Letters to the Editor

Considerably on the Tensilon test, six had detectable anti-AChR abs, five showed decremental pattern on RNS performed on proximal muscles (deltoide and biceps brachii). In the patient without fluctuations of clinical signs, in whom the Tensilon test was negative, the diagnosis of myasthenia gravis was confirmed by the presence of serum anti-AChR antibody and muscle biopsy performed in this patient as well in another case with no detectable anti-AChR abs, showed normal findings. All patients had a follow-up period of at least two years and alternative diagnoses were excluded.

Eight patients had chronic progressive external ophthalmoplegia, 3 males and 5 females, aged 35-71, mean (SD) 56 (14.9) with a duration of disease ranging from 1-5-17 years mean (SD) 5.3 (5.1). All patients had clinical weakness restricted to ocular muscles and five complained of mild fluctuations of ptosis. In this group, serum anti-AChR ab titration and repetitive nerve stimulation gave negative results, while the response to the Tensilon test was negative in four cases and equivocal (minimal improvement of ptosis) in the other four. The diagnosis of progressive external ophthalmoplegia was confirmed by the presence of typical ragged red fibres on muscle biopsy in all cases.

Single fibre EMG (Stalberg 4) was performed in the orbicularis oculi muscle during slight voluntary contraction. A single fibre EMG electrode (Medelec SF 25) was inserted in the inferior and lateral portion of the muscle and recordings performed on a Medelec Myster electromyograph. For each muscle tested, 20 action potential pairs were analysed and the overall mean jitter as mean consecutive difference (MCD), the percentage of potential pairs with blocking and from the percentage of potential pairs with blocking were evaluated. In agreement with other authors, we considered a jitter study pathological when either a mean MCD was above the 95% upper limit for age or a jitter was above the 95% upper limit for potential pairs in more than 10% of pairs. The reference values for these parameters in age groups, were derived from literature data.

Single fibre EMG studies were performed, at least 48 hours after discontinuing therapy, on patients with myasthenia. Single fibre EMG in the orbicularis oculi muscle was pathological in 13 of 14 patients (93%) with purely ocular myasthenia gravis. Eleven patients showed abnormal mean jitter and more than 10% potential pairs had prolonged jitter (in 8 patients blocking was recorded); two patients had mean MCD below the 95% upper limit and respectively 20% and 40% potential pairs with prolonged jitter were found. In all patients with chronic progressive external ophthalmoplegia, single fibre EMG studies showed values within the normal limits. The mean jitter did not exceed 25 μsec. Only 5 had 5% of potential pairs with prolonged jitter and no blocking was recorded.

In patients with ocular myasthenia gravis, the positivity rate for single fibre EMG was higher than with the Tensilon test (86%), RNS (36%) or anti-AChR abs (43%).

Serum anti-AChR abs, which are present in many patients with or without myasthenia gravis, are detectable in a much lower percentage of patients with the ocular form of the disease. Single fibre EMG has proved the most sensitive technique in detecting the neuromuscular transmission defect, especially when facial muscles are tested. The orbicularis oculi muscle shows abnormal responses even more than the frontalis muscle. Unfortunately, single fibre EMG abnormalities can also be seen in primary neuropathic and myopathic disorders as a result of abnormal conduction of the impulse in degenerating or reinervating nerve terminals and re-formed neuromuscular plates. In CPEO patients, Kendel and Sanders described increased jitter and blocking in facial (frontalis) and arm muscles and a primary defect of neuromuscular transmission has been suggested in this condition. In our study, single fibre EMG performed in the orbicularis oculi muscle revealed a neuromuscular transmission defect in 93% of patients with purely ocular myasthenia gravis and so demonstrated a diagnostic capacity significantly higher than the Tensilon test, repetitive nerve stimulation or anti-AChR ab titration. Also, in our experience, the sensitivity of single fibre EMG is higher than that of other tests, particularly in the orbicularis oculi, than in the extensor digitorum communis muscle.

