Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis

Alasdair J Coles, Mark Wing, Sheila Smith, Francesca Coraddu, Sandra Greer, Craig Taylor, Anthony Weetman, Geoff Hale, V Krishna Chatterjee, Herman Waldmann, Alastair Compston

Summary

Background Multiple sclerosis results from T-cell-dependent inflammatory demyelination of the central nervous system. Our objective was long-term suppression of inflammation with short-term monoclonal antibody treatment.

Methods We depleted 95% of circulating lymphocytes in 27 patients with multiple sclerosis by means of a 5-day pulse of the humanised anti-CD52 monoclonal antibody, Campath-1H. Clinical and haematological consequences of T-cell depletion, and in-vitro responses of patients' peripheral-blood mononuclear cells were analysed serially for 18 months after treatment.

Findings Radiological and clinical markers of disease activity were significantly decreased for at least 18 months after treatment. However, a third of patients developed antibodies against the thyrotropin receptor and carbimazole-responsive autoimmune hyperthyroidism. The depleted peripheral lymphocyte pool was reconstituted with cells that had decreased mitogen-induced proliferation and interferon gamma secretion in vitro.

Interpretation Campath-1H causes the immune response to change from the Th1 phenotype, suppressing multiple sclerosis disease activity, but permitting the generation of antibody-mediated thyroid autoimmunity.

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University of Cambridge Neurology Unit (A J Coles PhD, M Wing PhD, F Coraddu MD, Prof A Compston FRCP) **and Department of Medicine** (V K Chatterjee MRCP)**, University of Cambridge; Cambridge Centre for Brain Repair** (A Compston)**; Tissue Typing Laboratory, Addenbrooke's Hospital, Cambridge** (S Smith BSc, C Taylor PhD)**; Therapeutic Antibody Centre** (S Greer BSc, G Hale PhD, Prof H Waldmann FRS) **and Sir William Dunn School of Pathology, Oxford** (H Waldmann)**; and Department of Medicine, University of Sheffield Clinical Sciences Centre, Sheffield, UK** (Prof A Weetman MD)

Correspondence to: Dr Alasdair J Coles, University of Cambridge Neurology Unit, Box 165, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, UK
(e-mail: alasdair_coles@hotmail.com)

Introduction

Inflammation of the central nervous system causes demyelination and neurological impairment in multiple sclerosis. The inflammatory activity is mediated by CD4 T cells directed against unknown antigens. We tested a strategy for the treatment of multiple sclerosis that draws on the experimental demonstration of long-term allograft acceptance after short-term therapy with monoclonal antibodies against T cells.[1] We used Campath-1H, which targets the CD52 antigen found on lymphocytes and monocytes; this monoclonal antibody is humanised to minimise antiglobulin responses. Campath-1H has been used in transplantation and in autoimmune diseases, especially rheumatoid arthritis. We have previously reported that a single pulse of Campath-1H suppresses magnetic-resonance markers of multiple sclerosis disease activity for at least 6 months;[2] we have now extended the follow-up in 27 additional patients, treated with Campath-1H alone, or in combination with a humanised non-depleting antibody to CD4. Details of the results have been published elsewhere[3] but, in brief, radiological markers of disease activity were significantly suppressed for at least 18 months in all patients, yet half experienced progressive disability, probably due to axonal degeneration conditioned by high disease activity before treatment. Here we report an unprecedented adverse effect of treatment with Campath-1H, and explore its pathogenesis.

Methods

29 patients with secondary progressive multiple sclerosis, worsening disability over the previous year, and at least one gadolinium enhancing lesion on one of 4-monthly magnetic-resonance-imaging scans, received 20 mg Campath-1H intravenously for 5 consecutive days, after which 14 were randomly assigned a humanised non-depleting antibody to CD4 (40 mg daily for 5 days; Therapeutic Antibody Centre). Patients were also randomly assigned a single dose of methylprednisolone (500 mg), soluble tumour-necrosis-factor α (TNFα) receptor (4 mg; Therapeutic Antibody Centre), or nothing before the first Campath-1H infusion in an attempt to eliminate the first dose effect.[4] None of these additional therapies affected clinical, radiological, or immunological variables after 24 h, so all treatment groups were combined for this analysis. Two patients were excluded from the study: one did not complete treatment, and one was lost to follow-up. The mean age of the remaining 27 patients was 38·6 years, mean disease duration was 12·6 years, and mean Expanded Disability Status Score at entry was 5·4. Normal controls were 21 healthy age-matched non-atopic individuals. The trial protocol had local research ethics committee approval (LREC 92/49), and patients gave informed written consent.

