Evidence from Basic Research Justifying Immunotherapy in MS

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Many lines of circumstantial evidence support an immunopathogenesis of MS. Among them are formation of oligoclonal immunoglobulin bands in the cerebrospinal fluid, disturbances of regulator T cell equilibria, abnormalities of natural killer cells, occurrence of myelin-reactive T lymphocytes, and a linkage disequilibrium of MS susceptibility and certain HLA phenotypes. Perhaps the most convincing argument is, however, the morphological picture of the early, active MS plaque lesion. It is characterized by dense infiltration of postcapillary blood vessels by T lymphocytes, by activation of astrocytes of the 'protoplasmic' lineage, and by the disappearance of oligodendrocytes.

Recent progress in characterization of lymphocytes by monoclonal antibodies in definition of growth conditions on T lymphocytes have made it possible to isolate putatively pathogenic T cells recognizing myelin determinants from brain undergoing autoaggressive immune inflammation, and to establish pure monospecific permanent T lines [1]. These lines, which can be compared with isolates of pathogenic bacteria, are now used as probes to study in detail different phases of CNS autoimmune disease.

We have concentrated on the rat model of experimental autoimmune encephalomyelitis (EAE), which is induced in susceptible strains by immunization with myelin basic protein (MBP) in suitable adjuvants, and which serves as an excellent experimental model for the early inflammatory stages of MS plaque formation. We have isolated a panel of MBP-specific T lymphocyte lines, which in the activated state can transfer lethal EAE to normal recipient rats. The T lines express markers of the 'helper/inducer' subset, and recognize their antigen in the context of HLA molecules.

The phenotype of the restricting HLA epitope determines encephalitogenicity of MBP: MBP acts only as an encephalitogen if presented by accessory cells from EAE-susceptible rat strains. Then, only freshly activated autoimmune T cells can transfer disease. In this state, they express HLA antigens, which are derived from the antigen presenting cells. The capacity to specifically home to the CNS is a capacity intrinsic to the T line cells.

Recognition on MBP/HLA may play a role in determining homing specificity, either on the level of CNS vascular endothelium, or beyond the blood-brain barrier. While we have not been able to demonstrate HLA antigen on rat CNS endothelium, we found that astrocytes are induced to express enhanced levels of HLA [2].

The potential relevance of these findings for designing novel therapeutic strategies will be discussed. In addition, data will be shown which indicate that MBP-specific T lymphocyte lines can be isolated in vitro, which not only are lacking encephalitogenic potential, but are even able to suppress EAE transferred by other pathogenic T lines. Suppressor T line selection may become a new and promising tool to immunospecifically treat the autoimmune immune system without the side effects of cytostatic immunosuppression.


Immunotherapy of MS - The Clinician’s Viewpoint

W.I. McDonald, London

The practising physician must consider a number of questions before advising immunotherapy for the patient with MS. They include questions about the rationale for it, whether it should be stimulating or suppressive, the risks involved, the timing of treatment and its duration. There are several lines of evidence for an immunological abnormality in MS but how it relates to the mechanism of tissue damage is still unclear. In treatment, immunosuppression has generally been favoured over immune stimulation. The risks of the former are appreciable, but worth taking in the patient who is clearly deteriorating. Whether patients without disability should be treated is arguable. There is at present no reliable way either of predicting prognosis early in the course of MS or of deciding whether the treatment is more or less effective. NMR scanning and genetic characterization have some promise in this respect.

Completed Double-Blind Trial with Azathioprine. Final Results and Implications for the Design of Future Studies

J. Merlin, London

In a double-blind controlled pilot trial 43 patients with relapsing-remitting MS were randomly allocated either to group A (21 patients) receiving immunosuppressive treatment (antilymphocyte globulin, prednisolone and azathioprine) or group B (22 patients) receiving placebo preparations [1]. After a treatment
was possible to describe the individual disease course throughout the observation period by means of a polynomial transformation. As a measure of the progression of the disease throughout the period of observation the 1st, 2nd and 3rd degree of orthogonal polynomials were compared between the two therapy groups, using univariate and multivariate tests, taking into consideration the individual severity of the disease at onset of therapy. The duration of illness, the sex and the type of the disease course prior to the observation (intermittent, intermittent-progressive, chronic progressive).

