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Effective Therapy for a Murine Model of Adult T-Cell Leukemia with the Humanized Anti-CD52 Monoclonal Antibody, Campath-1H

Zhuo Zhang, Meili Zhang, Carolyn K. Goldman, Jeffrey V. Ravetch, and Thomas A. Waldmann[1]

Metabolism Branch, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland 20892-1374 [Z. Z., M. Z., C. K. G., T. A. W.], and Laboratory of Molecular Genetics and Immunology, The Rockefeller University, New York, New York 10021 [J. V. R.]

ABSTRACT

Adult T-cell leukemia (ATL) develops in a small proportion of human T-cell leukemia virus I-infected individuals. Presently, there is no effective therapy for ATL. A murine model of ATL was produced by introducing leukemic cells (MET-1) from an ATL patient into nonobese diabetic/severe combined immunodeficient mice. The MET-1 cells are activated T cells that express CD2, CD3, CD4, CD25, CD122, and CD52. We evaluated the efficacy of Campath-1H (alemtuzumab; a humanized monoclonal antibody directed to CD52), alone and in combination with humanized anti-Tac (HAT) directed to CD25 (interleukin 2 receptor α) or with MEDI-507 directed to CD2. We observed that four weekly treatments with 4 mg/kg HAT significantly prolonged survival of MET-1-bearing mice. However, the survival of mice receiving 4 weeks of 4 mg/kg Campath-1H was significantly longer than that of the group receiving four weekly treatments with HAT ($P < 0.001$). Treatment with Campath-1H for 4 weeks led to a striking prolongation of the survival of MET-1 ATL-bearing mice that was comparable with that of tumor-free nontreated controls. Using Fc receptor (FcR) $\gamma^{-/-}$ mice, we found that FcRγs on polymorphonuclear leukocytes and monocytes are required for Campath-1H-mediated tumor killing *in vivo*. These results demonstrate that Campath-1H has therapeutic efficacy on ATL *in vivo* in that the life span of the Campath-1H treatment group was comparable with that of mice that did not receive a tumor or therapy. The main tumor killing mechanism with Campath-1H *in vivo* involves FcRγ-containing receptors (*e.g.*, FcRγIII) on polymorphonuclear leukocytes and macrophages that mediate antibody-dependent cellular cytotoxicity and/or trigger cross-linking induced apoptosis. This study provides support for a clinical trial of Campath-1H in the treatment of patients with T-cell leukemias and lymphomas.

INTRODUCTION

ATL[2] develops in a small proportion of HTLV-I-infected individuals (1). At present, there is no effective therapy for ATL, and patients progress to death with a median survival duration of 9 months for those with acute ATL and 24 months for those with chronic ATL (2). The conventional therapies (*i.e.*, multidrug chemotherapy regimens or zidovudine with IFN-α) do not appear to prolong the life of patients with ATL (2, 3). A murine model of ATL was developed by introducing leukemic cells (MET-1) from an ATL patient into NOD/SCID mice (4). New therapeutic agents have been tested in this model before initiating clinical trials (4–6). The MET-1 ATL cells in this model are activated T cells that express CD2, CD3 dim, CD4, CD122, and CD25. They also highly express CD52. In earlier studies, anti-CD25 mAbs (*e.g.*, murine and HAT) were tested in this model with promising results. Furthermore, clinical trials showed that HAT-based immunotherapies manifested efficacy in the therapy of patients with ATL. In the present study, we targeted CD52 on the human xenograft MET-1 ATL cells using a humanized mAb, Campath-1H (alemtuzumab).

CD52 is a glycosylated protein with a large complex *N*-oligosaccharide that is attached to the cell membrane by a glycosylphosphatidylinositol anchor (7–9). The molecular weight of CD52 is approximately 21,000–28,000. The function of CD52 is unknown. Some data indicated that CD52 is involved in T-cell activation through the CD2 pathway or through T-cell receptor-dependent signal transduction (10, 11). CD52 is expressed on peripheral blood lymphocytes, monocytes, and macrophages with weak expression on neutrophils (11, 12). Campath-1H is a humanized antibody that is directed against CD52. It has been used for the treatment of refractory chronic lymphocytic leukemia (13) and for the prevention of graft-*versus*-host disease associated with bone marrow transplantation (14). It is also active against T-cell prolymphocytic leukemia (15). Infectious complications are the most significant side effects associated with its usage. Other side effects are fevers, chills, nausea, and vomiting.

