Effects of CAMPATH-1 Antibodies In Vivo in Patients With Lymphoid Malignancies: Influence of Antibody Isotype

By Martin J.S. Dyer, Geoffrey Hale, Frank G.J. Hayhoe, and Herman Waldmann

The CAMPATH-1 family of antibodies recognize an abundant glycoprotein expressed on virtually all human lymphocytes. All rat IgM and IgG antibodies of this specificity are lytic with human complement, but only IgG2b is active in antibody-dependent cell-mediated cytotoxicity (ADCC). We compared the ability of IgM, IgG2a, and IgG2b to deplete lymphocytes in vivo in two patients with prolymphocytic transformation of B-cell chronic lymphocytic leukemia (CLL). The IgM (CAMPATH-1M) produced transient depletion of blood lymphocytes with consumption of complement but had no effect on solid masses or bone marrow. Similar transient depletion of blood lymphocytes was noted with the IgG2a (YTH34.5). In contrast, the IgG2b (CAMPATH-1G) produced long-lasting depletion of lymphocytes from blood and marrow and improvement in splenomegaly but no detectable changes in complement levels. These differences probably reflect the importance of Fc receptor binding for effective clearance of target cells in vivo. We treated 16 more patients with a variety of lymphoid malignancies and noted consistent effects on blood lymphocytes, marrow infiltration, and splenomegaly. At this dose level, there was comparatively little improvement in affected lymph nodes or extranodal masses. Nevertheless, the in vivo lympholytic ability of CAMPATH-1G is very potent as compared with other monoclonal antibodies (MoAbs) and may have applications in therapy of lymphoid malignancies and as an immunosuppressive agent.

SOON AFTER the first descriptions of monoclonal antibodies (MoAbs) against cell surface differentiation antigens, interest in their application to treat leukemia and other malignant disease grew. Despite one report of a long-term remission induced in a lymphoma patient by therapy with an antibody isotype MoAb,1 most trials of unconjugated antibodies have produced less impressive responses.2-4 That therapy can be limited by the antoglobulin response which appears in about ten days in most patients other than those profoundly immunosuppressed by terminal disease or prior therapy was soon recognized. Within this short time, the effectiveness of antibody therapy is probably determined largely by the ability to activate human effector mechanisms such as complement, antibody-dependent cell-mediated cytotoxicity (ADCC) and opsonization. Many factors may influence this, including distribution and density of the target antigen, its tendency to modulate, the species and isotype of the antibody, and the extent to which natural effector mechanisms are compromised by disease or other therapeutic agents.

In animal models, several MoAbs are capable of efficient and long-term depletion of target cells in vivo. Therefore, we are optimistic that unconjugated antibodies can be used for therapy in humans if requirements of target antigen and antibody isotype are satisfied. We chose to study rat antibodies because of technical advantages in their production and large-scale purification.5 On the basis of in vitro assays, IgG2b appears to be the most suitable choice for serotherapy because it is the optimum IgG subclass for fixing human complement6 and is very active in ADCC with human effector cells,7 binding to the human Fc receptor FcRIII.8

As an initial target, we chose the antigen recognized by the antibody CAMPATH-1M,9 which is expressed at high density (∼5 × 10⁵ molecules per cell) on virtually all normal lymphocytes and most lymphoid malignancies and more weakly on monocytes and blast cells in some cases of acute myeloid leukemia.10 CAMPATH-1M is an IgM which is exceptionally lytic with human complement and has been widely used for removal of T cells from marrow grafts to prevent graft-v-host disease (GVHD).11-13 Infusion into patients with non-Hodgkin’s lymphoma and chronic lymphocytic leukemia (CLL) resulted in substantial complement consumption but only transient lowering of tumor cell counts in peripheral blood. No effects were observed on solid tissues or bone marrow.14-16 A rat IgG2 antibody (CAMPATH-1G, clone YTH34.5-HL-G2b) was obtained by selecting a class-switch variant of an IgG2a antibody (YTH34.5) that recognizes the same antigen cluster.22,23 All three antibodies fix human complement, but the IgG2b has the advantage of being active in ADCC. We compared the effects of these antibodies in vivo in two patients with prolymphocytic transformation of B-cell CLL and showed that unlike the other isotypes the IgG2b CAMPATH-1G can mediate substantial and long-lasting cell clearance. These initial findings were confirmed and extended in 16 other patients with a variety of lymphoid malignancies treated with CAMPATH-1G, and we report them briefly.