Further, the number of relapses during the observation period and the results of cerebrospinal fluid investigations after the treatment were compared between both therapy groups. Despite the randomization the two collectives differed concerning the severity of disease at the onset of the study, the azathioprine-treated group being more ill than the control collective. This pitfall had to be taken into account in the statistical evaluation. Differences between the therapy groups were only apparent when the type of the disease prior to the entrance of the patients in the study was taken into account. The patients were allocated into comparable azathioprine/placebo groups, according to the pretrial progression coefficient, histocompatibility (DR2), sex, duration of illness, age, and neurological deficit.

To conclude, long-term treatment with azathioprine in a dosage of 2–2.5 mg/kg body weight should be tried in patients with an intermittent-progressive disease course in order to diminish the further progression. The effect of azathioprine on the progression of MS for intermittent-progressive disease courses was the greater the shorter the disease lasted, but did not depend on the sex of patients. No medication effect could be registered concerning the relapse rate and the amount of the within the blood-brain barrier synthesized IgG and the cell count of the cerebrospinal fluid. Azathioprine reduced in this low dosage the number of lymphocytes, especially the number of T lymphocytes in the peripheral blood and in the cerebrospinal fluid.
As previously presented, repeated analysis according to neurological examination with signs and symptoms graded and converted into a computer program, this double-blind study was found to be without significant difference concerning the progression coefficient for the placebo- and azathioprine-treated groups. Consequently, the trial was stopped in December 1982. During the follow-up period 1 patient from the placebo group received high-dose corticosteroids for acute relapse.

In this investigation it is shown, by use of the Kurtzke disability status score, that - even an overall significant change (5% level) in disability deterioration in the placebo group (Friedman two-way analysis of variance) - at least 2 years' azathioprine treatment did not offer a significant effect over placebo (Fisher fourfold table test (Table I, II)).

Table I. Change in Kurtzke disability score for the two groups during the follow-up period (≥ 5- &lt; 18 months)

<table>
<thead>
<tr>
<th>Deterioration</th>
<th>Azathioprine</th>
<th>Placebo</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unchanged or improved</td>
<td>4</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>10</td>
<td>21</td>
</tr>
</tbody>
</table>

Fisher fourfold table test; no significant difference (5 % level) between the two groups in terms of deterioration assessed by disability system.

Results of Long-Term Therapy with Azathioprine in MS between 4 and 15 Years Later

F. Lhermitte, R. Marteau, E. Roulet, Paris

211 probable or definite (McAlpine) MS patients received azathioprine (100 mg daily) as primary immunosuppressive treatment between 1967 and 1982. 145 patients have been treated for 1 year or longer. 45 patients were male, 100 female. At the beginning of treatment, 97 patients were in the remittent phase of the disease and 48 in the progressive phase; mean age at onset of MS was 27 years, mean duration of treatment and follow-up were 67 and 115 months, respectively.

Treatment results are given according to disability which has been evaluated prospectively. In the 97 patients with the remittent form of the disease at onset of treatment, disability score was stable in 63 (65%), 22 of which (22%) had no further bout during therapy, and increased in 34 (35%), in 14 of which progression occurred. In the 48 patients with the progressive form of disease, the disability increased in 31 (65%) and was unchanged in 17 (35%); the number of stabilized patients decreased with duration of therapy. In the subgroup treated for 5–15 years, disability remained unchanged in 30 (47.6%) out of 63 patients.

