Neurology, Klinikum Großhadern,  
University of Munich and Department of  
Neuroimmunology, Max-Planck-Institute,  
Martinsried, Germany*  
TAREK YOUSRY  
*Department of Neuroradiology, Klinikum  
Großhadern, University of Munich*

Correspondence to: Dr R Voltz, Department of Neurology, Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York NY 10021, USA.

- Hohlfeld R, Lucas K, eds. Cytokine networks in multiple sclerosis. *Neurology* 1995;45 (suppl 6):S1–55.
- Olsson T. Critical influences of the cytokine orchestration on the outcome of myelin antigen-specific T-cell autoimmunity in experimental autoimmune encephalomyelitis and multiple sclerosis. *Immunol Rev* 1995;144: 245–68.
- Arend WP. Interleukin-1 receptor antagonist. *Adv Immunol* 1993;54:167–227.
- Fisher CJ, Dhainaut JFA, Opal SM, et al. Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome. *JAMA* 1994;271:1836–43.
- Gruss HJ, Dölken G, Brach MA, Mertelmann R, Herrmann F. High concentrations of the interleukin-1 receptor antagonist in serum of patients with Hodgkin's disease. *Lancet* 1992;340:968.

## Pathophysiology of the intermediate syndrome of organophosphorus poisoning

We report a patient with the intermediate syndrome with results of repetitive nerve stimulation studies and single fibre EMG.<sup>1</sup> A hypothesis for the pathophysiology of the intermediate syndrome is proposed.

A 28 year old previously healthy Asian woman drank a bottle of Fenthion (probably about 60 ml). She was admitted swiftly to hospital and treated with gastric lavage and intravenous atropine and a single dose of pralidoxime. After three to four days she developed respiratory weakness and was unable to lift her head. On the fifth day her vital capacity fell. Her facial muscles were weak, as was shoulder abduction and hip flexion. The distal muscles were normal. She had normal reflexes and no sensory deficit. She was intubated, respirated, and atropine was continued.

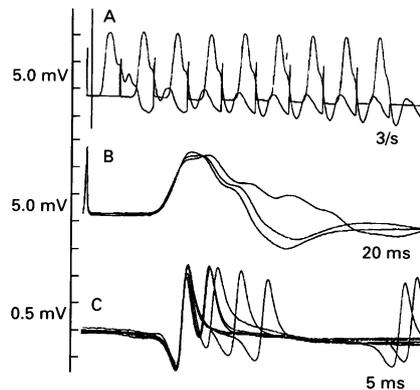
Her muscle strength slowly improved and she was weaned from the respirator and by the 15th day she was neurologically normal. Neurophysiological studies were carried out on the 7th, 14th, and 18th days. She recovered completely after three weeks. Results from motor and sensory nerve conduction studies on the median nerve on the 18th day were normal.

Repetitive nerve stimulation of the ulnar nerve were carried out at the wrist with recording from the abductor digiti minimi. On day 7 a single stimulus produced a repetitive discharge but the second and subsequent stimuli did not (figure). The repetitive discharge was present at every stimulus with rates at 1/2s but not at 3/s. It was present when the trial stimulus followed a few seconds after tetanus at 20/s for five seconds.

No decremental responses were seen at rates up to 50/s; nor was a decrement present after one minute of exercise or after 10 seconds of repetitive stimulation at 20/s or 50/s. Incremental responses were not seen. Single fibre EMG from the clinically normal extensor digitorum communis showed two single fibre pairs with borderline jitter values; the mean consecutive difference (MCD) was 59 and 63  $\mu\text{s}$  (normal  $< 55 \mu\text{s}$ ). Frontalis muscle was examined on day 7 and showed increased jitter with blocking. Of 17 fibre pairs, 12 had increased jitter and seven of these had greater than 10% blocking (figure). As expected, blocking was only seen in pairs with a considerably raised MCD.