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Soluble Interleukin-2 Receptor levels in serum of patients with demyelinating polyneuropathy associated with monoclonal gammopathy

Monoclonal gammopathies, especially monoclonal gammopathy of uncertain significance (MGUS) with IgM components, are associated with demyelinating polyneuropathy (PN). In approximately 80% of these patients, the IgM monoclonal component reacts with myelin associated glycoprotein (MAG) or other subcomponents of myelin and a pathogenic role of the M-component has been proposed. However, other mechanisms, besides the M-component acting as antibody, may be operating. In vitro studies have shown that monoclonal antibodies against MAG in a patient with monoclonal gammopathy were subject to T-cell regulation, but the role of a T-cell mediated response has not been further investigated.

Interleukin-2 (IL-2), which is synthesised by antigen or mitogen activated T lymphocytes, plays an essential role in triggering T-lymphocyte proliferation, T-cell differentiation, and surface expression of IL-2 receptors (IL-2R) on T-cells. It also induces maturation and proliferation of B-lymphocytes, and is required for the secretory function of the plasma cell. Therefore the measurement of soluble (s) IL-2 in serum is a feasible and simple method for evaluating T-cell activity. Elevated serum levels of sIL-2R have been demonstrated in several autoimmune disease states, for example, multiple sclerosis, and the Guillain-Barré syndrome.

We analysed sIL-2R levels in serum as a measure of T-cell activation in 19 patients (11 male and 8 female) with monoclonal gammapathy (15 with MGUS and 4 with Waldenström's macroglobulinaemia; 17 of the IL-2R were 2- of IgG, 1- of IgA, and 3- of IgM). Seventeen of these patients had anti-MAG and/or anti-peripheral nerve myelin (PNM) antibodies. Sera from 19 patients (13 men and 6 women, age 43-80 years, mean age 66) with monoclonal gammapathy (15 with MGUS and 4 with multiple myeloma; 13 of IgG, 2 of IgM, 2 of IgA-isotype, and 2 with light chain proteinuria) without PN and without antibodies against MAG or PNM, and 5 healthy individuals (5 men and 6 women, age 47-80 years, mean age 66) without monoclonal gammapathy served as controls. Antibodies against MAG and PNM were analysed in serum and urine using enzyme linked immunosorbent assays (ELISA) essentially as described previously. In 15 patients with monoclonal gammapathy and PN, 2 sample sera were analysed, one before treatment and/or shortly after tapering off the immunosuppressive treatment. Serum samples were stored at -70°C, and thawed only once. Serum sIL-2R levels were measured using a commercial ELISA (Cellfree, T Cell Diagnostics, Inc, Cambridge, MA) according to instructions from the manufacturer. Values exceeding the mean value ±2SD (> 919 U/ml) of 50 blood donors were considered pathological (manufacturers information).

Seven of 19 patients with M-component associated PN had increased sIL-2R levels in the first serum sample compared with 2 of 19 M-component patients without PN (p = 0.06 according to Fisher's exact test, one-tail) and 1 of 15 healthy controls (p < 0.05 according to Fisher's exact test, one-tail) (fig). At the follow-up of the sample 4 with M-component associated PN and 4 M-component patients without PN were receiving immunosuppressive treatment. Serum sIL-2R levels were detectable in 15 patients with M-component associated PN, subspectrum with 4 patients. One patient had elevated serum sIL-2R levels and 6 of 15 untreated patients had elevated serum sIL-2R levels.

In 15 patients with M-component associated PN, subspectrum with 4 patients, serum sIL-2R levels were detectable.
these patients showed decreased (>10% difference in OD value as compared with the first serum sample), 1 increased and 3 unchanged sIL-2R levels. In the remaining patients no judgement could be made because of continuous treatment in 3 patients and a serum sample taken several years after treatment in one patient. In 3 out of 4 patients with elevated sIL-2R levels at the first sampling occasion, consecutive samples showed remaining high sIL-2R levels.

In a study by Hartung et al. comparing serum sIL-2 levels in Guillain-Barré syndrome and other neurological diseases, patients with IgM M-component associated demyelinating PN (n = 6) did not have elevated IL-2 levels. However, their patient material was limited, and IL-2 has a rapid turnover making detection of transient elevated IL-2 levels difficult.

