Use of an Anti-Pan T-Lymphocyte Ricin A Chain Immunotoxin in Steroid-Resistant Acute Graft-Versus-Host Disease


Acute steroid-resistant graft-versus-host disease (AGVHD) after allogeneic bone marrow transplantation is frequently fatal. A new treatment for this T-lymphocyte-mediated condition uses an immunotoxin, H65-RTA, comprised of a monoclonal antibody that recognizes the CD5 lymphocyte differentiation antigen coupled to ricin A chain, a cytotoxic enzyme that inhibits protein synthesis. The safety and efficacy of this lymphocyte-targeted immunotoxin was evaluated in patients with severe AGVHD in a phase I-II dose escalation study with group expansion at the two middle doses. Thirty-four patients received up to 14 daily intravenous infusions of the immunotoxin. The principal side effects were constitutional symptoms such as fatigue and myalgias, and hypoalbuminemia with weight gain was seen at all doses. Thirty-two patients were evaluated for improvement or resolution of disease. Durable complete or partial responses were not dose-related and were seen in 16 patients. Skin GVHD had the highest incidence of response (73%), although improvement or resolution in gastrointestinal tract (45%) and liver (28%) GVHD was also noted. Survival in responding patients was significantly prolonged at all times as compared with those with no response (P = .03). Treatment was associated with a rapid decrease in peripheral blood T lymphocytes, which persisted for greater than 1 month after therapy. Anti-immunotoxin antibodies were seen in 6 of the 23 patients tested; these were of low titer and did not block immunotoxin binding to T cells. Results of this study indicate that anti-T-lymphocyte immunotoxins may form a new class of immunosuppressive agents useful in T-lymphocyte-mediated diseases.

Acute graft-versus-host disease (AGVHD) is a major limitation to the success of allogeneic bone marrow transplantation (BMT) as therapy for hematologic malignancies, aplastic anemia, and certain congenital disorders. AGVHD occurs within the first 100 days after marrow transplantation and presents as a dermatitis of variable severity often accompanied by gastroenteritis and hepatitis. The overall incidence of moderate to severe AGVHD in recipients of marrow from an HLA-identical sibling is 45% to 50%.

The risk of GVHD is influenced by HLA compatibility of the donor and recipient, posttransplant immunosuppressive regimen, age of the recipient, and by the age, sex, and parity of the donor.

GVHD is caused by donor T lymphocytes and ex vivo depletion of mature T lymphocytes from the marrow with various anti-T monoclonal antibody (MoAb) reagents before transplantation can eliminate the disease. Unfortunately, when the extent of depletion is sufficient to adequately control GVHD, the incidence of leukemia relapse and graft rejection become unacceptable.

Immunosuppressive agents such as corticosteroids, cyclosporine, and anti-thymocyte globulin (ATG) have been used as initial therapy in AGVHD with overall partial or complete responses occurring in 40% to 60% of patients. However, survival is poor with all regimens. In one randomized study, survival past 17 months of patients with moderate to severe AGVHD treated with either corticosteroids or cyclosporine was 24% to 28%.

Although death can occur from infection, leukemic relapse, or multiorgan failure, AGVHD remains an important cause of mortality. In a multicenter study of over 2,000 patients treated with a variety of immunosuppressive agents, 48% of patients who developed moderate to severe AGVHD died with this as a primary or contributory cause of death.

Second-line therapy in patients who do not respond to initial treatment is often unsuccessful. High-dose corticosteroids, ATG, or other immunosuppressive medications have been used as second-line treatment, but only 10% to 15% of such patients become long-term survivors.

The availability of anti-T-lymphocyte murine MoAbs (anti-T-MoAbs) offered a new modality for therapy of AGVHD. However, responses to treatment with unmodified antibodies have been relatively infrequent, of short duration, and incomplete. Characteristically, GVHD recurred shortly after MoAb therapy was discontinued. In addition, therapy with anti-CD3 MoAbs was associated with a high incidence of B-cell lymphoproliferative disorders. Because unmodified MoAbs may function by blocking receptor interaction or by accelerating clearance of target cells, it was reasoned that MoAbs may be more effectively used in GVHD as specific delivery systems for toxic agents. Killing of the targeted cells by such immunotoxins might then produce more durable responses.

