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ORIGINAL COMMUNICATION

The window of therapeutic opportunity in multiple sclerosis

Evidence from monoclonal antibody therapy

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Abstract From 1991–2002, we treated 58 patients with multiple sclerosis (MS) using the humanised monoclonal antibody, Campath-1H, which causes prolonged T lymphocyte depletion. Clinical and surrogate markers of inflammation were suppressed. In both the relapsing-remitting (RR) and secondary progressive (SP) stages of the illness, Campath-1H reduced the annual relapse rate (from 2.2 to 0.19 and from 0.7 to 0.001 respectively; both $p < 0.001$). Remarkably, MRI scans of patients with SP disease, treated with Campath-1H 7 years previously, showed no new lesion formation. However, despite these effects on inflammation, disability was differently affected depending on the phase of the disease. Patients with SPMS showed sustained accumulation of disability due to uncontrolled progression marked by unrelenting cerebral atrophy, attributable to ongoing axonal loss. The rate of cerebral atrophy was greatest in patients with established cerebral atrophy and highest inflammatory lesion burden before treatment (2.3 versus 0.7 ml/year; $p = 0.04$). In contrast, patients with RR disease showed an impressive reduction in disability at 6 months after Campath-1H (by a mean of 1.2 EDSS points) perhaps owing to a suppression of on-going inflammation in these patients with unusually active disease. In addition, there was a further significant, albeit smaller, mean improvement in disability up to 36 months after treatment. We speculate that this represents the beneficial effects of early rescue of neurons and axons from a toxic inflammatory environment, and that prevention of demyelination will prevent long-term axonal degeneration. These concepts are currently being tested in a controlled trial comparing Campath-1H and IFN-beta in the treatment of drug-naïve patients with early, active RR MS.

Key words multiple sclerosis · Campath-1H · cerebral atrophy

Introduction

The clinical course in multiple sclerosis is characterised initially by episodes with full recovery, later by a phase of cumulative deficits following individual attacks, and eventually by the onset of secondary progression. Transition between these three stages is often gradual and indistinct, making for many intermediate forms of the disease. This clinical course is the expression of focal tissue injury of the brain and spinal cord arising from the complex interplay of inflammation, axonal injury, demyelination, astrocytosis and tissue atrophy – partially compensated by physiological reorganisation of circuitry and remyelination [13]. The immediate aims of disease-modifying treatment are to limit the accumulation of

disability and prevent the onset of disease progression. Because the central pathology of multiple sclerosis has been understood to be inflammation that damages the myelin-oligodendrocyte unit and so disrupting saltatory conduction, the emphasis has been on immunological therapies. Recently, however, the importance of acute and chronic axonal injury, which was described by early writers on multiple sclerosis, has again been recognised [20, 63]. A critical therapeutic issue is the relationship between these processes. If axonal loss and inflammation are independent pathologies, then no immunotherapy may influence accumulation of disability due to multiple sclerosis. However, if axons degenerate directly as a result of the inflammatory process, or indirectly through loss of trophic support normally provided by cells of the oligodendrocyte lineage, the dividend from early suppression of inflammation may be considerable. And if the naked axon is resistant to the inflammatory milieu but has poor survival properties, strategically timed interventions leading to enhanced remyelination may be directly neuroprotective.

Since 1991, we have attempted to disrupt the inflammatory process in multiple sclerosis by systemic depletion of lymphocytes using Campath-1H, a humanised monoclonal antibody that targets the CD52 antigen present on all T and B lymphocytes, monocytes and eosinophils. Campath-1H was already known to be effective at achieving rapid and sustained lymphocyte depletion, and it has subsequently been shown to have useful effects in a variety of immunologically-mediated illnesses [25, 31–34, 37, 41, 42] but its efficacy and safety profile as a treatment for multiple sclerosis were unknown. We proceeded with caution, treating one patient in 1991, six more during 1993 [50] and a total of 36 up to 1999 [9]. Each had secondary progressive multiple sclerosis with Kurtzke scores of 6.0 or less at the time of entry into an MRI screening programme during which one gadolinium-enhancing lesion had to be present in the three months before patients were treated electively. The lessons learned from that cohort led to a change in strategy and we have since treated 22 patients earlier in the disease with activity confined to relapsing-remitting multiple sclerosis, and before onset of the secondary progressive phase. Here, we report how ideas concerning the pathogenesis of multiple sclerosis based on open uncontrolled therapy in these 58 individuals have evolved into a hypothesis for future management of the disease.

Methods

Patients

We treated two cohorts of patients with multiple sclerosis: a "secondary progressive" and a "relapsing" group. The secondary progressive cohort consisted of 36 patients (22 female) who had a sustained increase in disability in between few identifiable relapses but following an earlier period of episodes with full or partial recovery. These patients were made up of two cohorts of secondary progressive patients: those treated in 1991–3 (and reported by Moreau et al. in 1994), and those treated in 1994–7 (reported by Coles et al. in 1999; Fig. 1). At the time of treatment, disease duration was 11.2 years (SD ± 6.1 years) of which 3.6 years (±2.6 years) had been in the progressive phase, and mean EDSS was 5.8 (±0.8, range 3.5–7.0). One selection criterion for treatment was an increase in disability in the year before treatment of at least one EDSS point, during which annual relapse rate was shown to be 0.7/patient/year.

