Efficacy of an Anti-CD7-Ricin A Chain Immunoconjugate in a Novel Murine Model of Human T-Cell Leukemia

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ABSTRACT

In vivo efficacy testing of monoclonal antibody-based drugs specific for human leukemias is hampered by the paucity of suitable animal models, due in part to the inability of many anti-human monoclonal antibodies to cross-react with antigens expressed in animal tissues or cells. Moreover, human leukemic cells have proven difficult to establish in immunosuppressed mice except as solid tumors. We report here the establishment of a murine model for human leukemia displaying features in immunosuppressed mice only. Human T-lymphoid cancer cells (CEM) injected into cyclophosphamide-pretreated NIH-III mice engrafted in all mice (n = 41), with CEM cells detected in the bone marrow, spleen, and blood 4 weeks after injection. There was no evidence of solid tumors. Treatment of CEM-engrafted mice with 4A2-RTA30, an immunoconjugate of an anti-CD7 monoclonal antibody and ricin A chain (RTA30), resulted in a 100- to 200-fold overall depletion of CEM cells from the spleen and the bone marrow (P < 0.02). This depletion was specific and toxin-dependent, as a control immunoconjugate had no demonstrable effect (P > 0.5). Depletion of CEM cells was also observed after treatment with unconjugated anti-CD7 mAb, but the effect was not significantly different from controls (P > 0.1). Therefore, significant depletion of CEM cells required the presence of the ricin A chain moiety. Further investigations revealed that CEM cells recovered from NIH-III mice expressed less CD7 antigen, but remained sensitive to subsequent in vitro exposure to 4A2-RTA30. In conclusion, we have established a model for studying the efficacy of immunoconjugates and have successfully depleted human T-lymphomoid cells from lymphoid tissues in immunodeficient mice by treatment with an anti-CD7-RTA30 immunoconjugate.

INTRODUCTION

A recent development in the treatment of lymphocytic disorders such as GvHD,2 autoimmune diseases, or cancers has been the use of immunoconjugates, which are hybrid molecules composed of mAbs conjugated to toxins (1). In such compounds, the binding site of the toxin is replaced by the mAb, which directs binding of the immunoconjugate only to those cells expressing the antigen recognized by the mAb (2). The toxin moiety is then internalized into the cell, inhibits protein synthesis, and eventually causes cell death. For example, an immunoconjugate consisting of a mAb recognizing a pan T-cell marker, such as CD5 or CD7, is specifically cytotoxic for the majority of human T-cells. Applications of this targeted immunoconjugate approach have included ex vivo purging of malignant cells from autologous bone marrow (3), elimination of mature T-cells from allogeneic bone marrow (4), in vivo prophylaxis or treatment of GvHD (5, 6), and treatment of leukemias expressing T-cell antigens (7–9). The recognition of epitopes on non-human T-cells by anti-human T-cell immunoconjugates is limited to higher primates, which precludes much in vivo assessment of efficacy prior to clinical trials. Instead, the efficacy is evaluated either by in vitro cytotoxicity assays using such immunoconjugates with appropriate human target cells (10–12) or by in vivo treatment with analogous immunoconjugates consisting of mAb directed against homologous target cell antigens that are expressed in an animal model system (13, 14). Alternatively, human leukemia cell lines have been established as s.c. solid tumors in mice for the evaluation of efficacy (15–18). Such animal models are not representative of the leukemic condition, and treatment efficacy is difficult to demonstrate. Leukemic infiltrates have been described with a few cell lines injected into immunosuppressed mice. Only one of many human T-ALL cell lines showed engraftment in lymphoid tissues of nude mice, preconditioned with whole-body irradiation (19, 20). Animal models for human non-T-ALL in SCID and NIH-III mice (21) and for human mixed lineage leukemia in SCID mice (22) also have been reported. Such engrafted non-T-leukemia (22) and disseminated B-cell lymphoma (23) models have been recently shown to respond to treatment with immunoconjugates.