In this study, we investigated the efficacy of Campath-1H in a xenograft ATL model when used alone and in combination with HAT (an anti-CD25 antibody) or with MEDI-507 (humanized antibody directed to CD2). The scientific basis for these combinations is that Campath-1H, MEDI-507, and HAT target distinct cell surface receptors (CD52, CD2, and CD25, respectively) that are expressed on the MET-1 ATL cells, an observation that suggested that they might manifest additive or synergistic efficacy. We were particularly interested in the mechanism underlying the tumor killing action mediated by Campath-1H on ATL *in vivo*. We demonstrated that the efficacy was lost in FcR$\gamma^{-/-}$ mice, suggesting that the expression of the receptor FcRγIII that utilizes the FcRγ chain is required for the effective action of this mAb in the mouse leukemia model.

MATERIALS AND METHODS

Mouse Model of ATL. Female NOD/SCID mice were purchased from The Jackson Laboratory (Bar Harbor, ME). The mice were used in studies at the age of 6–12 weeks. Leukemia was established by i.p. injection of 15×10^6 freshly isolated MET-1 cells. Mice were randomly assigned to each group when their sIL-2Rα levels reached a range of 1,000–10,000 pg/ml serum. These levels were observed at approximately 10–14 days after tumor inoculation, at which time treatments were initiated. In the second set of studies, the tumor burdens were higher and involved double the sIL-2Rα level when compared with the first set of studies. sIL-2Rα levels were from 1,000 to 25,000 pg/ml serum in the second set of studies. The FcRγ knockout mice were generated in the laboratory of Jeffrey Ravetch (Rockefeller University, New York, NY). In the study directed toward defining the mechanism involved in tumor killing, very large tumor burdens were used in the FcRγ knockout and FcRγ intact NOD/SCID mice. In these latter studies, mice with sIL-2Rα levels of 20,000–90,000 pg/ml serum (mean 80,000 pg/ml) were randomly assigned to the study groups for the experiments.

Measurement of sIL-2Rα and Soluble $\beta_2\mu$ by ELISA. Throughout the therapy experiments, the serum concentrations of soluble human IL-2Rα and human $\beta_2\mu$, which were used as surrogate tumor markers, were measured using ELISA kits purchased from R&D Systems (Minneapolis, MN). The

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[1] To whom requests for reprints should be addressed, at Metabolism Branch, National Cancer Institute, NIH, Building 10, Room 4N115, 10 Center Drive, Bethesda, MD 20892-1374. Phone: (301) 496-6656; Fax: (301) 496-9956; E-mail: tawald@helix.nih.gov.

[2] The abbreviations used are: ATL, adult T-cell leukemia; HAT, humanized anti-Tac; HTLV, human T-cell leukemia virus; SCID, severe combined immunodeficient; NOD, nonobese diabetic; mAb, monoclonal antibody; IL, interleukin; sIL-2Rα, soluble IL-2 receptor α; IL-2Rα, IL-2 receptor α; FcR, Fc receptor; CDC, complement-dependent cytotoxicity; ADCC, antibody-dependent cellular cytotoxicity; $\beta_2\mu$, β_2-microglobulin; HuMikβ1, humanized Mikβ1; NK, natural killer.