Lymphocytes were phenotyped by flow cytometry; group data were compared by paired Student's *t* test. Antibodies against Epstein-Barr virus were detected by a commercial immunoassay (Sigma, UK), and those against Campath-1H were measured by a sandwich ELISA.[5] Antibodies against the thyrotropin receptor were measured by the inhibition of labelled-thyrotropin binding

Patient	Time of first biochemical abnormality (months after Campath-1H treatment)	Biochemistry at time of treatment			Antimicrosomal antibodies	Antibodies to TSH-receptor			Technetium-99 uptake
		Thyrotropin	Free T4	Free T3		TBII (%)	TSAb		
							Blocking	Stimulating	
Thyroiditis									
1	5	0·06	20·4	7·7	1/102 400	Negative	Negative	Positive	Decreased
2	8	<0·03	46·5	17·5	1/6400	71·7	Positive	Positive	Decreased
2	14	88·3	7·7	ND	1/6400				
Graves' disease									
1	11	<0·03	88·0	60·0	Negative	42·2	Negative	Positive	Increased
2	20	<0·03	31·2	14·4	1/1600	31·0	Negative	Positive	Increased
3	9	<0·03	76·0	33·7	1/1600	50·3	Negative	Negative	ND
4	12	<0·03	>80	ND	1/6400	Negative	Negative	Positive	Increased
5	14	<0·03	73·9	35·3	Negative	65·6	Negative	Negative	Increased
6	18	<0·03	79·7	53·6	Negative	53·7	Negative	Positive	Increased
7	23	<0·03	43·7	27·9	Negative	Negative	Negative	Positive	Increased
8	25	<0·03	77·5	46·6	Negative	67·8	Negative	Positive	Increased
9	31	<0·03	75·0	30·7	Negative	ND	Negative	Positive	Increased

Patient 2 had autoimmune thyroiditis with thyrotoxicosis, then hypothyroidism, before developing Graves' disease; all patients were negative for antimicrosomal antibodies before treatment except for patient 4; ND=not done; TSH=thyrotropin (normal range 0·4–4·0 mU/L); T4=thyroxine (normal range 9–20); T3=tri-iodothyronine (normal range 3·5–8·5 pmol/L).

Biochemical features of autoimmune thyroid disease after Campath-1H treatment

to solubilised thyrotropin receptors,[6] and by a bioassay of cAMP production from cells transfected with a human thyrotropin receptor.[7] Polymorphisms in HLA and cytotoxic T-lymphocyte-associated molecule 4 were identified by established techniques;[8,9] PCR and single-strand conformational polymorphism analysis was used to detect all phenotypically expressed DRB1 and DQB1 alleles, and G to A polymorphisms at positions –308 and –1082 of the TNF-α and IL-10 promoter genes respectively. Statistical analysis was by Fisher's exact test.

Peripheral-blood mononuclear cell cultures, in RPMI 1640 with 10% human serum, were incubated with 10 mg/L lipopolysaccharide, phytohaemagglutinin, or tuberculin purified protein derivative for 48 h or 5 days, after which the proliferation of the mononuclear cells was measured by incorporation of tritiated thymidine. These data were log-transformed, and compared by paired Student's *t* test. Cytokines were assayed in culture supernatants by customised ELISAs with monoclonal antibodies against human cytokines (Cambio, Cambridge, UK; MAB-1045) and the hybridomas 2-179-E11 (TNFα), 4SB3 (interferon gamma), and JES 3-19F11.1 (interleukin 10).

Results

Clinical features of Graves' disease

Nine of the 27 patients developed Graves' disease 6–31 months after Campath-1H treatment; this disorder was characterised by low thyrotropin concentrations, raised free thyroxine and tri-iodothyronine concentrations, positivity for antibodies against thyrotropin receptor, a diffuse pattern of increased thyroidal uptake of technetium-99 (table), and thyroid ophthalmopathy in two patients. Graves' disease was not associated with a particular treatment regimen: four of nine patients with Graves' disease received the anti-CD4, of whom two received methylprednisolone and two the soluble TNFα receptor before Campath-1H; of the remaining five patients, one was pretreated with methylprednisolone, and two with the soluble TNFα receptor.

