

Interferon- α and transfer factor in the treatment of multiple sclerosis: a double-blind, placebo-controlled trial

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SUMMARY The role of interferon- α (IFN- α) and transfer factor (TF) in the treatment of multiple sclerosis was investigated in a prospective, multi-centric, three year, double-blind, placebo-controlled trial. One hundred and eighty two patients with clinically definite multiple sclerosis were randomised into three treatment groups whose compositions were found to be similar for demographic and prognostic variables including HLA status. Subcutaneous injections of IFN- α (3×10^6 units), TF (0.5 units) manufactured from leucocytes of cohabiting donors, or placebo were given twice weekly for two months, once weekly for 10 months then fortnightly for 24 months. One hundred and fifty three patients completed the injection regimen. There was no significant difference in the progression of disability for multiple sclerosis patients in either the IFN- α or TF-treated groups compared with the placebo group. Similarly, change in visual evoked responses (VER), and in number of oligoclonal bands (OCB) and the level of myelin basic protein (MBP) in the cerebrospinal fluid (CSF) over the trial period did not differ significantly between the three groups. However, the IFN- α -treated group had significantly more reported adverse drug reactions and patient withdrawals than either of the other two groups.

Although the pathogenesis of multiple sclerosis is uncertain, current evidence suggests that the disease is immunologically mediated in genetically susceptible individuals in response to an environmental factor, probably a viral infection.^{1,2} Studies of longer term

treatment of the disease have therefore involved trials of immunotherapeutic and antiviral agents.^{2,3}

Use of interferons (IFNs), which have both antiviral and immunomodulatory properties^{4,5} has been advocated in multiple sclerosis. However, reported clinical experience of effects of IFN- α in multiple sclerosis has been conflicting.⁶⁻⁹ Transfer factor (TF), a dialysable leucocyte extract,¹⁰ which has been claimed both to restore cell-mediated immune reactivity and to have antiviral properties has also been under consideration as a therapeutic agent in multiple sclerosis although results of clinical trials have differed.^{11,12}

In order further to define the roles of IFN- α and TF in the treatment of multiple sclerosis, the AUSTIMS trial (Australian trial of TF and IFN- α in multiple sclerosis) was devised as a collaborative (multi-centre), prospective, randomised, double-blind, placebo-controlled, non-crossover study of TF and IFN- α involving initial enrolment of 182 multiple sclerosis patients and treatment for a 3-year period.

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Patients and methods

Patients

One hundred and eighty two patients (112 female, 70 male)

with symptoms and signs of clinically definite multiple sclerosis¹³ and without serious intercurrent illnesses were accepted into the trial. Eighty five per cent of patients had multiple sclerosis for less than 10 years and all patients had clinical evidence of disease activity, either one or more relapses or progression of more than 1 on the Kurtzke disability status scale (DSS),¹⁴ within the three pre-trial years. At the initial assessment, 82% of patients had DSS scores of less than 4 which represented mild to moderate disability. After fulfilling these selection criteria, patients were randomised into three groups; 60 patients received IFN- α , 61 TF and 61 placebo. Patients, attending staff and neurologists were blinded to the treatment given. The protocol for the trial was approved by Medical Ethics Review Committees in all participating centres and written informed consent was obtained from all patients.

The trial protocol allowed for short courses of corticosteroid or ACTH therapy to be given for relapses when indicated; it was recommended that the trial injection regimen be suspended for the duration of the steroid therapy and for two weeks thereafter. Immunosuppressive agents such as azathioprine and cyclophosphamide were not prescribed for any patient for 12 months prior to or during the trial. Throughout the course of the trial clinical progress of patients in each treatment arm and reports of adverse drug reactions were monitored by an Independent Review Committee.