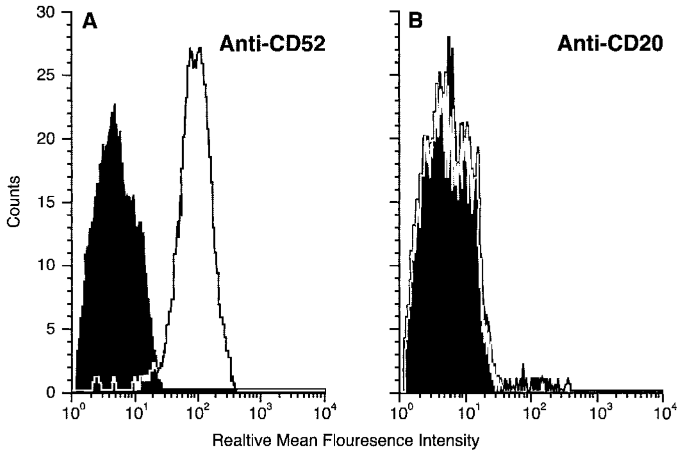


Fig. 1. The binding of Campath-1H to MET-1 ATL cells was evaluated by fluorescence-activated cell-sorting analysis. The MET-1 ATL cells were harvested as described in "Materials and Methods." The primary mAbs used were humanized anti-CD20 and Campath-1H. The secondary antibody was a rat antihuman IgG Fc fragment conjugated with FITC. In *A*, the primary antibody was Campath-1H directed to CD52. The isotype control is represented by the *solid area*, whereas the *line* represents the humanized anti-CD52. In *B*, the *solid area* represents the isotype control, and the *line* represents humanized anti-CD20.

ELISA tests were performed as suggested in the manufacturer's kit inserts. The more sensitive and more accurate marker human sIL-2Rα was used as the pretherapy entry parameter, but $\beta_2\mu$ was used posttherapy because HAT interacts with sIL-2Rα, precluding its accurate assessment.

Analysis of the Binding of Campath-1H to MET-1 ATL Cells. The binding of Campath-1H to CD52 was analyzed by flow cytometry before the therapeutic experiments were conducted. The MET-1 leukemic cells were prepared for phenotypic analysis in the same fashion used in the phenotypic analysis performed in Ref. 4. The cells were stained with the primary antibody, Campath-1H, or rituximab on ice for 30 min. They were washed and then stained with a FITC-labeled antibody directed against the human IgG Fc fragment. After washing, the cells were analyzed for the binding of Campath-1H directed to CD52 on the MET-1 cells using a Becton Dickinson FACSort Flow Cytometer.

mAbs. The humanized mAb Campath-1H that recognizes CD52 was obtained from Ilex Pharmaceuticals (San Antonia, TX), whereas MEDI-507 against CD2 was a gift from BioTransplant Inc. (Charlestown, MA). HAT (daclizumab, Zenapax), a humanized mAb directed toward the IL-2Rα chain CD25), and HuMikβ1, an antibody (anti-CD122) directed to the IL-2 and IL-15 binding sites of the IL-2/15Rβ subunit, were obtained from Hoffmann-La Roche (Nutley, NJ). Rituximab was obtained from IDEC Pharmaceuticals (San Diego, CA).

Treatment with Antibodies. For the evaluation of therapeutic efficacy, groups of 10 NOD/SCID mice each were injected with 10 million MET-1 leukemic cells i.p. and randomly assigned to groups that had comparable levels of the surrogate tumor marker, the serum sIL-2Rα (Tac, CD25). In the small tumor burden trial, the animals were treated when their sIL-2Rα levels ranged from 1–10,000 pg/ml (10–14 days after introduction of MET-1 leukemic cells into the mice). The groups of mice were given PBS, Campath-1H, HAT, or the combination of Campath-1H with HAT at a dose of 100 μg of each mAb i.v. weekly for 4 weeks.

In the large tumor burden trial, mice were treated when their sIL-2Rα levels ranged from 1,000 to 25,000 pg/ml. The groups of 10 mice were given PBS, Campath-1H, HAT, MEDI-507, or the combination of Campath-1H with MEDI-507 or with HAT at a dose of 4 mg/kg (100 μg/mouse) of each mAb i.v. weekly for 4 weeks. A final group of NOD/SCID mice was included that did not receive a tumor or a therapeutic agent to serve as a tumor-free and treatment-free control. In a study to define the mechanism of action of Campath-1H, the mAb was given weekly for 4 weeks by i.p. injection to FcR$\gamma^{-/-}$ mice and to FcRγ intact mice. Throughout the studies, the leukemic progression was evaluated using an ELISA assay for human $\beta_2\mu$ in the serum as well as by monitoring the survival of the mice using Kaplan-Meier analysis.