We report here the establishment of a novel murine model to study the efficacy of immunoconjugates on human T-cell leukemias in immunosuppressed NIH-III mice engrafted with CEM leukemia cells following i.v. administration.

MATERIALS AND METHODS

mAb and Immunoconjugates. mAbs Leul (IgG2a anti-CD5), HLe-1 (IgG1 anti-CD45), and control (IgG2a anti-keyhole limpet hemocyanin) conjugated to FITC were obtained from Becton-Dickinson (San Jose, CA). FITC-4A2 (IgG2a anti-CD7) and FITC-S9.1 (IgG2b anti-CD7) were prepared at XOMA according to standard procedures (24).

Murine mAbs 4A2, IND1 (IgG2a anti-melanoma), and H65 (IgG1 anti-CD5) were conjugated to RTA30 with 5-methyl-2-iminothiolane (25) following standard procedures (26) to produce the immunoconjugates referred to as 4A2-RTA30, IND1-RTA30, and H65-RTA30, respectively. The toxin to mAb ratio was approximately 1.1 for 4A2-RTA30, 1.7 for H65-RTA30, and 1.3 and 2.7 for 2 lots of IND1-RTA30. There was no detectable free mAb in the immunoconjugate preparations, with the exception of one lot of IND1-RTA30, which contained 4% free mAb.

Mice. Outbred male NIH-III (N:NIH-bg-nu-xid) mice (27) aged from 4 to 11 weeks were obtained from Charles River Laboratories (Raleigh, NC) and housed in our specific pathogen-free animal facility. The mice were kept in filter-top cages in a laminar flow hood and were given autoclaved food and water.

Cells. CEM cells, derived from a patient with ALL (28), were maintained in log phase growth prior to freezing. For each experiment, cells from a frozen aliquot were thawed and expanded for approximately 1 week prior to injection in RPMI 1640 (Gibco, Grand Island, NY).
supplemented with 10% heat-inactivated fetal bovine serum (Hyclone, Salt Lake City, UT), 25 mM N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid (Gibco), 2 mM L-glutamine (Gibco), 100 units/ml penicillin (Gibco), and 100 μg/ml streptomycin (Gibco), hereafter referred to as CM-FBS.

Establishment and Treatment of Human Leukemia Cells in NIH-III Mice. Groups of 4 to 10 mice were given i.p. injections of 300 mg/kg cyclophosphamide (Sigma, St. Louis, MO) in PBS 2 days prior to the i.v. injection of 1 × 10^6 CEM cells in PBS via the tail vein. The day that CEM cells were injected was study day 1. Beginning on day 19 or 20, mice were administered, by i.v. bolus, various doses of 4A2-RTA30, 4A2, IND1-RTA30, H65-RTA30, or buffer alone daily for 10 days. The maximum tolerated dose of immunoconjugate in these mice was 0.5 mg/kg/day for 10 days. Mice were sacrificed 1 day after receiving the last dose (day 29 or 30).

Preparation of Tissue Samples. Cells were removed aseptically from spleens; washed in PBS; resuspended in 16 mM ammonium chloride, 1 mM potassium bicarbonate, and 12.5 μM EDTA for red blood cell lysis; then washed and resuspended in PBS for subsequent enumeration of WBC. Bone marrow cells from the femurs and tibiae were suspended in RPMI 1640 and WBC were counted. Blood cells were washed twice with 16 mM ammonium chloride, 1 mM potassium bicarbonate, and 12.5 μM EDTA buffer to eliminate red blood cells and resuspended in PBS for WBC enumeration. The total number of WBC in blood was calculated, assuming a theoretical blood volume of 1.4 ml/mouse. WBC recovered from the spleen, bone marrow, and blood were assayed for antigen expression by flow cytometry.

The remaining cells from the bone marrow and spleen were cultured in CM-FBS. Cells recovered from these cultures were subsequently assayed for antigen expression by flow cytometry and in vitro sensitivity to immunoconjugates using cytotoxicity assays.

