An Immunotoxin With Therapeutic Potential in T Cell Leukemia: WT1-Ricin A


A conjugate of the monoclonal antibody WT1 and ricin A-chain was studied for its suitability for purging marrow of leukemic T cells for autologous transplantation in T cell acute lymphocytic leukemia (T-ALL). The conjugate was powerfully cytotoxic to the human T-ALL cell line, GH1, which expresses the WT1 antigen at a high density. Treatment of the cells with the conjugate at $10^{-11} \text{M}$ reduced their rate of protein synthesis by 50%, and the inclusion of 6 mM ammonium chloride in the cultures enhanced the potency of cytotoxic effect by 10–100-fold. Clonogenic assays indicated that less than 0.1% of GH1 cells survived 3-hr exposure to the conjugate in ammonium chloride. WT1 alone did not react with multipotent (CFU-GEMM) hematopoietic progenitors in normal human bone marrow, as measured by fluorescence-activated cell sorting. Under conditions giving maximal killing of GH1 cells, there was no toxicity to multipotent progenitors in normal human marrow.

The murine monoclonal antibody WT1 identifies a single-chain glycoprotein of approximately 40,000 molecular weight that is present on normal thymocytes and cells from T lymphoblastic leukemias (T-ALL) at presentation and in relapse. The antigen is more consistently expressed on T-ALL cells than other T cell antigens so far described and has been shown to be a reliable reagent for the differential diagnosis of T-ALL. WT1 and similar monoclonal antibodies, 3A1 and 4A, offer a possible means for removing leukemic cells from autologous marrow transplants for therapy.

Various strategies have been explored for using monoclonal antibodies to rid marrow autografts of leukemic cells in vitro prior to reinfusion into patients treated with high dose chemotherapy and irradiation. Physical separation systems using monoclonal antibodies attached to Sepharose, plastic, or magnetic microspheres appear to be suitable, but have not been tested clinically to date. Antibodies and heterologous complement selectively lyse leukemic cells added to normal marrow in experimental systems, and this approach has been successfully used to treat common ALL in relapse. More recently, studies in mice and rats have shown that immunotoxins—antibodies chemically linked to ricin or other highly poisonous toxins—can destroy 99.9% of leukemic cells in marrow with little or no harm to hematopoietic stem cells. Studies by Muirhead et al. using immunotoxins selectively to kill Daudi B lymphoblastoid cells mixed with normal human marrow suggest that this approach is applicable to man. The advantage of immunotoxins is their potent cytotoxic action, which maximizes the likelihood of achieving complete eradication of malignant cells.

In the present study, we show that WT1 antibody linked to the ribosome damaging A chain of ricin specifically kills cells bearing the WT1 antigen and is not harmful to any of the measurable hematopoietic progenitors in normal human marrow. Thus, the WT1-ricin A chain conjugate merits consideration for use in autologous marrow transplant procedures in man.

MATERIALS AND METHODS

Cell Lines

Four T-ALL cell lines with a range of WT1 expression were selected: GH1, CCRF-CEM, RPMI-8402, and HPB-ALL (Fig. 1). These were all provided by Dr. J. Minowada (Roswell Park Memorial Inst., Buffalo, NY) and were passaged in our laboratory in RPMI-1640 medium (Gibco Europe Ltd., Paisley, Scotland) supplemented with 5% fetal calf serum (Sera-Lab, Sussex, England), 4 mM L-glutamine, and antibiotics. Cells were always passaged the day prior to use to ensure log phase growth.

Bone Marrow

Normal human bone marrow was obtained from consenting healthy donors, usually by aspiration from the iliac crest into preservative-free heparin. Occasionally marrow from allogeneic transplant donors was used. In all cases, the marrow was processed within 12 hr of aspiration. Mononuclear fractions were separated by centrifugation over Lymphoprep (Nyegaard and Co., A/S, Oslo, Norway) and were resuspended in Iscove's modification of Dulbecco's medium (IMDM) with 5% fetal calf serum.

Immunotoxin

The WT1 antibody was provided as purified IgG3, and had been prepared as described by Tax et al. Details of its selectivity of binding to normal and leukemic T cells have been published elsewhere. Ricin A chain, trace labeled with $^{125}\text{I}$, was a gift from Dr. J. A. Forrester, Chester Beatty Research Institute, London.