Hemological, cutaneous, digestive and infectious side effects were always reversible. 10 epitheliomas were diagnosed in the subgroup of 131 patients who had received no other immunosuppressive treatment than azathioprine and in whom information was obtained at the end of 1982; 5 patients died from cancer (4 of 6 deaths in the remittent group), and the frequency of malignancy in the patients followed for 5 years or more was 10%. It is suggested that azathioprine therapy should be restricted to patients who cannot be enrolled in controlled trials.

Table II. The variable in Kurtzke disability score for each of the two groups during the total period

<table>
<thead>
<tr>
<th>Friedman two-way analysis of variance</th>
</tr>
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<tbody>
<tr>
<td>at the beginning of treatment</td>
</tr>
<tr>
<td>at the end of treatment</td>
</tr>
<tr>
<td>at the present follow-up state</td>
</tr>
<tr>
<td>Sum of ranks</td>
</tr>
<tr>
<td>Azathioprine Placebo</td>
</tr>
</tbody>
</table>

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Combined Acute and Chronic Immunotherapy with Cyclophosphamide (Endoxan) in MS R.E. Gonsette

Belgian National Center for Multiple Sclerosis, Melsbroek, Belgium

Since 1967, 265 MS patients have been treated with cyclophosphamide (CY, Endoxan) at the Belgian National Center for MS. During the first part of this clinical trial, an acute immunosuppression was obtained after intravenous infusion of 4–12 g CY (mean dose 6 g). This short-term intensive immunotherapy appeared to reduce the annual relapse rate (ARR) as well as the progression of the disease in nearly 70% of the patients. In the long run, however, this beneficial effect appeared transient and in most cases the disease seemed reactivated 2 or 3 years later.
In order to prolong the therapeutic effect, it was decided to combine acute intravenous and chronic oral CY immunotherapy. Recruitment was limited to patients experiencing frequent relapses (mean ARR 1.39), a short evolution (mean duration of the disease 4 years) and presenting a disability no greater than a Kurtzke 6 at entry (mean K 2.27).

Combined CY immunotherapy was performed in 112 patients. Due to frequent subjective side effects (gastric intolerance), a prolonged treatment was possible in only 69 cases. An evaluation of the results was performed in 59 MS patients (36 males, 23 females) with a follow-up for 2 years or more (mean follow-up 4.76 years).

Cy dosage was 50 mg/day in 25 patients, and 50 mg ever other day in 34, totalling 281 patient years of observation (cumulative annual dose 9-18 g). A long-term drop-out (mean 4.5 years) was observed in 13 patients. The main reasons for discontinuation were cystitis (5 cases) and other adverse reactions (4 cases). Biological tests, neurological examination and evaluation of the functional disability (Kurtzke DSS) were performed every 2 months. The group of chronically treated patients was compared to a group of 59 patients treated with an single immunosuppression and retrospectively matched as close as possible according to sex, age at onset, handicap when entering the trial, interval of time between onset and treatment and type of course. After a mean follow-up period of 4.76 years, 47/59 patients (80%) remained stable in the group treated with combined acute and chronic CY immunotherapy compared to 23/59 (39%) in the group treated with a single immunosuppression. Chronic administration of CY appears therefore to be effective in preventing the disease progression in some patients.

In order to demonstrate potential CY effects on immune functions, lymphocyte subset determination was performed in 21 chronically treated patients. Compared to the results obtained in 33 nontreated MS patients, three parameters appear significantly different (p < 0.01): H cell count is lower (36.85 vs. 45.92); S cell population is higher (30.41 vs. 19.14), and as a result the H/S immunoregulatory ratio is lower (1.28 vs. 2.78) in treated patients. No significant changes were noted concerning K-NK, B and total lymphocyte cell populations.

After a follow-up for 18 years, 37 deaths (14%) were reported in our group of 265 MS patients treated with CY and cancer was diagnosed in 4 patients (10.8%). In a group of 3,000 nontreated MS patients followed at the National Center, 330 deaths were reported (11%). The exact cause of death was known in 257 cases and malignant diseases were diagnosed in 27(10.5%).