Flow-Cytometric Analyses. Five hundred thousand cells in 100 μl of a 0.1% (w/v) sodium azide, 1% (w/v) bovine serum albumin in PBS were stained on ice using saturating amounts of various FITC-conjugated antibodies for 30 min. Cells were then washed in cold 0.1% sodium azide and 1% bovine serum albumin in PBS, fixed in 0.37% (w/v) formaldehyde in PBS, and analyzed promptly on a FACScan using log amplifiers. Regions to quantitate positive cells were set based on staining with the nonbinding control mAb-fluorochrome. Regions to quantitate positive cells were set based on staining with the nonbinding control mAb-fluorochrome.

To determine the average antigen density, a standard curve of mean channel of fluorescence versus number of fluoresceins was constructed using fluorescent microbeads (Flow Cytometry Standards Corp., Research Triangle Park, NC). The equivalent number of fluoresceins bound per cell was determined from the standard curve, and the average number of antigens per cell was determined by dividing that number by the fluorescein to protein ratio for that particular mAb-FITC.

Detection of Human Cells in Murine Tissues. FITC anti-CD45 was used to detect and quantitate CEM cells. The absolute numbers of CEM cells recovered from bone marrow, spleen, and blood were determined by multiplying the percent CD45+ cells by the number of cells recovered from each murine tissue sample. The detection limit for accurate quantitation of CEM cells was less than 0.1% CD45+ cells. The treatment effect was evaluated based upon the total number of CEM cells from the 3 tissues combined. All statistical comparisons were made with the Mann-Whitney U test.

Cytotoxicity Assay. As previously reported (12), 1 × 10^5 CEM cells were treated in triplicate with immunoconjugates (diluted in CM-FBS) in microtiter plates and incubated for 48 h at 37°C in a 10% CO2 incubator with humidified air. The cells were pulsed with 1.0 μCi/well of [3H]dThd (6.7 Ci/mmol; New England Nuclear, Wilmington, DE) 8 h prior to harvest. Uptake of [3H]dThd was quantitated by gas ionization chromatography (Trace 96 Detection System INB-384; Inotech, Lansing, MI). IC50 was determined from a concentration-response curve using control (no immunoconjugate) [3H]dThd uptake as 100%. In a similar assay using melanoma cell lines, IND1-RTA30 inhibited protein synthesis with an IC50 value of approximately 15 ng/ml (12).

RESULTS

In Vitro Sensitivity of CEM Cells to Immunoconjugates. As shown in Fig. 1, the proliferation of CEM cells was inhibited by 4A2-RTA30 in a concentration-dependent manner, with an IC50 of 1.2 ng/ml (6.7 pm) and complete inhibition at 100 ng/ml (560 pm). The isotype-, linker-, and toxin-control immunoconjugate, IND1-RTA30, was ineffective even at the highest concentrations examined (3,000 ng/ml or 13,000 pm) as was the unconjugated mAb, 4A2 (1,000 ng/ml or 6,700 pm). A second anti-T-cell immunoconjugate directed against CD5, H65-RTA30, also inhibited proliferation, with an IC50 of 90 ng/ml (450 pm), and demonstrated maximal inhibition at 1,000 ng/ml (5,000 pm).

Detection of CEM Cells Pre- and Postinjection. mAbs specific for human antigens (e.g., CD45, CD7, and CD5) were used to distinguish CEM cells from murine cells. These mAbs reacted with all in vitro cultured (preinjection) CEM cells as evidenced by positive fluorescent staining for CD5, CD7, and CD45 (Fig. 2A), but did not bind to murine bone marrow (Fig. 2B), spleen, or blood cells (data not shown) isolated from naive NIH-III mice. Human cells were detectable in bone marrow obtained from mice 30 days after injection of CEM cells (Fig. 2C). The reactivity of anti-CD45 and anti-CD5 mAbs, as measured by the fluorescence intensity, did not change significantly upon passage of CEM cells through NIH-III mice. However, the reactivity of anti-CD7 mAb was much lower when compared to in vitro passaged CEM cells and most likely arose from a partial loss of the CD7 antigen (see below).

