Removal of T Cells From Bone Marrow for Transplantation: A Monoclonal Antilymphocyte Antibody That Fixes Human Complement

By Geoffrey Hale, Susan Bright, Gill Chumbley, Trang Hoang, Donald Metcalf, Alan J. Munro, and Herman Waldmann

Graft-versus-host disease is one of the major problems in clinical bone marrow transplantation. Many experiments in animals have shown that it could be greatly reduced if mature T lymphocytes were removed from the donor marrow. Here we describe a new rat monoclonal antibody, CAMPATH 1, which is suitable for depleting lymphocytes from human marrow grafts. CAMPATH 1 is an IgM that fixes human complement. It binds to both T and B lymphocytes and some monocytes but not to other hemopoietic cells. When peripheral blood mononuclear cells were treated with CAMPATH 1 and complement, more than 99% of lymphocytes were killed and viable T cells could no longer be detected. Under these conditions, in vitro multipotential erythroid and myeloid colony-forming cells were unaffected. As well as being used for in vitro treatment of bone marrow to remove T cells, CAMPATH 1 could potentially be applied to other experimental and clinical situations where depletion of lymphoid cells is required, including serotherapy to achieve immunosuppression for organ transplants or to treat lymphocytic leukemias.

One of the major problems in clinical bone marrow transplantation is the high incidence of graft-versus-host disease, which can affect 50%-70% of the recipients of transplants from fully matched (HLA-A,B,C, and DR compatible) siblings and is a barrier to the performance of transplants with unmatched donors. Experimental studies in many animal models have shown that acute graft-versus-host disease can be abolished if mature T cells are removed from the bone marrow. A number of different methods have been tried to deplete human marrow of T cells, but to date, none have proved completely satisfactory.

In one approach, T cells have been removed by agglutination with soybean lectin followed by rosette formation with sheep erythrocytes. HLA-A,B nonidentical parental bone marrow thus treated has been used in a transplant without causing graft-versus-host disease. The disadvantage of this method at present is the substantial number of complex manipulative steps required. Furthermore, only about 20% of colony-forming cells are recovered, which has restricted its use to young recipients of grafts from adult donors.

Others have incubated marrow with antilymphocyte sera in order to opsonize the T cells and promote their elimination by the recipient. However, it is possible to shelter that some of the coated cells will escape destruction by capping and shedding of the antibody or because the recipient's reticuloendothelial system has been compromized by the cytotoxic conditioning regimen. Moreover, by the very nature of the maneuver, it is impossible to monitor the extent to which this occurs.

A better method would be to destroy the T cells in vitro by incubating the marrow inoculum with antibody plus complement. It would then be possible to assess the extent of T-cell depletion and ensure that it was optimal. The ideal for this purpose would be to have a monoclonal antibody specific for human lymphocytes that could fix human complement. A monoclonal antibody of appropriate specificity would be much better than a polyclonal antilymphocyte serum, because it would be available in essentially unlimited amounts and extensive absorptions to remove undesired reactivity against stem cells and other normal tissues would be unnecessary.

Unfortunately, to date, the most suitable monoclonal antibodies, for example OKT3,9 have been described to fix rabbit complement only, and individual batches of rabbit complement may themselves be toxic to stem cells. This would cause difficulties in the widespread use of such antibodies for bone marrow transplants because it would require much time and expense to screen batches of rabbit complement for toxicity.

In this article we describe a rat monoclonal antibody, designated CAMPATH 1, which appears to meet all of the criteria. It fixes human complement and reacts with all lymphocytes (T cells and B cells) both by complement-mediated cytolysis and by immunofluorescence. It spares all colony-forming cells (including the mixed erythroid/myeloid colony-forming cells) and seems, therefore, to be the most appropriate reagent for use in a bone marrow transplant program.

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MATERIALS AND METHODS

Chemicals, Culture Media, and Sera

These were obtained from the following sources: radiochemicals from Amersham International, Amersham, U.K.; culture media and fetal calf serum from GIBCO Laboratories, Grand Island, NY, and Flow, Irvine, Scotland. Human AB serum was kindly provided by Dr. D. Voak, Regional Transfusion and Immuno-Haematology Centre, Cambridge. Human urinary erythropoietin was prepared by Dr. N. N. Iscove, Basel Institute for Immunology, Switzerland, or by Dr. N. Nicola and Dr. G. Johnson, Walter and Eliza Hall Institute, Melbourne, Australia.