Immunotoxins form a new class of pharmaceutical agents consisting of cytotoxic molecules coupled to MoAbs. Such
immunotoxins have been used in clinical trials as anti-cancer agents, and in this study, as a specific immunosuppressive agent targeted to T lymphocytes.

H65-RTA is an immunotoxin composed of an MoAb directed against the CD5 antigen present on mature T lymphocytes conjugated to ricin A chain (RTA), a cytotoxic enzyme. RTA is a potent inhibitor of protein synthesis; its mechanism of action is intracellular inactivation of an adenosine residue at the ribosomal binding site for elongation factor II, causing cell death. The anti-CD5 MoAb, when coupled to RTA, forms a compound that is specifically cytotoxic for T lymphocytes.

A multi-institutional clinical study was designed in which patients suffering from steroid-resistant AGVHD were treated with H65-RTA, an anti-T cell RTA immunotoxin, for up to 14 daily doses. A sequential patient phase I-II dose escalation design was used to assess safety and efficacy.

MATERIALS AND METHODS

Drug Preparation

H65-RTA (XomaZyme-H65; XOMA Corp, Berkeley, CA) is composed of a CD5-specific murine immunoglobulin IgG1 MoAb coupled by a disulfide bond to RTA. The CD5 antigen is present on greater than 95% of mature human T lymphocytes and on no other normal human adult or fetal tissues, as assessed by immunoperoxidase studies (XOMA Corp, unpublished observations, July 1985). All detectable ricin B chain is removed from the RTA preparation before conjugation. The generation of the H65 MoAb and preparation of the immunotoxin have been previously described. The immunotoxin used in the clinical study contained an average 8.1% free MoAb, had an average RTA:MoAb molar ratio of 2.0, and retained an average of 82.7% binding activity to MOLT-4 cells (ATCC, Bethesda, MD), a T-cell acute lymphocytic leukemia (ALL)-derived cell line that expresses CD5. Under appropriate conditions, H65-RTA can produce up to a 98% depletion of T lymphocytes from human bone marrow without decreasing the number of committed hematopoietic progenitor cells assayed as colony forming unit (CFU) or colony forming unit (BFU). For clinical use, the dose was diluted in 50 to 150 mL of normal saline and infused intravenously (IV) over a 1-hour period.

Clinical Protocol

The clinical trial was conducted under a US investigational new drug exemption with approval of the investigational review board of each institution. Written informed consent was obtained. Patients had received an allogeneic marrow transplant as treatment for leukemia, lymphoma, myeloma, severe aplastic anemia, or genetic disorders of hematopoiesis, and were entered into the study irrespective of the method of GVHD prophylaxis. Patients had persistent or progressive grade II through IV AGVHD of the liver or gut, or stage II through IV skin disease despite at least 5 days of steroid therapy, and H65-RTA therapy was started within 100 days after therapy; evaluation of GVHD was performed on study days 5 through 7 after the initial infusion. Titer of antibodies against MoAb H65 and RTA were measured on study days 15, 18, 21, and 28, and thereafter periodically to day 100.

All patients were evaluated for safety as assessed by subjective symptoms, physical examination, and laboratory parameters during and 7 days after therapy; evaluation of GVHD was performed on study days 5 through 7 after infusions. Serum chemistries and complete blood counts were performed daily and 7 days after therapy; evaluation of GVHD was performed on study days 5 through 7 after the initial infusion. Titers of antibodies against MoAb H65 and RTA were measured on study days 15, 18, 21, and 28, and thereafter periodically to day 100.