Statistics. StatView was used to generate Kaplan-Meier cumulative survival plots. The unpaired *t* test was conducted in the analysis of $\beta_2\mu$ levels.

RESULTS

Demonstration of Campath-1H Binding to CD52 Expressed on MET-1 ATL Cells

Using fluorescence-activated cell-sorting analysis, we demonstrated that Campath-1H binds to MET-1 ATL cells (Fig. 1*A*), in contrast with the lack of reactivity of the B-cell-specific, anti-CD20 mAb, rituximab (Fig. 1*B*). In additional studies, the levels of expression of CD2 and CD25 were comparable with those of CD52.

Effective Treatment of ATL Was Obtained Using Campath-1H Directed toward CD52

The Small Tumor Burden Treatment Trial in the MET-1 Model. A 4-week course of treatment with Campath-1H, HAT, and the combination of Campath-1H (100 μg i.v./week) with HAT (100 μg i.v./week) manifested therapeutic efficacy as demonstrated by the effect on the serum levels of human $\beta_2\mu$, a surrogate tumor marker in the murine model (Fig. 2) and on the survival of ATL-bearing mice (Fig. 3). When compared with the serum concentration of human $\beta_2\mu$ in the PBS control group of mice, on day 14 and 28, there was a significant reduction of $\beta_2\mu$ in the 4-week Campath-1H-treated animals on day 14 ($P < 0.05$) and day 28 ($P < 0.001$), as well as in the 4-week HAT-treated animals ($P < 0.05$ on day 14 and $P < 0.01$ on day 28) and in animals treated with the 4-week combination of Campath-1H with HAT ($P < 0.05$ on day 14 and $P < 0.001$ on day 28). Furthermore, there was a significant ($P < 0.001$) prolongation of survival of groups of mice that were treated with Campath-1H, HAT, and the combination of Campath-1H and HAT when compared with the PBS control (Fig. 3). There was a significant difference between the combination of Campath-1H with HAT and HAT alone ($P < 0.05$). The mean survival duration of the control group (PBS) was 50 days, and the mean survival of the HuMikβ1 group was 50 days. In contrast, the mean survival durations were 139 days in the 4-week Campath-1H-treated group ($P < 0.001$ *versus* PBS), 95 days in the 4-week HAT-treated group ($P < 0.05$), and 178 days in the group treated with the 4-week combination of Campath-1H with HAT ($P < 0.001$).