MATERIALS AND METHODS

Patients. Approval for use of the MoAbs was given by the ethical committee of Addenbrookes Hospital. Written informed consent was obtained from each patient. Patients were considered eligible if (a) all their tumor cells expressed the CAMPATH-1 antigen (>95% positive by immunofluorescence), (b) tumor cells were sensitive to complement-mediated lysis in vitro by CAMPATH-1M and CAMPATH-1G, and (c) the disease was no longer responding to conventional chemotherapy (except for patients 5 and

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Submitted July 26, 1988; accepted December 15, 1988.

Supported by grants from the Medical Research Council UK, Wellcome Biotech, and St John’s College, Cambridge (MRES studentship to M.J.S.D.).

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0006-4971/89/7306-0008$3.00/0

Blood, Vol 73, No 6 (May 1), 1989; pp 1431-1439

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the antibody infusions were started. Baseline investigations included full blood count, urea and electrolytes, liver function tests, urate, serum immunoglobulins, autoimmune profile, complement CH50, C3 and C4 components, chest radiograph, electrocardiogram, and urine analysis. During antibody infusions full blood count, urea, electrolytes, and liver function were measured at least daily.

**Antibody administration.** Antibodies were purified from rat ascitic fluid by precipitation with ammonium sulfate, redissolved in phosphate-buffered saline (PBS), sterile-filtered, and checked for sterility and absence of pyrogens. Patients were prehydrated overnight with 2L intravenous (IV) fluid and in initial studies were premedicated with 125 mg methylprednisolone IV on the first one or two days of CAMPATH-1G infusion in an attempt to ameliorate the rigors, fevers, and sweating associated with the first two doses of this antibody. Subsequent patients received oral paracetamol (1 g four times daily) and, if necessary, IV meperidine (demerol) to control these reactions, thus avoiding any possible tumor lytic effect of the steroid (Table 1). In addition, 500 mL saline was administered before the antibody infusion. The antibody was diluted in 500 mL 0.9% saline and administered IV by an infusion pump in incremental doses, usually 1 mg in the first hour, 4 mg in the second hour, and 20 mg in the third hour. Temperature, pulse, and BP were recorded every 15 minutes during antibody infusions.

**Immunofluorescence.** Mononuclear cells were isolated by centrifugation of heparinized blood over Ficoll/Hypaque. Approximately 2.5 × 10^6 cells were incubated on ice with 50 L antibodies in phosphate-buffered saline (PBS), sterile-filtered, and checked for antibody purity on tumor cells, we used fluorescein-labeled CAMPATH-1M (FITC-CAMPATH-1M). To measure cells coated with antibody in vivo, we used fluorescein-labeled F(ab')2 fragments of rabbit anti-(rat Ig) (FITC-anti-(rat Ig)). To measure the antibody concentration in serum samples, we incubated them with normal lymphocytes and detected bound antibody with FITC-anti-(rat Ig). The mean fluorescence was compared with standard curves obtained using known concentrations of CAMPATH-1 antibodies diluted in heat-inactivated normal human serum. Labeled cells were fixed with 1% formaldehyde and analyzed on a FACS II cell sorter (Becton Dickinson, Sunnyvale, CA) or a Cytofluorograf (Ortho, Raritan, NJ).

**Complement-mediated lysis.** Cells were labeled with 11Cr and incubated with serial dilutions of CAMPATH-1M or CAMPATH-1G and human AB serum at 37°C for 45 minutes. Released radioactivity was counted as a measure of cell lysis, and the concentration of antibody giving 50% of maximal lysis was calculated.

**Anti-(rat Ig) responses.** Patients' sera were tested for anti-(rat Ig) by agglutination of RBCs coupled with CAMPATH-1G.

**Immunoglobulin and T-cell receptor (TCR) gene rearrangements.** High-molecular-weight DNA prepared from blood or marrow mononuclear cells was digested with the restriction endonucleases according to the manufacturer's instructions. The DNA was electrophoresed in 0.8% agarose and blotted overnight onto nylon membranes. The filters were probed with 32P-oligo primer-labeled gel-purified DNA fragments (specific activity > 10^9 cpm/μg) and finally washed in 0.1 x SSC at 68°C. Filters were autoradiographed for a minimum of 16 hours. Details of probes and restriction

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<th>Marrow Infiltration (%)</th>
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Table 1. Summary of Patients Treated With CAMPATH-1 Antibodies

Patients usually received treatment MoAbs for ten days. Disease activity was assessed immediately before and at the end of therapy by blood cell count and, when appropriate, by marrow infiltration and size of tumor masses, which were confirmed by computed tomography scanning whenever practicable. In general, lymph nodes and extranodal masses were only slightly reduced, but patients 9 and 14 did show some improvement.