The clinical protocol was started 1 through 4 for GVHD: skin by amount of surface involved with rash, gastrointestinal tract by the volume of diarrhea, and liver by serum bilirubin levels. Patients with blistering were considered to have stage 4 skin disease, and patients with ileus, melena, or visible blood in the stool were considered to have stage 4 gastrointestinal disease. Documentation of a second disease process in the liver or gastrointestinal tract excluded the respective organ from evaluation. An organ response was defined as achievement by at least one organ above 25% decrease in serum bilirubin by at least 25%. Patients were also given an overall grade based on severity of organ involvement. At study entry, those with only stage 1 and 2 skin disease were designated grade 1, and those with either stage 3 skin disease only or stage 1 visceral involvement were grade II. Grades III and IV included stage 2 through 4 visceral disease and/or stage 4 skin disease.

Safety Assessment

Side effects associated with administration of H65-RTA were assessed on each infusion day and for 7 days thereafter by physical examination, EKG, chest x-ray, serum chemistries, urinalysis, and complete blood count. The skin, gastrointestinal tract, and liver were not assessed for drug toxicity if those organs were involved with GVHD. Reasons for premature discontinuation of drug were determined. Hypoalbuminemia was defined as a decrease to less than 75% of the starting value on 2 successive days despite albumin infusions. Weight gain was defined as increase of at least 1 kg in body weight during or within 7 days after infusions. Fevers were defined as temperature greater than 38°C occurring during or within 6 hours after any infusion. Renal dysfunction was defined as a serum creatinine that increased to at least twice the baseline on at least 2
Flow Cytometry

Lymphocyte phenotyping was performed at each participating institution. Peripheral blood mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation. Cells were stained by direct or indirect immunofluorescence using Leu 1 or Leu 4 (CD5 or CD3) antibodies (Becton-Dickinson Co, Mountain View, CA). The percent of CD3 + and CD5 + lymphocytes was determined by forward and side scatter-gated flow cytometry, and converted to absolute numbers based on the absolute lymphocyte count. To be evaluable, the lymphocyte phenotyping had to include a prestudy sample obtained no more than 2 days before the start of immunotoxin infusions, and three or more samples obtained after starting treatment. Samples containing less than 95% lymphocytes were excluded. Six patients had complete studies for CD3 + lymphocytes, and two additional patients had complete studies for CD5 + lymphocytes.

Pharmacokinetics

To measure serum levels of intact immunotoxin, an affinity-purified goat anti-RTA antibody in carbonate buffer (pH 9.6) was adsorbed to microtiter plates, and residual sites were blocked with 1% bovine serum albumin (BSA). Test samples was diluted 1:10 to 1:160 in phosphate-buffered saline containing 1% BSA and 0.05% polysorbate 20, and incubated in the wells for 1 hour at 37°C. Bound immunotoxin was detected using an alkaline-phosphatase conjugated goat anti-mouse IgG, antibody, and the reaction was developed using p-nitrophenylphosphate. Optical densities were read at 405 nm in a MICROELISA autoreader (Dynatech, Alexandria, VA). Levels of circulating immunotoxin were calculated from a standard curve.14

Antibody Response

An enzyme immunoassay was used to detect and titrate antibody responses to RTA or H65 MoAb.14 Test samples were diluted from 1:50 to 1:2,040,800 and added to microtiter plates containing adsorbed H65 MoAb or RTA. Human antibodies were detected using alkaline-phosphatase conjugated goat anti-human IgG (Zymed Labs, South San Francisco, CA), and the reaction was developed using p-nitrophenylphosphate. Optical densities were read at 405 nm in a MICROELISA autoreader (Dynatech, Alexandria, VA). Levels of circulating immunotoxin were calculated from a standard curve.14

RESULTS

Between May 1986 and December 1987, 34 patients were entered onto the protocol in eight institutions in the United States and Canada. Five patients had stage 2 through 4 skin GVHD only, whereas the others had visceral involvement. Severity of GVHD at initiation of therapy is shown in Table 1. Overall, the average grade of GVHD at start of therapy with H65-RTA was 3.3. Most patients had leukemia and received marrow transplants from HLA-identical siblings (Table 2). Eleven patients had received a second- or third-line agent before immunotoxin treatment.