The relapsing group consisted of 22 patients (17 female) with active relapsing-remitting multiple sclerosis. They received Campath-1H either following the failure of licensed treatments to control their disease or because a high relapse rate early in the disease raised the prospect of a poor prognosis. Disease duration ranged from 9 months to 12 years (mean 2.7 years ± 2.9 years) before elective treatment with Campath-1H, at which time mean EDSS was 4.8 (± 2.0, range 1.0–7.5). As a group, they had experienced a total of 133 relapses over 60 patient-years of combined disease history before treatment, giving an annual relapse rate of 2.2/patient. This relapse rate rose to 2.94/patient in the immediate year before Campath-1H. This cohort included 17 drug naïve patients in whom disease duration ranged from 9–41 months (mean 1.7 ± 0.9 years). During that time, the annualised relapse rate of this group was 2.8/year, rising to 3.4 in the year before treatment, during which disability had increased by 0–7.5 (mean 2.1 ± 2.0) EDSS points. Five additional patients had failed treatment with IFN-β. Their disease duration was longer, ranging from 17 months to 12 years (mean 6.3 years ± 4.9 years). Their increase in EDSS ranged from 0–5.5 (mean 2.4 ± 2.3) EDSS points in the previous year during which relapse rate was 2.0/patient.

Patients were assessed every 3 to 6 months for the first three years after Campath-1H treatment, and then annually, but with additional visits triggered by clinical events. A standard definition of relapse was used [45]. Sustained increase in disability was defined as an increase in the Kurtzke score of at least 1.0 point on consecutive examinations over six months, if the baseline EDSS was less than 6.0, or an increase in the Kurtzke score of 0.5 point on consecutive examinations over six months, if the baseline EDSS was 6.0 or greater.

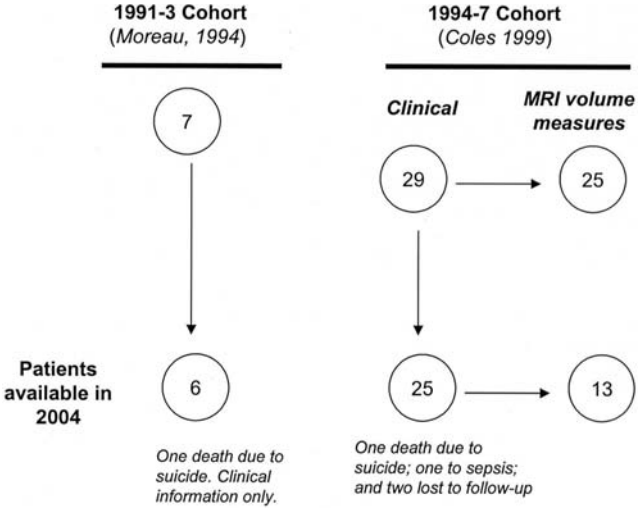

Fig. 1 The sources of long-term follow-up data on the patients with secondary progressive multiple sclerosis available

Campath-1H

Campath-1H® (Alemtuzumab or MABCAMPATH®, ILEX Pharmaceuticals, LP) was approved by the US Food and Drug Administration (FDA: as CAMPATH) for treatment of patients with chronic lymphocytic leukaemia who have failed alkylating agents and fludarabine therapy, and by the European Medicines Evaluation Agency (EMEA: as MABCAMPATH) for similar use, in May and July 2001, respectively. Its use in the present off-licence study was approved by a local ethics committee (Cambridge LREC 02/315) and the (United Kingdom) Medicines Control Agency. Our early experience was with Campath-1H made by the Therapeutic Antibody Centre, Oxford.

Administration of Campath-1H

Based on experience from open-label use in other disorders, we administered 100 mg of Campath-1H as five daily doses of 20 mg given by intravenous infusion over four hours. Most patients were pre-medicated with intravenous methylprednisolone, given as 1 gram over one hour preceding the Campath-1H doses on days 1, 2, and 3 in order to ameliorate the cytokine release syndrome (see below). Seven of 36 patients in the secondary progressive cohort were re-treated with Campath-1H in order to maintain or improve perceived improvements. Subsequently, our policy has been to offer elective re-treatment after 12–18 months giving a fixed total dose of 60 mg over three consecutive days (20 mg/day), again pre-medicated with corticosteroids; three patients declined this offer and so 19/22 of the acute relapsing group have now received a second course of Campath-1H. Three of these have gone on to receive a third dose of 60 mg Campath-1H 12–30 months after the second dose, in response to a relapse.