Each trial patient underwent a full neurological assessment performed by a neurologist before entry, after two months and six months, and every three months of treatment thereafter for the duration of the trial. All neurological assessment forms were later reviewed by one neurologist (JGMcL). At each examination the patients' disability was assessed on the Kurtzke DSS. The DSS ranges from 0 (normal) to 10 (dead); grades 0-3 represent only mild or moderate disability; grade 6 indicates assistance required with walking and grade 7 restriction to a wheelchair. Abnormalities in each of the functional systems (FSS) (pyramidal, cerebellar, brainstem, sensory, bowel-bladder, visual, mental, and other)¹⁴ were also graded to indicate neurological impairment. Patient disability was further assessed on an Ambulatory Status Scoring System (ASSS) which, as an adaptation of the DSS, emphasised the impact of the patient's neurological dysfunction on performing the tasks of daily living (Appendix 1). The number of relapses since the previous examination and adverse drug reactions were recorded.

Preparation and administration of interferon- α , transfer factor, and placebo

IFN- α (human leucocyte IFN) was produced by the Commonwealth Serum Laboratories (CSL) according to the method of Cantell and Hirvonen¹⁵ from the buffy coats of healthy blood donors and purified by monoclonal antibody affinity chromatography (Celltech Ltd, UK). Potency was expressed in units of antiviral activity using GM2504 cells and the EMC virus obtained from the Institute for Medical Research, New Jersey, and Professor Grossberg, Medical College of Wisconsin, respectively.

Transfer factor was produced as described previously.¹¹ Buffy-coat preparations rich in mononuclear cells were obtained by an Aminco continuous-flow cell-separator from

relatives living in the same house as patients suffering with multiple sclerosis. The mononuclear cells were separated on "Ficoll-Hypaque" gradients and a crude cell-free extract was prepared by repeated freeze-thawing (10 times). After ultrafiltration through a PM 30 Amicon filter, the dialysate was placed in a freeze drying apparatus and the contents lyophilised for storage as a powder at -20°C . When needed, the powder from several donors was pooled and reconstituted in sterile pyrogen free water. The reconstituted product was filtered through a Pall 0.2 μm membrane and samples taken to test for sterility, pyrogens, protein, HBsAg and human immunodeficiency virus antibodies. The chromatographic profile of TF obtained in this way was very similar to that observed with the more classical dialysis procedure, the advantage of the present method being that sufficient amounts of TF could be produced for a trial of this size and duration.

The concentration of TF was adjusted so that each 1 ml contained 2.0×10^8 cell-equivalents and one unit of TF was arbitrarily defined as 4×10^8 cell-equivalents.

Placebo consisted of a sterile buffered salt solution containing 0.5% human albumin which was also present in the IFN preparation. IFN- α , TF and placebo were stored in 1 ml vials at -20°C until used. Each patient received 3×10^8 units of IFN- α , 0.5 units of TF or 1 ml of placebo subcutaneously twice weekly for two months, then once weekly for 10 months, then fortnightly for 24 months. Thus each patient who completed the therapeutic regimen had received approximately 100 injections over the three year period of the trial. This dosage schedule was based in part on those previously used for trials of IFN- α and TF.^{8,11} Two hours before and two hours after each injection, it was recommended that the patients take paracetamol 1g orally, to reduce febrile reactions thereby assisting with blinding of the study. Despite therapy with paracetamol¹⁶ febrile reactions to injections necessitated a reduction in the dose of injections in four patients and indomethacin¹⁷ cover in five patients.

Laboratory investigations

Routine studies Before randomisation, haematological parameters including erythrocyte sedimentation rate, haemoglobin quantitation, differential leucocyte count and platelet count, a blood biochemistry profile including urea, creatinine, electrolytes and liver function tests, and a chest x-ray were performed. The haematology and biochemistry profiles were repeated monthly for the first six months, then three monthly thereafter. If the neutrophil count was reduced below $1 \times 10^9/\text{l}$, injections were suspended until the count had returned to within the normal range ($2.5-7.5 \times 10^9/\text{l}$).

IFN- α has been shown to reduce the levels of plasma iron and zinc¹⁸ and as iron and zinc deficiency could result in impaired lymphocyte function^{19,20} thereby interfering with the immunopotentiating effect of therapy, full iron studies and serum zinc levels were performed on patients in the New South Wales branch of the trial at the 12, 18 and 24 month assessments. Results of these studies were analysed by an independent observer during the trial in order to identify the development of iron or zinc deficiency and instigate appropriate treatment if necessary.