Two patients experienced a transient autoimmune thyroiditis 6–12 months before Graves' disease: clinical features were thyrotoxicosis (followed in one case by hypothyroidism), decreased thyroidal ^{99}Tc uptake, and positivity to thyrotropin receptor antibodies; in patient 1, some of these antibodies were inhibitory but curiously, given the low ^{99}Tc uptake, in patient 2 they were only stimulatory. At the onset of Graves' disease, all ^{99}Tc antibodies to thyrotropin receptor were stimulatory; one patient developed blocking antibodies 14 months later. All patients with Graves' disease had normal thyroid function and no antibodies to thyrotropin receptor before treatment with Campath-1H. All responded initially to carbimazole, but when it was withdrawn 6 months later, seven patients relapsed and were treated medically (two patients), by partial thyroidectomy (one), and by radioactive iodine (four; two courses were required in two patients).

One of the remaining 18 patients with multiple sclerosis had antibodies to thyrotropin receptor; she had undergone a thyroidectomy for Graves' disease 20 years earlier. No anti-thyrotropin-receptor antibodies were found in serum from 50 randomly selected untreated patients with multiple sclerosis, nor from eight patients with rheumatoid arthritis treated with Campath-1H. After Campath-1H treatment, antibodies against smooth muscle were detected in six patients (at titres of 1/25 in three, and at 1/100 in the others) and antibodies against nuclear antigens were present in five patients (all at 1/25). There were no antibodies to DNA, epithelial neutrophil-activating protein, reticulin, gastric parietal cell, mitochondria, endomysium, gliadin, the acetylcholine receptor, or the voltage-gated calcium channel; rheumatoid factor was also not detected.

Genetics

Apart from the patient with a history of Graves' disease before treatment with Campath-1H, there was no overexpression, in the patients who developed Graves' disease, of HLA class II alleles known to be associated with autoimmune thyroid disease (compared with 395 organ donors). Six of 26 patients (three with Graves' disease) had DR17(3), DRB1*0301; four (three with Graves' disease) had DR7, DRB1*0701; nine (five with Graves' disease) had DQ2, DQB1*0201; and eleven (six with Graves' disease) had DQA1*0501/0502/0503. The cytotoxic-T-lymphocyte-associated molecule 4 106 allele frequency was nine in this cohort, which was not significantly different from that of controls.[9] Five of nine patients with Graves' disease, but only three of 17 patients with euthyroidism had the G/A TNFα^{-308} promoter polymorphism associated with intermediate TNFα production (p=0·054), although this may be due to the strong linkage disequilibrium between the TNFα^{-308} A allele and HLA-DR17(3).

Immunology

Campath-1H caused an extended T lymphopenia; counts of CD4 and CD8 were 30–40% of pretreatment values

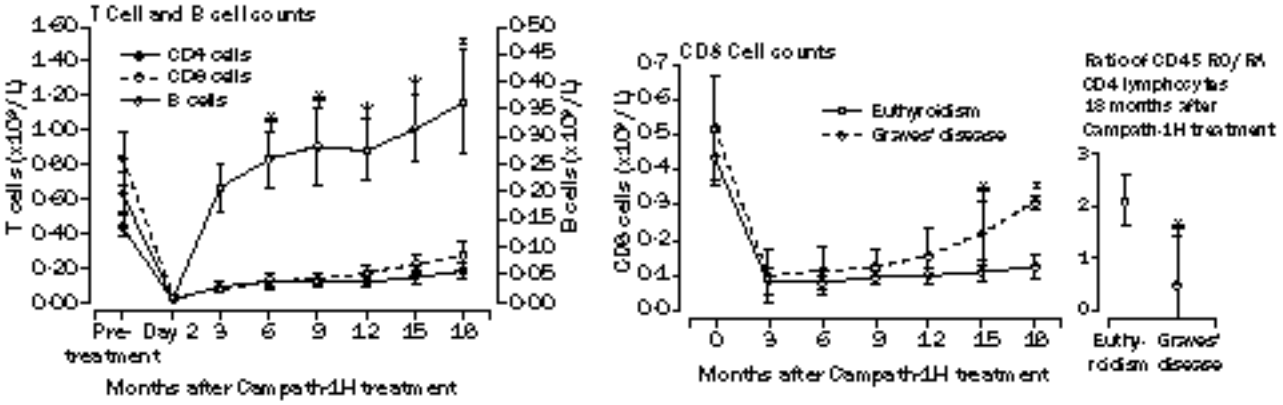

Figure 1: **Haematological changes after Campath-1H treatment**
Bars indicate 95% CI. *p<0·005, †p<0·01, ‡p<0·05, compared with baseline.