Cell Handling Medium

Unless otherwise stated, cells were suspended and washed in Iscove's modified Dulbecco's medium, buffered with N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES) and containing 1% bovine serum albumin and 1% fetal calf serum. This will be referred to simply as "medium."

Sources of Normal Cells

Studies on human tissue were performed with the informed consent of the donors and the approval of the Ethics Committee of Cambridge University Medical School. Blood was collected into heparin (10 U/ml) or defibrinated by shaking with glass beads and depleted of red cells by sedimentation for 20 min at 37°C in the presence of 20% Dextran (dextran solution). Tonsils were from routine tonsillectomies at Addenbrooke's Hospital, Cambridge, and a sample of thymus tissue was kindly provided by R. Cory-Pearce, Papworth Hospital, Cambridge. These were cleaned, cut into small pieces, and a single cell suspension was prepared by forcing the cells through a wire gauze. Mononuclear cells were isolated from these cell suspensions by centrifugation at 800 g for 20 min at room temperature over a layer of Lymphoprep or Ficoll-Hypaque, density 1.077 g/ml, consisting of Ficoll 400 (63.5 g/liter) plus sodium diatrizoate (100 g/liter).

Bone marrow cells were obtained from two sources. Small portions of aspirates for marrow transplants from normal donors were provided by Drs. E.C. Gordon-Smith and J. Hows of the Royal Postgraduate Medical School, Hammersmith Hospital, London. Sternal scrapes from heart-operated patients were provided by R. Cory-Pearce and colleagues. Nucleated cells were prepared by centrifugation at 300 g for 40 min over Ficoll-Hypaque, as above.

Antisera and Monoclonal Antibodies

Antiseras to rat Ig were raised in rabbits hyperimmunized with a mixture of rat monoclonal antibodies and normal rat Ig. Antiseras to mouse Ig were made analogous. These were kindly provided by Dr. T. Ternynck. Reactivity against human cells was removed by absorption with human red blood cells and human Ig coupled to Sepharose. The Ig fraction of antisera was isolated by precipitation with ammonium sulphate. F(ab')2 fragments were prepared by digestion with 2% (w/w) pepsin for 6 hr at 37°C and coupled with 1% (w/w) fluorescein isothiocyanate for 2 hr at 20°C. The substitution ratio of the coupled protein was estimated spectrophotometrically to be about 2 mole fluorescein/mole F(ab')2.

Class- and subclass-specific antisera to rat immunoglobulins were from Miles Laboratories, Stoke Poges, U.K.

The monoclonal antibody UCHT1 was kindly donated by Dr. P. Beverley, Imperial Cancer Research Fund Laboratories, London.

Immunization, Cell Fusion, and Production of Monoclonal Antibodies

A DA rat was immunized subcutaneously with 5–10 x 10⁶ human peripheral mononuclear cells enriched for T cells. One month and 2 mo later, it received similar cells intravenously. Three days after the final dose, the rat spleen cells were fused, using polyethylene glycol,11 with the rat myeloma line Y3 Agl.2.3.12 which was kindly provided by Dr. C. Milstein, MRC Laboratory of Molecular Biology, Cambridge. The fusion was labeled YTH. Hybrid cells were grown in selective medium, and the hybrid myelomas were cloned and reconstituted on soft agar.

To obtain antibody, cells were grown to stationary phase in Iscove's modified Dulbecco's medium containing 1% fetal calf serum, or as ascitic tumors in (DA x LOU)F₁ rats. Immunoglobulin fractions were isolated from the ascitic fluids by precipitation with (NH₄)₂SO₄ and were redissolved in phosphate-buffered saline (PBS) containing 0.02% sodium azide for storage at 4°C or –30°C.

The hybrid myeloma YCL 6.8 was obtained from a fusion of Y3 Agl.2.3 with spleen cells from a DA rat immunized with cells from a patient with chronic myeloid leukemia in blast crisis. It was shown to be specific for HLA-DR by immunoprecipitation and by its reactivity with various hemopoietic cells and cell lines.

Determination of Cell Viability

Cell suspensions were mixed with an equal volume of PBS containing 2 mg/ml trypan blue or 10 μg/ml fluorescein diacetate plus 50 μg/ml propidium iodide before counting in a hemocytometer. Using the latter reagents, live nucleated cells appeared bright green and dead nucleated cells bright red when viewed with a fluorescence microscope.