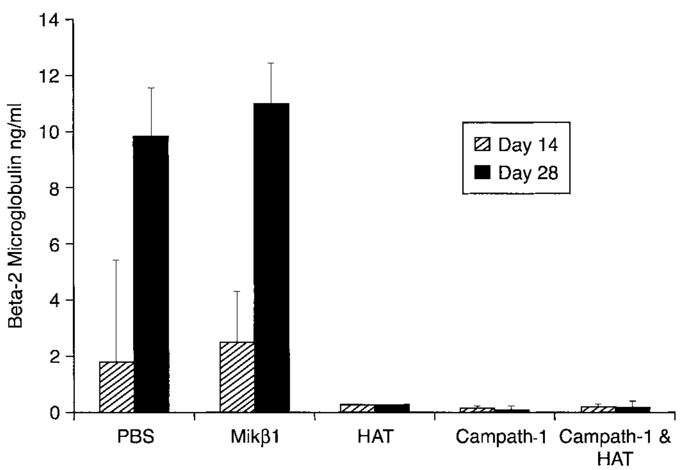


Fig. 2. Inhibition of the growth of MET-1 ATL cells in NOD/SCID mice with a small tumor burden by HAT and Campath-1H. MET-1 ATL cells were transferred into mice. The groups (10 mice/group) included those receiving PBS, four weekly doses of 4 mg/kg (100 μg/mouse) Campath-1H, four weekly doses of 4 mg/kg HAT, four weekly doses of 4 mg/kg HuMikβ1 (anti-IL-2Rβ), and a 4-week combination of 4 mg/kg each of Campath-1H and HAT. The data represent the mean concentration of human $\beta_2\mu$ in ng/ml. The 4-week Campath-1H-treated group, 4-week HAT-treated group, and the group treated with a 4-week combination of Campath-1H with HAT had significantly decreased values of $\beta_2\mu$ when compared with those of the PBS control group ($P < 0.05$). There was no significant difference in survival between the groups receiving four weekly dose of HuMikβ1 and PBS (P = nonsignificant).

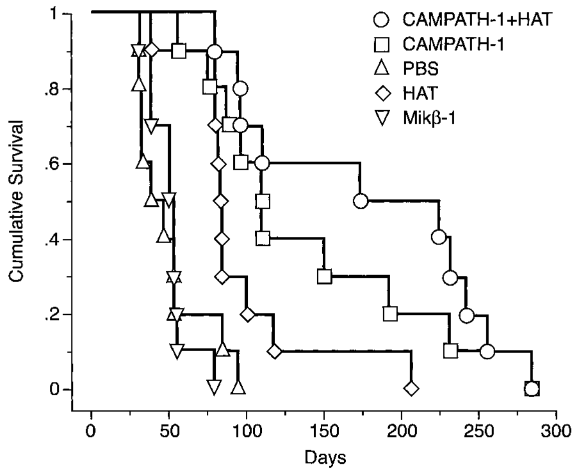


Fig. 3. Kaplan-Meier survival plot of MET-1-bearing NOD/SCID mice with a small tumor burden. The groups (10 mice/group) included those receiving i.v. PBS, 4 mg/kg HAT, 4 mg/kg HuMikβ1, 4 mg/kg Campath-1H per week for 4 weeks and the combination of 4 mg/kg Campath-1H with 4 mg/kg HAT, respectively, for 4 weeks. Survival in the treated groups was followed out to 284 days. The 4-week HAT-treated group, the 4-week Campath-1H-treated group, and the group treated with the 4-week combination of Campath-1H with HAT had significantly prolonged survival when compared with the PBS control group ($P < 0.001$). The combination treatment of tumor-bearing mice with Campath-1H and HAT for 4 weeks significantly prolonged the survival of the group when compared with the 4-week HAT treatment group ($P < 0.05$).

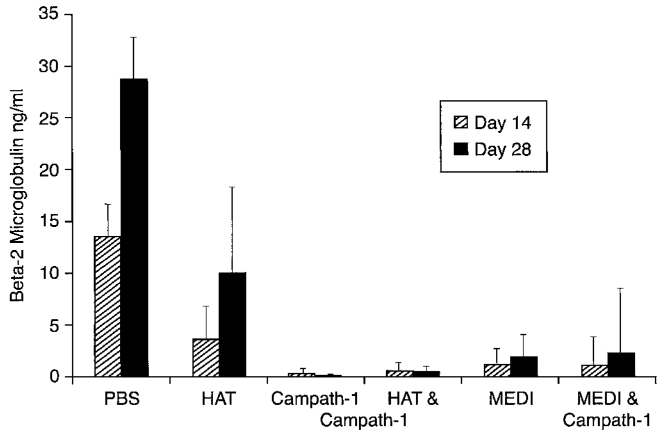


Fig. 4. Inhibition of the growth of MET-1 ATL cells in NOD/SCID mice with a large tumor burden by Campath-1H, MEDI-507, and HAT. MET-1 ATL cells were transferred into mice. The groups (10 mice/group) included those receiving PBS, four weekly doses of 4 mg/kg Campath-1H, four weekly doses of 4 mg/kg MEDI-507, four weekly doses of 4 mg/kg HAT, and a 4-week combination of 4 mg/kg each of Campath-1H with MEDI-507 or with HAT. The data represent the mean concentration of human $\beta_2\mu$ in ng/ml. The 4-week Campath-1H-treated group, the 4-week HAT-treated group, and the group treated with the 4-week combination of Campath-1H with MEDI-507 or with HAT had significantly decreased values of $\beta_2\mu$ when compared with those of the PBS control group ($P < 0.001$).