18 months later (figure 1; p<0·0001). The monocyte count, although initially depleted, returned to within the normal range after 3 weeks (data not shown), as did the B cell count, which rose progressively throughout the study to 179% of pretreatment values at 18 months after Campath-1H treatment (figure 1; p<0·001). This finding was not due to Epstein-Barr virus infection; only one patient had a significant rise in antibodies to Epstein-Barr virus after Campath-1H treatment. There was no change in serum immunoglobulin concentrations, and anti-idiotypic antibodies to Campath-1H were not seen in 13 serum samples tested 15–17 days after the first dose of Campath-1H. In more than 100 patient-years of follow-up, there were only three episodes of infection after Campath-1H: two of herpes zoster (6 and 22 months after Campath-1H) and one of measles. The latter occurred 11 days after treatment, and the patient's circulating lymphocyte count rose from 0·10 to $0{\cdot}62\times10^9$/L.

The development of Graves' disease was associated with significantly higher CD8 lymphocyte counts 15–18 months after Campath-1H treatment (figure 1). Retrospectively, CD4 lymphocytes from 17 patients (seven with Graves' disease and ten with euthyroidism) were typed for the CD45RO/RA markers at about 18 months after treatment; the ratio of CD4+CD45RO:RA cells was significantly lower in those with Graves' disease euthyroidism (figure 1).

In-vitro proliferative responses to the T-cell mitogen, phytohaemagglutinin (figure 2), and Th1 recall antigen, purified protein derivative (data not shown), had decreased by 90–95% at 3 months after Campath-1H treatment, and remained suppressed despite restoration of the circulating lymphocyte count. By contrast, proliferation in response to the B-cell mitogen, lipopolysaccharide, was not significantly affected (data not shown). 3 months after Campath-1H treatment, the stimulation index of each patient's phytohaemagglutinin response, normalised for the number of CD3 cells in cultures of peripheral-blood mononuclear cells,[10] was unchanged from that at baseline; however, the stimulation index fell thereafter to 45% of the pretreatment value at 9 months, and to 18% at 18 months (p<0·001, figure 2).

We also normalised for T-cell number the supernatant concentrations of interferon gamma and interleukin 4 in phytohaemagglutinin-stimulated peripheral-blood mononuclear cells from treated patients at different times after Campath-1H treatment; this study is justified by our previous work showing that supernatant concentrations of interleukin 4 and interferon gamma are linearly dependent on T-cell number in such cultures.[10] Compared with healthy controls, patients' mononuclear cells produced higher concentrations of interferon gamma (by 146%, p=0·006) and lower concentrations of interleukin 4 (49%, p=0·050; figure 3) before treatment, but equivalent concentrations of TNFα and interleukin 10 (data not shown). When these assays were carried out,

