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Repopulation of blood lymphocyte sub-populations in rheumatoid arthritis patients treated with the depleting humanized monoclonal antibody, CAMPATH-1H

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SUMMARY

Patients with severe rheumatoid arthritis who had failed treatment with conventional therapies were treated with a course of five or 10 daily intravenous infusions of CAMPATH-1H, a humanized antibody against the CD52 antigen, resulting in profound depletion of peripheral blood mononuclear cells. During the subsequent 18 months, lymphocytes were analysed for sub-populations by fluorescence-activated cell sorter (FACS) and for proliferation in response to polyclonal T-cell stimulation with anti-CD3 or staphylococcal enterotoxin B (SEB). Treatment resulted in almost complete depletion of lymphocytes from the blood followed by gradual repopulation. $CD16^+$ natural killer (NK) cells and $CD14^+$ monocytes returned to pretreatment levels within 1–2 months. $CD19^+$ B cells returned to within 50% of pre-treatment levels by day 66 and to within normal range by day 150, whereas $CD8^+$ T cells recovered to 50% of pretreatment levels by day 66, but did not show any further increase during the rest of the study period. The most profound effects were on the $CD4^+$ T lymphocyte sub-population, as the mean $CD4^+$ count did not increase above 20% of pre-treatment level at any time during the study period (550 days), at all the doses tested. The T cells which initially repopulated the blood 1–2 months after treatment, nearly all expressed the activation markers human leucocyte antigen (HLA)-DR and CD45RO, although the percentage of T cells expressing these molecules gradually declined to normal levels over time. Proliferative responses to polyclonal T-cell stimulation (anti-CD3 and SEB) were also significantly reduced in the first few months after treatment, but recovered to pre-treatment levels by day 250. The relationship between these observations and the clinical response is discussed.

INTRODUCTION

CAMPATH-1H is humanized monoclonal antibody created by transplanting the hypervariable regions from a rat anti-human CD52 monoclonal antibody onto a human immunoglobulin G (IgG) framework.[1] The resultant antibody is efficient at complement- and cell-mediated lysis of human cells expressing the CD52 antigen.[1,2] The humanized antibody is also extremely effective at depleting lymphocytes from peripheral blood following *in vivo* infusion.[3] This property appears to require the interaction of the antibody with FcR bearing effector cells.[4] The structure and density of the CD52 antigen is also important, as other immunoglobulin G1 (IgG1) antibodies, for example against the CD4 molecule, do not necessarily deplete lymphocytes *in vivo* (J. Isaacs, personal communication). The CD52 antigen is expressed at high levels on B and T lymphocytes, at lower levels on monocytes and is absent on polymorphonuclear leucocytes and bone marrow colony-forming cells.[5] Thus the CAMPATH-1H antibody has potential therapeutic utility for the specific reduction of lymphocyte populations. Humanized antibodies should also appear less foreign to humans than conventional rodent antibodies,[1] and indeed by comparison with previous experience using the rat antibody CAMPATH-1G, the humanized CAMPATH-1H antibody is less immunogenic than its rodent counterpart, after a single course of therapy.[3]

The structure of the CD52 antigen has been fully characterized and found to be a very small glycosylphosphatidylinositol (GPI)-anchored glycoprotein with a mature peptide containing only twelve amino acids.[6,7] There is a single N-linked glycosylation site, and the carbohydrate structure is complex and comprises over 50% of the apparent molecular weight of 21–28 kDa. The CAMPATH series of antibodies appear to recognize an epitope close to the cell membrane, which could be one reason why they are such effective mediators of cell lysis.[8] The function of the antigen and its natural ligand *in vivo* have not yet been elucidated, but as with other GPI-linked molecules, it has been shown that

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cross-linking the CD52 antigen in the presence of phorbol esters results in T cell activation and proliferation.[9]