Establishment of CEM Leukemia in NIH-III Mice. CEM cells were injected into cyclophosphamide-pretreated NIH-III mice and allowed to engraft for 4 weeks prior to sacrifice. At this time, CEM cells were detected in bone marrow of all CEM-injected NIH-III mice (16 of 16) and in the spleen (13 of 16) and blood (11 of 16) of most animals (Table 1). There were no solid tumors observed in these mice. In addition, in vivo expansion of CEM cells was evident, as more CEM cells were recovered from most (14 of 16) CEM-engrafted mice (ranging from a total of 2 to 210 × 10^6 cells) than had been injected (1 × 10^6 cells). The number of CEM cells recovered from bone marrow was moderately uniform from mouse to mouse, whereas the number of CEM cells in the spleen varied considerably, ranging from less than 0.05 to 205 × 10^6. In addition, among all 3 tissues in animals with engraftment, the spleen typically had more CEM cells than the bone marrow, with blood having the least number of CEM cells.
Treatment of CEM-engrafted NIH-III Mice. Having demonstrated reproducible leukemic infiltrates in the bone marrow of all mice and in the spleen and blood of most mice, CEM-engrafted NIH-III mice were used to assess the efficacy of various immunoconjugates and mAbs. Mice treated for 10 consecutive days with 4A2-RTA_{30} at a dose level of 0.1 mg/kg/day showed a 100-fold depletion of CEM cells for all 3 tissues combined (Fig. 3), which was statistically significant when compared to the buffer control group (P < 0.02). In 2 additional experiments with the same dose level and dosing regimen (Table 2), there was approximately a 75-fold (P < 0.002) and a 30-fold (P < 0.02) depletion of CEM cells from all tissues examined.

The specificity of 4A2-RTA_{30} treatment was demonstrated by the lack of depletion of CEM cells (P > 0.5) when CEM-engrafted NIH-III mice were treated with 0.1 mg/kg/day of the isotype-, linker-, and toxin-matched control immunoconjugate, Table 1 Recovery of CEM cells from NIH-III mice

<table>
<thead>
<tr>
<th>Experiment no.</th>
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<th>Spleen</th>
<th>Blood</th>
<th>Total</th>
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</thead>
<tbody>
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<td>1.22</td>
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<td>&lt;0.01*</td>
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</tr>
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<td>165</td>
<td>0.56</td>
<td>169</td>
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</table>

* Tissue samples from individual mice were stained for the presence of CEM cells as described in "Materials and Methods."

**Mice with CEM cells below the detection limit (0.05 \times 10^6 in spleen and 0.01 \times 10^6 in blood).**

IND1-RTA_{30}, following the same dosing regimen (Fig. 3). A slight depletion of CEM cells resulted from the treatment with unconjugated 4A2 mAb, but this effect was not significantly different from controls (P > 0.1). Therefore, the RTA moiety of 4A2-RTA_{30} was essential for depletion of CEM cells. This differential activity of 4A2-RTA_{30} versus 4A2 was reproduced in a second experiment using a lower doses of each agent, 0.01 mg/kg/day (Fig. 4). There was a 200-fold decrease of total CEM cells recovered in the 4A2-RTA_{30}-treated mice (P < 0.01) and only a 3-fold decrease in the 4A2-treated mice (P > 0.2).

Examination of individual tissues (Fig. 5) revealed that, in the bone marrow, only 4A2-RTA_{30} depleted CEM cells. In the spleen, both 4A2-RTA_{30} and 4A2 depleted CEM cells, but 4A2 was much less effective. Depletion of CEM cells in the blood occurred with 4A2 and IND1-RTA_{30} as well as with 4A2-RTA_{30} and thus appeared to be independent of toxin and mAb specificity.