The Larger Tumor Burden Treatment Trial in the MET-1 Model. The tumor burden was double that of the small tumor burden group. A 4-week course of treatment with Campath-1H, HAT, and MEDI-507 alone (4 mg/kg/week i.v. of each mAb) and the combination of Campath-1H (4 mg/kg/week i.v.) with HAT (4 mg/kg/week i.v.) or MEDI-507 (4 mg/kg/week i.v) also demonstrated therapeutic efficacy as demonstrated by the effect on the serum levels of human $\beta_2\mu$ a surrogate tumor marker in the murine model (Fig. 4) and on the survival of ATL-bearing mice (Fig. 5). When compared with the serum concentration of human $\beta_2\mu$ in the PBS control group of mice, on day 14 and 28, there was a significant reduction of $\beta_2\mu$ in the Campath-1H-treated animals ($P < 0.001$), 4-week MEDI-507-treated animals ($P < 0.001$), 4-week HAT-treated animals ($P < 0.001$), animals treated with the 4-week combination of Campath-1H with HAT ($P < 0.001$), and animals treated with the 4-week combination of Campath-1H with MEDI-507 ($P < 0.001$). Furthermore, there were significant reductions in $\beta_2\mu$ levels when 4-week treatment of Campath-1H and 4-week treatment combinations were compared with 4-week treatment with HAT alone ($P < 0.05$). Human $\beta_2\mu$ levels were undetectable in 80% of surviving mice in each group that received the four weekly treatments with Campath-1H alone and its combination groups when measured on day 60 of the posttreatment period. The mean survival duration of the control group (PBS) was 32 days. All of the mice in the PBS group died by day 40 of the study. In contrast, 100% of the mice in the 4-week Campath-1H group, 4-week MEDI-507 group, 4-week HAT group, and 4-week combination groups receiving Campath-1H and MEDI-507 or HAT were alive at that time. The mean survivals of treated groups were 203 days in the 4-week Campath-1H group, 151 days in the 4-week MEDI-507 group, 73 days in the 4-week HAT group, 239 days in the group treated with the combination of Campath-1H with MEDI-507, 224 days in the group treated with the combination of Campath-1H with HAT, and 241 days in life span control group. Furthermore, survival in the 4-week Campath-1H group and its combination groups was comparable with that of the tumor-free life span control group of mice that did not receive either tumor or a therapeutic agent.

FcRγ Expression Is Required for Effective Campath-1H Action

Campath-1H clearly had a therapeutic effect in our murine model of an ATL. We wished to define its mode of action. Several mechanisms could theoretically be involved. It could involve CDC, or, alternatively, it could use ADCC. As discussed below, CDC does not appear to be involved, and we focused on ADCC as a potential effector mechanism. Because NOD/SCID mice were deficient in both T and NK cells, we considered the hypothesis that monocytes and granulocytes expressing FcRs were the potential effector cells *in vivo*. An experiment involving parallel groups of mice that differed in their expression of FcRγ was conducted to define the mechanism involved in the tumor killing by Campath-1H. FcRγ knockout NOD/SCID mice bearing MET-1 leukemia were used in one study group, whereas the FcRγ was wild-type in the other study group of mice. In the FcR$\gamma^{-/-}$