Magnetic resonance imaging

Twenty-nine patients in the secondary progressive cohort were scanned intensively for the first 18 months after treatment, as previously described; however cerebral volume measurement was only possible in 25 of these because of technical limitations [52] (Fig. 1). The entire cohort was invited to volunteer for a late follow-up scan. Of the 25 patients potentially available, 13 chose to attend for this repeat scan at a mean of 5.8 years from the last scan in the previous series (range 5.0–6.6 years). Those scanned were representative of the entire secondary progressive cohort. They had a mean EDSS of 6.5 (SD ±1.0), compared with a mean EDSS of 7.1 (±2.1; p 0.44) in those who did not volunteer. Six of the 13 patients who were scanned had already demonstrated progression of cerebral atrophy within 18 months after treatment. Scans were performed on a 1.5-T system (General Electric Signa, Milwaukee, WI) using a standard quadrature head coil and the following sequences were performed: axial proton density- (PD) and T2-weighted fast spin-echo sequence (repetition time [TR], 3,500 ms; echo time [TE], 18/90 ms; with an echo train length of 8) and axial T1-weighted spin-echo (TR, 600 ms; TE, 20 ms), obtained 10 minutes after IV injection of 0.1 mmol/kg gadolinium diethylene-triamine penta-acetic acid. Contiguous axial slices were obtained using a 5-mm thickness, 24-cm field of view, and 256 × 256 image matrix. Repositioning was performed based on standardised anatomic landmarks. All MRI measurements were performed blinded to the patients' clinical status and the analysis methods were identical to those used in the original quantitative MRI study [52]. Lesion volumes and cerebral volume were all expressed in millilitres. Gadolinium-enhancing lesions, T2-hyperintense and T1-hypointense lesions were all identified and marked on hard copies from baseline and follow up scans. Each of the T1-hypointense lesions selected had a corresponding hyperintensity on T2-weighted imaging. A single rater (SAT) subsequently measured the volume of marked lesions on electronic data. All lesion volume measurements were performed with a semi-automated local thresh-holding technique. Brain extraction and volume calculation were performed on the Gd-enhanced, T1-weighted images, using an algorithm integrated in a window-based image analysis package [43]. Prior to brain extraction, the scans were examined to ensure adequate repositioning. Four contiguous slices from each scan were selected with the most caudal at the level of the *velum interpositum*.

Statistics

Relapse rate between groups was compared using parametric statistics (Student's t test) and disability scores were treated as numeric ordinals and analysed with non-parametric statistics (Mann-Whitney Test) using SPSS. In analysing serial data with missing values (such as comparing disability and MRI brain volume over time in the secondary progressive patients), only cohorts with all data points intact were compared. The statistical analysis of serial data at varying time points, such as of lymphocyte subsets after Campath-1H treatment, is not standardised [44]. We chose to describe a model derived from raw data which, for CD4+ and CD8+ lymphocyte regeneration after Campath-1H, assumes that each individual has a usual mean CD4 count about which their actual CD4 count fluctuates; that this falls to zero immediately after Campath is administered, and then begins to recover, at a constant rate, eventually reaching its usual level. The individual's usual mean cell count, time to recovery, and mean and variance of the distribution of log times to recovery were all unknown quantities estimated simultaneously for each individual from the data using a Bayesian approach and WinBUGS v1.3 software. The changes in B lymphocyte numbers after Campath-1H were too complex to model and have simply been described.

Results

Campath-1H achieves prolonged depletion of circulating lymphocytes

Within an hour of receiving 5–10 mg of Campath-1H intravenously, cells that carry the target antigen (lymphocytes and monocytes) were no longer detectable in the circulation. Neutrophils increased transiently for a few days after treatment, perhaps due to concomitant corticosteroid administration, but were otherwise unaffected. Monocyte numbers returned to normal by three months. Median recovery time to baseline levels for CD4+ and CD8+ T cells was 61 months (95% Bayesian confidence interval: 47–78) and 30 months (19–46) respectively. B-cell numbers initially fell, then returned to baseline by three months, only to rise further to 124% (SD ±74%) pre-treatment levels at 27 (±15) months after Campath-1H. When last measured, at a mean of 62 (±17) months after treatment, B cell numbers had returned to baseline levels in 13 patients. These elevations in B cell count rarely rose above the upper limit of the normal range.

Adverse effects of Campath-1H treatment of multiple sclerosis

In common with other monoclonal antibodies that deplete T cells, Campath-1H caused an acute cytokine release syndrome, consisting of pyrexia, headache,

malaise and urticarial rash. We have previously described the transient exacerbation of current or past neurological symptoms associated with this syndrome (Moreau et al. 1996) and their amelioration by pre-treatment with corticosteroids [9]. This practice has since been routinely adopted when using Campath-1H in multiple sclerosis.

The 58 patients with multiple sclerosis treated to date have received a total of 87 courses of Campath-1H and have been followed prospectively for 316 patients-years. Eight infections that probably or possibly represent adverse effects of Campath-1H have occurred. Three developed acutely during treatment. One patient each experienced spirochaetal gingivitis at 10 days after treatment and primary measles at 11 days in an individual without previous immunity to measles. The third complained of a frontal headache before receiving Campath-1H for the second time; by day three of treatment typical ophthalmic shingles developed with a superimposed bacterial facial infection, suggesting that we had inadvertently immunosuppressed a patient in the earliest stage of shingles. A fourth patient developed chickenpox at one month after Campath-1H. There have been two cases of herpes zoster (at 6–24 months after treatment), and one each with recurrent aphthous mouth ulcers (from 6–9 months) and pyogenic granuloma (at 22 months). None of the eight patients had residual disability from these infections. One patient with secondary progressive multiple sclerosis died of sepsis seven years after Campath-1H treatment. At this point, her disability was 8.5 and she had experienced several urinary tract infections. Following one of these, she failed to respond to antibiotic therapy, developed septic shock and died. Her total lymphocyte count and T cell subsets, which had been normal for at least three years before this event, were also normal at the time of this agonal infection (CD4+ lymphocyte count 0.92×10^9/L, CD8+ 0.34×10^9/L), so we judge it unlikely that Campath-1H contributed to the terminal illness.