To assess the potency of 4A2-RTA_{30}, varying doses of immunoconjugate, 0.001 to 0.5 mg/kg/day, were studied in several experiments. While there was variation in the extent of depletion in different experiments, when compared to the buffer controls, all doses greater than 0.001 mg/kg/day 4A2-RTA_{30} were considered effective (Figs. 3 and 4; Table 2). Thus, the minimum effective dose of 4A2-RTA_{30} was established as approximately 0.01 mg/kg/day.
Effect of in Vivo Treatment on CEM Cells Cultured from NIH-III Tissue Samples. As noted in Fig. 2, CEM cells detected in untreated or buffer control NIH-III mice 4 weeks after injection expressed much less CD7 antigen than either preinjection CEM cells or CEM cells maintained in culture for the same amount of time. Cells recovered from buffer control mice continued to express low levels of CD7 antigen as measured by mean channel of fluorescence even after additional growth in vitro for 2 to 4 weeks (Table 3). While CD7 density decreased from approximately 60,000 antigens/cell on continuously cultured cells to approximately 20,000 on cells recovered from NIH-III mice, there was no effect on CDS (Table 3) or CD45 expression. Even though the CD7 density was significantly decreased when CEM cells were passaged through buffer control NIH-III mice, such cells were only slightly less sensitive to 4A2-RTA\textsubscript{30} in in vitro cytotoxicity assays as assessed by percent maximal inhibition (Table 3); the IC\textsubscript{50} values were essentially identical.

Treatment of mice with 4A2-RTA\textsubscript{30} resulted in even lower expression of CD7 on the remaining CEM cells in a dose-dependent manner. Thus, CEM cells cultured from mice treated with doses of 4A2-RTA\textsubscript{30} that depleted CEM cells (greater than or equal to 0.04 mg/kg/day) consistently showed significantly lower expression of CD7 antigen as compared to those cells recovered from buffer controls (Table 4). In contrast, therapeutic regimens that were ineffective at CEM depletion (0.001 mg/kg/day 4A2-RTA\textsubscript{0}, 4A2, IND1-RTA\textsubscript{0}, or H65-RTA\textsubscript{0}) also resulted in no further reductions in expression of CD7 on CEM cells. The observed loss of CD7 was not an artifact arising from blocking of the epitope by 4A2-RTA\textsubscript{30} or 4A2 because the anti-CD7 mAb, S9.1, used to monitor CD7 expression recognizes a nonoverlapping epitope on CD7.\textsuperscript{3} Neither 4A2-RTA\textsubscript{30}, 4A2, IND1-RTA\textsubscript{30}, nor H65-RTA\textsubscript{30} treatment caused a significant reduction in CD5 expression. In many cases, those CEM cells that had shown further decreases in CD7 expression as compared to the buffer control were slightly less sensitive to 4A2-RTA\textsubscript{30} in vitro as determined by the percent maximal inhibition, but without a concomitant change in the IC\textsubscript{50} values (Table 4). The treatment of CEM-engrafted NIH-III mice with a CD5-specific immunoconjugate, H65-RTA\textsubscript{30}, had no effect on sensitivity of CEM cells to 4A2-RTA\textsubscript{30} in vitro.

### DISCUSSION

Imunoconjugates have been used as anticancer agents for a variety of tumors. However, solid tumors have proven intrinsigent to the effects of immunoconjugates probably due to

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**Table 2** Effect of 4A2-RTA\textsubscript{30} on CEM cell depletion at various doses

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Dose of 4A2-RTA\textsubscript{30} (mg/kg/day)</th>
<th>CEM cells recovered (\texttimes 10^6)</th>
<th>Fold depletion</th>
<th>P</th>
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<tbody>
<tr>
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<tr>
<td>3</td>
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<td>0.25</td>
<td>75</td>
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</tr>
<tr>
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<tr>
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<td>11</td>
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</tr>
<tr>
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</tr>
<tr>
<td>5</td>
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<td>1.12</td>
<td>9</td>
<td>&lt;0.2</td>
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</table>

\textsuperscript{a} Ten daily doses of 4A2-RTA\textsubscript{30} were administered to CEM-engrafted NIH-III mice 18 days after CEM injection as described in "Materials and Methods."

\textsuperscript{b} n = number of mice per group.

\textsuperscript{c} Number of CEM cells recovered from bone marrow, spleen, and blood combined.

