Transient increase in symptoms associated with cytokine release in patients with multiple sclerosis

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Summary
Fourteen patients with multiple sclerosis were treated with the humanized monoclonal antibody CAMPATH-1H which targets the CD52 antigen present on all lymphocytes and some monocytes; four also received anti-CD4 antibody. Lymphopaenia developed rapidly and was sustained for at least 1 year. In 12 patients, the first infusion of antibody was characterized by significant exacerbation or re-awakening of pre-existing symptoms lasting several hours. These clinical effects of antibody treatment correlated with increased levels of circulating cytokines. Peak levels of tumour necrosis factor (TNF)-α and interferon (IFN)-γ occurred at 2 h, whereas the rise in interleukin-6 (IL-6) was significantly delayed and peaked at 4 h after starting antibody treatment. There was a decline in CH50, indicating complement activation. The neurological symptoms could not be attributed directly to pyrexia and were not provoked (in one patient) by an artificial rise in temperature. In the remaining two patients, a single pre-treatment with intravenous methylprednisolone (500 mg) prevented both the transient increase in neurological symptoms and the cytokine release. Our results, involving 14 intensively studied patients treated with humanized monoclonal antibodies, suggest that soluble immune mediators contribute to symptom production in multiple sclerosis; the mechanism remains uncertain but, on the available evidence, we favour the interpretation that cytokines directly affect conduction through partially demyelinated pathways.

Keywords: multiple sclerosis; CAMPATH-1H; lymphocytes; cytokines; symptoms

Abbreviations: CRP = C-reactive protein; Gd-DTPA = gadolinium-diethylenetriaminepentaacetic acid; IFN = interferon; IL = interleukin; TNF = tumour necrosis factor

Introduction
The clinical manifestations of multiple sclerosis can be attributed both to perivascular inflammation, which initially characterizes the disease process, and to the resulting demyelination. The early clinical course is marked by relapses from which symptomatic recovery is usually complete; subsequent episodes may affect the same or different myelinated pathways. Clinical deficits, which correlate with abnormalities in saltatory conduction of the nerve impulse (McDonald, 1986: Yol et al., 1991) usually accumulate with time. Even when functional recovery is complete, relapse may be followed by persistent delay in electrical conduction through affected pathways; and transient recurrence of previously experienced symptoms may follow exercise or a change in temperature (Uhthoff, 1889; McDonald, 1986). These clinical characteristics can be attributed to partial demyelination leaving a reduced safety factor for conduction of the nerve impulse (McDonald and Sears, 1970; McDonald, 1986). There is a propensity for the rate of conduction to decrease with body cooling and the proportion of blocked fibres to increase with warming, or a change in the local free
Recruitment of patients

4.0 and 7.0, which had increased (under observation) by at

Antibodies

et al., prepare humanized anti-CD4 monoclonal antibody.

protein A. The antibody was formulated in phosphate buffered

complementarity determining regions, as previously described

was made in Chinese hamster ovary cells and purified on

1988). The therapeutic grade antibody

targeted immunological therapies (Ebers, 1994). We treated

are involved has prompted the use of non-specific and more

understood, the evidence that immunological mechanisms

least 1.5 points in the preceding 2 years (Table 1). Thirteen

Table 1 Characteristics of patients

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Age (years)</th>
<th>History of disease (duration in years)</th>
<th>EDSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>45</td>
<td>RR 2</td>
<td>6.0</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>43</td>
<td>Prog 9</td>
<td>5.5</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>40</td>
<td>7</td>
<td>5.5</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>43</td>
<td>–</td>
<td>6.5</td>
</tr>
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<td>5</td>
<td>F</td>
<td>41</td>
<td>–</td>
<td>6.0</td>
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<td>M</td>
<td>35</td>
<td>1</td>
<td>6.0</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>29</td>
<td>12</td>
<td>6.0</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>23</td>
<td>1</td>
<td>6.0</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>38</td>
<td>0.5</td>
<td>4.0</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>42</td>
<td>10</td>
<td>4.0</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>40</td>
<td>10</td>
<td>4.0</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>36</td>
<td>8</td>
<td>6.0</td>
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<tr>
<td>13</td>
<td>M</td>
<td>40</td>
<td>17</td>
<td>6.0</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>48</td>
<td>7</td>
<td>7.0</td>
</tr>
</tbody>
</table>

RR = relapsing/remitting; Prog = progressive.

Methods

Antibodies

The humanized CD52 monoclonal antibody CAMPATH-1H was prepared for clinical use in the Therapeutic Antibody Centre in Cambridge. Briefly, the rat anti-human CD52 monoclonal IgG2b antibody CAMPATH-1G was used to produce a humanized IgG1 antibody with anti-human CD52 complementarity determining regions, as previously described (Riechmann et al., 1988). The therapeutic grade antibody was made in Chinese hamster ovary cells and purified on protein A. The antibody was formulated in phosphate buffered saline, and stored at -70°C after checking for sterility and the absence of endotoxins. Similar methods were used to prepare humanized anti-CD4 monoclonal antibody.