Autoimmunity after Campath-1H

The principal adverse effect of Campath-1H therapy in patients with multiple sclerosis is Graves' disease, as previously reported [10]. One patient had experienced Graves' disease prior to Campath-1H treatment but to date, we have observed 15 new cases after Campath-1H in the remaining 57 patients (27%), with one additional case of autoimmune hypothyroidism. The incidence of Graves' disease has fallen considerably since we adopted the practice of electively re-treating patients with a second dose of Campath-1H at 12 months, for reasons which are unclear, although we cannot discount the possibility that we have simply delayed emergence of the disease. More recently, one patient in the relapsing-remitting group developed acute renal failure due to Goodpasture's syndrome, with no lung involvement. This occurred 10 months after treatment with Campath-1H and was associated with the development of high titre anti-glomerular basement membrane antibodies, which were not detectable in serum taken before Campath-1H treatment, nor one month before her illness. She had characteristic changes on renal biopsy and was treated with plasma exchange, pulsed cyclophosphamide and corticosteroids. There has been no renal recovery and she is therefore currently dependent on renal dialysis. Systematic screening of sera from all other patients for auto-antibodies against glomerular basement membrane, reticulin, gastric parietal cell, endomysial, anti-ACH receptor and anti-voltage gated calcium channel have shown no abnormalities. One patient has developed positive anti-double-stranded DNA antibodies without any clinical evidence of SLE, impaired renal function, or arthritis.

Campath-1H suppresses inflammation in secondary progressive multiple sclerosis but does not protect from disease progression

We have shown in two previous studies that Campath-1H treatment of secondary progressive multiple sclerosis was associated with a very significant reduction of new gadolinium-enhanced magnetic resonance imaging lesions – maximally by > 90% for at least 18 months after a single pulse of treatment [9, 50]. This correlated with a reduction in new clinical relapses. However, even during the first eighteen months after treatment, dissociation emerged between the suppression of inflammation and disease progression [9] which has become even more apparent with prolonged follow-up. This cohort has now been observed for a total of 263 patient-years. Two patients have been lost to follow up and three others have died (one suicide, one possible suicide and one death through sepsis in a severely disabled patient seven years after Campath-1H, see above). The remaining patients have been systematically reviewed by the same investigator (AJC) for a mean of 8.8 years (±1.4 years, range 6.9–12.6 years).

One year after Campath-1H, 33/36 patients in our progressive cohort had maintained their pre-treatment EDSS. With time, this proportion decreased; at last follow-up, only 4/36 had no sustained worsening of disability from their pre-treatment EDSS 8.5 years (±0.5) after treatment (7/36 if the more lax criterion for disability progression of just one EDSS point confirmed at six months throughout the EDSS scale is used). As a group, the mean rate of increase in disability after treatment was +0.2 EDSS points/patient/year (Fig. 2a), with a significantly reduced rate of progression compared to the year before treatment ($p < 0.001$). There was no dif-