In our relatively large experience, the risk of malignant diseases does not seem to be increased in MS patients treated with CY. Nevertheless, we think that this treatment is indicated in selected cases only and that there is a real need for more benign immune treatment alternatives.

Cyclophosphamide Treatment of Progressive MS

Stephen L. Hauser

In an initial study [New Engl. J. Med. 308: 173—180, 1983] 58 patients with severe, progressive MS were prospectively randomized to one of three treatments: 20 received ACTH by intravenous route, 20 received high-dose intravenous cyclophosphamide plus ACTH, and 18 were placed on a regimen consisting of plasma exchange, low-dose oral cyclophosphamide, and ACTH. The three groups were similar in age, sex, duration and type of disease, and in degree of disability. Before treatment, and 6 months and 1 year after treatment, a disability status score, ambulation index and functional status score were determined, and a quantitative neuologic examination was performed. In the ACTH group, the number of patients stabilized or improved was 8 of 20 at 6 months and 4 of 20 at 1 year in the cyclophosphamide-ACTH group, 18 of 20 at 6 months and 16 of 20 at 1 year; and in the plasma exchange group, 13 of 18 at 6 months and 9 of 18 at 1 year. High-dose cyclophosphamide plus ACTH was most effective in halting progression of the disease at both 6 and 12 months (at 12 months, cyclophosphamide-ACTH vs. ACTH, p = 0.0004; cyclophosphamide-ACTH vs. plasma exchange, p = 0.087). Thus, progressive MS may be stabilized with short-term, intensive immunosuppression with cyclophosphamide plus ACTH.

The majority of patients treated with a single course of cyclophosphamide-ACTH develop recurrent disease progression between 1 and 3 years following treatment. Current experimental protocols are designed to test whether repeat treatments or maintenance therapy with cyclophosphamide can modify the late progression of disease observed following initial treatment. A Northeastern Cooperative Multiple Sclerosis Treatment Group has been formed in order to test new immunosuppression protocols, to assess the long-term toxicity of treatment, and to evaluate the usefulness of current laboratory markers of disease activity in predicting clinical course and treatment response.

Short-Term High-Dose Therapy with Cyclophosphamide in Chronic Progressive MS Patients with Special Attention to Side Effects

O.R. Hommes, Nijmegen

This is an open, uncontrolled study of the effect of an intensive short course of immunosuppression (IS) with 400 mg cyclophosphamide and 100 mg prednisone per day during 20 days, on the course of the disease in chronic progressive MS patients. Since 1971 we have treated 150 such patients, and followed them over a total of 700 patient years. Improvement 12 months after treatment is found in 30%, stabilization in 30% of the patients. Progression is not stopped in 30% of the patients. Seven to 11 years after the first treatment, 70% of the patient are still better than before the treatment. The conclusion is that IS may halt or reduce progression in chronic progressive MS. Clear effects on immunological parameters in CSF are present. Short-term and long-term side effects are discussed, with emphasis on malignancy and fertility. In our patients no malignancies occurred. Effects on fertility are present. No effects on 9 children born after treatment were detected.

Antilymphocyte Serum in Severe MS Rene Marteau, Paris

Antilymphocyte serum (ALS), a powerful immunosuppressive drug, has been used in MS for over 15 years. Since 1968, three therapeutic trials with ALS have been conducted in Salpetriere Hospital in Paris.