\textsuperscript{d} Calculated by dividing the median number of CEM cells recovered from mice receiving 4A2-RTA\textsubscript{30} by the median number of CEM cells recovered from the buffer control mice.

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\textsuperscript{3} D. M. Fishwild and S. Aberle, unpublished observations.
problems associated with decreased accessibility to the tumor, arising from decreased vascularity and necrosis of the tumor. In contrast, immunoconjugate therapy has been successful when directed against single cell targets, such as T-cells in patients with GvHD (5, 6) or rheumatoid arthritis. Given these results, immunoconjugates targeting T-cells would be predicted to be effective against T-cell leukemia. An anti-CD5 immunoconjugate, H65-RTA, has already been shown to be effective against T-cell leukemia. An anti-CD5 immunoconjugate intended for leukemic therapy. We have expanded in nude (16-20), BALB/c (33), and SCID (21) mice, although human leukemia cell lines have survived and expressed in other animal models either by the transient depletion of circulating (nonmalignant) T-cells in rhesus monkeys treated with anti-human CD2 mAbs (32) or the extension of the survival time of mice with an ascitic EL4 leukemia by treatment with anti-murine immunoconjugates (13). The direct extrapolation of such animal data, in which non-human T-cells serve as targets and/or the homologous anti-murine immunoconjugate is used, to potential efficacy in humans is questionable. The model described in this paper uses human leukemia cells expressing the CD7 antigen (29). However, as most human T-cell leukemias do express CD7 (29), an anti-CD7 immunoconjugate was evaluated for efficacy. We report here the development of a novel murine model system for human T-leukemia in which human leukemic cells, CEM, were engrafted into cyclophosphamide-immunosuppressed NIH-III mice. These cells reside and replicate in the bone marrow, spleen, and blood of the recipient mice as individual cells, with no solid tumors evident. When an anti-CD7 immunoconjugate, 4A2-RTA30, was administered to these mice parenterally, the leukemic CEM cells were depleted. This therapeutic effect was mAb- and toxin-dependent, as an isotype control immunoconjugate had no effect and the unconjugated mAb was only minimally (but not significantly) effective.

Other investigators have administered anti-human T-cell mAbs or immunoconjugates to animals. Intrathecal administration of an anti-CD7 immunoconjugate, WT1-SMPT-dgRTA, resulted in high levels of drug in the cerebrospinal fluid and also in serum (30). In addition, following i.v. administration, localization of an anti-CD5 mAb and immunoconjugate to human leukemic cells grown either in nude mice as solid tumors (17) or in BALB/c mice as ascites (31), respectively, was also observed. These reports suggested the feasibility of immunoconjugates for the treatment of human leukemia. The efficacy of mAbs and immunoconjugates was demonstrated in other animal models either by the transient depletion of circulating (nonmalignant) T-cells in rhesus monkeys treated with anti-human CD2 mAbs (32) or the extension of the survival time of mice with an ascitic EL4 leukemia by treatment with anti-murine immunoconjugates (13). The direct extrapolation of such animal data, in which non-human T-cells serve as targets and/or the homologous anti-murine immunoconjugate is used, to potential efficacy in humans is questionable. The model described in this paper uses human leukemia cells expressing the CD7 antigen as targets and 4A2-RTA30 as the anti-CD7 immunoconjugate intended for leukemic therapy. We have shown not only that 4A2-RTA30 reaches its intended target (as evidenced by CD7 modulation), but also that 4A2-RTA30 successfully depletes human leukemic cells.