In an in vitro study it was shown that B-cells from a patient with neuropathy and anti-MAG IgM M-protein were stimulated by pokeweed mitogen activated T-helper cells, and also partially by T-cells in the absence of pokeweed mitogen. T-cells might thus act as regulators of B-cell activation and secretion of immunoglobulins reacting with myelin components. Our findings of activated T-cells in some patients may be due to the action of such regulatory T-cells. We found no statistically significant correlation between serum sIL-2R levels and amount of anti-MAG IgM antibodies. Some of the blood samples, however, were taken at different occasions for analysis of sIL-2R concentration and levels of anti-MAG IgM antibodies.

A substantial number of PN patients, however, had normal sIL-2R levels, arguing for mechanisms other than activated T-cells in the pathogenesis of PN. Also some M-component patients without PN showed

signs of T-cell activation, possibly in response to antigens other than peripheral nerve myelin. 2 patients with M-component associated PN and elevated sIL-2R levels did not differ from the 12 patients with M-component associated PN with normal sIL-2R levels regarding disease activity or duration of disease.

Factors other than activation of autoreactive (or immunoregulating) T-cells, for example, viral infection, might have been responsible for the elevated levels of sIL-2R in this study. However, no signs of symptoms of infection were noted in our patients. Furthermore, repeated blood samples on different occasions showed persistently elevated sIL-2R levels in three out of four tested patients.

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HLA class II susceptibility and resistance genes in patients with multiple sclerosis from Northern Spain, by DNA-RFLP genotyping

The association between susceptibility to multiple sclerosis (MS) and the HLA system has been shown in previous population studies, but the associated HLA antigens have varied in different ethnic groups. 1 Southern Europe is an area of interest for the HLA-MS association, since the associations observed in most Northern European populations are usually absent. Previous studies have reached conflicting conclusions on whether particular HLA types influence the clinical course of the disease.

HLA typing has traditionally been accomplished serologically or immunologically, but some subtypes have so far evaded serological recognition. An alternative method is typing at the restriction fragment length polymorphism (RFLP). This HLA genotyping detects further subtypes of serologically defined -DR and -DQ specificities, which will permit a better understanding of the HLA-MS association.

We examined MS associated HLA-DR and DQ alleles, characterised by RFLP, at the genomic level in 96 MS patients (63 women and 33 men) from Asturias, in the northwestern region in Northern Spain with a medium MS prevalence of 24,000/100,000. MS was defined clinically or by laboratory support using the Poser et al criteria. 2 Eleven had previously chronic progressive MS, and 85 had relapsing-remitting MS. The latter group also included patients with a secondary progressive evolution of symptoms. A total of 123 healthy unrelated Spanish individuals were used as controls.

To carry out the HLA typing by DNA-RFLP analysis, the DNA from peripheral blood leukocytes was digested with the restriction enzymes Dr, Dq and Dq alpha genes, using standard methodological and analytical procedures. Haplotypes DR-DQ were assigned according to the pattern of bands following the Bidwell method. 3 The results of HLA-class II frequencies in MS patients and controls were compared by using the Chi-square test with Yates's correction and ρ values were multiplied by the number of allotypes tested.

DR15 (p < 0.05) and DR15/DQw6 (p < 0.05) were significantly increased in the whole MS group compared with the controls (Table). DQw13 was significantly decreased (p < 0.05). Dqw5 and DRw13/DQw5 were also decreased but the differences were not significant when corrected for the number of allotypes tested.

The frequency of MS patients that were positive for DR15 and/or DR16 (splits of DR2) appears increased compared with the

Table HLA-DR and DQ allele and haplotype frequencies (%) in patients with multiple sclerosis and controls from Asturias (Northern Spain), by DNA-RFLP genotyping

<table>
<thead>
<tr>
<th>Alleles</th>
<th>All MS patients (n = 96)</th>
<th>Remitted MS (n = 85)</th>
<th>Progressive MS (n = 11)</th>
<th>Controls (n = 123)</th>
<th>p</th>
<th>p*</th>
<th>p‡</th>
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<tr>
<td>DR15/DR2</td>
<td>39</td>
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<td>42</td>
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<tr>
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</table>

NS not significant. p All MS patients vs controls. p* Remitted MS vs controls. p‡ Progressive MS vs controls.