Cytospin Preparations

Cell suspensions containing 2–10 x 10⁶ cells in 20–100 μl were centrifuged onto microscope slides at 700 rpm for 5 min in a Cytospin (Shandon Southern Products, Runcorn, U.K.). The slides were dried and stained for 15 min with 0.3% May-Grünwald in methanol, then 15 min with 3% Giemsa (Gurr's improved R66) in tap water, and cells were classified according to their apparent morphology. Dead cells were sometimes removed from the original suspensions by centrifugation through Ficoll-Hypaque, but this did not significantly alter the differential cell counts because dead cells tended to disintegrate during preparation of the smears and were not counted.

Rosette Assays

Mononuclear cells (50 μl, 5 x 10⁶/ml) were mixed with 50 μl of washed sheep red cells (1% suspension in PBS) and 50 μl fetal calf serum, briefly centrifuged, and left overnight at 4°C. The pellet was resuspended in 50 μl of supernatant and mixed with 50 μl of PBS containing 50 μg/ml fluorescein diacetate. Rosettes containing viable lymphocytes were counted by fluorescence microscopy.

Complement-Mediated Cytotoxicity

About 10⁶ cells were incubated at 37°C for 1 hr in 0.3 ml of medium containing 150 μCi sodium [³⁵Cr] chromate. The labeled cells were washed and resuspended at 0.5–2 x 10⁶/ml. Aliquots (50 μl) of the suspension were added to U-well microtiter plates containing 50 μl of monoclonal antibody in medium. After incubation for 10 min at room temperature, 100 μl of complement (autologous plasma or AB serum, diluted in medium) was added. (Sometimes excess antibody was removed before addition of complement, with identical
results.) The cells were incubated at 37°C for 45 min, then centrifuged at 100 g for 2 min, and 100 μl of the supernatants was collected for measurement of released radioactivity.

**Immunofluorescence and Flow Cytometry**

Cells (1 × 10^7/ml) were incubated with monoclonal antibody (20–100 μg/ml) at 4°C for 30 min, then washed and resuspended at 2–8 × 10^7/ml in medium containing fluorescein-labeled F(ab')2 fragments of rabbit anti-rat Ig (0.5 mg/ml) and incubated for a further 30 min. (When mouse monoclonal antibodies were used, the second reagent contained an equimolar mixture of anti-rat and anti-mouse Ig.) The cells were washed and resuspended in PBS containing 1% bovine serum albumin and 0.02% sodium azide. Just before fluorescence analysis, propidium iodide was added to a final concentration of 25 μg/ml. This caused dead cells to fluoresce bright red and enabled the green fluorescence of live cells to be measured independently. Cells were analyzed in a Cytofluorograf model 50-H (Ortho, Westwood, MA), using a 50 mW Argon ion laser operating at 488 nm. Data were processed online with an Ortho 2150 computer. For the cell sorting experiments, we used a fluorescence-activated cell sorter (FACS II, Becton-Dickerson, Sunnyvale, CA) with a 300 mW Argon Ion laser operating at 488 nm. Both machines were operated using standard methods.

**Colony-Forming Cell Assay**

Bone marrow or peripheral mononuclear cells were plated in 35-mm Petri dishes at 5 × 10^4 cells/ml in Iscove's modified Dulbecco's medium containing 0.9% methylcellulose, 50 μM 2-mercaptoethanol, 360 μg/ml transferrin, 10 mg/ml bovine serum albumin, 10% fetal calf serum, 1 U/ml erythropoietin, and 2% human placental conditioned medium. After 2,500 rad. Stimulators were added to the cultures as appropriate: (when mouse monoclonal antibodies were used, the second reagent contained an equimolar mixture of anti-rat and anti-mouse Ig.) The cells were washed and resuspended in PBS containing 1% bovine serum albumin and 0.02% sodium azide. Just before fluorescence analysis, propidium iodide was added to a final concentration of 25 μg/ml. This caused dead cells to fluoresce bright red and enabled the green fluorescence of live cells to be measured independently. Cells were analyzed in a Cytofluorograf model 50-H (Ortho, Westwood, MA), using a 50 mW Argon ion laser operating at 488 nm. Data were processed online with an Ortho 2150 computer. For the cell sorting experiments, we used a fluorescence-activated cell sorter (FACS II, Becton-Dickerson, Sunnyvale, CA) with a 300 mW Argon Ion laser operating at 488 nm. Both machines were operated using standard methods.