Electrophysiological studies Visual evoked responses (VER) elicited by pattern reversal, full field stimulation were

performed on 155 patients at the commencement and conclusion of the trial.

Cerebrospinal fluid studies Samples of cerebrospinal fluid (CSF) were obtained for analysis from 32 patients, before commencement and at completion of the trial. Another nine patients had CSF studies performed at the start of the trial only. On each specimen a cell count and culture and sensitivity were performed. To demonstrate oligoclonal bands (OCBs), samples of unconcentrated CSF from 36 patients were stained with a silver-stain after isoelectric focusing on polyacrylamide gel.²¹ Seventeen patients had OCB studies done pre- and post-trial. The level of myelin basic protein (MBP) in the CSF before and after treatment was assessed in 25 patients using the method of Cohen *et al*;²² the only technical variation from this methodology was in the preparation of the anti-human MBP antibody in the rabbit where the technique of Bernard *et al*²³ was used.

Tissue typing HLA typing was carried out with 120 sera for HLA-A, B and 60 sera for HLA-DR. All sera had been standardised against cells typed at the Ninth International Workshop²⁴ and previous workshops. In the case of anti-DR sera, platelet adsorption was carried out to remove micro-aggregates and any anti-Class I activity.²⁵ For HLA-DR typing, an enriched population of B lymphocytes was obtained by depletion of monocytes on nylon-wool columns²⁶ followed by removal of T cells by 2-aminoethyl-isothiuronium bromide hydrobromide (AET) rosetting.²⁵ Typing was performed using standard lymphocytotoxicity assay,²⁷ with modified incubations for B lymphocytes in HLA-DR typing.²⁵ Class III antigens (C2, C4, Bf) were also measured and the results will be reported elsewhere.

Statistical analysis

The base-line characteristics of the study population in the three treatment arms were compared with analysis of variance for continuous variables and Chi-square tests for discrete variables.

Differences in accumulation of disability between treatment groups were statistically evaluated in a number of ways. First, the significance of the differences in Kurtzke DSS, ASSS and FSS scores between treatment groups was assessed with unconditional multiple logistic regression²⁸ using odds ratios (OR) as the parameters with 95% confidence intervals (CI). The simple dichotomy of worse versus no-worse was chosen as the primary outcome variable for the study but worse on the Kurtzke DSS score by 2 or more versus worse by 1 or less, worse by 2 or more versus worse by 1, and better versus same, were also examined. Treatment effects were estimated before and after adjustment for potential confounding variables. Age and initial Kurtzke DSS score were the only variables found to be related consistently to the change in Kurtzke DSS score during the trial and consequently they were included as covariates in the analysis.

Secondly, each patient's disability after 3 years of treatment was compared with his own initial disability and recorded as "better" (by 1 or more points on the Kurtzke DSS), "unchanged" or "worse" (by 1 or more points on the Kurtzke DSS). The difference in distribution between treatment arms of patients within the categories, better or unchanged, and worse, was compared by Chi-square analysis with two degrees of freedom.

Thirdly, the mean of the change in DSS score for the

patients in each group was compared by analysis of variance. The data for the three groups were also analysed using the Kruskal-Wallis statistic.

Finally, treatment groups were subdivided into HLA DR2 positive or negative subgroups and Chi-square analysis, analysis of variance and the Kruskal-Wallis statistic applied to test for an effect of this antigen on prognosis and treatment effect.

The mean number of types of adverse drug reactions per patient in each treatment group and the difference in the mean number of exacerbations of multiple sclerosis experienced by patients in each group was assessed for significance with 95% confidence intervals. An exacerbation was defined as a worsening of symptoms or signs or occurrence of new neurological symptoms or signs that persisted for at least 24 hours.²⁹

The data on the evoked potential studies from before and after treatment were evaluated in three ways. First of all, patients were recorded as having normal or abnormal VERs on the basis of the normal range of P100 latency and interocular latency for their centre of testing. The number of patients in each treatment arm who improved (abnormal to normal response), remained unchanged or deteriorated (normal to abnormal response) was recorded but, because of small expected values, Chi-square analysis was not performed. Secondly, the change in latency of the P100 over the trial period was calculated and 10 ms was accepted as a significant change, this figure comfortably exceeding the variability in control patients recorded recently in one of our laboratories³⁰ and being in agreement with other workers.³¹ Each patient's evoked potential results were assessed as "better", "unchanged" or "worse"; the distribution of patients in each group was compared between treatment arms by Chi-square analysis. Finally the mean change in latency for each treatment group was compared by analysis of variance.