WT1 antibody was coupled by a disulfide bond to freshly prepared ricin A chain by means of the SPDP reagent (Pharmacia Ltd., Piscataway, NJ), as described previously. The component of


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Fig. 1. Staining of selected T-ALL cell lines with WT1. Cell lines were stained with either WT1 (---) or nonimmune ascitic fluid (---), followed by a second layer of fluoresceininated goat anti-mouse F(ab')2. Cells were then analyzed by flow cytofluorimetry and the percentage of cells staining more heavily than the control were recorded.

Cytotoxicity to Cell Lines

Cytotoxicity assays were carried out in microtiter plates, with each well containing 4 x 10⁴ cells in IMDM supplemented with 5% FCS. Solutions of WT1-rinic A, ricin, ricin A chain, WT1 monoclonal antibody, and, in some experiments, ammonium chloride were added to give a final incubation volume of 200 µl. Cultures were incubated for 24 hr at 37°C in a 5% CO₂ humidified incubator. Other cultures were incubated for 1 hr and the cells washed 3 times prior to incubating for a further 23 hr in fresh medium. Ten microliters (=1 µCi) ³H-leucine (TRK. I 70; Amersham International PLC., Bucks, England) was added, and the plates were incubated for a further 24 hr. The cells were harvested onto glass fiber paper using a Skatron automated cell harvester, and their ³H-leucine incorporation was measured by scintillation counting.

Cytotoxicity to Bone Marrow

Normal human bone marrow mononuclear cells were resuspended in IMDM at 4 x 10⁶/ml. Two hundred microliter volumes of the suspension were mixed with 22 µl of various solutions of WT1-rinic A chain conjugate in the presence or absence of ammonium chloride and incubated at 37°C in 5% CO₂. Samples containing 3 x 10⁶ cells were removed at 4 and 24 hr, washed, and assayed in the mixed colony assay.

Cell Sorting and Analysis

Normal bone marrow mononuclear cells were incubated with WT1, or nonimmune murine ascitic fluid as a negative control, for
30 min on ice. They were washed twice with ice-cold medium and incubated for 30 min with fluoresceinated F(ab')2 fragments of affinity-purified goat anti-mouse Ig that had been absorbed with human Ig. The cells were again washed twice and resuspended in IMDM on ice. Cell separation was performed with either a FACS I (Becton Dickinson, Sunnyvale, California) or a cell sorter built at the I.C.R.F. The WT1-treated cells were separated into one positive fraction, with fluorescence intensity greater than that of the control, and the remaining cells were arbitrarily split into three fractions containing approximately equal percentages of the remaining cells, designated “upper gap,” “lower gap,” and “negative” in accordance with their intensity of fluorescence (cf., Fig. 6).

Cells being analyzed for surface antigen were similarly incubated for 30 min at 4°C with the test antibody and, after washing, for a further 30 min with the FITC second layer as described above. The percentage of cells staining more brightly than cells treated with the control ascitic fluid and the median fluorescence intensity were recorded.

**Mixed Colony Assays**

Bone marrow cells fractionated on the cell sorter or treated with immunotoxin were resuspended at $2 \times 10^6$/ml in IMDM and set up for mixed colony assay according to a modification of the method described by Fauser and Messner. Briefly, 0.3 ml of the cell suspension was mixed with 1.2 ml 2% methylcellulose in IMDM, 0.6 ml normal human plasma, 0.3 ml fetal calf serum, 0.3 ml phytohemagglutinin-stimulated leukocyte conditioned medium (PHA-LCM) (made by stimulating normal blood mononuclear cells with 1 μg/ml PHA for 5 days in IMDM plus 10% donor serum) and 30 μl $1.4 \times 10^{-2}$ M 2-mercaptoethanol. Aliquots of 0.9 ml of this mixture were added to 40-mm petri dishes containing 0.1 ml (1 U) human erythropoietin (provided by Dr. A. Eaves, Terry Fox Labs., Vancouver, Canada) and incubated at 37°C in 5% CO₂ for 14 days prior to scoring. CFU-GM, BFU-e, CFU-Meg, and CFU-GEMM were all counted on the same plates using an Olympus inverted microscope.