Abbreviations: NHL, non-Hodgkin lymphoma; HCL, hairy cell leukemia; cALL, common acute lymphoblastic leukemia; PLL, prolymphocytic leukemia; B-ALL, B-cell acute lymphoblastic leukemia.
enzymes and size of germline fragments are as follows: probe Ig J\textsubscript{H} (clone C76R51A\textsuperscript{14}), restriction enzyme BglII; germline fragment size in kilobases (kb) 4.0; probe TCR\textsubscript{\delta} (clone M13H60), restriction enzyme EcoR1, and germline fragment (in kb) 3.2, 1.5.

RESULTS

Table 1 summarizes the results in 18 patients treated with CAMPATH-1G. Also included for comparison are similar data for two other patients, previously reported,\textsuperscript{21} treated with CAMPATH-1M. We describe the first two patients treated with CAMPATH-1G in more detail below because we were able to compare the effects of different antibody isotypes in them.

Case 1: Treatment with IgM and IgG2b antibodies. A 67-year-old man was diagnosed with stage A(0) B-cell CLL in 1979 at a routine medical examination. He remained well on no treatment until January 1986 when prolymphocytic transformation occurred. His WBC count, previously stable at 60 $\times$ 10\textsuperscript{9}/L increased to 360 $\times$ 10\textsuperscript{9}/L, and his spleen enlarged to 10 cm below the costal margin. He was started on IV combination (CHOP: cyclophosphamide, hydroxydannorubicin, vincristine, and prednisolone) chemotherapy; six courses induced only a partial remission (WBC count 85 $\times$ 10\textsuperscript{9}/L, spleen palpable to 6 cm); he was therefore considered suitable for a trial of antibody therapy.

CAMPATH-1M was administered to the patient on days 1 and 2, followed by CAMPATH-1G from days 3 to 12. Infusion of CAMPATH-1M resulted in prompt lowering of the WBC count (Fig 1A) associated with a decrease in serum complement levels (Fig 1B). At the end of the second infusion, the CH50 and C4 component were below detectable levels. Prolymphocytes reappeared in the blood within five minutes after the CAMPATH-1M infusions were stopped, and within 24 hours the cell count had returned to the pretreatment level. Similar results have been obtained in other patients with lymphoid malignancies (Table 1).\textsuperscript{21}

Infusion of CAMPATH-1G resulted in an equally profound lowering of the tumor cell count but no demonstrable effect on serum complement (Fig 1A and B). The rebound was now less rapid, and repeated doses resulted in a lowering of the cell count until by day 6 no prolymphocytes or lymphocytes could be detected in the peripheral blood. This was achieved with no major effect on hemoglobin concentration or platelet or neutrophil count (Fig 2). By day 10, the splenomegaly had also resolved.

Cells present in the blood during and after the antibody infusions on days 1 through 4 were studied by immunofluorescence to assess the degree of labeling with antibody in vivo. The staining with FITC-anti-(rat Ig) was weak as compared with controls that had first been labeled with CAMPATH-1M or CAMPATH-1G in vitro, indicating that the in vivo labeling was unlikely to have been saturating. This was not surprising since the highest plasma concentration that could have been obtained was only $\approx$10 $\mu$g/mL, not a saturating dose.\textsuperscript{22}

A bone marrow aspirate taken on day 10 showed no detectable lymphoid cells but hematopoiesis was otherwise active. A buffy coat preparation of peripheral blood taken on the same day showed both neutrophils and monocytes but no
lymphocytes (Fig 3B and C). DNA prepared from mononuclear fractions of marrow aspirated on days 10 and 24 and probed with an immunoglobulin JH probe revealed only germline configuration under conditions in which as few as one in 300 cells bearing a rearrangement might have been detected (Fig 4).

Serum levels of antibody were measured with an immunofluorescence assay calibrated with control samples of known concentration (Table 2). The minimum concentration detectable was -0.1 μg/mL IgM or 0.02 μg/mL IgG2b. During the first four days of therapy, antibody was rapidly cleared (presumably by binding to tumor cells), but by day 7 it was still detectable 21 hours after infusion, although at a low level. This study did not provide enough information to calculate a half-life ($t_{1/2}$), but by comparison with levels of several other patients (not shown), we conclude that the plasma levels are significantly influenced by the tumor burden.