Analysis of results was performed on an intention to treat basis; all patients who commenced therapy, regardless of whether they completed the trial, were included in the statistical analysis of neurological status. The analyses were based on changes in status between the commencement of therapy and the final assessment, which, for most patients, was 36 months after commencing therapy. For all statistical analyses the level of significance was taken as $p < 0.05$ and two-tailed tests were used.

Results

Clinical evaluation

One hundred and eighty two multiple sclerosis patients were randomised into one of the three treatment arms: the IFN- α -treated, TF-treated or placebo group. The subjects in each of the treatment arms were similar with respect to age ($F = 0.182$, NS), sex (Chi-square = 1.258, 2DF, NS), clinical course (Chi-square = 0.124, 2DF, NS), clinical disease type (Chi-square = 4.356, 8DF, NS), mean duration of disease ($F = 1.075$, NS), initial Kurtzke DSS ($F = 0.300$, NS) and the presence of HLA DR2 (Chi-square = 0.989, 2DF, NS) (table 1). There was an

Table 1 Initial clinical and HLA data

Data	IFN- α -treated group	TF-treated group	Placebo group
Number of patients	60 (25M, 35F)	61 (25M, 36F)	61 (20M, 41F)
Age at trial outset			
—range (years)	19–50	20–49	18–48
—mean (years)	36.1	35.8	35.3
Clinical course			
—relapsing disease	55	55	56
—progressive disease	5	6	5
Clinical disease type*			
—disseminated	40	34	35
—spinal	5	9	12
—spinal and optic	11	12	9
—spinocerebellar	2	3	2
—brainstem	2	3	2
Mean duration of disease (years)	5.5 [†]	5.0	6.0
Disability score at randomisation			
—range	0–6	0–5	0–5
—mean DSS	2.35	2.38	2.51
HLA phenotype			
—DR2 positive	32/52 62%	31/57 54%	31/59 53%
—A3 B7 DR2 positive	12/51 24%	7/57 12%	15/59 25%
—A3 B7 positive	13/51 25%	7/57 12%	17/59 29%

No significant differences between treatment arms.

*Data unavailable on one patient.

Table 2 Better, Unchanged, or Worse analysis of change in clinical disability

	Interferon- α †	Transfer factor	Placebo
Better*	9	10	16
Unchanged	26	15	17
Worse*	24	36	28

*Change by ≥ 1 on Kurtzke DSS.

†One patient in IFN- α group withdrew after the initial assessment and no further assessments were performed, therefore no change in disability is available.

analysis of the ASSS revealed no significant difference between treatment groups (Chi-square = 1.559, 2DF, $p < 0.5$).

The mean change in DSS over the trial period of 0.59 (SD, 1.30), 1.10 (SD, 1.61), and 0.52 (SD, 1.70) for IFN- α , TF and placebo groups respectively was not significantly different ($F = 2.540$, $p > 0.05$). Similarly the mean change in ASSS over the trial period of 0.58 (SD, 1.28), 1.15 (SD, 1.64), and 0.68 (SD, 1.62) for IFN- α , TF and placebo groups respectively was not significantly different ($F = 2.384$, $p > 0.05$). The change in the DSS and the ASSS, when analysed with the Kruskal-Wallis statistic, also revealed no significant difference between treatment groups. When the data were reanalysed using only the results of patients who were HLA DR2 positive, HLA DR2 negative, had an initial DSS of less than 4, or had relapsing-remitting disease, the findings were the same.