Treatment and management

Two patients (4 and 8) were treated with pulsed intravenous methylprednisolone 5 and 4 months before CAMPATH-1H, respectively, and another (Patient 2) had earlier received intravenous cyclophosphamide on two occasions, separated by 1 year. Treatment with CAMPATH-1H (with or without anti-CD4) was given electively to patients who had shown at least one Gd-DTPA enhancing lesion during screening, and was not scheduled to coincide with a phase of clinical activity. The patients were assessed daily, and more often during the first 24 h of treatment; neurological symptoms and adverse effects were documented and the Kurtzke Expanded Disability Status Score was recorded. Patients were admitted to hospital, after the monthly series of preliminary scans, for treatment with monoclonal antibodies. These were administered intravenously over 3-4 h on a daily basis; the protocol and duration of therapy changed with time, and not every patient received the same dose or combination of antibodies. In the first cohort, Patient 1 received CAMPATH-1H, 60 mg over 10 days (2 mg daily for 5 days followed by 10 mg daily for 5 days); Patients 2-7 received CAMPATH-1H 120 mg over 10 days (12 mg/day); Patients 4 and 5 were
given a single intravenous bolus of methylprednisolone (500 mg) 30 min before the first infusion of monoclonal antibody. After analysis of the MRI results (Moreau et al., 1994) and consideration of the immediate adverse effects which form the basis for this report, a new group of seven patients was recruited and randomized to receive CAMPATH-1H (100 mg over 5 days; Patients 9, 10 and 14), CAMPATH-1H (100 mg over 5 days) followed by anti-CD4 [200 mg over 5 days; Patients 3 (re-treated after an interval of 24 months), 8, 11, 12 and 13]; no member of this group was given methylprednisolone before the first infusion of monoclonal antibody. The first seven patients were scanned at 1, 3, 6, 9 and 12 months after therapy (Moreau et al., 1994) and the protocol for radiological follow-up will be extended to 18 months in the remaining seven.

Visual evoked potential recordings
Visual evoked potentials were recorded in selected patients using electrodes placed 5 cm above the inion and in a horizontal array at 5 cm intervals on each side, with Cz as the reference point. A 1.4 cm pattern reversal stimulus was used, subtending an angle of 50° and placed at 1 m; 128 responses were averaged. The upper normal limit for the laboratory is 107 ms and 112 ms for individuals aged <50 and >50 years, respectively.

Cell counts
Full blood count with differential estimation of white cells and platelets was recorded daily during antibody administration and at each subsequent clinical assessment. The CD4, CD8, CD16 and CD19 lymphocyte sub-populations were estimated once the total lymphocyte count was greater than 0.4×10⁹/l.

Antibody levels
Levels of CAMPATH-1H were assayed by incubating CSF and serum samples taken from the non-perfused arm at the conclusion of daily therapy, with normal human lymphocytes followed by FITC-monoconal anti-human IgG1 antibody (Sigma, Poole, Dorset, UK); antibody labelled cells were enumerated by flow cytometry and calibrated against defined concentrations of CAMPATH-1H (Isaacs et al., 1992).

Antiglobulin response
The presence of a specific antiglobulin response to CAMPATH-1H was monitored using a double capture enzyme linked immunoabsorbent assay, as previously described (Cobbold et al., 1990). Briefly, samples were incubated in CAMPATH-1H coated microtitre wells. Bound antiglobulin was visualized with biotinylated CAMPATH-1H, followed by streptavidin–horseradish peroxidase and substrate; the CAMAPTH-1H anti-idiotype monoclonal antibody YID13.9 was used as a positive control. The sensitivity of this assay was equivalent to 0.3 μg/ml of YID13.9.

Interleukin-6 assay
Interleukin-6 was detected in serum and CSF by proliferation of the IL-6 dependent B-lymphocyte cell line MH60. Samples were serially diluted in RPMI with penicillin and streptomycin, 2-mercapto-ethanol and 2.5% AB positive normal human serum, and incubated with 2×10⁶ MH60 cells which had not been exposed to IL-6 for 3 days. After 48–72 h, IL-6 was determined by comparing the number of viable cells in test samples with a standard curve achieved using recombinant human IL-6 in known concentrations. The specificity of the assay was confirmed using a neutralizing antiserum to IL-6.