Florid CNS disease became evident by day 14. Hindsight showed that symptoms of cervical root involvement had been present for several weeks, although at the time they were ascribed to a coincident severe vincristine neuropathy. The cytology of these cells was distinct from the original blood prolymphocytes; they had deeply basophilic cytoplasm with very large nucleoli (Fig 3D). Because they were recognized by CAMPATH-1 in vitro, we attempted to treat this disease using 1 and 10 mg CAMPATH-1G intrathecally on consecutive days. This was well tolerated but produced little change in the cerebrospinal fluid (CSF) cell count. All cells obtained 24 hours after intrathecal injection could be stained brightly by FITC-anti-(rat Ig), suggesting that there had been little or no clearance. Treatment with alternate-day intrathecal methotrexate and craniospinal irradiation was started, but the patient rapidly became paraplegic from a lesion in the lower cervical spine despite further radiotherapy to that area. The patient received no further systemic therapy, and eventually systemic relapse occurred 7 weeks after the antibody therapy. The patient died of staphylococcal pneumonia shortly afterward.

Morphologically, the relapse cells resembled those obtained from CSF more closely than the original prolymphocytes with deeply basophilic cytoplasm (Fig 3E). They expressed much more surface Ig than the original prolymphocytes but comparable amounts of CAMPATH-1 antigen and were still equally sensitive to complement-mediated lysis. Southern blot analysis with the Ig JH probe showed that
Fig 3. Cytology of cells from patient 1. (A) Blood prolymphocytes before treatment. (B) Blood buffy coat on day 10. (C) Marrow aspirate on day 10. (D) CSF cells on day 14. (E) Blood relapse cells on day 56.
the relapse cells had the same Ig gene rearrangement as the original tumor cells, although there was an extra weaker restriction fragment at 2.0 kb (Fig 4, left panel, track D). In contrast, analysis with the TCR \( \gamma \) probe revealed substantial differences, since the original prolymphocytes were of germline configuration whereas the relapse cells exhibited rearrangement of one allele and deletion of all others (Fig 4, right panel). Unfortunately, insufficient cells were present in the CSF sample to permit cytogenetic or Southern blot analysis.

**Table 2. Serum Level of Antibody in Patient 1**

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Serum samples were assayed for rat antibody by incubation with normal lymphocytes which were then stained with FITC-anti-(rat Ig). The fluorescence intensity was compared with controls incubated with known concentrations of CAMPATH-1M or CAMPATH-1G.

*Some serum samples were not available.

**Case 2: Treatment with IgG2a, IgM, and IgG2b antibodies.** A 67-year-old woman with B-cell CLL had previously been treated with both oral and IV cyclophosphamide as well as chlorambucil and prednisone. In December 1985, she was treated with both CAMPATH-1M and YTH 34.5 (IgG2a), but neither produced a lasting effect (Fig 5) although as in other patients a marked drop in complement levels was noted with the IgM and, to a lesser extent, with the IgG2a antibody. She subsequently received three courses of CHOP chemotherapy followed by daily oral cyclophosphamide and prednisolone in varying doses. By January 1987, the leukemia had undergone prolymphocytic transformation (56% of the cells expressing CD9); she had a rapidly rising lymphocyte count and was severely neutropenic. Because no anti-rat antibody was detectable, a second therapeutic attempt was made using CAMPATH-1M and CAMPATH-1G.

CAMPATH-1M was administered in incremental doses with no premedication. The patient developed angioedema of lips and tongue during the third hour of infusion, by which time she had received ~25 mg antibody; this may have been caused by complement activation. The antibody infusion was stopped, and she was administered 100 mg hydrocortisone IV. The edema resolved in the next three hours. For the next seven days, CAMPATH-1G was infused at 25 mg/day. No initial side effects other than fever and rigors were associated with the first two infusions. However, on days 7 and 8, the patient experienced asymptomatic infusion-related fever which was believed to be due to an anti-(rat Ig) response; therefore, antibody therapy was stopped. In fact, no anti-(rat...
EFFECTS OF CAMPATH-1 ANTIBODIES IN VIVO

Fig 5. Effects of antibodies of different isotype in patient 2. Patient 2 with BCLL was treated in December 1985 with YTH34.5 (IgG2a) and CAMPATH-1M (IgM). By January 1987, the disease had progressed into prolymphocytic transformation, and the patient received further treatment with CAMPATH-1M and CAMPATH-1G. Antibodies were infused in three hours at the times and doses shown, and peripheral blood lymphocytes were monitored.