**Colony Assay on GH1 Cells**

The method used to grow GH1 cells in semisolid conditions was similar to the lymphoid cell assay described by Izaguirre et al. A quantity of 0.6 ml of a suspension of cells in IMDM was mixed with 1.2 ml 2% methylcellulose in IMDM, 0.6 ml fetal calf serum, 0.6 ml PHA-T cell conditioned medium (PHA-TCM), and 30 μl $1.4 \times 10^{-2}$ M 2-mercaptoethanol. The mixture was then distributed in 100-μl volumes into the wells of 96-well flat-bottomed microplates (Linbro, Flow Laboratories, Irvine, Scotland) and incubated at 37°C in a humidified atmosphere of 5% CO₂ in air. Colonies of greater than 8 cells were scored on day 6 of incubation.

**RESULTS**

**WT1 Binding by T Cell Leukemic Lines**

A number of human T cell lines were screened for expression of the WT1 antigen with the cell sorter. Four cell lines were selected that ranged from very weak to strong in their expression of the antigen. The lines with the highest density of the antigen were GH1 and CCRF-CEM. Compared to controls treated with nonimmune ascitic fluid, brighter fluorescence was seen in 95% of GH1 and 79% of CCRF-CEM cells (Fig. 1). The two weakly staining lines were RPMI-8402 and HPB-ALL and, these, only 26% and 13% of the cells, respectively, showed greater fluorescence than the controls. Cells that showed no reaction with WT1 may lack the antigen or may express it at a level too low for detection. Binding of the conjugate to GH1 cells was indistinguishable from the binding of native WT1 as judged by FACS analysis (Fig. 2).

**Effect of Immunotoxin on T Cell Leukemic Lines**

The two strongly staining cell lines, GH1 and CCRF-CEM, were efficiently killed by the WT1-ricin A conjugate. Their rate of $^3$H-leucine uptake was diminished by 50% upon prolonged incubation with the immunotoxin at a concentration of $10^{-11}$ M (Fig. 3). The use of progressively higher concentrations of immunotoxin continued to reduce the $^3$H-leucine uptake of the GH1 and CCRF-CEM cells until minimal levels of 2% and 8%, respectively, were reached. By contrast, the weakly stained lines, RPMI-8402 and HPB-ALL, were much less sensitive to the immunotoxin. The maximal decrease in $^3$H-leucine incorpora-
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Cytotoxicity was 20% for HPB-ALL and 40% for RPMI-8402.

All four cell lines were highly sensitive to native ricin. Treatment of GH1 and CCRF-CEM cells with ricin at $10^{-13}$ M and RPMI-8402 and HPB-ALL cells with ricin at $10^{-12}$ M (equivalent to 300 and 3,000 molecules per cell, respectively) reduced their rate of $^3$H-leucine incorporation by 50%. Ricin A was without effect except at the highest concentration tested of $3 \times 10^{-8}$ M when a decrease in leucine uptake of up to 10% was observed. Native WT1 produced no adverse effect at $3 \times 10^{-8}$ M.

Ammonium chloride has been reported to potentiate the toxicity of antibody-ricin A conjugates, suggesting that its inclusion should be of benefit when purging leukemic marrow with immunotoxin. Concentrations of ammonium chloride greater than 6 mM caused a decline in the rate of protein synthesis in the cell lines being studied (results not shown). Continuous incubation of GH1 cells in 6 mM ammonium chloride reduced the concentration of immunotoxin needed for a 50% decrease in protein synthesis by 100-fold (Fig. 4). The maximal reduction in $^3$H-leucine incorporation was not improved by adding ammonium chloride to any of the lines (data not shown for CCRF-CEM, RPMI-8402, or HPB-ALL), nor was the weak nonspecific toxic effect of free ricin A chain enhanced.

The 24-hr period of exposure of leukemic cells to immunotoxin could be unacceptably long when treating human leukemic bone marrow for autografting. Analysis of WT1 uptake by GH1 cells has shown that WT1 rapidly disappears from the cell surface and can be demonstrated in the cytoplasm within 1 hr (Fig. 5). Accordingly, the above experiments were repeated using a 1-hr incubation with immunotoxin. Cells were then washed and incubated in fresh medium for a further 23 hr before adding the $^3$H-leucine. Under these conditions, the maximal inhibition of protein synthesis caused by immunotoxin without ammonium chloride was only 88% for GH1, and the concentration of immunotoxin required for 50% inhibition was about double that required during continuous incubation. The potentiating effect of ammonium chloride was less pronounced with this shorter incubation period. A tenfold enhancement in toxicity was gained by applying the conjugate in the presence of ammonium chloride for 1 hr, as opposed to 100-fold enhancement on continuous incubation. However, the presence of ammonium chloride during the 1-hr period of incubation improved the maximal reduction of protein syn-