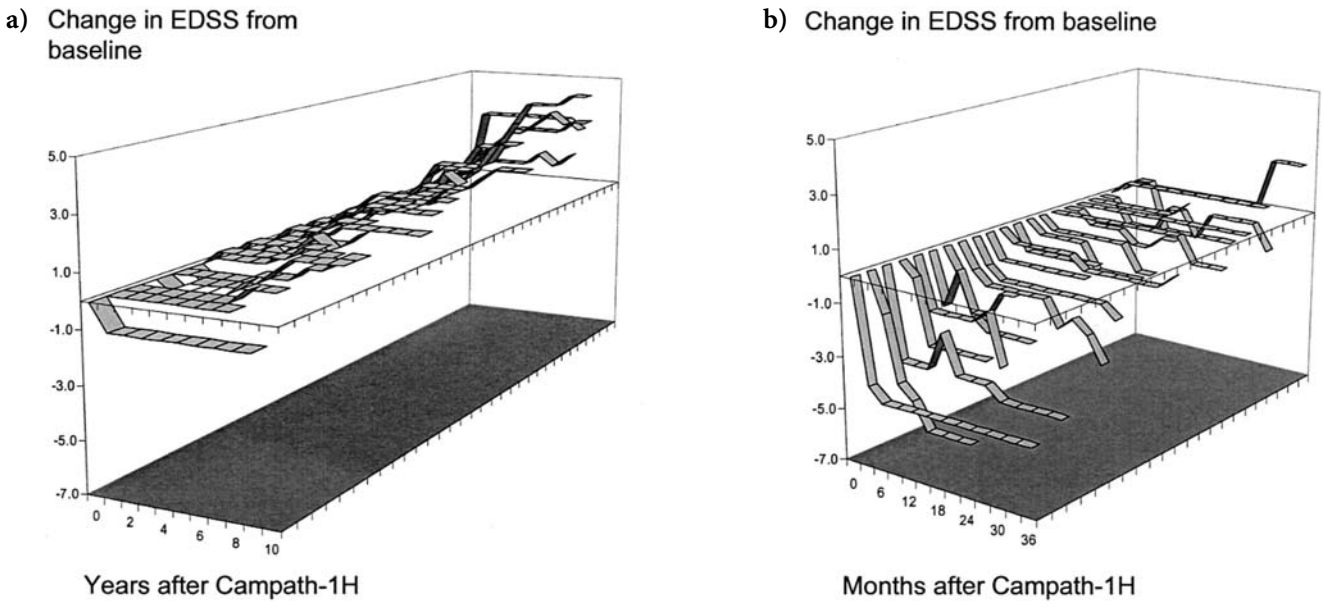

Fig. 2 Comparison of the change in accumulation of disability between the secondary progressive (**a**) and relapsing-remitting (**b**) cohorts treated using Campath-1H. Gradients above the equator represent increasing disability and below represent reducing disability. Note the different time scale between a and b; the data are annualised to allow comparison between time epochs of different duration

ference in the rate at which disability accumulated between patients with early progression after treatment and those who were initially stable. Relapse rate, which normally declines with time in the secondary progressive phase of multiple sclerosis, fell from 0.7/patient/year before treatment to an annualised rate of 0.01/patient/year: over the entire follow-up period of 263 patient-years; this group of 36 patients has experienced just seven episodes, of which three occurred in the first two months after Campath-1H treatment and none has been associated with a persistent increase in disability.

Of the secondary progressive cohort, we had baseline MRI measurements of cerebral volume in 25 patients (Fig. 1) [9]. Patients who had already progressed from baseline at the first follow-up interval (18 months) showed reduced brain volume at the time of initial treatment with Campath-1H by comparison with patients showing initial stability of clinical progression. These patients who progressed early had the greatest magnetic resonance inflammatory activity prior to treatment but no distinguishing clinical features, as previously reported (Coles et al. 1999). When 13 representative patients from this original cohort of 25 were re-examined 5.8 years (±0.5) after their last scan (which was itself 18 months after Campath-1H), there was no evidence for an increase in proton density or T1 lesion volume in the intervening period (Table 1). However, 11/13 patients had evidence for further cerebral atrophy. The two with stable brain volumes were both amongst the group without atrophy in the first 18 months; one had nonetheless shown significant progression of disability. The mean percentage change in cerebral volume was –0.48 (±0.46) %/year; the mean absolute change was –1.37 (±1.28) ml/year ($p = 0.002$). The two patients in this group with stable disability after Campath-1H both had measurable cerebral atrophy. The 6/13 patients who had already shown increased cerebral atrophy at 18 months after Campath-1H had a mean further loss of 2.13 mls/year (±0.65), compared to only 0.7 (±1.4) mls/yr in those whose cerebral volume was stable for the initial 18 months after treatment ($p = 0.042$). Lesion volume (PD or T1 hypointense) did not distinguish these groups. A single enhancing lesion was seen in one individual on the long-term follow-up scan. Five patients had new T2 lesions at follow up and 8 patients had not.

The treatment of early active multiple sclerosis with Campath-1H reduces relapse rate and prevents accumulation of disability

We addressed the dissociation between successful suppression of new lesion formation and failure to arrest disease progression by treating the early cohort of 17 drug naïve patients and five who had already failed licensed therapy. This group has been observed for a mean of 29 months (range 18–82 months) after treatment, representing 53.5 patient-years of follow-up. Before treatment, their relapse rate was 2.21/patient/year

Table 1 Comparison of MRI parameters seen at long-term follow-up in 25 patients treated with Campath-1H, compared to imaging at 18 months after treatment

	Proton Density Lesions	T1 hypointensity Lesions	Cerebral volume
Mean volume at 18 months (mls ± SD)	14.6 (±10.1)	4.2 (±5.6)	287.2 (±21.9)
Mean volume at 7 years (mls ± SD)	13.9 (±7.5)	4.5 (±4.88)	279.3 (±22.8)
Mean annual absolute change in volume (mls/year ± SD)	−0.1 (±0.85)	+0.051 (±0.29)	−1.37 (±1.28)
Mean annual percentage change in volume (%/year ± SD)	3.4 (±11.3)	10.8 (±16.7)	−0.48 (±0.46)
P (absolute values at 18 months and 7 years)	0.66	0.611	0.002

(2.94/patient in the immediate year preceding treatment). After treatment this cohort has had 10 investigator-confirmed episodes, giving a relapse rate of 0.19/patient/year and a significant 91% reduction in relapse rate ($p < 0.0001$; Fig. 3). Of these new episodes, one occurred in the first three months and two were experienced by the same patient at 15 and 30 months after her first dose of Campath-1H; she had declined our routine re-treatment at 12 months because she perceived further treatment to be unnecessary. Her disability had improved compared with before treatment, and this status was maintained despite the two subsequent relapses. Only one of the ten relapses was associated with any persistent increase in disability. The extent of relapse rate reduction is the same if patients previously treated with IFN-β are excluded.

In the year before treatment, the relapsing patients showed a mean annual increase of disability by +2.2 EDSS points. Mean annualised disability changes showed a marked reduction in disability at 6 months after Campath-1H (by –1.2 EDSS points, representing an annualised change of –2.36 EDSS points) (Fig. 2b). Sixteen out of twenty-two patients observed at one year had an improved EDSS; all but one of the others was stable, and the mean effect was an improvement by –1.4 points compared to baseline. This improvement was sustained in all but one of the 14 patients observed at 24 months, whose mean EDSS was –0.2 points from mean month 12 disability. One patient had a sustained deterioration from EDSS 6.0–6.5 within the first three months after Campath-1H, but no subsequent change in disability. At 36 months, when data from only seven patients are available, there is an impression of continued improvement in disability in all but one patient.