Table 1. Severity of GVHD at the Initiation of H65-RTA Treatment

<table>
<thead>
<tr>
<th>Organ Stage</th>
<th>0</th>
<th>I</th>
<th>II</th>
<th>III/IV</th>
<th>NE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>6</td>
<td>2</td>
<td>8</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>10</td>
<td>4</td>
<td>3</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>Liver</td>
<td>14</td>
<td>3</td>
<td>3</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Overall grade</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: NE, nonevaluable.

Safety

All patients were evaluated for safety. Clinical observations noted during therapy are shown in Table 3, and were evenly distributed across all doses. Symptoms thought to be related to drug were constitutional symptoms such as depression, lethargy and fatigue, and hypoaalbuminemia with weight gain, which generally resolved within 1 week of cessation of therapy, absent other complicating medical conditions. The etiology of other observations was obscured by multiple medical complications and concomitant medications.

Weight gain was seen at all dose levels during the first 11 infusion days in 23 of the patients, but in 11 patients it resolved during or within 7 days after therapy. The amount and rate of weight gain were similar in patients treated at 0.1 and 0.2 mg/kg/d. The small size of the other groups did not permit statistical analysis. Hypoaalbuminemia was also noted, but was easily controlled by albumin infusions and never caused premature discontinuation of the infusion course. Sporadic large temperature fluctuations occurred during and between infusions for most patients. Infections were common and probably accounted for most of the fevers, since no characteristic pattern was discernible. Twenty-two patients were treated with amphotericin during H65-RTA therapy. Onset of microscopic or macroscopic hematuria during immunotoxin therapy was noted in 13 patients and was not associated with casts or proteinuria; this was attributed to
hemorrhagic cystitis associated with use of cyclophosphamide in the conditioning regimen.

All patients in the study were receiving at least one potentially nephrotoxic drug (cyclosporine, aminoglycoside antibiotic, or amphotericin) during immunotoxin treatment, and renal insufficiency occurred in six patients. The mean serum blood urea nitrogen and creatinine levels (48.5/1.35) at the onset of immunotoxin treatment were significantly higher in this group than in those with stable renal function (31.1/1.0). Renal dysfunction reversed in two patients and was a cause of death in one; three patients died of other causes with continued renal dysfunction; one patient required dialysis.

Fourteen of the 34 patients received less than the 14 prescribed immunotoxin doses because of deterioration in pre-existing transplant-related conditions. Reasons for drug discontinuance were death from infection in 5 cases, death from multi-organ failure in 3 cases, or increasing renal dysfunction in 5 cases. Immunotoxin treatment was discontinued on study day 13 in one patient because of a seizure that occurred while anti-convulsant medications were being tapered. No other indication of neurotoxicity was noted in this patient. No patients developed B-cell lymphoproliferative disorders.

Efficacy

GVHD response at last assessment: survival to study day 100. Of the 34 patients entered in the study, 32 were evaluable for efficacy. The other two patients, both of whom had grade IV disease, died within the first 7 days of therapy, before the first evaluation for GVHD response. Twenty of the 32 patients received a full 14-day course of immunotoxin therapy, 5 received 7 to 13 doses, and 7 received fewer than 7 doses. No apparent relationship was observed between the dose level and response rate; hence, data for all dose levels have been pooled for analysis.

Evaluation of individual organ response was performed at study days 45 through 60, at the initiation of subsequent additional immunosuppressive treatment, or at the time of death, whichever occurred first (Table 4). Six patients had concomitant gastrointestinal or liver dysfunction that precluded evaluation of GVHD in the respective organs. Responses occurred in all three organ systems: 73% in skin, 45% in gastrointestinal, and 28% in liver. Twenty-two of the 32 evaluable patients (69%) responded in at least one organ system. Two patients showed initial improvement but had subsequent recurrence of GVHD. These patients are recorded in Table 5 as nonresponders. The other 20 patients had durable responses in their responsive organs.

In addition to individual organ response, overall improvement in AGVHD was assessed. Nine patients had complete resolution of disease in all evaluable organ systems at last evaluation (complete response) and received no additional immunosuppressive medications (Table 5). Six survived past study day 100. The other two died on study days 16 and 38, respectively, of hepatic deterioration associated with documented cytomegalic inclusion virus hepatitis.