Rheumatoid arthritis (RA) is a common, progressive and crippling disease. The rationale for using CAMPATH-1H in the therapy of rheumatoid arthritis is based on favourable results with other anti-lymphocyte therapies, such as total lymphoid irradiation,[10] thoracic duct drainage,[11] and cyclosporin A.[12] The disease association with HLA,[13] and the observations that a number of mAbs that target T cells[14,15] have shown benefit in RA, albeit of limited duration, provide strong evidence that T cells play a role in the pathogenesis of the disease. Early results with the use of CAMPATH-1H to treat RA patients were promising, as 7/8 patients treated with a cumulative dose of 60 mg over 10 days showed initial clinical improvement, although the duration of response varied from 1–6 months.[3] However, the results from this study,[3] and also from a single dose escalation study[16] revealed long-term depletion of $CD4^+$ T cells in the peripheral blood. The present study was undertaken to examine in detail the kinetics of lymphocyte subset repopulation and function following almost complete depletion of peripheral blood with various doses of CAMPATH-1H, as part of larger Phase II clinical trial to examine the efficacy of this antibody in RA. The clinical data from this trial have been reported in more detail elsewhere.[17]

MATERIALS AND METHODS

Study design and patient population

Treatment with CAMPATH-1H was performed in a multicentre (USA and Europe), open labelled, dose escalation Phase I/II study of this antibody in rheumatoid arthritis. Details of the patients treated and the effects of CAMPATH-1H on their clinical course have been published elsewhere.[17] Patients with active RA who had previously failed or were intolerant of at least one disease modifying anti-rheumatic drug (DMARD) were entered into the study. Stable doses of non-steroidal anti-inflammatory drugs and low dose oral prednisone (< 10 mg/day) were permitted during the study period. The results reported here were obtained from the five and 10 day i.v. dosing study. In the five-day i.v. study, cohorts of six patients each were treated with five consecutive, daily i.v. doses of 12, 20, 50, or 80 mg (cumulative doses of 60, 100, 250, and 400 mg per cohort, respectively). In the 10-day i.v. study, the first cohort of six patients was dosed with 4 mg for 5 days, followed by two days rest, and then a further 8 mg/day for 5 days (60 mg cumulative dose). Daily doses in the remaining cohorts were 10, 25, or 40 mg providing cumulative doses of 100, 250, and 400 mg per cohort, respectively. Fifty-four patients from 10 centres in Denmark, the Netherlands, the United Kingdom and the USA were enrolled into the study. Forty-seven patients were considered evaluable since they received at least 80% of their prescribed dose. Baseline demographic and disease characteristics of this population are shown in Table 1.

CAMPATH-1H

Therapeutic grade antibody (> 99% monomeric IgG) was produced from Chinese hamster ovary (CHO) cells, grown in serum free medium in large fermenters. After harvesting, the antibody was concentrated by ultrafiltration and purified by sequential Protein A affinity chromatography, ion exchange chromatography on an S-Sepharose column (Pharmacia, Uppsala, Sweden) and size exclusion chromatography through Superdex 200 (Pharmacia). The antibody was sterilised by filtration and formulated at 10 mg/ml in phosphate buffered saline (PBS) containing 0·05 mM ethylenediamine tetra-acetic acid (EDTA) before storing at 2–8° ready for use. Patients were admitted to hospital for antibody therapy, which was administered by intravenous infusion over at 2–4 hr. The first dose of CAMPATH-1H was associated with a 'flu-like syndrome (fever, chills, nausea) in most patients. This infusion related toxicity appeared to be ameliorated after the first treatment day.

Immunophenotyping of peripheral blood leucocytes

For immunophenotyping of peripheral blood by fluorescence-activated cell sorter (FACS), 3 ml of EDTA anti-coagulated venous blood was washed three times in PBS and resuspended to the initial volume in PBS. This was to remove any anti-CAMPATH-1H antibodies present in the serum, which would interfere with the staining. The cells (100 μl/sample) were incubated with a panel of directly conjugated fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-labelled antibodies for 20 min. The cells were then treated with 2 ml red blood cell lysing solution (Becton-Dickinson) for 10 min followed by three washes with PBS and finally fixed with 1% paraformaldehyde. The cells were dual stained with FITC-labelled CAMPATH-1H $F(ab)_2$ antibody and PE-labelled anti-CD3, anti-CD4, anti-CD8, anti-CD14, anti-CD16 and anti-CD19, obtained from Becton-Dickinson. Directly conjugated isotype matched antibodies were used as negative controls. 'Activated' T cells were determined by dual staining with FITC-anti-CD3 and PE-anti-human leucocyte antigen (HLA)-DR. Memory and naive $CD4^+$ T cells were determined by dual staining with FITC-anti-CD4 and PE-anti-CD45RO and FITC-anti-CD45RA and PE-anti-CD4, respectively. Analysis was performed using a FACScan flow cytophotometer