Although human leukemia cell lines have survived and expanded in nude (16-20), BALB/c (33), and SCID (21) mice, most of the human leukemic cells grew either as solid tumors s.c. or i.p., or as leukemic infiltrates with macrometastases. In addition, the co-injection of feeder cells such as HT-1080 was necessary in the establishment of some solid tumor models (16-18). Animal models of disseminated human leukemia (22) and

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**Table 3** Comparison of CEM cells passaged in vitro versus in vivo in NIH-III mice: immunoconjugate sensitivity and antigen expression

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Cell source</th>
<th>Mean channel of fluorescence</th>
<th>4A2-RTA&lt;sub&gt;30&lt;/sub&gt; cytotoxicity</th>
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<tr>
<td></td>
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<td>In vitro</td>
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<td>174 ± 19</td>
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<tr>
<td></td>
<td>In vivo</td>
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<tr>
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</table>

<sup>a</sup> CEM cells were obtained from either continuous in vitro culture or from NIH-III bone marrow of buffer control animals and then maintained in culture an additional 2 to 4 weeks. Once sufficient numbers of cells were obtained, cells were examined for antigen expression and immunoconjugate sensitivity. Similar results were observed for CEM cells obtained from spleens. * n = number of animals.

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**Table 4** Comparison of the effect of various treatment regimens on CEM cells: immunoconjugate sensitivity and antigen expression

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Treatment groups</th>
<th>Mean channel of fluorescence</th>
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<td>1</td>
<td>Buffer</td>
<td>218 ± 19</td>
<td>64 ± 12</td>
</tr>
<tr>
<td>0.1 mg/kg 4A2-RTA&lt;sub&gt;30&lt;/sub&gt;</td>
<td>202 ± 23</td>
<td>42 ± 9</td>
<td>5.01 ± 1.78</td>
</tr>
<tr>
<td>0.1 mg/kg 4A2</td>
<td>207 ± 19</td>
<td>65 ± 20</td>
<td>5.72 ± 1.55</td>
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<tr>
<td>0.1 mg/kg IND1-RTA&lt;sub&gt;7&lt;/sub&gt;</td>
<td>193 ± 10</td>
<td>61 ± 11</td>
<td>3.87 ± 2.48</td>
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<tr>
<td>4</td>
<td>Buffer</td>
<td>232 ± 19</td>
<td>81 ± 14</td>
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<tr>
<td>0.01 mg/kg 4A2-RTA&lt;sub&gt;30&lt;/sub&gt;</td>
<td>190 ± 37</td>
<td>55 ± 13</td>
<td>4.40 ± 3.29</td>
</tr>
<tr>
<td>0.04 mg/kg 4A2-RTA&lt;sub&gt;30&lt;/sub&gt;</td>
<td>183 ± 39</td>
<td>44 ± 13</td>
<td>12.90 ± 2.50</td>
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<tr>
<td>0.1 mg/kg 4A2-RTA&lt;sub&gt;30&lt;/sub&gt;</td>
<td>171 ± 25</td>
<td>37 ± 6</td>
<td>4.85 ± 2.40</td>
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<tr>
<td>6</td>
<td>Buffer</td>
<td>209 ± 8</td>
<td>70 ± 17</td>
</tr>
<tr>
<td>0.5 mg/kg H65-RTA&lt;sub&gt;30&lt;/sub&gt;</td>
<td>186 ± 45</td>
<td>62 ± 17</td>
<td>1.29 ± 1.23</td>
</tr>
</tbody>
</table>

<sup>a</sup> CEM cells cultured from NIH-III bone marrow were tested for sensitivity to immunoconjugates and for antigen expression as described in "Materials and Methods."

<sup>b</sup> n = number of animals.

<sup>c</sup> IC<sub>50</sub> concentration (ng/ml) of immunoconjugate required for 50% inhibition of [3H]dThd uptake (mean ± SD).

<sup>d</sup> NA, not applicable.

<sup>e</sup> P ≤ 0.01.

<sup>f</sup> P ≤ 0.05.
lymphoma (23), which parallel the human clinical experience, have been recently described, but with B- or mixed lineage cell lines. The CEM/NIH-III model system described herein more accurately reflects human T-cell leukemia as supported by the recovery of leukemic cells from lymphoid compartments, the absence of any solid tumors, and the lack of requirement of feeder cells for engraftment.