**Proliferation Assays for T-Cell Function**

Cells were cultured in 150 μl of RPMI 1640 medium containing 20% (v/v) heat inactivated human serum, 100 μg/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine in microculture plates at 37°C in 5% CO2 for 4–7 days. Each well contained 3 × 10^7–10^8 responder cells plus 5 × 10^5 autologous cells that had been irradiated at 2,500 rad. Stimulators were added to the cultures as appropriate: (when mouse monoclonal antibodies were used, the second reagent contained an equimolar mixture of anti-rat and anti-mouse Ig.) The cells were washed and resuspended in PBS containing 1% bovine serum albumin and 0.02% sodium azide. Just before fluorescence analysis, propidium iodide was added to a final concentration of 25 μg/ml. This caused dead cells to fluoresce bright red and enabled the green fluorescence of live cells to be measured independently. Cells were analyzed in a Cytofluorograf model 50-H (Ortho, Westwood, MA), using a 50 mW Argon ion laser operating at 488 nm. Data were processed online with an Ortho 2150 computer. For the cell sorting experiments, we used a fluorescence-activated cell sorter (FACS II, Becton-Dickerson, Sunnyvale, CA) with a 300 mW Argon Ion laser operating at 488 nm. Both machines were operated using standard methods.

**RESULTS**

Isolation of Hybrid Myeloma and Production of Monoclonal Antibody

Culture supernatants of cloned hybrid myelomas from the YTH fusion were screened using a number of techniques, including enzyme-linked binding assays to various types of normal and tumor cells fixed to plastic, indirect immunofluorescence, and microcytotoxicity assays on peripheral mononuclear cells. Several different antibodies were identified that appeared to have comparable specificity in that they bound to T and B lymphocytes and monocytes but not to other hemopoietic cells, including colony-forming cells. One of these, YTH 66.9 (subsequently designated CAMPATH 1), was selected for further study because it showed a high titer in the complement-mediated cytotoxicity assay (Fig. 1). It was shown to be an IgM by Ouchterlonely diffusion with class-specific antisera and by dodecylsulphate/polyacrylamide gel electrophoresis of culture supernatant from cells grown in the presence of [U-14C]lysine. Two immunoglobulin light chains of slightly different electrophoretic mobilities could be detected, consistent with the continued synthesis by the hybrid myeloma of the kappa light chain from the parental myeloma, as well as the specific heavy and light chains.

For most of the experiments described here, the antibody used was an immunoglobulin fraction from ascitic fluid, but essentially identical results were obtained with unfraccionated culture supernatants.

**Killing of Mononuclear Cells With CAMPATH 1 and Human Complement**

Peripheral mononuclear cells labeled with 51Cr were incubated with the antibody, followed by diluted auto-
logous plasma (Fig. 1). The maximal release of $^{51}$Cr occurred at antibody concentrations above 10 µg/ml and at a final plasma concentration of 10%–20% and corresponded to a residual viability of 5%–10% determined in parallel experiments when viability was measured by counting cells stained with fluorescein diacetate. Most of the residual viable cells were monocytes (see Table 5). When the plasma concentration was increased above 30%, killing became less efficient, and this was particularly marked at lower antibody concentrations (Fig. 1B).

**Binding of CAMPATH 1 to Hemopoietic Cells Analyzed by Indirect Immunofluorescence**

Suspensions from peripheral blood, tonsil, thymus, and bone marrow were incubated with monoclonal antibody (50 µg/ml), washed, and labeled with fluorescent F(ab')$_2$, fragments of rabbit anti-rat Ig. Just before analysis, dead cells were counterstained with propidium iodide, which enabled viability to be determined (95%–99%) and dead cells to be eliminated from the analysis. Particles with low forward light scatter (i.e., platelets and cell debris) were also excluded. In control experiments, cells were treated with the fluorescent reagent alone and a threshold of fluorescence intensity was set that was exceeded by 1%–2% of these cells. Most of the mononuclear cells from peripheral blood were stained under these conditions, but very few cells in bone marrow were (Fig. 2). CAMPATH 1 also labeled, with similar intensity, the majority of cells from tonsil and thymus (Table 1). No binding to red cells or platelets could be detected. When nucleated cells from bone marrow aspirates were used instead of the material from sternal scrapes, about 40%–45% of cells were labeled (Table 1 and Fig. 3); these were lymphocytes and monocytes, probably mainly from the peripheral blood that contaminates such aspirates (see Table 3).