IgG2a could be detected in the serum taken at that time (possibly due to clearance by the infused IgG2a), but it was clearly detected 21 days after the last antibody infusion. The anti-(rat IgG2a) titer of the latter serum sample was 1:120, whereas the titer of the serum taken before treatment or at the end of antibody infusions was <1:10.

This therapy induced long-lasting clearance of lymphocytes from the peripheral blood but only a slight reduction in bone marrow infiltration—from 97% to 87%. However, there was a rise in blood neutrophil count from $0.2 \times 10^9/L$ to $0.7 \times 10^9/L$. The rate of reappearance of blood lymphocytes was very slow in contrast to the previous therapy with CAMPATH-1M and YTH 34.5 (IgG2a). The patient remained well on no therapy for 6 months; severe autoimmune hemolytic anaemia then developed and was treated with high-dose oral prednisolone. Later, she acquired a multiresistant Staphylococcus aureus infection which proved impossible to eradicate despite repeated courses of IV antibiotics; eventually, she died of staphylococcal septicemia 17 months after therapy with CAMPATH-1G.

Cases 3 through 18: Treatment with IgG2b. Table 1 shows brief details of 16 other patients treated with CAMPATH-1G as a single agent according to the protocol. We intentionally treated a variety of lymphoproliferative disorders in which the tumor cells expressed the CAMPATH-1 antigen, and we also introduced some variation in the dosing schedule to gain experience for the design of a larger trial. The main findings were that, irrespective of the absolute count, malignant and normal lymphocytes were always cleared from the peripheral blood (16 of 16), marrow infiltration was reduced (five of nine) or cleared (four of nine), and splenomegaly was improved (six of six). The reduction in tumor load was often accompanied by an improvement in normal marrow function as measured by peripheral blood neutrophil counts. For example, in patient 3, the neutrophil count rose from $0.5 \times 10^9/L$ at the beginning of treatment to $2.7 \times 10^9/L$ by day 17. Significant effects on blood lymphocyte count were observed with daily doses as low as 5 mg. The maximum total dose was 300 mg administered in ten days or fewer. At these doses, palpable lymph nodes and extranodal masses showed little or no regression. Two patients had CNS disease (besides case 1 above) which was unaffected by systemic antibody treatment.

Side effects of CAMPATH-1G. A spectrum of short-term reactions was observed. In the first two days, all but one patient experienced fever and rigors which were minimized by slowing the rate of administration. Oral paracetamol and, if necessary, IV meperidine appeared to be equally as effective as methylprednisolone in ameliorating these effects. One patient (patient 10) had fever, malaise, nausea, and vomiting with most doses. Occasionally, fever developed following the final doses (days 8 through 10), which we considered suggestive of an antiglobulin response since this was later confirmed serologically in two of three patients who suffered from this symptom. During the next infusion of antibody, two of these patients suffered bronchospasm which responded to inhaled bronchodilators and oxygen but precluded further treatment. No other physical or biochemical abnormalities were induced by CAMPATH-1G except a transient rise in liver transaminases in all patients to a maximum of three times the upper limit of normal. No opportunistic infections occurred.
DISCUSSION

We have obtained several rat MoAbs with the same specificity as CAMPATH-1M but of different isotype.22,23 Of these, the IgM, IgG2a, and IgG2b have been tested in vivo. The IgM and IgG2a produced only transient lowering of the blood lymphocyte count but no demonstrable effect on solid tissues. This type of response with rapid "rebound" of cells into the circulation has been observed with a variety of MoAbs in vivo and may result mainly from temporary sequestration of antibody-coated cells. In contrast, the IgG2b CAMPATH-1G (a class-switch variant of the IgG2a) produced relatively long-lasting lymphopenia in all patients; in certain cases, there were also pronounced effects on spleen and bone marrow.

The data suggest that CAMPATH-1G causes cell destruction rather than sequestration. Several patients had long-lasting clearance of tumor cells; in patient 1, marrow and blood remission lasted 6 weeks, during which no tumor cells or lymphocytes could be detected either morphologically or by Southern blot analysis. At eventual relapse, the cells differed from the original prolymphocytes in cytology, expression of surface Ig, and in organization of the TCR-γ gene. We suspect that the final systemic relapse may have arisen from resistant CNS disease. In cases in which marrow infiltration was not cleared but only reduced (eg, case 2), the recruitment of tumor cells into the circulation occurred in weeks rather than minutes as had occurred with the IgM antibody. In several cases, normal hematopoiesis improved during antibody therapy.