The mechanism for depletion of CEM cells from NIH-III mice by 4A2-RTA30 treatment appeared to result primarily from cell death caused by the RTA moiety as a consequence of inhibition of protein synthesis after immunonoconjugate had bound to and been internalized by the target cells. Even though the unconjugated mAb, 4A2, had no cytotoxic effect on CEM cells in vitro, it was somewhat effective in vivo. Fewer CEM cells were recovered from the spleens of treated mice as compared to controls, probably as a result of mAb binding to mouse effector cells through Fc receptors or activation of murine complement. The effect of unconjugated mAb in humans is thus difficult to predict because it would be dependent upon host (i.e., human) effector mechanisms. In contrast, the specific cytotoxicity of 4A2-RTA30 arising from the toxin moiety would be independent of host effector functions.

In similar animal models of non-T-cell leukemia (22) and lymphoma (23), anti-B-cell immunonoconjugates have been shown to improve survival. An anti-CD19-pokeweed antiviral protein immunonoconjugate also decreased the number of engrafted mixed lineage leukemic cells recovered from SCID mice (22). However, in this study, immunonoconjugate treatment was begun the day after leukemic cells were injected. We have observed that 4A2-RTA30 also results in complete depletion of engrafted leukemic cells if given one day after CEM cells. In the B-cell lymphoma model, the authors noted that even if treatment was delayed for 3 weeks after Daudi cell inoculation, an anti-CD22-RTA immunonoconjugate was still effective, although not as potent as when administered within 4 days after inoculation (23). These results are similar to ours in the T-cell leukemia model.

Passage of CEM cells through NIH-III mice resulted in a loss of approximately 66% of CD7 antigen, without a significant effect on CD5. Other investigators reported a decrease in CD5 expression when CEM cells were passaged through nude mice as a solid tumor (17). The decrease in CD7 was not a transient effect and was maintained by cells recovered from CEM-engrafted NIH-III mice during subsequent in vitro culture for many weeks. We hypothesize that there was either a growth advantage for the low-expressing CD7 cells or a selective loss of high-expressing CD7 cells.

It has been reported that, for a given antigen, the extent of cell death induced in vitro by immunonoconjugates is a function of the antigen density (34, 35) and that below a certain level of antigen expression immunonoconjugate is ineffective. This threshold effect could explain why the extent of maximal killing was lower for CEM cells recovered from mice that expressed less CD7 antigen as compared to cells maintained in vitro, without a significant change in the IC50 values.

CEM cells were depleted by doses of 4A2-RTA30 as low as 0.01 mg/kg/day, corresponding to a theoretical peak serum level of 240 ng/ml. A 10-fold lower dose of 4A2-RTA30 was essentially ineffective, even though such doses would theoretically lead to concentrations sufficient to inhibit cell growth by greater than 50% in vitro. These data indicated that efficacy was governed by peak drug concentration in vitro rather than steady state drug concentration as maintained in vitro.

In summary, we have established a novel model that can be used in the study of efficacy of various therapeutic regimens on human leukemia in immunodeficient mice and have successfully depleted those leukemic cells from lymphoid compartments by treatment with an anti-CD7-RTA30 immunonoconjugate. Moreover, we have clearly demonstrated the requirement for the targeted RTA moiety in order to maximize the depletion of tumor target cells. Applications of this model system may extend beyond demonstration of efficacy for various antileukemic agents; such an animal model will aid in the study of the mode of action of immunonoconjugates. The pharmacokinetics and toxicities of various potential therapeutics can be assessed in CEM-engrafted NIH-III mice, rather than using antigen-bearing large animals such as nonhuman primates. Based on the in vivo efficacy in the CEM-engrafted NIH-III leukemia model, 4A2-RTA30 should be a potent drug in the treatment of human leukemia and other immune disorders involving human CD7 positive T- and/or natural killer cells.

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REFERENCES


IN VIVO EFFICACY OF AN ANTI-CD7 IMMUNOCONJUGATE


Efficacy of an Anti-CD7-Ricin A Chain Immunoconjugate in a Novel Murine Model of Human T-Cell Leukemia

Dianne M. Fishwild, Sharon Aberle, Susan L. Bernhard, et al.