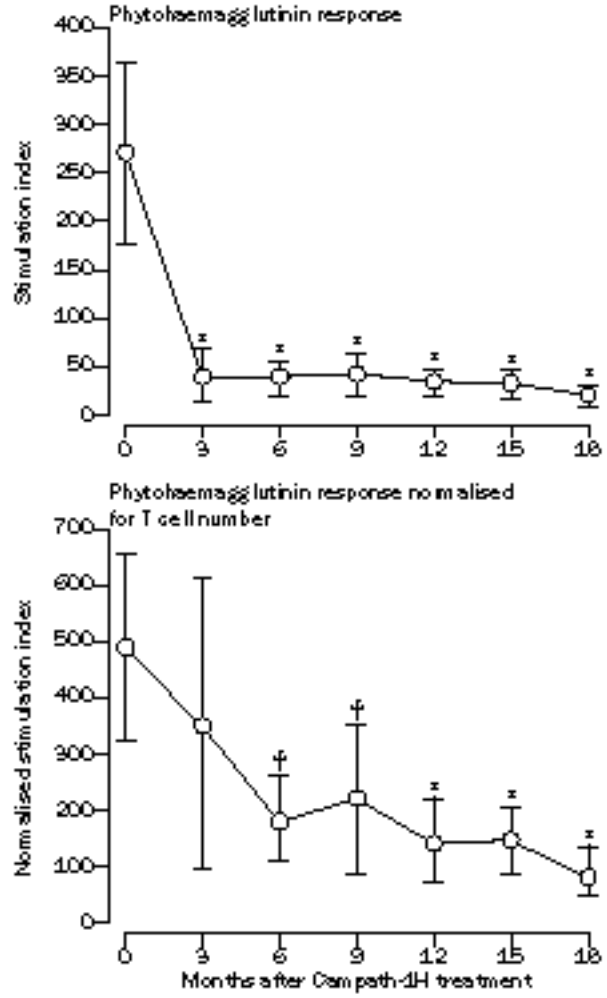

Figure 2: **Proliferative response of peripheral-blood mononuclear cells from patients treated with Campath-1H to T-cell mitogen, phytohaemagglutinin**
Bars indicate 95% CI. *p<0·005, †p<0·01, compared with baseline.

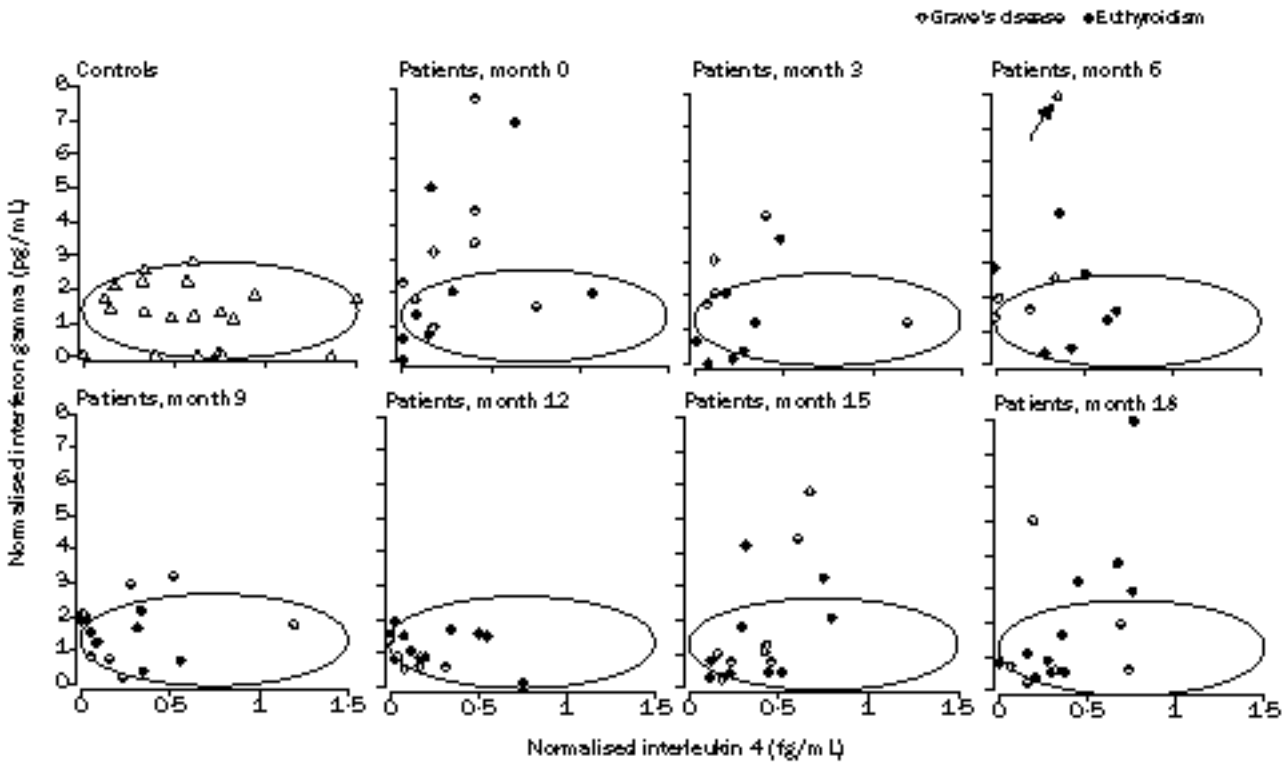

Figure 3: **Cytokine response of peripheral-blood mononuclear cells exposed to phytohaemagglutinin (10 mg/L) in controls and patients after Campath-1H treatment**

Each point represents cytokine profile of peripheral-blood mononuclear cell response normalised for T cell number from an individual patient. Ellipse was drawn arbitrarily by hand to define range of control responses. Arrow indicates outlier.