The accumulation of disability was compared in the relapsing and progressive groups (Fig. 4). In contrast to the patients with relapsing-remitting multiple sclerosis, the secondary progressive cohort showed increased mean annualised EDSS scores after treatment. These rates of change in accumulation of disability between the relapsing-remitting and secondary progressive groups are significantly different at all time points except 12 months (Fig. 4).

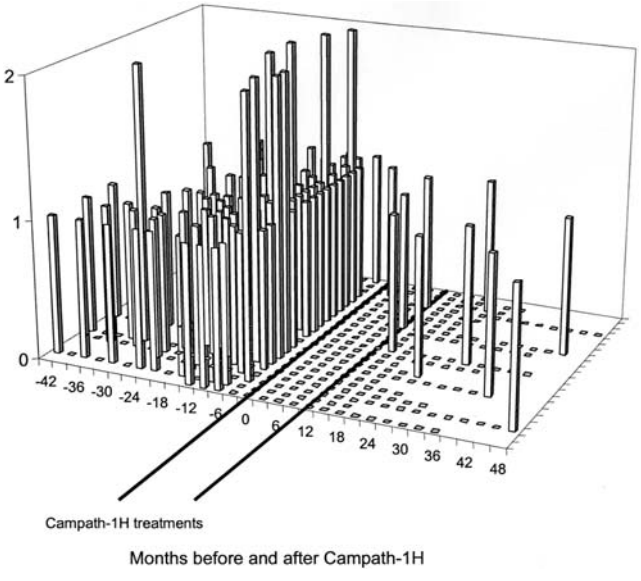

Fig. 3 A record of episodes in the relapsing-remitting cohort before and after treatment with two pulses of Campath-1H at months 0 and 12

Discussion

In 2003, recruitment started for a randomised controlled trial comparing the novel immunotherapy, Campath-

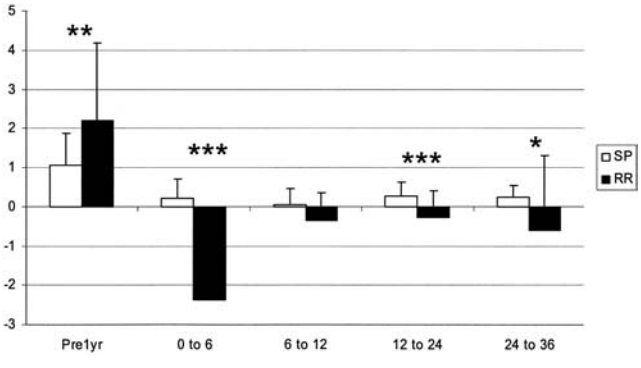

Fig. 4 Comparison of change in disability between the relapsing-remitting and secondary progressive cohorts. The data are annualised to allow comparison between time epochs of different duration * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ Mann-Whitney U test)

1H, and IFN-β in the treatment of early relapsing-remitting multiple sclerosis. Here we describe how the hypothesis underlying this trial evolved from our observational study of 58 patients treated between 1991 and 2002. At first, we assessed safety and efficacy in patients with relatively advanced secondary progressive disease. Although cerebral inflammation was suppressed, disease progression continued. Despite some adverse effects, we considered that safety data accumulated from this cohort were sufficiently encouraging to justify treating a group of patients with early clinically active multiple sclerosis. Inflammation was equally dramatically suppressed in this group, as evidenced by a significant reduction in relapse rate. However, in contrast with progressive patients, there was an accompanying improvement in disability. This dissociation, to which we first drew attention in 1998 [8], raises the prospect that there may be a window of opportunity, early in the course of multiple sclerosis, for immunotherapy to have a useful effect and lasting on long-term disability. This hypothesis is now being tested in the CAMMS223 trial.

Critical to our use of Campath-1H has been the unexpected lack of infections despite subjecting patients to prolonged lymphocyte depletion. *In vitro*, Campath-1H kills lymphocytes by antibody-dependent cytotoxicity and complement-mediated lysis [55], acting only on mature lymphocytes and not haematological progenitors [22]. The reasons for the very different rates of reconstitution between lymphocyte subtypes are unknown. The regeneration of peripheral T lymphocyte counts is certainly slower than would be expected from turnover of spared haematological progenitors. In the face of infections, our patients have generated a significant rise in numbers of peripheral lymphocytes [9]. Intriguingly, two patients were closely exposed to chickenpox by their children within a month of receiving Campath-1H but neither developed the illness. We conclude that peripheral lymphocyte counts do not accurately reflect immune competence after Campath-1H, perhaps because cells are sequestered in solid lymphoid organs.