Tumour necrosis factor-α and IFN-γ assays
Samples of serum and CSF were analysed for the presence of TNF-α in accordance with the manufacturers instructions, using a sandwich enzyme linked immunosorbent assay (R+D Systems, Abingdon, UK) which was sensitive down to 15 pg/ml. Interferon gamma was measured using a commercial immunoradiometric assay (Centocor, Malvern, Pa., USA), which was sensitive down to 0.1 NIH U/ml, or in a sandwich enzyme linked immunosorbent assay (Genzyme).

C-reactive protein assay
Serum C-reactive protein (CRP) levels were determined, in accordance with the manufacturers instructions, using a particle enhanced turbidimetric immunoassay supplied by Du Pont, Wilmington, which was sensitive down to 2 mg/l serum. Briefly, CRP was detected by incubating samples with latex beads coated with an anti-CRP antibody, and the turbidity resulting from antigen:antibody aggregation was determined at 340 nm in a spectrophotometer.

Determination of haemolytic activity
Complement haemolytic titres were estimated by incubating 20 μl of 10% v/v sheep erythrocytes, which had been optimally coated with a locally produced complement fixing IgM antibody, with serial dilutions of serum samples in a total volume of 200 μl complement fixing diluent with gelatine for 15 min at 37°C. Haemolysis was determined from the optical density (OD412) of the supernatant, and the dilution of serum which resulted in 50% lysis was determined.

Statistical methods
Differences in the timing of peak cytokine release were assessed in a one-tailed test but the statistical significance of
these results has been cited using the more stringent two-tailed test.

Results

Clinical effects of treatment with CAMPATH-1H therapy

The first infusion of CAMPATH-1H was associated with transient systemic and neurological adverse effects. They recurred, but with diminished intensity, in some patients following subsequent treatments but were rarely noticeable beyond the second or third day. These clinical observations are summarized in Table 2, in the order in which the 14 patients were treated.

With the exception of Patients 4 and 5 (who received methylprednisolone), each either experienced a worsening of persistent symptoms or a recurrence of clinical manifestations which had characterized previous episodes of demyelination. Each of the neurological exacerbations occurred 2–4 h after the start of the first infusion and usually recovered within 12 h, although improvement was delayed in three patients [3 (second treatment), 12 and 14]. In those who noticed a change in motor disabilities [Patients 2, 3 (both treatments), 8, 10 and 12–14], there was an initial phase of positive phenomena such as flexor spasms and the appearance of clonus, and this was followed by increased weakness. There were no neurological complications in the patients who received anti-CD4 antibody immediately after treatment with CAMPATH-1H. These clinical observations are illustrated by reference to selected cases.

Reactivation of pre-existing symptoms

Patient 2 (with secondary progressive spastic paraparesis and a past history of internuclear ophthalmoplegia but normal eye signs at the start of treatment) developed a significant increase in arm and lower limb weakness and was unable to stand, 3 h after treatment on the first day; he became numb below D7 on the left and developed diplopia—symptoms which had characterized his two most recent relapses. Examination showed a complete bilateral internuclear ophthalmoplegia, with decreased strength in the legs and subjective reduction in cutaneous and position sense. These neurological exacerbations lasted 6 h (Fig. 1).

Transient symptoms may arise from conduction block

Patient 6 (with a past history of optic neuritis and secondary progressive spastic paraparesis) described a tight sensation around the waist with segmental numbness (D8–10), which was reminiscent of his most recent relapse, and he was unable to walk due to poor balance within 180 min of the first infusion of CAMPATH-1H. The deterioration lasted 6 h during which visual acuity decreased in the left eye (from 6/12 to 6/18), and he showed nystagmus with poor adduction of the left eye. Visual evoked responses performed before, during and 48 h after the first infusion showed a complete but fully reversible loss of amplitude during the phase of symptomatic deterioration (Fig. 2). During the second infusion, he developed slight systemic effects, with blurred vision on the left and abdominal numbness, but these symptoms were less pronounced than on the previous day.