Because CAMPATH 1 bound to virtually all cells from tonsil, it seemed likely that it recognized both T and B lymphocytes. This was tested in subsequent experiments. Lymphocytes were prepared from tonsils as before and from peripheral blood by separation on Ficoll-Hypaque and treatment with carbonyl iron. They were labeled with CAMPATH 1, UCHT1, a mouse antibody that reacts with T cells, and YCL 6.8, a rat monoclonal antibody that recognizes HLA-DR and thus is a marker for B cells. We found that

<table>
<thead>
<tr>
<th>Source of Nucleated Cells</th>
<th>Percentage of Positive Cells (Above 20 Fluorescence Units)</th>
<th>Mean Fluorescence Intensity of Positive Cells (Arbitrary Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral blood</td>
<td>90 – 95</td>
<td>300</td>
</tr>
<tr>
<td>Tonsil</td>
<td>97 – 100</td>
<td>330</td>
</tr>
<tr>
<td>Thymus</td>
<td>95</td>
<td>200</td>
</tr>
<tr>
<td>Bone marrow (scrape)</td>
<td>2 – 4</td>
<td>280</td>
</tr>
<tr>
<td>Bone marrow (aspirate)</td>
<td>40 – 45</td>
<td>320</td>
</tr>
</tbody>
</table>

Leukocyte suspensions from normal tissues were stained with CAMPATH 1 and fluorescent anti-rat Ig, then analyzed by flow cytometry, using the Ortho Cytofluorograf. The range of values indicates the variation seen between different experiments.
virtually all lymphocytes from both peripheral blood and tonsil were stained by CAMPATH 1 and no extra cells were labeled when it was mixed with either of the other two antibodies (Table 2). This confirmed the conclusion that CAMPATH 1 reacts with virtually all T cells and B cells.

Cell Sorting of Bone Marrow Labeled With CAMPATH 1

Nucleated cells from a bone marrow aspirate labeled with CAMPATH 1 and a fluorescent detection reagent, as above, were sorted under sterile conditions into two populations according to whether their fluorescence intensity was above (positive) or below (negative) a threshold as defined before (Fig. 3). Cytospin preparations of each population and of the original cell suspension showed that the positive fraction contained only lymphocytes and monocytes, whereas the negative fraction was substantially depleted of these cells (Table 3). Samples of each fraction were cultured in methylcellulose with human placental conditioned medium, and after 7 and 14 days, erythroid and myeloid colony-forming cells were scored. Essentially all of the progenitor cells were recovered in the negative fraction, and none were found in the positive fraction (Table 3). Small numbers (about 5/10^6 cells) of multipotential mixed colonies (erythroid, granulocyte, and monocyte) were observed on day 14, in the control and in the negative fraction, though for simplicity, their numbers have been included with the day 14 erythroid colonies (BFU-E).

Survival of Erythroid and Myeloid Colony-Forming Cells After Treatment With CAMPATH 1 and Complement

The previous experiments showed that binding of CAMPATH 1 to erythroid and myeloid colony-forming cells was undetectable by immunofluorescence and cell sorting; this experiment was designed to check whether they were killed by treatment with the antibody plus complement. Nucleated cells from bone marrow scrapes or aspirates (4–10 × 10^6/ml) were incubated for 30 min at room temperature with either 50 μg/ml CAMPATH 1 (treated) or medium alone (control), washed, and resuspended in medium containing 10% (v/v) human AB serum and incubated for 45 min at 37°C.

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**Table 2. Fluorescent Staining of Human T and B Lymphocytes by CAMPATH 1**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Peripheral Blood Lymphocytes</th>
<th>Tonsil Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>CAMPATH 1</td>
<td>97</td>
<td>97</td>
</tr>
<tr>
<td>UCHT1 (T cells)</td>
<td>88</td>
<td>11</td>
</tr>
<tr>
<td>YCL 6.8 (B cells)</td>
<td>6</td>
<td>69</td>
</tr>
<tr>
<td>CAMPATH 1 + UCHT1</td>
<td>95</td>
<td>97</td>
</tr>
<tr>
<td>CAMPATH 1 + YCL 6.8</td>
<td>96</td>
<td>98</td>
</tr>
</tbody>
</table>

Lymphocyte suspensions from peripheral blood or tonsil were stained with monoclonal antibodies and fluorescent anti-Ig, then analyzed by flow cytometry, using the Ortho Cytofluorograf.