![Fig. 4. The potentiation of toxicity of immunotoxin by ammonium chloride. GH1 cells were incubated in immunotoxin with or without 6 mM ammonium chloride for 1 or 24 hr. Viability was assessed after 24 hr by measuring $^3$H-leucine incorporation. Standard deviations, omitted for clarity, were generally less than 10%. (O) Continuous incubation, no NH$_4$Cl; (i) continuous incubation, 6 mM NH$_4$Cl; (A) 1-hr incubation, no NH$_4$Cl; (A) 1-hr incubation, 6 mM NH$_4$Cl.](https://www.bloodjournal.org/content/118/5/1181/F4)

![Fig. 5. The kinetics of WT1 internalization by GH1 cells. GH1 cells were incubated with saturating concentrations of native WT1 for 30 min at 4°C, washed twice, and resuspended at 2 x 10$^5$/ml in IMDM at 37°C. Aliquots were taken at various times and tested for the presence of WT1 on the cell surface by reincubating cells in suspension with FITC-goat anti-mouse F(ab')$_2$ and FACS analysis, or for cytoplasmic staining by mounting cells on glass slides using a cytocentrifuge, staining with the same second layer, and analyzing by fluorescence microscopy. FACS results are expressed as median fluorescence intensity window, and the percentage of positive cells are compared to the control.](https://www.bloodjournal.org/content/118/5/1181/F5)
leucine incorporation assay does not give a reliable estimate of cell viability when percentage survival is low. To obtain a better estimate of cell killing with the immunotoxin, a colony assay was used. Control cells in this assay had a plating efficiency of 17%. Results shown are the percentage survival of GH1 cells after treatment compared with this control.

Although the WT1-ricin A conjugate was extremely effective at inhibiting protein synthesis by the GH1 cell line, the leucine incorporation assay does not give a reliable estimate of cell viability when percentage survival is low. To obtain a better estimate of cell killing with the immunotoxin, a colony assay was used. Control cells in this assay had a plating efficiency of 17%. Less than 1% of cells treated with immunotoxin at $3 \times 10^{-8} M$ in 6 mM ammonium chloride survived a 1-hr period of exposure and less than 0.1% survived 3 hr of exposure to the immunotoxin (Table 1). Neither WT1 antibody at $3 \times 10^{-8} M$ nor ricin A chain at $3 \times 10^{-6} M$ had any effect on the plating efficiency of the GH1 cells whether applied alone or together in the presence or absence of ammonium chloride.

In order to examine the expression of WT1 on the surviving cells, $5 \times 10^6$ cells from each cell line were treated for 24 hr with $3 \times 10^{-8} M$ WT1-ricin A and then washed and incubated for a further 24 hr in the absence of conjugate. In GH1 and CCRF-CEM cell lines treated in this way, no cells remained intact and no further regrowth was observed in a further 14 days of culture. With the treated HPB-ALL and RPMI-8402 lines, viable cells were collected by Ficoll-Hypaque centrifugation and were found to have WT1 staining profiles similar to those of the untreated cells (results not shown). This does not necessarily indicate that the cells surviving exposure to the conjugate expressed WT1 at the time of treatment, because GH1 cells sorted into fractions (7%) of the highest and lowest staining intensity were found to have reverted to a pattern of staining close to that of the unseparated cells after 24 hr in culture.

Lack of Binding to Hematopoietic Progenitors

In seven samples of normal bone marrow, no CFU-GEMM or CFU-Meg and less than 5% of the total BFU-e and CFU-GM were detected by mixed colony assays in the WT1-positive fraction obtained by cell sorting (Fig. 6 and Table 2). In the three WT1-negative or very weakly positive fractions (see Fig. 6), all the measurable colony types were represented (Table 2), although the distribution within these fractions was variable. Essentially all of the terminal deoxynucleotidyl transferase (TdT) positive cells were found in the lower gap and negative fractions (data not shown). These results suggest that the immunotoxin is likely to be free from any specific toxicity to progenitor cells. The total yield of progenitor cells was variable and always less than 100% (Table 2). The colony loss is probably attributable to the sorting procedure and not to an inhibitory effect of the antibody, as progenitor growth was not inhibited by antibody alone when cultured prior to sorting.