Transient symptoms are not directly an effect of elevated temperature

Most patients developed headache, rigors and pyrexia (up to 40°C). One patient had hypotension and urticaria coinciding with the onset of neurological symptoms, but the severity and duration of the transient neurological consequences of antibody infusion were not explained merely on the basis of the Uthoff phenomenon, as illustrated by Patient 3. One hour after the first infusion, she developed flu-like symptoms with headache, fatigue, chills and myalgia; she was not pyrexial but her left arm then became weak and she complained of paraesthesia in the hands and feet. On examination, the lower limb reflexes became absent during the period of neurological deterioration which lasted 5 h. She developed an extensive pruritic rash, which persisted during the first week of treatment, but there were no other complications. She was retreated after an interval of 24 months because of symptomatic deterioration, using CAMPATH-1H (100 mg given over 5 days) followed by humanized anti-CD4 (200 mg). She developed headache, fatigue, vomiting and an urticarial rash with the first dose of CAMPATH-1H. Sixty-five minutes after onset of the infusion, whilst still apyrexial, she reported paraesthesiae of the left hand; within 10 min, her left shoulder became weaker and 30 min later she developed a fever which peaked at 39.4°C. Within 4 h of starting the infusion, and at a time when her temperature was 39.2°C, she was unable to raise her left arm or stand, even with support; the tendon reflexes were unchanged. She became apyrexial within 13 h but did not regain her former clinical status for several days. In order to exclude temperature as the direct cause of these temporary clinical exacerbations, Patient 3 was artificially heated over 4 h during the second week of re-treatment from 36.0°C to 39.0°C, using space blankets in a raised ambient room temperature; throughout this exercise, there was no change in her neurological symptoms or signs. There was also a clear dissociation between neurological exacerbations and pyrexia in Patients 11 and 14. The relationship between temperature and the development of neurological symptoms in Patients 3, 9, 11 and 14 is also discussed in the section of Results describing cytokine release.

Corticosteroids prevent transient exacerbation of symptoms

Transient neurological exacerbations were prevented by pre-treatment with methylprednisolone. Patients 4 and 5 received...
### Table 2  Transient symptoms during treatment with CAMPATH-1H

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>EDSS</th>
<th>Previous symptoms (time before treatment)</th>
<th>Onset (min)</th>
<th>CAMPATH-1H induced symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.0</td>
<td>Ataxia and diplopia (1 year); L ON (2 years) and paraparesis (4 years).</td>
<td>120</td>
<td>Reduced L visual acuity.</td>
</tr>
<tr>
<td>2</td>
<td>5.5</td>
<td>Paraparesis with sensory level at L2 (1 month); ataxia and INO (2 years); weak L foot (9 years); numb L hand and R ON (13 years).</td>
<td>240</td>
<td>Increased weakness L leg; sensory loss below T7; bilateral ophthalmoplegia (Fig 1).</td>
</tr>
<tr>
<td>3</td>
<td>5.5</td>
<td>Spastic paraparesis with diminished position sense in fingers.</td>
<td>60</td>
<td>Weakness and numbness L arm; exacerbation of paraparesis; loss of lower limb reflexes.</td>
</tr>
<tr>
<td>4</td>
<td>6.0</td>
<td>Paraparesis (7 years); spastic paraparesis and L ON (23 years).</td>
<td>65</td>
<td>L hand numbness followed, 10 min later, by L arm weakness. Then, at 105 min, much more severe exacerbation of paraparesis than with the first treatment. No change in reflexes. L arm normal at 10 h, with some improvement in leg power. Full recovery by 3 weeks.</td>
</tr>
<tr>
<td>5</td>
<td>6.0</td>
<td>Paraparesis (1 year); diplopia and ataxia (18 months); leg paraesthesiae and L ON (16 years).</td>
<td></td>
<td>Given steroids; no exacerbation.</td>
</tr>
<tr>
<td>6</td>
<td>6.0</td>
<td>Paraparesis (8 months); L ON (2 years); urinary frequency (4 years).</td>
<td>180</td>
<td>Band of altered abdominal sensation, reduced visual acuity and conduction block L optic nerve (Fig. 2).</td>
</tr>
<tr>
<td>7</td>
<td>6.0</td>
<td>Limb ataxia (3 years); R VI palsy, R arm and leg weakness (4 years); L ON (4 and 11 years).</td>
<td>90</td>
<td>Lower limb weakness; numbness R hand; increased delay in conduction L optic nerve.</td>
</tr>
<tr>
<td>8</td>
<td>6.0</td>
<td>R ON (2 years); spastic paraparesis (3 years).</td>
<td>60</td>
<td>Violent flexor spasms, exacerbated by movement; no change in sensory level but legs markedly more spastic. Spontaneous ankle clonus for the next 30 min. Knee and ankle clonus for the next 150 min. At 4 h, worsening of paraparesis without change in sensory level. Visual acuities unchanged. Full recovery by 10 h.</td>
</tr>
<tr>
<td>9</td>
<td>4.0</td>
<td>Ataxia, diplopia and dysarthria (10 months); paraparesis L&gt;R (18 months).</td>
<td>150</td>
<td>Slight increased weakness L hip flexion, which returned to normal within 6 h.</td>
</tr>
<tr>
<td>10</td>
<td>5.0</td>
<td>Paraparesis with occasional paraesthesiae in arms (1 year); diplopia (1 and 5 years); L ON (3 years).</td>
<td>90</td>
<td>Reduced L visual acuity to 1/60. At 2 h, paraesthesiae then numbness and hyperreflexia of L arm with Hoffman’s sign, followed by flexor spasms of legs and then severe worsening of paraparesis.</td>
</tr>
<tr>
<td>11</td>
<td>4.0</td>
<td>L ON (9 years); spastic paraparesis and diplopia (10 years); ataxia (20 years).</td>
<td>110</td>
<td>Mild exacerbation of leg ataxia, spasticity and weakness, worse on the L, associated with lower limb hyperreflexia, clonus, cramps of the R foot, and numbness of the L hand at 4 h. Full recovery by 5 h.</td>
</tr>
<tr>
<td>12</td>
<td>6.0</td>
<td>Ataxia (9 years); paraparesis and L ON (10 years).</td>
<td>65</td>
<td>Paraesthesiae in hands, followed by spontaneous ankle clonus and back spasms. At 160 min, marked increase in leg ataxia, with development of nystagmus to the R, urinary retention and worsening of paraparesis. Walking was worse after treatment in that he required two, not one, sticks; recovered fully but not until 6 weeks later.</td>
</tr>
<tr>
<td>13</td>
<td>6.0</td>
<td>Paraparesis (17 years) developing into quparaparesis L&gt;R (6 years); leg paraesthesiae (19 years).</td>
<td>75</td>
<td>Cramps in L foot followed by pyramidal weakness of L arm and leg; no reflex change. Fully resolved by 8 h.</td>
</tr>
<tr>
<td>14</td>
<td>7.0</td>
<td>Arm weakness and diplopia (1 year); R ON (7 years) paraparesis (8 years).</td>
<td>135</td>
<td>Flexor spasms then marked paraparesis and complete loss of touch and vibration sensation to L1, with full recovery by 12 h but walking not returned to normal for 2 weeks.</td>
</tr>
</tbody>
</table>