Table 1. Patient characteristics at start of therapy

Cohort mg (*n*)	Age (median years)	Sex (M/F)	Duration RA (median years)	Prior DMARDs (median no./pt)	Concurrent steroids (no.)
60 (7)	53·9	1/6	19·3	5	4
100 (15)	51·8	5/10	12·2	4	5
250 (15)	45·9	3/12	13·6	3	5
400 (11)	51·2	5/6	9·2	4	6
All (48)	50·7	14/34	13·5	4	20

(Becton-Dickinson). Data acquisition was accomplished using LYSIS II software (Becton-Dickinson). Granulocytes, lymphocytes and monocytes were identified by characteristic forward and right-angle light scattering, and confirmed using the Simultest reagent FITC-anti-CD45/PE-anti-CD14. Immunophenotyping was performed prior to treatment and at various times after CAMPATH-1H treatment on 25 patients who were part of the European arm of the study. Identical analysis was also carried out on a group of 20 normal healthy volunteers and a group of nine control RA patients who had failed 3–4 DMARDs but did not receive CAMPATH-1H.

T-cell proliferation assays

Peripheral blood mononuclear cells were isolated by Lymphoprep (Flow) density gradient separation, and resuspended in RPMI-1640 medium supplemented with 10% heat-inactivated normal human serum, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin (ICN Flow, High Wycombe, UK). Cells (10^5/well) were incubated with medium alone, and anti-CD3 monoclonal antibody (mAb) (OKT3) at 1, 0·1 or 0·01 μg/ml or staphylococcal enterotoxin B (SEB; Sigma, St Louis, MO) at 100, 10, and 1 ng/ml final concentration, in 96-well microtitre plates for 3 days at 37°, 5% CO_2. [^{3}H]thymidine (1 μCi/well; Amersham International, Amersham, UK) was added for the last 6 hr of culture, followed by harvesting onto filters. Proliferation was determined by [^{3}H]thymidine incorporation by liquid scintillation spectroscopy on a Beta plate counter (LKB Pharmacia, Uppsala, Sweden). The results are expressed as mean c.p.m. of triplicate cultures (SEM < 15%).

CD4$^+$ T-cell purification and costimulation assay

Human CD4$^+$ T cells were isolated by a rigorous negative selection procedure as previously described.[9] Human peripheral blood mononuclear cells (PBMC) were left to adhere to plastic for 2 hr, followed by incubating non-adherent cells with a cocktail of mAb against CD20 on B cells (Leu16), CD16 on natural killer (NK) cells (Leu11), CD8 on CD8$^+$ T cells (Leu2a) and CD14 (M3) on monocytes (Becton-Dickinson) for 45 min at room temperature. After washing with PBS cells were incubated with sheep anti-mouse IgG-coated magnetic beads (Dynal, Skøgen, Norway) for 30 min, followed by removal of antibody-coated cells with magnet.

For the costimulation assay, 96-well microtitre plates were coated with either anti-CD3 (OKT3 1 ug/ml) alone or in combination with CAMPATH-1H, rat anti-CD28 (YTH 913.12), rat anti-CD18 (YTH 51.1) (kindly provided by Professor Herman Waldmann), anti-CD2 (39C15; Immunotech, Marseille-Luminy, France) or fibronectin (Sigma) all at 10 μg/ml) in PBS for 2 hr at 37°. After washing away free antibody with PBS, 10^5 purified CD4$^+$ T cells were added to the wells in complete RPMI-1640 medium containing 10% FCS (fetal calf serum), and cultures incubated for 4 days at 37°/5% CO_2. T-cell proliferation was determined as described above.