Analysis of the FSS scores by unconditional multiple logistic regression with both crude and adjusted OR revealed significantly less deterioration in the cerebellar score for patients receiving IFN- α (adjusted OR = 0.38, 95% CI 0.16–0.92) than those receiving TF (adjusted OR = 1.11, 95% CI 0.52–2.37) or placebo for the outcome worse versus no worse (table 3). Similarly when patients were assessed as better, worse or unchanged for each of the FSS, the only significant result was for the cerebellar FSS (Chi-square = 7.106, 2DF, $p < 0.05$) where there was a trend for fewer patients receiving TF to show improvement in their cerebellar function and fewer patients receiving IFN- α to deteriorate.

The mean number of relapses during the three years of therapy for each group was 1.53, 2.11 and 1.97 for IFN- α , TF, and placebo groups respectively. The ratio of observed to expected events (the number of expected events being the total number of relapses divided by the total number of trial participants) for the IFN- α treated group was 0.82 (95% CI 0.81–1.24) and for the TF treated group was 1.13 (95% CI 0.84–1.21). The 95% confidence intervals confirm there was no significant difference in the mean number of relapses in each treatment group.

The mean types of adverse drug reactions per

excess of female patients in the placebo-treated group but this was non-significant.

One hundred and fifty three (84%) of the 182 patients completed the trial (42/60, 53/61 and 58/61 patients in the IFN- α , TF and placebo groups respectively). Of the 29 patients who withdrew, 18 (62%) were receiving IFN- α , eight (28%) were receiving TF and three (10%) were receiving placebo. Reasons for patient withdrawal are given later.

When unconditional multiple logistic regression was performed on the Kurtzke DSS scores neither the adjusted OR for IFN- α (OR = 0.78, 95% CI 0.37–1.63) nor that for TF (OR = 1.78, 95% CI 0.85–3.71) reached a significant difference from the placebo group. Similarly with the ASSS scores, the adjusted OR for the two active treatment groups again did not reach a significant difference from the placebo group (adjusted OR for IFN- α = 0.81, 95% CI 0.37–1.74 and for TF = 1.54, 95% CI 0.72–3.27). Values for the crude OR were similar to the OR adjusted for age and initial Kurtzke score with the implication that the potential confounding variables did not prove to be actual confounding variables.

When patients were assessed as better, worse or unchanged on the DSS (table 2), improvement was seen in 15%, 16% and 26% of patients in the IFN- α , TF and placebo groups respectively. The difference was not significant (Chi-square = 4.293, 2DF, $p < 0.25$). Similarly better, worse or unchanged

Table 3 Adjusted odds ratios with 95% confidence intervals for Kurtzke Functional System Scale Scores

FSS	Transfer factor OR (95% CI)	Interferon- α OR (95% CI)	p Value
1 - Pyramidal	1.71 (0.82-3.59)	0.95 (0.45-2.03)	0.22
2 - Cerebellar	1.11 (0.52-2.37)	0.38 (0.16-0.92)	0.03
3 - Brainstem	0.52 (0.20-1.35)	1.17 (0.49-2.75)	0.19
4 - Sensory	0.88 (0.35-2.23)	0.91 (0.35-2.36)	0.96
5 - Bowel and Bladder	1.07 (0.47-2.45)	0.64 (0.27-1.55)	0.46
6 - Visual	1.66 (0.69-3.99)	0.96 (0.37-2.52)	0.39
7 - Mental	0.83 (0.26-2.65)	0.81 (0.25-2.60)	0.93
8 - Other	3.23 (1.18-8.84)	1.80 (0.66-4.92)	0.06

Adjusted OR for the outcome worse versus no worse for the Kurtzke FSS scores for patients treated with TF and IFN- α , relative to patients treated with placebo. The p value for the differences between the three treatment groups is from the likelihood ratio test.

patient were 5.42, 0.85 and 1.05 for the IFN- α , TF and placebo treated groups respectively. Patients in the IFN- α -treated group experienced significantly more types of adverse drug reactions than expected (the number of expected events being the total number of adverse drug reactions divided by the total number of trial participants) whereas the reverse was true for the TF-treated and placebo groups. In the IFN- α group there was a greater frequency of fever, rigors, headache, fatigue, generalised myalgia, nausea, anorexia, skin sensitivity, and abnormalities in laboratory tests compared with either the TF or placebo groups. In addition 30 (50%) patients in the IFN- α group reported transient exacerbations of neurological symptoms within the 24 hours following injections compared with 5 (8%) patients from each of the TF-treated and placebo groups who experienced such symptoms (Chi-square = 40.909, 2DF, $p < 0.001$).