ON = optic neuritis; INO = internuclear ophthalmoplegia.
methylprednisolone (500 mg given by intravenous injection) 30 min before the first infusion of CAMPATH-1H (10 mg) and neither noticed any adverse systemic or neurological effects following the first or subsequent treatments; Patient 5 developed a pruritic skin rash on the limbs and face, during the first 4 days of treatment.

**Cell counts**

Lymphopenia was rapid and sustained in all patients with counts of between 0 and $0.2 \times 10^9$ circulating lymphocytes/l following the first infusion of antibody (2–20 mg), and there was a transient reduction in platelet count. The rate of induction of lymphopaenia in Patient 1, who received only 2 mg of antibody for the first 5 days, was slower than for other patients and counts of below $0.2 \times 10^9$ lymphocytes/l were not reached until the seventh infusion (day 9). In all patients, the total lymphocyte count did not return into the normal range for at least 12 months (Fig. 3A); however, in contrast to this sustained lymphopenia, the number of circulating monocytes fell rapidly following the first antibody infusion but returned to within the normal range by 10–40 days (Fig. 3B). Immediately after the first infusion there was an increase in the number of circulating granulocytes (mean $15.6 \times 10^9$/l), except in Patient 1, who had received a reduced dose of antibody (data not shown).

After 3 months there were sufficient circulating
lymphocytes to enumerate subpopulations. Whilst the number of natural killer cells (CD16) and B-lymphocytes (CD19), had returned to normal by 3 months (data not shown), there was a reduction in CD4 and CD8 positive lymphocytes even 6 months after treatment. The mean number of CD4 positive lymphocytes was 0.154 and 0.18 × 10^9/l (normal range = 0.53–2.2 × 10^9/l) 3 and 6 months after treatment, respectively; corresponding values for CD8 positive T lymphocytes were 0.15 and 0.15 × 10^9/l (normal range = 0.3–1.44 × 10^9/l). CD4 counts remained below the normal range for 2 years, whereas in some patients CD8 counts were normal by the end of the first year (Fig. 3C and D). Patients who received CAMPATH-1H and anti-CD4 monoclonal antibody have not yet been followed for >6 months and the haematological consequences of combined treatment remain under investigation. There was no difference in the profile of lymphocyte counts seen between patients who received methylprednisolone and those given CAMPATH-1H alone (Fig. 3A). The observed distribution, pattern of recovery and duration of lymphopaenia was similar to that reported using CAMPATH-1H in other clinical settings (Hale et al., 1988; Isaacs et al., 1992; Lockwood et al., 1993).

Free antibody and antiglobulin levels during therapy
The serum concentration of free CAMPATH-1H antibody rose steadily during the course of treatment and peaked at 7–9 µg/ml on days 10–12 (Fig. 4); the dip in free antibody levels on day 8 reflects the break in therapy during the preceding 2 days. The inverse relationship between lymphopenia and concentration of free antibody is best illustrated by Patient 1 in whom the serum CAMPATH-1H concentration rapidly increased only after the establishment of lymphopenia on day 9. The concentration of CAMPATH-1H in CSF was below the detection limit for the assay (40 ng/ml). No antiglobulin responses were identified; samples from each patient contained less than the equivalent of 0.3 µg/ml of YID13.9 and these were therefore considered to be negative.