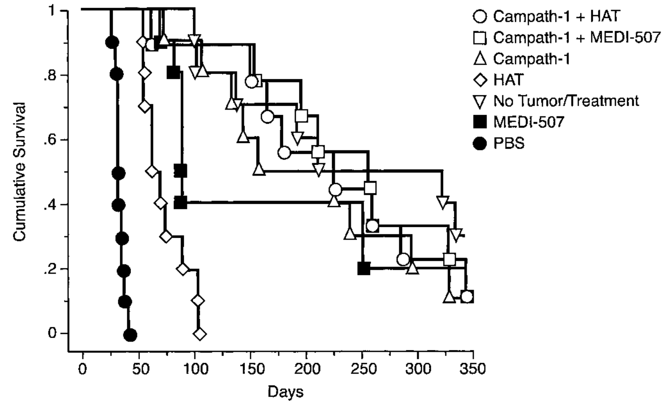
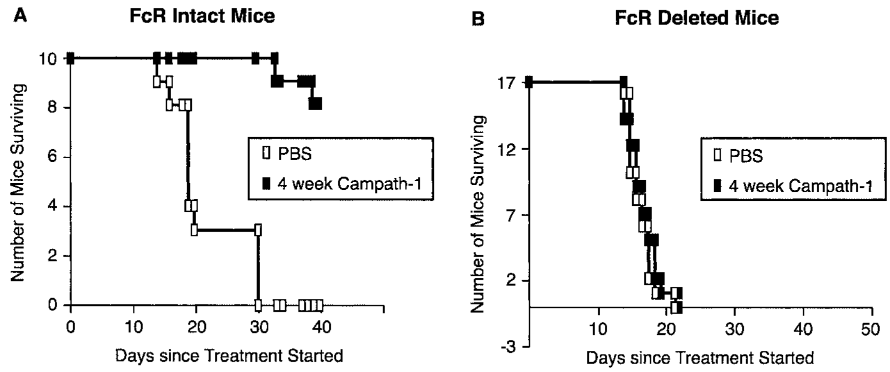


Fig. 5. Kaplan-Meier survival plot of MET-1-bearing NOD/SCID mice with large tumor burden. The groups (10 mice/group) included those receiving i.v. PBS, 4 mg/kg HAT, 4 mg/kg MEDI-507, 4 mg/kg Campath-1H per week for 4 weeks and combination of 4 mg/kg Campath-1H and 4 mg/kg MEDI-507 or with 4 mg/kg HAT, respectively, for 4 weeks. The survival in treatment groups was followed out to 350 days. The 4-week HAT-treated group, 4-week MEDI-507-treated group, 4-week Campath-1H-treated group, and the group treated with the 4-week combination of Campath-1H with MEDI-507 or with HAT had significantly prolonged survivals when compared with that of the PBS control group ($P < 0.001$). Treatment with Campath-1H alone, MEDI-507 alone, and combination treatment of tumor-bearing mice with Campath-1H and MEDI or HAT for 4 weeks significantly prolonged the survival of the group when compared with the 4-week HAT treatment group ($P < 0.05$).

Fig. 6. Kaplan-Meier survival plot of MET-1 FcγR knockout and FcγR intact ATL-bearing NOD/SCID mice. MET-1 ATL cells were transferred into mice. Once the mice developed sIL-2Rα levels of 20,000–90,000 pg/ml (reflecting a very large tumor burden), therapy with Campath-1H antibody was initiated. The groups (10 mice/group) included those receiving PBS or 4 weeks of 4 mg/kg/week Campath-1H in the FcRγ knockout ATL-bearing mice (*B*). In the parallel FcRγ intact ATL-bearing NOD/SCID mice, the groups (10 mice/group) included those receiving PBS or 4 weeks of i.p. (*A*) 4 mg/kg of weekly Campath-1H. Survival was followed out to 40 days. There was no significant statistical difference in survival between the group receiving four weekly doses of Campath-1H and that receiving PBS in the FcRγ knockout mice. In contrast, in FcRγ intact ATL-bearing NOD/SCID mice, the survival of the group receiving four weekly doses of Campath-1H was prolonged when compared with that of the control group.