CAMPATH 1, rat IgM, reacts with T and B cells.
UCHT1, mouse IgG, reacts with T cells.
YCL 6.8, rat IgG anti-HLA-DR, reacts with B cells.
Table 3. Cell Sorting of Human Bone Marrow Cells Labeled With CAMPATH 1

<table>
<thead>
<tr>
<th>Percent of Nucleated Cells</th>
<th>Differential Cell Count as Percent of Total Nucleated Cells Sorted</th>
<th>Colony-Forming Cells per $10^3$ Nucleated Cells Sorted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blasts</td>
<td>Promyelocytes and Myelocytes</td>
</tr>
<tr>
<td>Sorted</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>Negative</td>
<td>59</td>
<td>4</td>
</tr>
<tr>
<td>Positive</td>
<td>41</td>
<td>0</td>
</tr>
</tbody>
</table>

Cells from a bone marrow aspirate were stained with CAMPATH 1 and fluorescent anti-rat Ig. The cells were sorted into fluorescent and nonfluorescent fractions with a FACS II cell sorter and the morphology and colony-forming potential of these fractions were compared with unsorted cells as described in the text. Colonies were characterized morphologically as erythroid (CFU-E and BFU-E), granulocyte (G-CFC), monocyte (M-CFC), granulocyte/monocyte (G/M-CFC), or mixed (granulocyte, monocyte, and erythroid).

The cells were spun down, resuspended in the same volume of medium, and counted for viability by trypan blue exclusion. Cells in all groups were plated in duplicate at concentrations equivalent to $5 \times 10^4$ cells/ml in the control group. Cytospin preparations from the same cell suspensions were made for differential cell counts (Table 4).

The results were consistent with the cell sorting experiments, i.e., most lymphocytes and some monocytes were removed by treatment with monoclonal antibody and complement, but erythroid and myeloid colony-forming cells were unaffected. Cells treated with antibody alone or complement alone remained viable.

In six similar tests on bone marrow or peripheral blood mononuclear cells (not shown), we observed no significant inhibition of colony-forming cells by treatment with the antibody and complement compared with control samples, although the absolute number of colonies varied between experiments, as can be seen in Table 4. Granulocyte and macrophage colony-forming cells were also assayed in cultures prepared in soft agar with human placental conditioned medium; again, they were unaffected by the treatment (data not shown).

Table 4. Treatment of Human Bone Marrow Cells With CAMPATH 1 and Complement

<table>
<thead>
<tr>
<th>Source and Type of Cells</th>
<th>Percent viable Cells</th>
<th>Differential Cell Count as Percent of Total Nucleated Cells Input</th>
<th>Colony-Forming Cells per $10^3$ Nucleated Cells Input</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blasts</td>
<td>Promyelocytes and Myelocytes</td>
<td>Metamyelocytes and Polymorphs</td>
</tr>
<tr>
<td>Sternal control</td>
<td>99</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Sternal treated</td>
<td>78</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Aspirate control</td>
<td>99</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Aspirate treated</td>
<td>69</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

*Human bone marrow cells were treated with medium or CAMPATH 1 and complement for 45 min at 37°C, then assayed for viability, morphology, and colony-forming potential as described in the text. Results from two representative experiments are shown. Colonies were characterized as described in Table 3.*
Table 5. Morphology of Residual Human Peripheral Mononuclear Cells After Treatment With CAMPATH 1 and Complement

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Viable Cells as Percentage of Starting Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Total</td>
<td>99</td>
</tr>
<tr>
<td>Monocytes</td>
<td>14.5</td>
</tr>
<tr>
<td>Small lymphocytes</td>
<td>80.7</td>
</tr>
<tr>
<td>Large lymphocytes</td>
<td>3.2</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>0.2</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Mononuclear cells were treated with medium or CAMPATH 1 and complement. Dead cells were removed by centrifugation, though 35% (w/v) bovine serum albumin and cytospin preparations were made for differential cell counts. The results are expressed as a percentage of the initial number of cells.

the cells remained viable. Most of these were monocytes. About 0.5% of cells with apparent lymphocyte morphology remained viable, but most of these had a high ratio of cytoplasm to nucleus and frequently contained cytoplasmic granules. Only about 0.2% typical small lymphocytes could be seen.