The intermediate syndrome follows the acute cholinergic crisis of organophosphorus poisoning and is seen in up to 20%–50% of cases depending on the severity of poisoning and duration, and on the type of organophosphorus compound.<sup>1</sup> It differs from myasthenia gravis in that it is a constant rather than progressive weakness, responds adversely to neostigmine, and recovers within 18 days. There are no associated autoimmune phenomena. Decremental responses to repetitive nerve stimulation have been seen sometimes but usually it has been the clinically unaffected peripheral muscles that were studied.

We propose that down regulation of acetylcholine receptors (AChRs) could explain the syndrome and neurophysiological findings. These receptors have a half life of 10 days before undergoing endocytosis and proteolysis within the muscle fibre.<sup>2</sup> Regulation of the number of AChRs and



Trace A shows no decrement in the response of abductor digiti minimi with repetitive stimulation of the ulnar nerve at 3/s. B shows the first three responses from A on an expanded time scale and superimposed to demonstrate the repetitive discharge to the first stimulus. C is a single fibre EMG of frontalis showing increased jitter.

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.<sup>3</sup>

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 an increase in jitter at three days.<sup>4</sup> 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 desensitised, rendering them more readily endocytosed. The process may be related to the increased postjunctional non-contractile  $Ca^{2+}$ .<sup>5</sup> 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.

E M SEDGWICK  
Clinical Neurological Sciences,  
Faculty of Medicine,

University of Southampton,  
General Hospital,  
Southampton, SO16 6YD, UK  
NIMAL SENANAYAKE  
Faculty of Medicine,  
University of Peradeniya,  
Peradeniya, Sri Lanka

Correspondence to: Professor Sedgwick.

- 1 Senanayake N, Karalliede L. Neurotoxic effects of organophosphorus insecticides: an intermediate syndrome. *N Engl J Med* 1987; 316:761–3.
- 2 Duclert A, Changeux J-P. Acetylcholine receptor gene expression at the developing neuromuscular junction. *Physiol Rev* 1995;75: 339–68.
- 3 Martyn JA, White DA, Gronert GA, Jaffe RS, Ward JM. Up-and-down regulation of skeletal muscle acetylcholine receptors. Effects on neuromuscular blockers. *Anesthesiology* 1992; 76:822–43.
- 4 Baker DJ, Sedgwick EM. Single fibre electromyographic changes in man after organophosphate exposure. *Hum Exp Toxicol* 1996;15:369–75.
- 5 Tsuneki H, Kimura I, Kimura M. Independent regulation of activation and inactivation phases in non-contractile  $Ca^{2+}$  transients by nicotinic receptor at the mouse neuromuscular junction. *Brain Res* 1994;650:299–304.

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

Peripheral neuropathies associated with IgM monoclonal gammopathies may represent a particular subgroup of the dysglobulinaemic 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 other 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<sup>1</sup>; (2) nerve infiltration and stripping of myelin lamellae by macrophages, indicative of an inflammatory process, have been found in some biopsy specimens<sup>2</sup>; and (3) raised levels of soluble interleukin-2 receptors point to a role for a T cell mediated immune response.<sup>3</sup>

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.<sup>4</sup> 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 directed 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-cysteine 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) healthy subjects (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 paraproteinaemia (IgG, n = 21).

Antibodies to a chemically synthesised S-nitrosocysteine epitope carried by bovine serum albumin, NO-Cys-g-BSA, were measured by ELISA.<sup>5</sup> Polystyrene well plates were coated with a solution containing either the NO-Cys-g-bovine serum albumin (BSA) or BSA-g (10 µg/ml) in 0.05 mol/l carbonate buffer (pH 9.6) for 16 hours at 4°C. Free binding sites were saturated and the well plates filled with 200 µl serum at a dilution of 1:500 in phosphate buffered saline (PBS)-Tween containing 0.1% BSA and 0.1% BSA-g, and left for two hours at 37°C. The well plates were then incubated for one hour at 37°C with goat antihuman 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  $(OD_{\text{sample}} - OD_{\text{HS}})/OD_{\text{HS}}$  where  $OD_{\text{sample}}$  is the value of a patient's serum (triplicate measurement), and  $OD_{\text{HS}}$  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 (ALS ( $P = 0.56$ ) 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 sulphoglucoronyl 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 the anti-NO-cysteine 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