Lack of Toxicity of Immunotoxin to Normal Bone Marrow Progenitor Cells

To determine whether the immunotoxin is toxic to hematopoietic stem cells or other progenitors, bone marrow cells were incubated with immunotoxin for 4 or 24 hr in the presence or absence of 6 mM ammonium chloride. There was no toxicity to the CFU-GEMM demonstrable, nor were the committed progenitors affected by the immunotoxin (Fig. 7). The presence of 6 mM ammonium chloride had no toxic effect on the cells. A significant decrease in CFU-Meg and BFU-E colony counts was noted between 4 and 24 hr in control cultures and was probably due to aging of the marrow.

DISCUSSION

An immunotoxin must fulfill two criteria to be useful for destroying malignant cells in autologous

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**Table 1. The Percentage Survival of GH1 Cells After Treatment With WT1-Ricin A Chain**

<table>
<thead>
<tr>
<th>Test</th>
<th>1 hr</th>
<th>2 hr</th>
<th>3 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.71</td>
<td>0.23</td>
<td>0.11</td>
</tr>
<tr>
<td>2</td>
<td>0.48</td>
<td>0.23</td>
<td>0.04</td>
</tr>
</tbody>
</table>

GH1 cells were incubated in IMDM at 37°C with 6 mM ammonium chloride and $3 \times 10^{-9}$ M conjugate for 1, 2, or 3 hr prior to washing and assaying in the lymphoid colony assay. The plating efficiency of the control cells was 17%. Results shown are the percentage survival of GH1 cells after treatment compared with this control.
Table 2. Recovery of Hematopoietic Precursors in FACS-Separated Fractions of Normal Bone Marrow Stained With WTI

<table>
<thead>
<tr>
<th></th>
<th>CFU-GM</th>
<th>BFU-e</th>
<th>CFU-Meg</th>
<th>CFU-GEMM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>3%</td>
<td>&lt;1%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Upper gap</td>
<td>23%</td>
<td>19%</td>
<td>12%</td>
<td>10%</td>
</tr>
<tr>
<td>Lower gap</td>
<td>28%</td>
<td>19%</td>
<td>15%</td>
<td>22%</td>
</tr>
<tr>
<td>Negative</td>
<td>30%</td>
<td>23%</td>
<td>23%</td>
<td>21%</td>
</tr>
<tr>
<td>Recovery</td>
<td>84%</td>
<td>61%</td>
<td>50%</td>
<td>53%</td>
</tr>
</tbody>
</table>

The values given are mean results from three experiments where 4 fractions were collected in accordance with Fig. 2. Percentage recoveries are calculated from the absolute number of colonies recovered in each fraction and the number of the same colony type in the control:

\[
\text{Percentage recovery} = \frac{\text{Colonies in fraction}}{\text{Colonies in control}} \times 100\%
\]

Fig. 7. Survival of hematopoietic progenitors in normal bone marrow after incubation with immunotoxin. Bone marrow mononuclear cells were incubated with immunotoxin in the presence of 6 mM ammonium chloride and samples taken at 4 and 24 hr. The survival of progenitors was assessed using the mixed colony assay. Each point is the mean of counts from two 40-mm culture plates. Similar results were obtained in the absence of ammonium chloride. (Δ) CFU-GM, (φ) CFU-Meg, (■) CFU-GEMM, (○) BFU-e.

Bone marrow grafts. It must be both highly toxic to the clonogenic tumor cells and free from toxicity to the normal hematopoietic stem cells necessary for restoration of functional bone marrow.