Surface markers. When the remaining small number of viable cells were stained with fluorescent anti-rat Ig to reveal CAMPATH-1-coated cells, it was found that 40%–60% were labeled, and with a comparable intensity to that of mononuclear cells treated with CAMPATH 1 alone and similarly stained (Table 6). However, virtually all of these residual CAMPATH-1-positive cells had the high forward light scatter characteristic of monocytes. Therefore, it seems that some monocytes are not killed by CAMPATH 1 plus complement, despite the fact that antibody has been bound.

When the residual cells were stained instead with the mouse monoclonal antibody, UCHT1, which reacts with most peripheral human T cells, followed by the mixture of fluorescent reagents, no extra cells were labeled. We calculated that more than 99% of the cells that reacted with UCHT1 in the control sample were eliminated by treatment with CAMPATH 1 and complement (Table 6).

Table 6. Surface Markers on Residual Human Peripheral Mononuclear Cells After Treatment With CAMPATH 1 and Complement

<table>
<thead>
<tr>
<th>Surface Marker</th>
<th>Viable Cells as Percentage of Starting Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Total</td>
<td>99</td>
</tr>
<tr>
<td>CAMPATH 1</td>
<td>91–95</td>
</tr>
<tr>
<td>UCHT1</td>
<td>67</td>
</tr>
<tr>
<td>E-rosette</td>
<td>78</td>
</tr>
</tbody>
</table>

Mononuclear cells were treated with medium or CAMPATH 1 and complement. Viability was measured, and samples were stained with CAMPATH 1 or UCHT1 and fluorescent antihuman globulin for analysis using an Ortho Cytofluorograf. Other samples were used for enumeration of E-rosette-forming cells, as described in Materials and Methods.

None of the residual cells were able to form rosettes with untreated sheep red cells. However, incubation of lymphocytes with high concentrations (>10 μg/ml) of CAMPATH 1 was alone sufficient to inhibit formation of E-rosettes. This does not necessarily imply that CAMPATH 1 recognizes a specific sheep red cell receptor, but does suggest that it reacts with all peripheral lymphocytes that can form E-rosettes.

Proliferative responses. The proliferative capacity of the residual cells in response to various T-cell mitogens was compared with that of cells treated with complement alone (which itself had no effect on proliferation). Cultures were set up containing various numbers of responder cells plus a constant number of irradiated autologous cells as "fillers."

After incubation for an optimum time (4 days for phytohaemagglutinin and concanavalin-A, 7 days for tetanus toxoid and mixed lymphocyte cultures), proliferation was measured by incorporation of [6-³H]thymidine. Incorporation was plotted as a logarithmic function of cell input for the control cells, and from these standard curves, the proportion of responding cells in the treated samples were calculated by interpolation. An example of the results obtained using allodense lymphocytes as stimulators of a mixed lymphocyte response is shown in Fig. 4. The responses are expressed in terms of the starting cell numbers (Table 7), although in each culture, equivalent numbers of

![Image of Fig. 4](https://example.com/image.png)
viable cells were used as responders. The results are consistent with the elimination of most or all of the functional T cells by treatment with antibody and complement.

In contrast to their poor ability to respond to the T-cell mitogens, the residual cells remained good stimulators of a mixed lymphocyte response by allogeneic lymphocytes (Table 7).

**DISCUSSION**

It is widely accepted that graft-versus-host disease is caused by the immunocompetent T lymphocytes that are inevitably included in a bone marrow graft, and it is expected that removal of these cells will decrease the incidence and severity of the disease.\(^21,22\)

Serologic methods of specific cell depletion are among the simplest available, and monoclonal antibodies offer many advantages over polyclonal antisera in specificity, reproducibility, and availability. Mere coating with antibody alone is unlikely to be consistently effective because, for the reasons previously mentioned, small numbers of cells may survive to mount a graft-versus-host response.\(^3,4\) It is likely that this will be an even greater problem with HLA-mismatched transplants, since successful transplants across major histocompatibility barriers in animals require T cells to be removed very efficiently.\(^4,5\) Treatment with antibody and complement in vitro can achieve efficient and rapid killing, and the extent of removal of donor T cells can be accurately measured, which, moreover, is a prerequisite for testing whether mature lymphocytes are solely responsible for graft-versus-host disease in man.