RESULTS

Depletion and recovery of lymphocyte subsets following CAMPATH-1H treatment

The effects of CAMPATH-1H on the numbers of different peripheral blood lymphocyte subsets prior to and at various times after treatment are shown in Fig. 1. The results shown are the mean cell counts across all dose cohorts, as a similar pattern was found in all groups. The number of patients studied at each time point is shown above each column. The blood showed almost complete depletion of CD4$^+$ and CD8$^+$ T cells, CD19$^+$ B cells, CD16$^+$ NK cells and CD14$^+$ monocytes 4 days after the last dose of CAMPATH-1H, with no CD52 positive cells also being detected at this time. The CD16$^+$ NK cells had returned to pre-treatment levels by day 30, and CD14$^+$ monocytes had returned to 50% of normal by day 30 and to within the normal range by day 60. The CD19$^+$ B cells took slightly longer to recover, reaching 50% of pre-treatment levels by day 66 and to within the normal range by day 150. The CD8$^+$ T cell subset showed an initial recovery to 25% of normal by day 30, and a further increase to approximately 50% by day 66, however, this level did not increase further during the rest of the study period of 500–550 days. The most dramatic and long-term effects of the CAMPATH-1H treatment were found on the CD4$^+$ T cell subpopulation. By 30 days post-treatment CD4$^+$ counts were less than 10% of pre-treatment levels, and by 60 days this had increased to 17·5%. During the rest of the follow up period (> 500 days) the mean CD4$^+$ T cell count did not increase above 20% of pre-treatment values at any time. There was a fairly wide range in recovery levels of

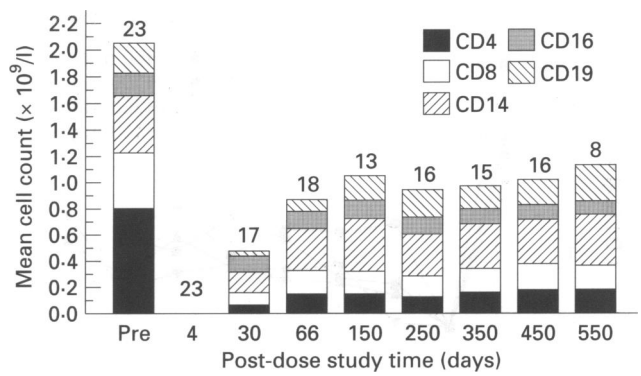

Figure 1. Depletion and recovery of peripheral blood mononuclear cell subsets following CAMPATH-1H treatment. The number of patients analysed at each time point is shown above each column.

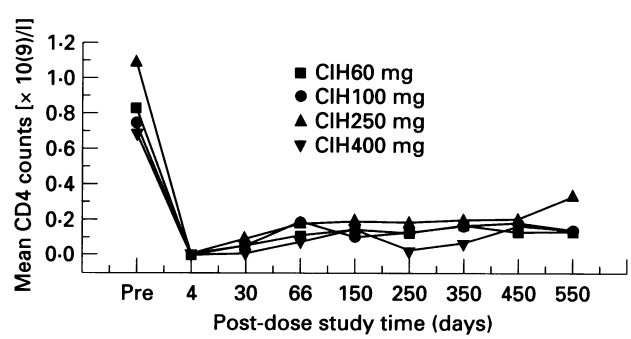

Figure 2. Effect of different doses of CAMPATH-1H on the CD4$^+$ T lymphocytes counts in peripheral blood. The results show peripheral blood mean CD4 count ± SD × 10^9/l. The number of patients for each dose was 60 mg, n = 4; 100 mg, n = 8; 250 mg, n = 7; and 400 mg, n = 4.

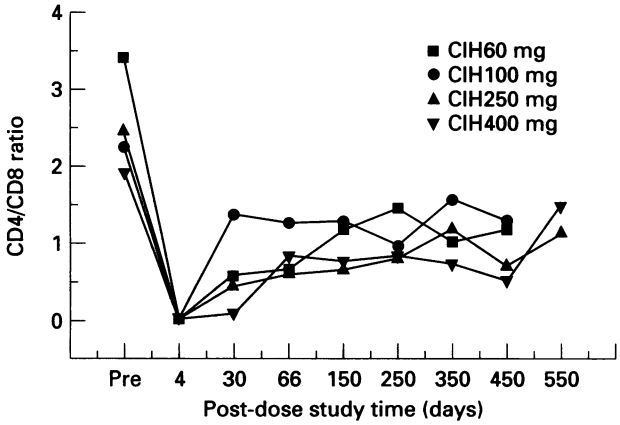

Figure 3. The CD4/CD8 ratio of peripheral blood T cells following CAMPATH-1H. 60 mg, $n = 4$; 100 mg, $n = 8$; 250 mg, $n = 7$; and 400 mg, $n = 4$.

between 10–40% of pre-treatment values, but no patient recovered to >40% of pre-treatment at any time. The results in Fig. 2 show the mean ± SD of the $CD4^+$ T-cell counts for each dose cohort (data from the 5 and 10 day dosing regimes were combined). The results for each dose show a very similar pattern, indicating that the saturating effects on peripheral blood $CD4^+$ T cells had been reached by the 60 mg dose. As a consequence of the differential recovery rates of $CD4^+$ and $CD8^+$ T cells, the CD4:CD8 ratios were significantly decreased and remained low during the entire study period (Fig. 3) and again these effects were independent of dose in the range studied.