group, there was no statistical difference in the survivals observed between the animals receiving four weekly doses of Campath-1H and those receiving PBS (P = nonsignificant). Both PBS-treated and 4-week Campath-1H-treated animals in the FcRγ knockout MET-1 ATL-bearing NOD/SCID mice died within 22 days of initiation of treatment (Fig. 6*B*). A contrasting pattern was observed in the FcRγ-expressing group as compared with FcR$\gamma^{-/-}$ group, where significant efficacy was manifested by the FcRγ intact group receiving Campath-1H (Fig. 6*A*). In particular, all of the animals in the PBS treatment group in FcRγ intact MET-1 ATL-bearing NOD/SCID mice died within 30 days of initiation of therapy, whereas all of the animals in the Campath-1H treatment group still survived at that time. Survival was followed out to 40 days, when 8 of 10 mice were still alive in the 4-week Campath-1H treatment group. Thus Campath-1H provides effective therapy for ATL in this model by a mechanism that requires the expression of FcRγIII, the receptor that involves FcRγ.

DISCUSSION

The MET-1 ATL model presents many features that parallel those observed in patients with ATL and thus represents a valuable model for the evaluation of the efficacy of therapeutic agents directed toward ATL (4). In earlier studies, HAT showed efficacy in the MET-1 ATL model. Furthermore, in human clinical trials, therapy with this anti-CD25, anti-Tac mAb proved effective for 6 of the 18 patients with ATL studied (16). We found that four weekly treatments with Campath-1H that is directed toward CD52 provided meaningful therapy for ATL in the MET-1 model. Furthermore, the efficacy of the four weekly Campth-1H treatments and the 4 weeks of MEDI-507 was better than that of 4 weeks of HAT treatment in terms of animal survival ($P < 0.05$). The combination of Campath-1H with MEDI-507 or with HAT was better than HAT alone in both studies ($P < 0.05$). Although the mean survival in combination groups appears better than that of Campath-1H alone, the differences did not reach statistical significance.

Several mechanisms could theoretically be involved in the action of Campth-1H in the MET-1 ATL model. These include CDC and ADCC. CDC appears to be excluded in the present model because the mice lack human complement, because there is only a limited amount of murine complement expressed in the NOD/SCID mice used, and because complement manifests poor lysing action on MET-1 ATL cells (4). Classical ADCC mediated by NK cells also does not appear to be a likely mode of action in this model because the NOD/SCID mice used as the recipients of ATL cells in our study virtually lacked functional NK cells.

The analysis of the efficacy of Campath-1H in FcR$\gamma^{-/-}$ mice was very instructive. FcRγ is required for the expression of FcRγIII, the stimulatory FcR. Efficacy of Campath-1H was observed in FcRγ intact MET-1 ATL-bearing mice but not in FcRγ knockout mice bearing MET-1 ATL. This observation supports the view that although multiple mechanisms have been suggested for the antitumor action of antibodies *in vivo*, in the case of Campath-1H, there is a dominant and necessary role played by a FcRγ-dependent mechanism. This FcR-dependent mechanism could theoretically involve ADCC mediated by FcRγIII-expressing macrophages or granulocytes. Alternatively, it could reflect FcR-bound antibody-mediated cross-linking of the target receptors leading to apoptosis. The efficacy of Campath-1H in the MET-1 ATL model parallels the previously reported requirement for FcRγ expression for an effective therapeutic response to trastuzumab and rituximab as well as to a mAb directed to a melanoma antigen in murine models of breast, B-cell, and melanoma malignancies, respectively (17, 18). In the present study comparing mAb efficacy in FcRγ knockout with FcRγ intact animal groups, the tumor burden at the onset of therapy was more than 10 times greater than that of mice in the initial trials of this study. Thus Campath-1H can significantly delay the progression of human leukemic xenografts in mice with very large ATL burdens, supporting the view that the application of Campath-1H to the therapy of ATL may be of value in the clinic. In summary, the humanized mAb Campath-1H effectively controlled leukemia in a human leukemia xenograft model through a FcRγ-requiring process presumably mediated by effector cells including monocytes and granulocytes that express FcRγIII. These studies provide support for a clinical trial of Campath-1H in patients with ATL and potentially in patients with other malignances that express CD52.

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