Withdrawals from the IFN- α group significantly exceeded withdrawals from the TF or placebo groups (Chi-square = 14.607, 2DF, $p < 0.001$). Consequently, the mean number of injections received by patients in the IFN- α -treated group was significantly fewer than that received by the TF-treated or placebo groups ($F = 3.673$, $p < 0.05$). Withdrawals from IFN- α therapy occurred because of unacceptable adverse effects reported as fever, rigors, fatigue and/or transient worsening of neurological symptoms (13), perceived progressive neurological deterioration (2), myocardial infarction (1), commencement of chemotherapy for carcinoma of the lung (1), or a belief that the therapy was of no benefit (1). Withdrawal from TF therapy occurred because of pregnancy (2), perceived neurological deterioration (1), sudden death (1), thrombocytopenia (1) or personal reasons (3). Withdrawal from the placebo group occurred because of perceived neurological deterioration (2), and loss to follow up (1).

Laboratory investigations

Significantly more patients in the IFN- α -treated group had neutropenia, and elevation of alanine transaminase, gamma glutamyl transpeptidase, and aspartate transaminase compared with the TF and placebo groups. These abnormalities were reversible with a lower, or less frequent dose of IFN- α , or both. There were no significant differences in frequency of any haematological or biochemical abnormalities between the TF and placebo groups.

Results of iron studies and zinc levels performed over one year on patients from New South Wales revealed no trends within treatment groups and no significant differences between groups. Thus the failure of IFN- α to influence the course of the disease could not be explained on the basis of immunological impairment secondary to iron or zinc deficiency.

Evoked potential studies

VER studies were performed before and after the trial on 155 patients. The VER latencies changed during the trial from normal to abnormal responses in five IFN- α , three TF and three placebo patients while VER latencies of two IFN- α , one TF and six placebo patients changed from an abnormal to normal response.

There was no significant difference between the treatment arms in relation to VER latency changes for either absolute values (table 4) (Chi-square = 6.377, 4DF, $p < 0.25$) or group mean values ($F = 0.568$, $p > 0.05$).

Cerebrospinal fluid studies

Oligoclonal bands were detected in 31 of the 36 patients (86%) who had CSF samples taken before or after the trial. In 17 patients, samples of CSF taken both before and after the trial were tested for OCBs; all patients, initially demonstrated to have OCBs, had OCBs in the post trial sample. Among these 16 patients in whom OCBs were present before and after the trial, more bands were detected in six patients (one IFN- α , three TF and two placebo patients) after treatment while fewer were found in one placebo-treated patient. One TF-treated patient, in whom no OCBs were demonstrated before the trial, remained negative for OCBs after the trial.

Table 4 VER results. Better, Unchanged, or Worse analysis of patients' change in latency

	Interferon- α	Transfer factor	Placebo
Better*	5	4	10
Unchanged	35	30	28
Worse*	12	19	12

*Change in latency of > 10 ms taken as a significant change.

Comparison of MBP levels in the CSF before and after the trial indicated that the level of MBP had increased in 15 patients (five patients from each group), decreased in nine patients (one IFN- α , five TF, three placebo) and remained unchanged in one placebo-treated patient.

Discussion

Neither IFN- α nor TF significantly altered the rate of progression of multiple sclerosis compared to that of controls as measured by the Kurtzke DSS, the ASSS or the relapse rate. Furthermore there was no significant difference between treatment modalities when the patients who were HLA DR2 positive, HLA DR2 negative, who had an initial DSS of less than 4, or had relapsing-remitting disease, were considered separately. These conclusions were unaffected by the method of statistical analysis used. However analysis of the FSS revealed that patients receiving IFN- α had significantly less deterioration in cerebellar function but this did not appear to be clinically significant in the light of the lack of significant change in overall disability.