The cytotoxicity of CAMPATH-1G may depend on several features, including the high density of the target antigen (~5 × 10^5 molecules/cell), its inability to modulate in the presence of antibody, and the effective interaction of rat IgG2b with human effector mechanisms. Redistribution of antigen in the presence of antibody can render cells insensitive to further antibody; this has been a significant limitation to therapy with other MoAbs.24-28 We previously showed that CAMPATH-1M does not modulate even after 24-hour culture in vitro,21 and the present results obtained from intrathecal administration of CAMPATH-1G suggest that modulation does not occur in vivo. The importance of antibody isotype is demonstrated in patient 2, who received IgM, IgG2a, and IgG2b antibodies; only the IgG2b produced long-lasting effects. This probably reflects the ability of rat IgG2b to interact with human cellular effectors since all three antibodies are lytic with human complement but only the IgG2b can bind to human Fc receptors and mediate ADCC.15,22,23 The IgM caused complement activation in vivo yet was comparatively ineffective. However, this does not imply that complement activation is unimportant since C3 fixation may contribute to effective opsonization in combination with an antibody that binds to Fc receptors. The IgG2b antibody might give adequate C3 fixation without a measurable effect on total hemolytic complement. Cellular effectors could include killer cells, natural killer cells, monocytes, or the phagocytic cells of the reticuloendothelial system. One potential problem is that these cells themselves could be targets for the antibody. Since so little is known about the key effector mechanisms in vivo, determination of the limiting factors in patients who made only a partial response is difficult, although the possible appearance of anti-(rat Ig) could have been a factor in some cases.

Therapy with CAMPATH-1G in patient 2 was limited by generation of an anti-(rat Ig) response apparently by day 8. This is earlier than usual and may have resulted from a degree of priming by the IgG2a and IgM antibodies that had been administered a year earlier. Because clear evidence for an antiglobulin response was observed only in one other patient, more effective therapy might have been achieved by longer-term administration of antibody in the other patients. Recently, a reshaped human IgG1 antibody was constructed by genetic engineering using the hypervariable regions from CAMPATH-1G29; this may be even less immunogenic. The human antibody is at least as potent as the rat in vitro,29 and preliminary results suggest it has equivalent or superior activity in vivo,30

We showed that as a consequence of the wide expression of the CAMPATH-1 antigen,14 a broad range of lymphoid malignancies may be treatable. An advantage of the wide specificity is that the clonogenic cells may be more likely to express the target antigen than might antibodies of more restricted specificity. A disadvantage is that normal T and B lymphocytes will also be destroyed. Theoretically this could result in loss of "immune surveillance" and an increased susceptibility to infections. Ultimately, we would expect normal lymphocytes to develop again from stem cells spared by the antibody,19,22 and we know that normal lymphocytes ultimately return in patients treated with CAMPATH-1G for kidney graft rejection (P.J. Frient, R.Y. Calne, G. Hale, and H. Waldmann, unpublished observations, 1987-1988).

On the other hand, in some cases depletion of normal lymphocytes could be desirable (eg, in conditioning for bone marrow transplantation when added immunosuppression could help overcome the problem of graft rejection).11,20 CAMPATH-1G might be particularly useful to destroy minimal residual disease in patients receiving marrow transplantation for lymphoid malignancies. We showed that injected MoAb can mediate substantial cytoreduction of lymphoid cells. This approach, using unconjugated antibody, has important implications for immunosuppressive therapy and treatment of lymphoid tumors.

ACKNOWLEDGMENT

We express our gratitude to the patients and their families, and to nursing staff and medical colleagues at Addenbrooke’s Hospital, particularly Dr P. Carey, who participated in the early studies with patient 2. We thank Drs D. Gilmore and H.S. Kruger-Gray for help with fluorescence analysis, Dr T.H. Rabbits for DNA probes, and Dr D. Brown for complement assays. We also thank Caroline Buckie, Gill Martin, and Mike Barber for skilled technical assistance. CAMPATH is a trademark of Wellcome Foundation.

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Effects of CAMPATH-1 antibodies in vivo in patients with lymphoid malignancies: influence of antibody isotype

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