The emergence of antibody-mediated autoimmune disease was unexpected. We cannot explain why the thyroid gland seemed particularly vulnerable to this complication. Although there are reports of an increased prevalence of anti-thyroglobulin and thyroid microsomal antibodies in patients with multiple sclerosis [1, 30], there is no evidence for increased thyroid dysfunction in patients with multiple sclerosis compared with healthy controls [19]. However, two studies show that the prevalence of autoimmune disease, especially Graves' disease, is increased among family members of patients with multiple sclerosis [5, 28] suggesting that families may inherit common autoimmune susceptibility genes with the specific autoimmune phenotype determined by additional genetic and/or environmental factors. Thyroid autoimmunity following the treatment of multiple sclerosis is not confined to the use of Campath-1H. Some [18, 49, 56], but not all [18, 64] authors report a significant increase in anti-thyroid microsomal antibodies after starting IFN-β. There is one case report of Graves' disease after treatment with glatiramer acetate [27]. The recent development of Goodpasture's disease in one patient treated with Campath-1H raises the possibility that there is a general increase in antibody-mediated complications of CD52– determined lymphocyte depletion. To date, we estimate that 800 individuals have received one or more courses of Campath-1H for autoimmune disease. One other patient treated with Campath-1H for systemic vasculitis developed Goodpasture's disease with no recovery of renal function. There is also a case report of a patient with untreated multiple sclerosis developing Goodpasture's disease [29]. We did not detect anti-glomerular basement membrane antibody in our patient before the development of acute renal failure, nor were these antibodies present in samples from other cases screened as part of our surveillance for adverse effects. The possibility remains that this concurrence of multiple sclerosis with a rare renal disorder is co-incidental but, given the antibody-mediated mechanism, we are at present treating this as a related and serious adverse effect. The mechanism of autoimmunity after Campath-1H treatment of multiple sclerosis remains mysterious. It is not due to preferential depletion of regulatory T cells as, paradoxically, for several months after Campath-1H the depleted lymphocyte pool is dominated by $CD4^{+}CD25^{high}$ cells, a recognised regulatory T cell phenotype [16].

Clinical and radiological data from our patients with secondary progressive multiple sclerosis suggest that just one or two pulses of Campath-1H significantly suppress cerebral inflammation for at least six years. Our 58 patients have together experienced only 17 episodes during 316 patient-years of follow up during both relapsing remitting (53.5 years) and secondary progressive (263 years) phases of the disease. There was no appreciable increase in the T1 hypointense, or proton density, lesion volume in a representative subgroup of patients with secondary progressive disease who agreed to an MRI scan some seven years after treatment. However, there was evidence for progressive cerebral atrophy at a volume loss of +1.37 (±1.28) ml/year. For comparison, in a previous cohort of comparable cases treated with the apparently ineffective anti-CD4 antibody, brain volume reduced by +1.8 ml/year in patients with stable EDSS scores and by 6.4 ml/year in patients with accumulating disability (relapsing-remitting and secondary progressive cases) [43]. We observed greater cerebral atrophy in those patients who already had established cerebral atrophy at the time of Campath-1H treatment: 6/13 patients had a mean further loss of 2.13 mls/year (±0.65), compared to only 0.7 (±1.4) mls/yr in those whose cerebral volume was normal at treatment

($p = 0.042$). A similar dissociation between effective suppression of new lesions and continued cerebral atrophy in progressive patients has also been seen in a trial of the lymphocytoxic drug cladribine, a purine nucleoside analogue resistant to the action of adenosine deaminase [21, 54], and of IFN-β [40, 47, 48].

Our previous studies with MR spectroscopy suggest that the majority of cerebral atrophy in these patients is mainly accounted for by axonal loss [9]. Immunohistochemical staining for amyloid precursor protein confirms that axonal injury occurs as part of the acute demyelinating lesion [20]. Axonal damage in acute inflammatory plaques is shown histologically [63] and radiologically through reduction in the neuronal spectroscopic marker, N-acetyl aspartate [17]. This acute axonal damage mainly occurs early in multiple sclerosis and is correlated with the degree of inflammation (specifically CD8+ and microglial numbers) [39]. However, at first glance the axonal loss in our patients appears unrelated to inflammation.

One interpretation of these observations is that axonal loss and inflammation are independent pathologies – an interpretation supported by epidemiological evidence that relapse rate during the progressive phase of multiple sclerosis does not alter disability outcomes [14]. If so, immunotherapy may not influence progression of disability due to multiple sclerosis, however early it is deployed. That said, several epidemiological studies have shown that relapse rate early in the course of the disease is associated with time to reach fixed disability milestones [15, 65]. A relationship has also been reported between the load of early inflammatory lesions on MRI and subsequent clinical disability [4] and cerebral atrophy [6]. Patients in our secondary progressive cohort who progressed had more inflammatory load before treatment; our data therefore may equally be interpreted as evidence for a link between inflammation and axonal injury. This relationship is complex, for some inflammatory mediators are certainly capable of promoting remyelination and repair. For instance activated lymphocytes may secrete neurotrophins in the brain [61] and microglial activation in animal models promotes oligodendrocyte precursor recruitment [38].