Expression of activation markers on T cells following CAMPATH-1H treatment

The expression of HLA-DR is considered to be a phenotypic marker of T cell activation, as resting human T cells do not usually express this antigen. It is however rapidly expressed,

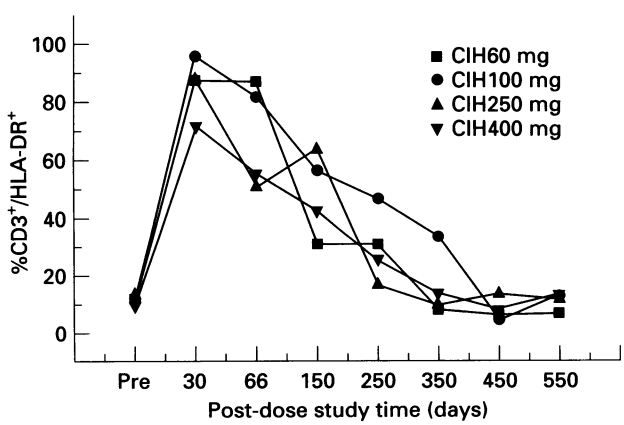

Figure 4. Expression of HLA-DR on peripheral blood T lymphocytes following CAMPATH-1H treatment. The percentage of peripheral blood T cells expressing HLA-DR at each time point was determined by dual staining with FITC-anti-CD3 and PE-anti-HLA-DR. The number of patients tested at each dose was 60 mg, $n = 4$; 100 mg, $n = 8$; 250 mg, $n = 7$; and 400 mg, $n = 4$.

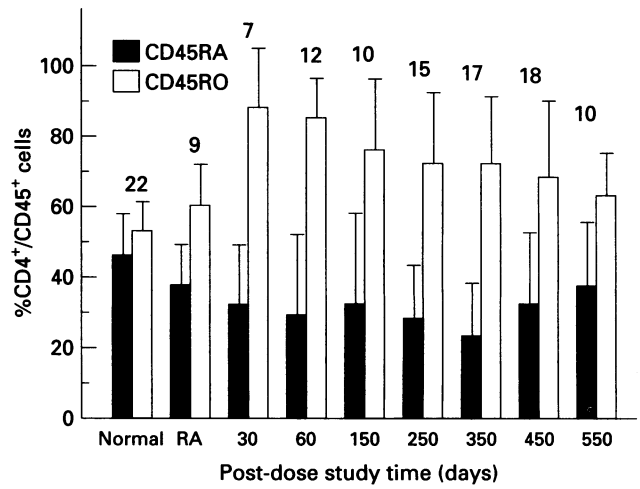

Figure 5. Expression of CD45RA and CD45RO on $CD4^+$ T lymphocytes following CAMPATH-1H. The percentage of peripheral blood $CD4^+$ T lymphocytes (mean ± SD) expressing CD45RA was determined by dual staining with FITC-anti-CD45RA and PE-anti-CD4 and CD45RO was determined by staining with FITC-anti-CD4 and PE-anti-CD45RO. The number of patients tested is shown above at each time point.

within a few days following antigen or polyclonal stimulation.[18] The results in Fig. 4 clearly show that the majority of $CD3^+$ T cells repopulating the blood in the first 30–60 days following CAMPATH-1H treatment express this activation marker. The percentage of HLA-DR positive peripheral blood T cells slowly declines with time, so by 250–350 days post-treatment, this is in the normal range.