The WTI-ricin A chain conjugate was a potent cytotoxic agent for two human T-ALL cell lines, GH1 and CCRF-CEM, which express the WTI antigen at high density. The conjugate was rapidly internalized by the cell with concomitant disappearance (i.e., modulation) of the antigen at the cell surface. Endocytosis may be essential for toxicity. This was indicated by the finding that, as with other A chain conjugates, ammonium chloride markedly enhanced toxicity, probably by inhibiting the fusion of endocytic vesicles with lysosomes and so giving the conjugate (or the A chain moiety) greater opportunity to escape to the cytosol. In a clonal assay of GH1 cells treated with optimal concentrations of conjugate with ammonium chloride for 1 hr, less than 1% of the cells survived and less than 0.1% survived 3 hr of treatment. The conjugate induced only partial inhibition of the other T-ALL cell lines, RPMI-8402 and HPB-ALL, which show weak and heterogeneous reactivity with WTI antibody. The partial cytotoxic effect may reflect a proportion of RPMI-8402 and HPB-ALL cells that lack the antigen or express it at levels too low for toxicity to occur. There is evidence from the work of Casellas et al.\(^{17}\) with melanoma cells that a threshold level of antigenic expression must be surpassed for a cell to be susceptible to an immunotoxin.

T-ALL cells from patients at diagnosis and in relapse usually express the WTI antigen (gp40) at high levels.\(^{2}\) In a series of 10 uncultured T-ALL samples analyzed with WTI (C. Myers, unpublished observations), there was a wide variation in the staining level, both between and within samples, that was similar to that shown by the cell lines reported here. It was difficult to make direct comparison between leukemic cells and the cell lines because of differences in cell size; however, the median staining of fresh leukemic cells was usually at least equal to that of CCRF-CEM and, in some cases, was far higher than that of GH1. In view of the tentative correlation between antigenic density and susceptibility to WTI-ricin A seen with the cell lines, any patient considered for therapy would have to have leukemic cells that express high levels of WTI antigen. The possible shedding of immunotoxin from leukemic cells, either before or after reinfusion of the marrow, is unlikely to have any dangerous consequences. Even if all the toxin used to treat a grafted marrow (expected to be less than 1 mg) were injected directly into the patient, it would represent less than a thousandth of the estimated human LD\(_{50}\).\(^{10}\)

WTI was chosen for these studies because it has
been shown to bind to almost all leukemic cells from patients with T-ALL at presentation and relapse and some, but not all, mature T cell leukemias, but it is not known whether the WTI antigen is expressed consistently on those leukemic cells with self-renewal or clonogenic capacity. The fact that WTI and similar monoclonal antibodies react well with the most immature T-lineage cells identifiable does, however, increase the likelihood of reactivity with clonogenic thymic leukemia cells. It is also clear from the present work that T-ALL clones, at least as established cell lines, can contain resistant cells with either low density or negligible expression of the WTI antigen. An improved strategy may therefore be to use WTI-ricin A in conjunction with immunotoxins prepared with other monoclonal antibodies that recognize different determinants on leukemic T cells but which are also absent from the measurable hematopoietic progenitors.

No toxicity to the measurable human hematopoietic precursors was observed when using the immunotoxin at a concentration sufficient for maximal destruction of GH1 cells. Not only are the CFU-GEMM unharmed, which bodes well for the maintenance of the marrow’s long-term repopulating capacity, but the committed progenitors also survive. The TdT-positive cells, representing lymphocyte precursors in normal marrow, are unreactive with the antibody. These observations extend those of Muirhead et al., who showed that ricin A chain linked to a rabbit anti-light chain antibody was not toxic to CFU-GM, BFU-e, and CFU-GM, while killing 99% of Daudi cells admixed with marrow. These findings suggest that marrow treated in vitro with WTI-ricin A should repopulate the patient rapidly and so reduce the time between treatment and regeneration of normal hematopoietic function. The CFU-GEMM assay may not, however, truly represent the pluripotent stem cell, which is ultimately required for restitution of full hematopoietic function. Lack of WTI binding to all measurable progenitors is encouraging, but there is currently no method to establish the capacity of treated bone marrow to repopulate in vivo other than by clinical trial.

There is evidence that it may not be necessary to eliminate every leukemic cell from the patient for “clinical cure.” Krolick et al. obtained prolonged remission in mice bearing the BCL1 tumor by total lymphoid irradiation, splenectomy, and intravenous administration of anti-δ-chain immunotoxin. Adoptive transfer of tissues (e.g., marrow, lung, liver) from these mice to non-tumor-bearing mice produced tumor. This suggests that massive reduction of the tumor burden may allow a few remaining tumor cells to be held in check by immune regulation or other mechanisms, which might also be operative in man.

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An immunotoxin with therapeutic potential in T cell leukemia: WT1-ricin A
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