In the first trial (1969–1971), ALS was given by the intramuscular route. Patients were randomly allocated to corticosteroids alone (21 patients) or corticosteroids + ALS (16 patients). The mode of preparation of ALS changed during the study and adverse effects were numerous; however, encouraging
results were seen in the treated patients and purified ALS (associated with azathioprine and corticosteroids) was used during a few years in an uncontrolled way. A pilot study (50 patients) was then done (1973—1978) using intravenous infusions of ALS. Daily dosage was 10 mg/kg, duration of treatment was 6 weeks, ALS was associated with azathioprine and corticosteroids. The incidence of side effects was low [12]. Immunosuppression, as measured by cutaneous tests, was effective and lasted 2 months after completion of ALS treatment. Therapeutic efficacy was very difficult to assess: there was transient stabilization and improvement in some patients; 2 years after treatment, more than one third of patients were still improved or stabilized, but this did not statistically differ from a nonrandomized control group; 4 years after treatment, 22% of patients were still improved or stabilized. This relatively low risk-to-benefit ratio warranted a controlled study. In the third trial (1978–1984), 45 treated patients (ALS + azathioprine + corticosteroids) were compared to 22 controls who received azathioprine + corticosteroids. All patients had severe progressive MS (with or without supervened relapses) of less than 5 years duration; treatment was given during 1 year; immunological monitoring included cutaneous tests, total seric complement, circulating immune complexes and E rosettes. The incidence of side effects was lower than in the previous studies. Therapeutic efficacy was assessed by disability score rating: when compared to controls, ALS-treated patients were improved at 1 and International MS Conference: Scientific Symposium 439 4 but not at 2 and 3 years of onset of therapy; the frequency of relapses was not modified by the treatment. ALS therapy may be used in severe MS patients resistant to azathioprine and corticosteroids; progression is stopped in a significant number of patients. Prolonged (1 year) low-dose and slurred (6 weeks) high-dose ALS cures give similar results. Lhermitte, F.; Marteau, R.; De Saxce, H.: Traite-ment de la sclerose en plaque par le serum antily-mphocytaire; in Schuller, Immunopathologie du systè-me nerveux, vol. 134, pp. 281–300 (Inserm, Paris 1975). Lhermitte, F.; Marteau, R.; De Saxce, H.: Traite-ment des formes graves de sclerose en plaques par le serum antilymphocytaire. Revue Neurol., Paris 135: 389–390 (1979). De Saxce, H.; Marteau, R.; Lhermitte, F.: Treatment of recent, grievous progressive multiple sclerosis by the combined application of antilympho-cyte serum, azathioprine and prednisone. Symposium of the Belgian Research Group for MS, 1983. Follow-Up Observations on MS Patients after Treatment with Intrathecal Interferon Lawrence Jacobs, Judith O’Malley, Arnold Freeman, Roslyn Ekes, Peter Reese Dent Neurologic Institute and Roswell Park Memorial Institute, Buffalo, N.Y., USA Long-term follow-up observations on MS patients who were treated with interferon administered intra-thecally over a 6-month period of time reveals a persisting beneficial effect in terms of a reduction in their exacerbation rates. At the time of our last report in 1982 [1], 10 interferon recipients had shown a reduction in their mean exacerbation rate for 18 months prior to the study to 0.2/year during the study (p < 0.01) (t test) while 10 MS control patients showed no change in their rates during the study (0.69/year) compared with before it (0.68/year). That report was based on observations made for means of 1.9 years in the recipients and 1.6 years in the controls. The recipient patients have now been followed for a mean of 4.4 years and their exacerbation rates have continued to decrease to a current mean level of 0.16/year (p < 0.003) (Wilcoxon signed rank test). The control patients were crossed-over and began receiving intrathecal interferon after they had been on the study for 2 years without showing any change in the exacerbation rate. During the 2 years since crossover they also have shown a reduction in exacerbation rate to a mean of 0.30/year currently (p < 0.04) (Wilcoxon signed rank test). The recipients had a higher mean prestudy exacerbation rate than the controls (vagary of randomization). However, this was adjusted for my multivariate regression analysis which confirmed the effect of interferon in reducing exacerbation rate (p < 0.05). Two of the initial recipients were retreated with single injections of interferon for acute exacerbations that occurred 25 and 37 months after their initial 6-month treatment phases. The other patients continue to benefit without retreatment. The toxic side effects of intrathecally administered interferon were acceptable in view of the benefit achieved. Interferon was identified in the cerebrospinal fluid (but not sera) of 2 patients prior to treatment which is probably a manifestation of de novo production of interferon by the central nervous system in response to the MS disease process. The initial study was not blinded. However, we are now in the midst of a multicenter, double-blinded study that will assess the efficacy of intrathecal administration of interferon in a larger number of MS patients with exclusively exacerbating-remitting disease and high exacerbation rates [2]. This study (now ongoing) is supported by the US Public Health Service (National Institutes of Health) and includes 80 patients at three centers (Dent Neurologic and Roswell Park Memorial Institutes, Buffalo; Walter Reed Army Medical Center, Washington; University of Rochester Medical Center, Rochester) [3]. Previously it was impossible to conduct double-blind clinical trials of interferon because of this substance’s toxic side effects which are not mimicked by benign placebo. However, small doses of indomethacin administered for 24 h after interferon treatment greatly reduce the toxic side effects of interferon [4]. We have achieved successful double blinding in the currently ongoing study by administration of small doses of indomethacin to both recipients and controls. While the final results of the study will not be known for another year, data regarding tolerance and toxicity will be presented. Slight reduction of interferon dosage and frequency of administration (from 40 treatments 440 International MS Conference: Scientific Symposium in initial study to 9 in the current one) as well as administration of indomethacin have resulted in lessening of the clinical side effects and lowering of the pleocytosis that accompanies intrathecal interferon administration.
Hyperbaric Oxygen Therapy of MS