After activation, naive T cells, as defined by expression of the CD45RA isoform of the CD45 molecule, tend to lose this antigen, and express the CD54RO isoform.[19] The expression of CD45RA and CD45RO isoforms on repopulating T cells following CAMPATH-1H treatment is shown in Fig. 5. Unfortunately expression of CD45RA and CD45RO isoforms was not determined on patients' peripheral blood T cells prior to CAMPATH-1H treatment. The results following CAMPATH-1H were therefore compared with a control group of healthy volunteers and a group of RA patients who had also failed 3–4 DMARDs and were similar to those selected for CAMPATH-1H treatment. The results with either normal healthy individuals or untreated RA patients showed approximately equal proportions of $CD4^+$ T cells expressing CD45RA and CD45RO. In RA patients not treated with CAMPATH-1H there was a slight decline in the percentage of CD45RA and a slight increase in CD45RO $CD4^+$ T cells in the peripheral blood. In contrast, the cells repopulating the peripheral blood, at 30–60 days following CAMPATH-1H appeared to be predominantly of the CD45RO isoform, as there was an increase in the percentage of CD45RO and a slight decrease in the percentage of CD45RA cells. This pattern persisted throughout the entire study period.

Responsiveness to polyclonal T cell activation following CAMPATH-H1 treatment

The proliferation response to the polyclonal stimulation with

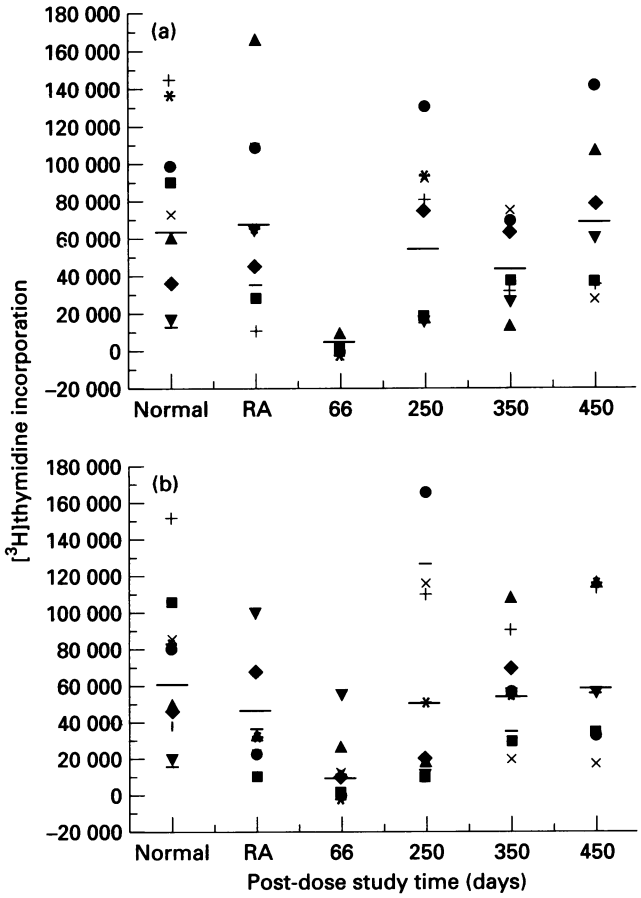

Figure 6. Proliferative responses to T cell mitogens following CAMPATH-1H treatment. PBMC (10^5/well) from normal healthy volunteers, untreated RA patients or RA patients at different times following CAMPATH-1H were incubated with optimal concentrations of (a) anti-CD3 (1 μg/ml), or (b) SEB (100 ng/ml) for 3 days at 37°, 5% CO_2. Proliferation was determined by [^{3}H]thymidine incorporation. Results are shown as mean c.p.m. for each individual patient at each time point.

either anti-CD3 or SEB at various times post-therapy with CAMPATH-H1 is shown in Fig. 6. Unfortunately proliferative responses of RA patients in the trial was not determined prior to therapy. Instead responses of a group of normal healthy volunteers and a control group of RA patients who had failed 3–4 DMARDs, equivalent to those entered into the trial, were included for comparison. The results show that in the first 2–4 months following CAMPATH-1H, peripheral blood T cell proliferative responses to both SEB and anti-CD3 are greatly reduced. Responses return to within the normal range by 250 days post-treatment.