It is possible that complete removal of T cells will have disadvantageous effects. Experiments in monkeys showed that engraftment was delayed and required a large marrow inoculum when T cells were removed.\(^6\) Perhaps graft-versus-host disease contributes to immunosuppression of the recipient. It has also been suggested that graft-versus-host disease could have a beneficial antileukemic effect.\(^1\) However, these hypotheses have not yet been widely tested in humans because no simple, effective method of removing T cells is generally available.

CAMPATH 1 is the first antilymphocyte monoclonal antibody described to have the desirable property of fixing human complement. All of the samples of human complement tested were found to be equally effective, whether used with allogeneic or autologous cells. Therefore, the cytolytic effect of CAMPATH 1 is unlikely to depend on synergy with antibodies present in the source of complement. It is expected to be particularly suitable, in conjunction with donor complement, for in vitro treatment of bone marrow, because problems with the availability, testing, and nonspecific toxicity of rabbit complement can be avoided. Treatment with CAMPATH 1 and complement had no demonstrable effect on erythroid or myeloid colony-forming cells (Table 4), nor could binding to those cells be detected by indirect immunofluorescence (Table 3). Based on mouse studies, it is unlikely that present cloning techniques are able to assay in vitro the human stem cells responsible for marrow repopulation after transplantation. However, we were able to monitor in vitro mixed colony-forming cells and the levels of these cells were unaffected by antibody treatment. These observations provide strong indirect evidence that the repopulating capacity of human marrow cell suspensions is not impaired by treatment with CAMPATH 1.

After treatment of peripheral mononuclear cells with CAMPATH 1 and complement, viable T cells could not be detected by markers or proliferation (Tables 6 and 7). This is in contrast with results obtained by Granger et al.\(^23\) using the monoclonal antibodies OKT3, MBG6, and OKT11A, where 5%–10% of T cells remained viable after treatment with the individual antibodies plus rabbit complement and a cocktail was necessary to achieve removal of all identifiable T cells.

Nevertheless, in mice, as few as 0.3% T cells in a bone marrow inoculum can cause graft-versus-host disease,\(^24\) and if sufficient T cells are spared by CAMPATH 1, it might be still necessary to use a cocktail of antibodies recognizing different antigens. To this end, we are investigating methods of obtaining other monoclonal antibodies against different antigens on human lymphocytes that also can fix human complement.
CAMPATH 1 appears to recognize all human T and B lymphocytes, for it stained virtually all lymphocytes from peripheral blood, tonsil, and thymus (Table 1) and no additional cells were labeled when T-cell or B-cell-specific reagents were mixed with it (Table 2). Treatment with CAMPATH 1 and complement killed more than 99% of the small lymphocytes in peripheral blood, though some large lymphocytes remained (Table 5). The properties of these cells have not yet been investigated in detail. The only other type of cell to which binding of CAMPATH 1 was detected was monocytes (Table 3). However, they were not efficiently killed (Table 5).

This antibody could potentially be of use in any situation where depletion of lymphocytes is required in experimental or clinical maneuvers. Preliminary experiments (G. Hale, D. Swirsky and H. Waldmann, unpublished work) have shown that it can cause complement-mediated lysis of leukemic cells from patients with chronic and acute lymphocytic leukemia. Therefore, it can be considered as a reagent for eliminating malignant cells from remission for autotransplantation and for serotherapy of these diseases, as well as for immunosuppression for organ transplants. Furthermore, CAMPATH 1 reacts with cells from various monkey species (G. Hale and H. Waldmann, unpublished results), and so it is possible to test proposed therapeutic protocols in primate models.

Early results from in vivo administration of CAMPATH 1 to a cynomolgus monkey and to two patients, one with non-Hodgkin’s lymphoma and the other with chronic lymphocytic leukemia, have shown that even repeated doses of large amounts (up to 100 mg in humans) of the antibody have no observable toxic effect other than causing removal of lymphocytes and consumption of complement (G. Hale, D. Swirsky, and H. Waldmann, unpublished work). There is now a need for more extensive trials to establish its efficacy in vivo in comparison with other antibodies of different isotypes in order to know whether it will be useful for serotherapy as well as in vitro removal of lymphocytes from bone marrow grafts.

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Removal of T cells from bone marrow for transplantation: a monoclonal antilymphocyte antibody that fixes human complement

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