Seven patients had partial responses, defined as durable improvement, in at least one evaluable involved organ system without progression in any other evaluable organ. Two of these received additional immunosuppressive therapy after completion of immunotoxin therapy, despite continued improvement, as consolidation therapy. The others received no additional immunosuppressives. Four of the eight died of non-GVHD complications by study day 28 and four lived past study day 100. Overall, sixteen patients (50%) had durable improvement in their GVHD (Table 5).

Sixteen patients were classified as nonresponders at study exit. Six who showed neither improvement or progression of GVHD in any evaluable organ at study exit were classified as having stable disease. Three of these died by study day 25; one died on study day 62 of multi-organ failure, and two lived past study day 100. Four patients showed progressive GVHD in at least one evaluable organ without improvement in other

Table 3. Clinical Observations Noted in Patients Treated With H65-RTA

<table>
<thead>
<tr>
<th>Observation</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain (&gt; 1 kg)</td>
<td>23 (68)</td>
</tr>
<tr>
<td>Hypoalbuminemia (≤ 75% of baseline)</td>
<td>11 (32)</td>
</tr>
<tr>
<td>Depression, lethargy, fatigue</td>
<td>14 (42)</td>
</tr>
<tr>
<td>Myalgia, arthralgia</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Tremor</td>
<td>12 (29)</td>
</tr>
<tr>
<td>Fever</td>
<td>18 (53)</td>
</tr>
<tr>
<td>Hematuria*</td>
<td>13 (38)</td>
</tr>
<tr>
<td>Renal Dysfunction</td>
<td>6 (18)</td>
</tr>
</tbody>
</table>

During or within 7 days after therapy.

*Onset after start of immunotoxin infusion.

Table 4. Response to H65-RTA Treatment at Study Exit Categorized by Individual Organs

<table>
<thead>
<tr>
<th>Involvement and Outcome</th>
<th>Skin</th>
<th>Gastrointestinal Tract</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluable</td>
<td>26</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>Resolution 11 (42%)</td>
<td>6 (27%)</td>
<td>3 (17%)</td>
<td></td>
</tr>
<tr>
<td>Improved</td>
<td>8 (31%)</td>
<td>4 (18%)</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>Stable</td>
<td>4 (15%)</td>
<td>11 (50%)</td>
<td>8 (44%)</td>
</tr>
<tr>
<td>Progression 3 (12%)</td>
<td>1 (4%)</td>
<td>5* (28%)</td>
<td></td>
</tr>
<tr>
<td>Not evaluable</td>
<td>0</td>
<td>2†</td>
<td>3†</td>
</tr>
<tr>
<td>Not involved</td>
<td>6</td>
<td>8</td>
<td>11</td>
</tr>
</tbody>
</table>

*Progression of liver disease was not documented by biopsy but was generally accompanied by documented progression in skin or gastrointestinal disease.

†Documented cytomegalinclusion virus hepatitis, gastroenteritis, or c. difficile gastroenteritis.

Table 5. Overall Response at Last Evaluation

<table>
<thead>
<tr>
<th>Response</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete response</td>
<td>9 - 50%</td>
</tr>
<tr>
<td>Partial response</td>
<td>7</td>
</tr>
<tr>
<td>Stable disease</td>
<td>6</td>
</tr>
<tr>
<td>Progression</td>
<td>4</td>
</tr>
<tr>
<td>Mixed response</td>
<td>6 - 50%</td>
</tr>
<tr>
<td>Not evaluable</td>
<td>2*</td>
</tr>
</tbody>
</table>

* Died before day of first evaluation for GVHD.
organs. Three of the four died by study day 34 of infection or renal failure, and one remains alive. Six patients showed improvement in at least one evaluable organ, but had progressive GVHD in at least one other organ. One of the six received additional immunosuppressive medications and remained alive past study day 100. One died on day 68 of leukemic relapse. The other four died of GVHD or respiratory failure.