J. Merlin

Convincing evidence indicates that a disturbed metabolism and/or action of essential fatty acids (EFA and their derivatives, prostaglandins, may contribute to MS pathogenesis [1, 2]. Lowered concentrations of EFA have been found not only in the CNS but also in the white and red blood cells of MS patients. These observations led to attempts to influence the clinical course of MS by EFA treatment. Recently, Dworkin et al. [3] have reanalyzed the data from three double-blind, controlled trials of EFA supplementation. EFA-treated patients with minimal or no disability at entry to the trials had significantly smaller increase in disability than control patients receiving nonessential fatty acid, a finding reminiscent of similar observations in trials of immunological treatment [4, 5]. In addition, EFA treatment significantly reduced the severity and duration of relapses in patients at all levels of disability at entry to the trials [3]. Based on studies in animals with experimental allergic encephalomyelitis, it has been argued that the beneficial effect of EFA may be exerted through the immunosuppressive effects of their derivatives. Prostaglandins of type E are widely accepted as immunomodulatory mediators and increased concentrations of PGE have been shown to be immunosuppressive in experimental animals [6].


Hyperbaric oxygen (HBO) therapy has been claimed to be of benefit primarily in chronic progressive MS in several uncontrolled studies originating in several countries. One relatively small double-blind, controlled trial also reported benefit from HBO therapy, however the improvement was generally modest and
Transient. Such studies must be evaluated with great caution because of the dramatic effect which HBO or placebo may induce in MS patients. Occasionally, individual MS patients have apparently had dramatic improvement from HBO therapy. No clear hypothesis of how HBO therapy affects the course or pathology of MS has been proposed.

Limited studies of experimental allergic encephalomyelitis (EAE), a possible animal model of MS, in rats and guinea pigs have indicated that HBO therapy may be of benefit in this autoimmune disease of the central nervous system (CNS). These and other studies suggest that HBO may have an immune modulating effect possibly by reducing phagocytic function and delayed hypersensitivity reactions in the CNS. Such studies have value because they may provide some rationale for HBO activity in CNS inflammatory lesions. Further study of HBO in appropriate animal models and in well-defined MS patients required to determine if true benefit occurs if such benefit is worth the cost of therapy and by what mechanism such improvement occurs.

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