Complement activation and CRP levels
Complement activation, measured using antibody sensitized sheep erythrocytes, was studied only in the first cohort of seven patients and occurred in Patients 2, 3, 6 and 7 within 2–4 h of antibody infusion (mean consumption 48%); there was no activation of complement in Patients 4 and 5, who both received methylprednisolone, and in these two cases the CH50 increased marginally (Fig. 5). Serum CRP levels were elevated in all 14 patients following antibody infusion and peaked 24 h after the start of therapy (Fig. 6). Methylprednisolone pre-treatment was associated with a reduction in circulating levels of CRP (38 mg/l in Patients 4 and 5 compared with 134 mg/l in the remaining patients).

Cytokine release in patients during therapy
The serum concentrations of TNF-α, IL-6 and IFN-γ were measured before, during and after CAMPATH-1H infusion in 11 out of 12 patients (and twice in Patient 3) experiencing transient neurological deterioration (Patients 2, 3 and 6–14) and in both individuals whose symptoms did not change.
Fig. 6 Serum levels of C-reactive protein (CRP; mg/l) in 14 patients receiving CAMPATH-1H, two of whom (Patients 4 and 5) were pre-treated with a single pulse of methylprednisolone (closed circles).

(Patients 4 and 5), using a bioassay (IL-6), enzyme linked immunosorbent assay (TNF-α) and immunoradiometric assay (IFN-γ). With the notable exception of Patients 4 and 5, a very substantial increase in circulating TNF-α, IFN-γ and IL-6 was observed in the first cohort (Patients 1–7) within 4 h of the first infusion in patients experiencing neurological deterioration (Fig. 7A–C). Different time points were chosen in the second group of patients, and correlations were made with serial rise in temperature. Similar profiles of cytokine release were observed with respect to the peak serum concentrations (Fig. 8). These experiments also showed the sequence in which cytokines were released and showed a significant delay in peak concentration of IL-6 compared with TNF-α ($P < 0.004$) and IFN-γ ($P < 0.004$; Table 3). In this cohort, cytokine concentrations all returned to baseline levels within 8 h (see Fig. 8). Cytokines were not detected in samples of CSF taken from different patients between 1 and 8 h after the start of treatment to coincide with and follow the period of symptomatic deterioration.

Discussion

Fourteen patients with multiple sclerosis were treated with the humanized anti-CD52 monoclonal antibody CAMPATH-1H; subsequently, some were electively given an anti-CD4 monoclonal antibody. Twelve experienced transient worsening of existing symptoms, or recurrence of previous clinical manifestations, coinciding with the first infusion of CAMPATH-1H. The onset of these reversible symptoms coincided with rapid reduction in numbers of circulating lymphocytes, and with cytokine release. There were no clinical or laboratory effects in two patients who were immediately pre-treated with methylprednisolone. In Patient 1 the increase in pre-existing symptoms recurred after the second infusion; she received a low dose of CAMPATH-1H and, unlike the remaining patients, full depletion of lymphocytes was not achieved until later in the course. In Patient 3, identical transient neurological symptoms developed during her second course of treatment (CAMPATH-1H followed, on this occasion, by anti-CD4 antibody) after an interval of 2 years, and with increased severity and duration. Cytokines could only be detected for

Fig. 7 Serum concentrations of TNF-α (A), IL-6 (B) and IFN-γ (C) measured before, during and after the first CAMPATH-1H infusion (10 mg) in Patients 2–7, two of whom (Patients 4 and 5) were pre-treated with a single pulse of methylprednisolone (closed circles).
Exacerbations coinciding with pyrexia

Patient 9

Exacerbations not coinciding with pyrexia

Patient 11

Fig. 8 The relationship between neurological exacerbations, serum cytokine concentration and pyrexia in Patients 3, 9, 11 and 14 during the first infusion of CAMPATH-1H; arrows indicate the onset of neurological exacerbations.
factor-a release, with or without IL-6 and IFN-γ, has also
mediated cytotoxicity. Tumour necrosis
released from damaged mononuclear cells during
and IFN-γ, suggests that cytokines are actively secreted and
hence delay in peak detection of IL-6, compared with TNF-a
to be cytokine mediated, correlate with sequential release of
neurological exacerbations was often no greater than 40°C. The
et al., 1990; Waagea/., 1990).
mechanism of cytokine release, and its suppression
by methylprednisolone, has yet to be fully characterized. Complement activation was observed in all patients tested
who received CAMPATH-1H without methylprednisolone,
as expected from the known ability of this antibody to
mediate complement lysis (Xia et al., 1993). Since the
complement intermediates C3a and C5a can induce monokine
secretion (Okusawa et al., 1988; Schermuck and Burger,
1993), this provides a potential stimulus for cytokine release
by monocytes. However, Fc receptor ligation may also cause
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### Table 3 The timing of peak serum cytokine concentration following CAMPATH-1H administration