Two processes account for axonal degeneration in the post-inflammatory phase. First, acutely transected axons undergo Wallerian degeneration over the subsequent eighteen months [58, 59], but this seems not to produce a progressive clinical deficit. Secondly, axons that escape injury in the acute phase may later degenerate through a non-inflammatory mechanism, dependent on prior inflammation. Specifically, we favour the interpretation that axon degeneration results from the loss of trophic support for neurons and axons normally provided by oligodendrocytes and myelin [24, 46]. The influence of oligodendrocytes on axonal calibre and function is well described; oligodendrocytes myelinate axons, increase axonal stability and induce local accumulation and phosphorylation of neurofilaments within the axon [3, 7, 57]. Neuronal function is further influenced by oligodendrocyte-derived soluble factors that induce sodium channel clustering along axons, necessary for efficient saltatory conduction, and maintain this clustering even in the absence of direct axon-glial contact [35]. We have shown that soluble factors produced by cells of the oligodendrocyte lineage support neuronal survival [66]. Insulin-like growth factor-1 contributes significantly to this effect acting through the PI3 kinase, Akt signalling pathway. Conversely, differentiated oligodendrocytes increase neurofilament phosphorylation and axonal length due to an effect of glial cell derived nerve growth factor (GDNF) acting through the MAP kinase/Erk pathways [67]. Thus, factors released by oligodendrocyte precursors and oligodendrocytes induce the activation of two intracellular pathways within neurones and these support different structural and functional properties of the neuron and its axon.

An unexpected finding was the sustained improvement in apparent fixed disability of the relapsing-remitting cohort, with EDSS still reducing three years after stabilisation of disease activity. This has rarely been seen in therapeutic trials of multiple sclerosis, although this cohort had atypically active multiple sclerosis. The only recent comparable example is the modest improvement (–0.22 EDSS points at 24 months) seen in the subgroup of patients with relapses in the MIMS mitoxantrone trial of worsening multiple sclerosis [26]. There are several potential explanations. The immediate improvement in the first few months after Campath-1H may represent release from residual physiological conduction block due to previous relapses, and on-going inflammatory activity. But it is less easy to offer this as the mechanism for the subsequent improvement in disability. We suggest that suppression of the inflammatory environment leads to protection of axons from secondary degeneration.

The circumstantial evidence suggesting that recently demyelinated axons are vulnerable to the inflammatory environment of acute lesions is supported by experimental studies. Nitric oxide causes reversible conduction block in rat dorsal roots axons, especially if they are demyelinated [53], and promotes Wallerian degeneration when the axons are conducting impulses at high physiological frequencies [60]. The suggestion that the mechanism underlying this is the intraaxonal accumulation of Na^+ and Ca^{2+} ions, secondary to inhibition of mitochondrial respiration, is supported by the protective effects of Na^+ channel blockade on dorsal root ganglia [36] and animals with chronic relapsing experimental autoimmune encephalomyelitis [2]. Soluble factors released by activated microglia impair mitochondrial (cytochrome oxidase) activity of neurons *in vitro*. These functional effects are blocked by antibodies to TNFα and cell death is prevented by an intracellular

increase of NK-κB [51]. Although this neuronal dysfunction is initially reversible, a separate and lethal sequence of events follows more prolonged exposure to microglial soluble factors. In co-cultures of rat microglia and embryonic cortical neurones, iNOS-derived NO alone is responsible for neuronal death from IFNγ and LPS-activated microglia. Neurones allowed to mature *in vitro* remain sensitive to NO but, whereas blocking NMDA-receptor activation with MK801 has no effect on NO-mediated toxicity of immature neurones, MK801 rescues 60–70 % of neurons matured in culture for 12 days. This increase in protection from toxicity matches increased neuronal expression of NMDA receptors. MK801 also delays the death of more mature neurons caused by the NO-donor DETA/NO indicating that NO elicits an excitotoxic mechanism, most likely through neuronal glutamate release. Thus, similar concentrations of nitric oxide cause neuronal death by two distinct mechanisms: NO acts directly upon immature neurons but indirectly, via NMDA receptors, on more mature neurones [23].

The lesson is clear. Once the cascade of events leading to tissue injury is established, effective suppression of inflammation does not limit brain atrophy or protect from clinical progression. It follows that there may only be an opportunity early in the disease course to suppress those components of the inflammatory process that initiate the cascade leading to loss of tissue integrity expressed as disease progression. There have been two placebo-controlled studies of IFNβ-1a in the earliest identifiable form of the disease; these involved 241–383 patients with a single clinical demyelinating event and multiple lesions on MRI, a poor prognostic sign for a subsequent diagnosis of multiple sclerosis [11, 12, 62]. The known effect of IFNβ-1a on relapse rate was replicated; treatment reduced the chance of developing a second episode over 2–3 years by 25–44 %. However, there was no difference in disability between the groups and, with such a short observation period, no likelihood of detecting any difference in the rate of transition to secondary progressive disease. Thus, the critical issues in the therapy of multiple sclerosis – whether early effective anti-inflammatory therapy reduces the proportion of patients who ever enter the secondary progressive phase or usefully influences the slope of that progression – have not yet been addressed. These ideas are being tested in CAMMS223, a randomised single-blind trial comparing the efficacy of two doses of Campath-1H and IFN-β in the treatment of drug-naïve patients with early, active relapsing-remitting multiple sclerosis. The hypothesis is that patients receiving effective anti-inflammatory treatment before the cascade of events leading to uncontrolled destruction of the axon-glial unit is irretrievably established will not subsequently accumulate disability over three years, develop cerebral atrophy or enter the secondary progressive phase of the illness. The results are expected in 2007.

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