The lack of clinical efficacy of IFN- α and TF was reflected by no significant change in VER latencies, and OCB number and MBP levels in the CSF of patients in each treatment arm during the trial.

The present study was prospective, double-blind, and placebo-controlled with large numbers of patients observed over a long period of time. The composition of patients within each of the treatment arms was similar for demographic and prognostic variables. The adjusted OR (0.78) for IFN- α indicated there was a 22% reduction in disease progression when 24/60 IFN- α treated patients deteriorated by 1 or more on the Kurtzke DSS compared with 28/61 of the patients on placebo. However the CI (0.37–1.63) showed the need for more than 60% reduction in disease progression (that is, where less than 15/60 patients on IFN- α deteriorated by 1 or more points on the Kurtzke DSS) to demonstrate a significant treatment effect. It is possible that small treatment effects could have remained undetected in this study; larger sample sizes with corresponding narrower confidence intervals would detect smaller treatment effects but the clinical significance of such effects would be controversial.

It is possible that multiple observers of the Kurtzke DSS and ASSS may have influenced the result. Inter-observer agreement on the Kurtzke DSS has recently been demonstrated to be 30% to 50%.³² A similar assessment of reliability for the ASSS has not been performed but to overcome the potential source of bias from multiple observers, all neurological assessment forms were reviewed by one neurologist who was experienced in the use of the Kurtzke DSS and ASSS.

Patients treated with IFN- α had significantly more different types of adverse drug reactions than those treated with TF or placebo. These reactions contributed to the significantly greater number of withdrawals in the IFN- α -treated group which led to the consequent reduction in mean number of injections per patient for this group.

With the increased availability of IFN for clinical trials, each of the three classes of IFN (IFN- α , IFN- β , IFN- γ) has been investigated as possible therapy for multiple sclerosis. Fog,⁶ using large doses of IFN- α (5×10^6 units daily for 2 weeks then 2.5×10^6 units daily for 5 to 15 months) in five patients with chronic progressive disease showed "that in all cases the disease process continued and the patients' clinical states deteriorated". Ruutiainen *et al*⁷ reported the use of intrathecal IFN- α weekly for three months in five patients with chronic progressive disease. Protean side effects of IFN- α administration were noted. The authors were unable to draw conclusions regarding the therapeutic value of intrathecal IFN- α due to the small numbers of patients involved and the brevity of follow-up.

Systemic human leucocyte IFN- α was first used in a randomised, double-blind, placebo-controlled trial by Knobler *et al*.⁸ The study had a crossover design with 24 patients receiving intramuscular injections of either IFN- α (5×10^6 i.u.) or placebo daily for six months then after a 6 month washout period, crossing to the alternate treatment for a further 6 months. The authors found that the rate of exacerbations was reduced in both placebo and IFN- α groups but the reduction was greater in the group receiving IFN- α . However, because of the apparent learning phenomenon associated with the trial design they saw a need for a placebo-controlled, non-crossover study. IFN- α in this study did not influence disease progression as measured by the change in Kurtzke DSS score.

In 1986, Camenga *et al*⁹ published the results of a randomised, double-blind, placebo-controlled trial of recombinant IFN- α -2. Ninety eight patients were given low dose IFN- α -2 (2×10^6 i.u.) or placebo three times per week for 52 weeks and observed for the duration of therapy and for 3 months after. No clear therapeutic benefit was detected.

In keeping with these substantially negative findings, our double-blind, placebo-controlled trial of low dose systemic IFN- α (3×10^6 i.u. fortnightly) administered over three years confirmed the lack of therapeutic benefit of this regimen in the treatment of multiple sclerosis.

All side effects experienced by our IFN- α group have been documented in previous trials of IFN- α therapy and were dose dependent.