In order to determine whether this low response was due to unresponsiveness in the CD4$^+$ T cell subset, the ability of purified CD4$^+$ T cells to respond in a costimulation assay was assessed. CD4$^+$ T cells were purified from two CAMPATH-1H patients after 2 (IW) and 4 months (EW) post-treatment, an untreated RA patient (SN) and a normal healthy individual (HD). The cells were incubated in 96-well microtitre plates coated with anti-CD3 in combination with anti-CD28, anti-CD2 anti-CD18, fibronectin or CAMPATH-1H. The results

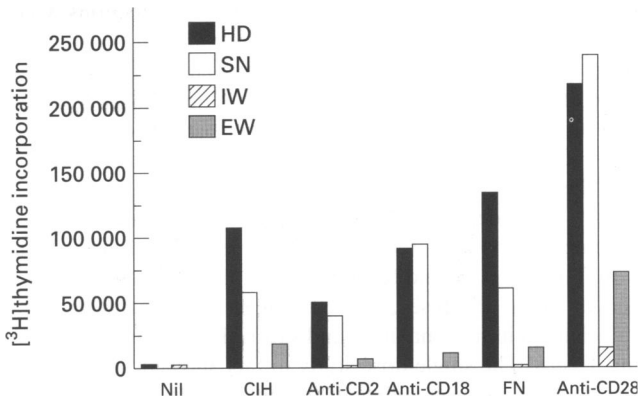

Figure 7. Responsiveness to different costimulatory stimuli by purified CD4$^+$ T lymphocytes 2–4 months after CAMPATH-1H treatment. CD4$^+$ T lymphocytes were purified from healthy donor (HD), an untreated RA patient (SN), or RA patients treaded 2 months (IW), or 4 months (EW) previously with CAMPATH-1H. Cells were incubated in 96-well microtitre plates coated with anti-CD3 alone (1 μg/ml), or in combination with anti-CD52 (CIH), anti-CD2, anti-CD19, fibronectin (FN) or anti-CD28 (all at 10 μg/ml), for 4 days at 37°, 5% CO_2. Proliferation was determined by addition of [^{3}H]thymidine, and results are shown as mean c.p.m. of triplicate cultures (SEM < 15%).

show that CD4$^+$ T cells from both CAMPATH-1H treated patients responded less well to all the stimuli than cells from the untreated RA patient and the normal healthy individual (Fig. 7).

DISCUSSION

The results of this Phase II multiple (5–10 day) dose escalation study of the effects of CAMPATH-1H showed that the antibody conferred significant initial clinical benefit for 55% of RA patients, with the duration of the response being longer in patients receiving the highest doses (250 and 400 mg).[17] The results agree well with the previously reported smaller scale study giving 60 mg over 10 days to eight RA patients.[3] However, as also found in the latter study, CAMPATH-1H treatment produced prolonged (> 18 months) depletion of circulating CD4$^+$ T lymphcytes. CD8$^+$ T-cell levels also did not return to pre-treatment levels over the study period, whereas NK cell, B cells and monocytes returned to within the normal range within 2–3 months post-treatment, confirming the results of the previous study. The reason for the differential rates of recovery of the different sub-populations probably reflects the turnover and replenishment from bone marrow colony-forming cells.[20] The long-term depletion of CD4$^+$ T lymphocytes is likely to be a consequence of the age of the patients (median age 55 years) in the clinical trial. Several studies have shown that production of new CD4$^+$ T cells after irradiation or chemotherapy correlates inversely with the age of the patient.[21,22] It is unclear whether this decrease in regenerative capacity represents a decline in the intrinsic capacity of haematopoetic stem cells to develop into T lymphocytes or whether the microenvironment of the thymus can no longer support the differentiation of new T cells as well as in younger individuals.

Although the CD4$^+$ T cell counts remained low for prolonged periods there was not an increased incidence of

infection in the long term, as the majority of infections which did occur were in the first 8 weeks after treatment. In addition, the majority of infections were well controlled and self-limiting, indicating that sufficient T cells, NK cells and monocytes were present to control opportunistic or more pathogenic infections in the CAMPATH-1H treated patients.