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>TNF-α</th>
<th>IFN-γ</th>
<th>IL-6 peak</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time (h)</td>
<td>Conc. (ng/ml)</td>
<td>Time (h)</td>
</tr>
<tr>
<td>3 (2nd)</td>
<td>2</td>
<td>0.704</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>1.123</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>0.735</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>1.75</td>
<td>0.694</td>
<td>1.75</td>
</tr>
<tr>
<td>9</td>
<td>2.03</td>
<td>1.200</td>
<td>1.5</td>
</tr>
<tr>
<td>10</td>
<td>2.83</td>
<td>0.884</td>
<td>2.5</td>
</tr>
<tr>
<td>11</td>
<td>3.5</td>
<td>0.685</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>3.5</td>
<td>1.064</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>1.91</td>
<td>0.560</td>
<td>1.66</td>
</tr>
<tr>
<td>14</td>
<td>2.3</td>
<td>1.310</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Mean: 2.33*± 2.07† 4.68*†
SD: 0.54 0.41 1.29

*Mean TNF-α versus IL-6, P = 0.004; †mean IFN-γ versus IL-6, P = 0.004; #mean TNF-α versus IFN-γ, P = NS. NT = not tested.

8 h following treatment with CAMPATH-1H, and were not
present after the use of methylprednisolone.

CAMPATH-1H (or the rat IgM isotype) has been used in
the treatment of rheumatoid arthritis (Isaacs et al., 1992) and
vasculitis (Lockwood et al., 1993), to deplete malignant cells
of lymphoid origin (Hale et al., 1988) and to prevent graft
rejection (Waldmann et al., 1984). In each of these clinical
settings, adverse neurological effects have not been observed,
despite (in patients with rheumatoid arthritis) an equivalent
release of TNF-α (J. Isaacs and M. Wing, unpublished
observations). Thus, whilst the neurological exacerbations
following CAMPATH-1H are specific to patients with
multiple sclerosis, the release of cytokines following
lymphocyte depletion with CAMPATH-1H is not. The
severity of exacerbations was not clearly related to the peak
concentration of serum cytokines but did correlate with the
severity of earlier exacerbations and with disability at the
time of treatment. For example, Patient 3, who had a very
severe increase in paraparesis at the start of the second course
of treatment, had a peak level of TNF-α in the middle of the
range observed in other patients, whereas Patient 9 had a
much greater elevation in all cytokines despite only slight
unilateral increase in leg weakness.

The neurological changes were accompanied by systemic
symptoms, similar to those described during anti-human
OKT3 therapy (Charpentier et al., 1992; Urrea et al., 1992).
They were reminiscent of the deterioration which may follow
exercise or a rise in temperature in patients with multiple
sclerosis (Uthoff, 1889) but clinically more severe, and the
associated pyrexia was often no greater than 40°C. The
systemic manifestations of OKT3 administration are known
to be cytokine mediated, correlate with sequential release of
circulating TNF-α and IFN-γ followed by IL-6 and are
suppressed using methylprednisolone (Peces et al., 1993).
The delay in peak detection of IL-6, compared with TNF-α
and IFN-γ, suggests that cytokines are actively secreted and
not merely released from damaged mononuclear cells during
CAMPATH-1H mediated cytotoxicity. Tumour necrosis
factor-α release, with or without IL-6 and IFN-γ, has also
been observed in patients with multiple sclerosis treated
openly in two studies with a non-humanized OKT3 and
anti-CD4 antibody, respectively (Weinshenker et al., 1991; 
Racadot et al., 1993).

The mechanism of cytokine release, and its suppression
by methylprednisolone, has yet to be fully characterized.
Complement activation was observed in all patients tested
who received CAMPATH-1H without methylprednisolone,
as expected from the known ability of this antibody to
mediate complement lysis (Xia et al., 1993). Since the
complement intermediates C3a and C5a can induce monokine
secretion (Okusawa et al., 1988; Schermuck and Burger,
1993), this provides a potential stimulus for cytokine release
by monocytes. However, Fc receptor ligation may also cause
cytokine production (Anegon et al., 1988; Bazzoni et al.,
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release (Ellison and Merchant, 1991), but since repair
mechanisms could not match the time-scale of recovery from
CAMPATH-1H induced symptoms in patients with multiple sclerosis, this does not provide a convincing explanation for our observations. A further interpretation is that the clinical effects associated with the use of CAMPATH-1H result from change in temperature but this is excluded by the time course of symptomatic deterioration and the failure in Patient 3 to reproduce symptoms experienced during the second course of CAMPATH-1H with an artificial pyrexia. Failure to detect cytokines in CSF may merely reflect the short half-life of these biologically active products following release, or the low concentrations which would be predicted on the basis of passive transfer across the blood–brain barrier; however, it has been reported that TNF-α can be transported from plasma to the CNS (Gutierrez et al., 1993).