IFN- β has been used in therapeutic trials of multiple sclerosis. In 1979 Ververken *et al*³³ published one of

the first articles describing the use of IFN- β in multiple sclerosis. They treated three patients with chronic progressive multiple sclerosis with IFN- β intramuscularly for two weeks but found no effect on the course of disease which was not unexpected in view of the short duration of the study. Jacobs *et al*³⁴⁻³⁸ have investigated the effect on relapse rate of intrathecal IFN- β given in a dose of 1×10^6 i.u. weekly for 4 weeks, then monthly for 5 months. In a randomised, double-blind, placebo-controlled trial involving 69 patients with exacerbating-relmitting multiple sclerosis observed over 2 years they found that IFN- β was effective in reducing exacerbations of multiple sclerosis but led to no significant change in progression of disease as measured by the Kurtzke DSS score. No comment was made regarding the difficulty and risk of repeat lumbar puncture.

A pilot study of recombinant IFN- γ ³⁹ given by slow intravenous infusion to 18 patients with relapsing-relmitting disease showed that a significant increase in exacerbations occurred during treatment compared to pre- and post-treatment rates ($p < 0.01$). In retrospect this result may have been predictable owing to the capacity of IFN- γ *inter alia* to increase expression of HLA-DR on antigen presenting cells thereby enhancing the reactivity of T cells with anti-self specificity. Further trials of IFN- γ do not appear to be warranted.

With regard to TF, prior to 1980 most reports of the use of TF in the treatment of multiple sclerosis⁴⁰⁻⁴⁵ have shown little or no benefit, despite conversion of cell-mediated reactivity to marker antigens in some instances. Three of the six trials lacked a placebo group and the duration of follow-up in controlled studies was 13 months or less.

Basten *et al*¹¹ reported the results of a double-blind, placebo-controlled, study showing that TF from cohabiting donors over a 2 year period slowed the rate of progression of disease in 30 patients with mild to moderate disability at treatment outset. In the present study in which 61 patients received TF, the leucocyte extract did not alter the natural course of disease. The reasons for this difference may be explained on the basis of a smaller number of patients in the pilot study and the more aggressive disease experienced by the placebo group in that study. The mean change in DSS for the placebo group in the first year of the pilot study was 1.22 which was considerably greater than the rate of 0.52 observed over three years for the placebo group in the present study. No serious side effects of therapy have been recorded in any patients on TF.

Lamoureux *et al*⁴⁶ assessed the response of multiple sclerosis patients to TF obtained from random donors and demonstrated a slight but not significant clinical improvement of the Kurtzke FSS and DSS for the TF group over a one year period. Stecchi *et al*⁴⁷ found no significant difference in change in DSS or relapse rate

between groups receiving either TF or azathioprine. A three year, prospective, double-blind, controlled trial¹² revealed no difference between groups treated with TF prepared from random donors, TF from family members living with the patients, and placebo.

As a result of our double-blind, placebo-controlled study involving 182 multiple sclerosis patients observed prospectively over three years, it appears that neither IFN- α nor TF from cohabiting donors given subcutaneously modifies significantly the course of multiple sclerosis. It is possible that this result may reflect the need for a more intensive dosage schedule or an alternative route of administration of therapy. With both treatments, efficacy presumably could be enhanced if their action could be directed specifically against the environmental agent that contributes to the disease. Clarification of the aetiology of multiple sclerosis is needed to enable more specific immunomodulating and antiviral agents to be developed for trial in the disease.

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Appendix I

Ambulatory Status Scoring System (ASSS)

- 0 – Normal neurological examination
- 1 – No disability and minimal signs
- 2 – Minimal disability
- 3 – Moderate disability though fully ambulatory
- 4 – Relatively severe disability though fully ambulatory and able to be self-sufficient and up and about for some twelve hours a day
- 5 – Disability severe enough to preclude ability to work a full day without special provisions. Maximal motor function: walking unaided no more than several blocks
- 6 – Assistance required for walking
- 7 – Restricted to a wheelchair but able to wheel self and enter and leave chair alone
- 8 – Restricted to bed but with effective use of arms
- 9 – Totally helpless bed patient
- 10 – Death due to multiple sclerosis

Assessment of the ASSS is based on the clinical status of the patient disregarding the Functional System Scale Scores.

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