Of the 32 evaluable patients, 27 had overall grade 3 and 4 AGVHD at study entry. Fourteen of those were responders, and 13 were nonresponders. The five remaining patients entered the study with skin disease only, and four were responders. The likelihood of response was not correlated with time to immunotoxin therapy or dose of immunotoxin. Two of 7 patients receiving fewer than 7 doses had complete responses, and two patients had partial responses. Thirteen of the 32 evaluable patients had partial or complete resolution of disease by day 7 of therapy.

Long-term survival and cause of death. There was a significant survival advantage at all times in patients who attained complete or partial responses (P = .03, log-rank test) (Fig 1). Deaths in responding patients were generally caused by late infection. Of the six patients with complete responses who survived past study day 100, three remain alive and disease-free 300 to 877 days after immunotoxin therapy, and three patients died of infection, veno-occlusive disease and leukemic relapse, respectively. All three of the patients with partial responses who survived past study day 100 died of infection. One patient with stable disease remains alive at study day 475 with mild chronic GVHD of the gastrointestinal tract. One patient with progressive disease remains alive at study day 320 after having responded to additional immunosuppressive medications. All six of the patients with mixed responses died.

Flow Cytometry

Seven patients had flow cytometric enumeration of CD5+ peripheral blood lymphocytes at least three times during or immediately after immunotoxin treatment (Figure 2A). Clearance of lymphocytes was not correlated with dose. Counts in four patients decreased to less than 20% of the starting values after three infusions (just before the fourth infusion). Two patients required eight infusions to reach this level, and one reached a nadir of 50% of the starting value after six infusions. Six of these patients had enumeration of CD3+ cells to determine whether the decrease in CD5+ cells was a reflection of antigen modulation or removal of cells from the circulation, since CD5 and CD3 are generally coexpressed on peripheral T lymphocytes. The results for CD3+ lymphocytes were very similar to those for CD5+ T lymphocytes (Fig 2B). Counts in four patients decreased to less than 20% of starting values after three infusions. One patient treated at the lowest dose developed a
population of CD3+/CD5- lymphocytes on study day 7. This corresponded to a relapse of his disease. This was not noted in the other patients.

**Pharmacokinetics**

Serum levels of immunotoxin were followed in 13 patients (Table 6). Peak serum levels were attained within 5 minutes after the end of infusion, followed by a rapid decrease during the following 2 hours. At or above doses of 0.1 mg/kg, immunotoxin could still be detected at 6 hours, but not at 24 hours. Reliable terminal half-lives could be calculated at the 0.1 and 0.2 mg/kg doses and were in the range of 1.5 to 3.9 hours. Peak serum levels ranged between 0.95 and 5.1 μg/mL and correlated with the dose.

**Anti-Immunotoxin Antibody Response**

Before treatment with immunotoxin, no patient had antibodies to H65 MoAb or to RTA. Of twenty-three patients tested between 13 and 103 days after therapy, six (26%) developed antibodies to H65 MoAb and to RTA (Table 7). In patients who did develop an antibody response, titers against H65 MoAb and RTA were comparable, and the human antibodies did not block binding of H65 MoAb to CD5. No patient developed an infusion-related allergic reaction.

**Comparison of H65-RTA to Historical Controls Treated With ATG**

The survival of patients in this study were compared with that of 84 patients who had failed steroids and received ATG as second-line therapy for AGVHD, accrued in one institution (Fred Hutchison Cancer Research Center, Seattle, WA) between 1975 and 1987, and in another (Johns Hopkins, Baltimore, MD) between 1978 and 1988. Survival of patients treated with H65-RTA was longer than that of patients treated with ATG, although this difference was not statistically significant (P = 0.07) because sample sizes were small (data not shown).