The pattern of release for the different cytokines, and collaterel evidence from experimental studies, makes us favour TNF-α, acting alone or synergistically with IFN-γ, as the most likely candidate for interfering with conduction through partially demyelinated pathways. Increased concentrations of TNF-α and IFN-γ are detected in the lesions of multiple sclerosis (Hofman et al., 1989), and an increased relapse rate has been associated with the therapeutic use of IFN-γ (Panitch et al., 1987). There are reports of elevated levels of TNF-α in the spinal fluid during relapse (Sharief and Hentges, 1991) and TNF-α production may predict relapse in multiple sclerosis (Chofflon et al., 1992; Hartung, 1993). Whilst TNF-α may be directly cytotoxic in vitro only at high concentrations (Selmaj and Raine, 1988), oligodendrocytes are more sensitive to membrane bound TNF-α on activated microglia (Zajicek et al., 1992).

The blocking effects of perivascular inflammation per se on conduction through myelinated pathways have previously been demonstrated in patients with acute optic neuritis. Youl et al. (1991) showed that impaired visual function correlated with increased blood–brain barrier permeability (Gd-DTPA enhancement) and reduced amplitude of the visual evoked potential, implying partial conduction block in the affected optic nerve. Recovery of vision and increase in amplitude of the visual evoked response occurred with cessation of Gd-DTPA enhancement. Delay in the visual evoked response, indicating demyelination, was also present early but persisted beyond the period of reduced visual acuity. Youl and colleagues concluded that inflammation made a significant contribution to conduction block and visual symptoms.

Experimental evidence suggests that a number of cytokines, including TNF-α, can directly impair nerve conduction; electrophoretically administered TNF-α reversibly reduces the discharge rate of rat glucose-sensitive neurons (Plata-Salaman et al., 1988); intraocular injection of TNF-α and IFN-γ reversibly delays nerve conduction through the anterior visual pathway (Brosnan et al., 1989), and exogenous TNF-α reduces potassium and sodium conductance in neurons of Aplysia kurodai (Sawada et al., 1990, 1991). The mechanisms of these effects are not fully established, but may relate to the ability of TNF-α to inhibit secretion of norepinephrine by neurons in culture following repeated potassium-induced depolarizations (Soliven and Albert, 1992). This interpretation does not preclude the possibility that the effects of cytokines are indirect and result from damage to the blood–brain barrier; Patient 1 was scanned on two occasions during the first course of treatment (days 4 and 8) and shown to have 24 independent enhancing (i.e. active) lesions; although anecdotal, this observation is consistent with an early physiological consequence of altered blood–brain permeability with appropriate imaging characteristics persisting for several days.

Anti-CD6 (Hafler et al., 1986), CD2 (Hafler and Weiner 1988), CD3 (Weinshenker et al., 1991) and CD4 (Racodot et al., 1993; Lindsey et al., 1994a, b) antibodies have each been administered to patients with multiple sclerosis. In some instances, antiglobulin responses and acute adverse effects have limited the usefulness of one or other of these monoclonal antibodies and an additional problem has been modulation of the targeted lymphocyte antigen, allowing some cells to survive. CAMPATH-1H offers theoretical advantages over other therapeutic monoclonal antibodies.

Lymphopaenia is rapid and prolonged since the CD52 antigen is expressed in high density on the target cell membrane; due to its isotype, CAMPATH-1H is exceptionally good at activating complement and mediating antibody dependent cell mediated cytotoxicity (Xia et al., 1993). CD52 does not lose its potential for lysis through modulation by antibody. Although in this study a single course did not elicit an antiglobulin response, this may not be the case if repeated courses of antibody have to be given, as has proved necessary in other clinical situations (Isaacs et al., 1992; Lockwood et al., 1993).

Our study was not designed to assess the clinical efficacy of CAMPATH-1H in patients with multiple sclerosis; we chose a surrogate outcome measure of disease activity and showed a reduction in new lesion formation using Gd-DPTA enhanced MRI (Moreau et al., 1994). The results of this pilot study only provide preliminary evidence for the therapeutic role of CAMPATH-1H, and a placebo-controlled study is needed to establish whether there is a disease modifying effect. However, our clinical studies have proved informative with respect to an immediate mechanism of symptom production in previously affected, and presumably partially demyelinated, pathways in patients with multiple sclerosis.

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References


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