only 17 patients had completed 18 months of follow-up. At 3 months and 6 months after Campath-1H treatment, five patients were so lymphopenic that we could not obtain sufficient peripheral-blood mononuclear cells. 3 months after Campath-1H treatment, the cytokine phenotype of patients' responses was unchanged, but subsequently patients' mononuclear cells produced progressively less interferon gamma. By months 9–12, the normalised interferon-gamma secretion had fallen to 59% and 40% of pretreatment values (p=0·039 and 0·006, respectively). This change started to reverse in months 15–18. Normalised interleukin 4 production was unchanged. Patients who developed Graves' disease after Campath-1H treatment did not have a distinct lymphocyte cytokine phenotype.

Discussion

We report the observation that Campath-1H induced Graves' disease in a third of patients with multiple sclerosis. Of ten patients treated with Campath-1H outside this study, three developed Graves' disease; thus 12 of 37 (32%) patients have developed Graves' disease after Campath-1H, compared with the incidence of 1–2% in untreated multiple sclerosis patients[11] and those treated with interferon beta 1b.[12] Graves' disease has not been reported in over 600 patients treated with Campath-1H for various other disorders; this finding suggests that patients with multiple sclerosis are uniquely susceptible to this complication.

The fact that one patient generated a 60-fold rise in peripheral lymphocyte count in response to viral infection suggests sequestration of lymphocytes still available for host defence after Campath-1H therapy. This mechanism may explain the low incidence of infections. The progressive rise in circulating B cells after Campath-1H may be similar to that after total lymphoid irradiation or HIV infection. As expected,[13] before treatment, the phytohaemagglutinin-induced cytokine secretion from patients' peripheral-blood mononuclear cells was biased towards the Th1 phenotype, in contrast to controls. However, lymphocytes that reconstituted the peripheral lymphocyte compartment after treatment had decreased mitogen-induced proliferation, and their cytokine phenotype was no longer characterised by excessive interferon-gamma production seen before treatment. Unlike Campath-1H, other T-cell-depleting antibodies do not suppress multiple sclerosis disease activity; none depleted peripheral T-cell counts to the same extent as Campath-1H, nor had the same immunomodulatory actions.[14,15] The specificity of Graves' disease after Campath-1H treatment of multiple sclerosis may merely reflect the extent of lymphocyte depletion.

Autoimmune thyroiditis develops in certain rat strains after lymphocyte depletion by neonatal thymectomy and sublethal irradiation.[16] The concept has emerged that autoimmunity arises when normal autoreactive lymphocytes escape thymic clonal deletion from the "dominant tolerance" of suppressor cells. Perhaps a similar effect explains the report of three cases of Graves' disease after highly active antiretroviral therapy in patients with HIV infection.[17] In our cohort of patients, the development of Graves' disease was associated with a quicker recovery of CD8 T cells, which are implicated in the pathogenesis of thyroid autoimmunity,[18] and a low production of memory CD4 cells, which suppress autoimmunity after lymphopenia in experimental models.[19] The emergence of other autoantibodies, in clinically insignificant titres, after Campath-1H treatment also suggests a breakdown in self-tolerance mechanisms.

We cannot account for the organ specificity of the autoimmune response other than by an unidentified genetic predisposition in patients with multiple sclerosis. However, we have identified a unique situation in which biochemical hyperthyroidism can be detected several months before symptoms develop, allowing presymptomatic treatment and unprecedented access to the earliest immunological events in the pathogenesis of Graves' disease.

Contributors

Alasdair Coles managed patients, administered monoclonal antibodies, carried out immunological assays, identified clinical phenomena, and wrote the paper with Alastair Compston. Mark Wing devised immunological experiments, assisted in their interpretation, and helped write the paper. Francesca Corradu and Sheila Smith HLA-typed patients and carried out assays to identify the cytokine promoter alleles; Craig Taylor analysed these data. Krishna Chatterjee managed patients' thyroid problems clinically and, with Anthony Weetman, coordinated investigations into patients' thyroid dysfunction and CTLA-4 type. Sandra Greer coordinated CD45RO/RA phenotyping. Geoff Hale was responsible for the production of therapeutic-grade Campath-1H and humanised anti-CD4 antibody. Herman Waldmann and Alastair Compston were responsible for the development of Campath-1H in multiple sclerosis, and planned the strategy of the study with Geoff Hale.

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