There appeared to be no correlation between the extent of $CD4^+$ T-cell depletion in the peripheral blood and the clinical outcome, as patients relapsed despite persistent $CD4^+$ depletion. The depletion and recovery of peripheral blood T cells was similar with all doses studied, suggesting that 60 mg was sufficient to induce maximal and saturating effects on circulating T cells. The single-dose study in fact showed that doses as low as 3–10 mg were sufficient to deplete cells from the blood, although this conferred only transient clinical benefit in 65% patients lasting a median of 2 weeks (as assessed by 20% Paulus response).[16] However higher doses given over a 5–10-day period induced more prolonged clinical benefit,[17] perhaps indicating that the blood is not a reliable indicator of the extent of lymphoid depletion in lymph nodes or synovial fluid or tissue. This is supported by a previous study of synovial fluid from two patients who received a single dose of CAMPATH-1H, which demonstrated persistence of lymphoid infiltrate at a time when circulating lymphocytes are markedly depleted.[23] Thus the higher doses may result in deeper penetration of the antibody to the lymphoid tissue or synovium with more efficient depletion and longer clinical benefit.

The FACS analysis of lymphocyte subsets showed that following the initial depletion from peripheral blood, there was an increase in $CD4^+$ T lymphocyte counts in the first 2–4 months after therapy to 20% (±10%) of pre-treatment levels, after which time levels of $CD4^+$ T cells remained constant. Interestingly the majority of the T cells which initially repopulated the blood expressed markers of T cell activation such as HLA-DR or CD45RO. This contrasts with results of a study of $CD4^+$ T lymphocyte regeneration in children after intensive chemotherapy, in which $CD4^+$ recovery correlated quantitatively with the appearance of $CD45RA^+$ T cells.[22] Expression of the CD45RA isoform is associated with T cells of 'naive' phenotype, including recent thymic emigrants.[18,19] The mechanism underlying the expression of activation markers on T cells following CAMPATH-1H therapy is unclear. It has been shown that extensive cross-linking of the CD52 antigen with CAMPATH-1H, in the presence of phorbol esters, as with other GPI-linked molecules, induces T cell proliferation and cytokine release *in vitro*.[9] It is therefore possible that in certain microenvironments *in vivo*, binding of CAMPATH-1H to T cells results in activation rather than depletion. This is also supported by the observation that the first dose reaction to CAMPATH-1H is accompanied by release of cytokines including tumour necrosis factor α (TNFα) and interferon γ (IFNγ) which may be released from T cells.[24] The expression of activation markers could also result from an antigen 'non-specific' homeostatic mechanism to at least partially replenish available space in the lymphoid compartment[20] or an antigen-specific proliferative response to environmental, opportunistic or pathogenic organisms.[25] As discussed above many of the patients developed self-limiting viral or bacterial infections after CAMPATH-1H treatment. It has recently been shown that following acute viral infections such as cytomegalovirus or Epstein–Barr virus, there is a transient increase in circulating activated T cells in the peripheral blood.[26] Many of these T cells express decreased bcl-2 expression and are programmed to die by apoptosis unless rescued by interleukin 2 (IL2) or other stimuli, and appear anergic to polyclonal stimulation.[26] This is believed to be part of the normal homeostatic mechanism to regulate T cell numbers. An alternative possibility may be that activated T cells preferentially survive CAMPATH-1H treatment or return to the recirculation pathway more rapidly than non-activated T cells.

T cells taken from patients 2–4 months post-treatment also appear poorly responsive to T cell activation. This does not appear to be a consequence of the change in relative proportions of different PBMC sub-sets (e.g. monocytes, CD4/CD8 ratio) as T-cell proliferation returned to within the normal range despite the continued imbalance of sub-sets. In addition, results with two patients with purified $CD4^+$ T cells suggested that low responses to a variety of costimulatory molecules in combination with anti-CD3 was intrinsic to the $CD4^+$ T-cell population and not a consequence of 'suppressor' $CD8^+$ T cells or excessive numbers of monocytes in the cultures. The period of low responsiveness correlated with maximal expression of the T-cell activation markers CD45RO and HLA-DR. Several studies have shown that 'activated' T cells are more prone to antigen or mitogen stimulated cell death than normal resting T cells.[27] Thus a similar mechanism may explain the low responsiveness to polyclonal T-cell stimulation for 2–4 months following CAMPATH-1H treatment.

In conclusion, this study has shown that although a short course of treatment with CAMPATH-1H is associated with significant clinical benefit,[17] this is accompanied by phenotypic changes in the composition of circulating peripheral blood mononuclear cells and persistent low $CD4^+$ T cell counts. It will be important to consider any long term consequences of these findings when assessing the risk–benefit profile of this treatment.

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