**DISCUSSION**

In this study, patients with moderate to severe AGVHD were treated with immunotoxin H65-RTA at four escalating doses for up to 14 days. Side effects were noted at all doses, primarily constitutional symptoms such as depression, lethargy and fatigue, as well as hypoalbuminemia with weight gain but without pulmonary edema. Similar side effects have been noted in clinical trials with other RTA immunotoxins. Renal dysfunction was seen in some patients, but the relationship to immunotoxin administration was unclear since volume depletion, and other drugs such as cyclosporine, amphotericin, and aminoglycosides represented concomitant risk factors for nephrotoxicity. Renal dysfunction related to H65-RTA administration has not been seen in less ill populations of BMT patients (Henslee et al, unpublished data), in rheumatoid arthritis patients, or in preclinical nonhuman primate models (XOMA Corp, unpublished data). Fifty-four percent of the deaths in this study were related to viral, bacterial, or fungal infection. Infections are a leading cause of death in all BMT patients: in one study, 67% of deaths listed fungal or bacterial infection or interstitial pneumonia as a primary cause of death, and it is particularly high in patients with GVHD, where 61% of deaths have been reported due to interstitial pneumonia alone. Thus, there was no indication that H65-RTA increased incidence of infection.

With follow-up from 7 to 60 days after starting treatment, 50% of patients had complete or partial overall responses without recurrence of GVHD. There was a 73% response in skin, 45% in gastrointestinal tract, and 28% in liver. None of the patients attaining complete responses required subsequent additional immunosuppressive treatment, and only two patients with partial response had a recurrence of GVHD. Patients with partial responses had a reduction in severity of GVHD, which improved the quality of life and reduced or eliminated the need for systemic immunosuppressants. Patients with stable disease, initial response with relapse, or response in one organ system but deterioration in another were classified as nonresponders. Survival analyses demonstrated the validity of this classification system since responders had significant improvement in survival as compared with nonresponders. BMT is a high-risk procedure with infection, multi-organ failure, and GVHD all causing mortality. These data suggest that an agent intended as treatment for only one of these problems, AGVHD, can influence survival.

In general, maximum reduction in circulating T lymphocytes, as measured by flow cytometry, occurred by day 7 (Fig 2). In two of these patients, recovery of T-lymphocyte number and function was followed for up to 14 months. In one patient, absolute T-lymphocyte counts returned to normal values within 2 months after therapy. In the other patient, T-lymphocyte counts remained low but normalized by 14 months. Both patients studied regained normal response to mitogen by 2 months after therapy, as measured by peripheral blood lymphocyte responsiveness to phytohemagglutinin.
glutinin (N.A. Kernan, personal communication, August 1989). There was no correlation between immunotoxin dose and clinical response, side effects, or clearance of CD5+ cells from the blood. Pharmacokinetic studies showed that peak serum levels at or above 1 μg/mL were attained at all doses. These results suggest that all the doses used in the study were above the minimal effective dose for this patient population. Studies with other anti–T-lymphocyte MoAbs have reported lymphocyte saturation to be achieved at similar serum levels. Furthermore, in vitro studies have shown that H65 MoAb and H65-RTA saturate peripheral blood mononuclear cells at levels between 0.1 and 0.5 μg/mL (XOMA Corp, unpublished observations, December 1988). On the basis of these results, a dose of 0.1 mg/kg/d can be recommended for future clinical studies. Although the kinetics in individual patients were complex, it was possible to calculate the terminal half-life for immunotoxin in the blood for nine patients receiving doses of 0.1 or 0.2 mg/kg. The values obtained, 1.5 to 3.9 hours, were considerably shorter than the 6- to 58-hour T ½ estimates reported previously for anti–T-cell MoAb in patients with AGVHD. Rapid clearance of RTA immunotoxins may be the result of uptake by Kupffer cells, which bind mannose residues on the RTA.

A variety of immunosuppressive medications have been used as first- or second-line therapy for AGVHD, and, in general all have some effect, especially on mild to moderate AGVHD, although none were clearly superior to steroids alone. Of the second line agents currently used for treatment of AGVHD, H65-RTA most closely resembles ATG in its mechanism of action and dosing schedule. ATG is a polyclonal antibody generally administered daily or every other day as an IV infusion for up to 14 days without tapering of the dose. One study reported that this agent had a relatively high incidence of transient improvement when used as second-line therapy.

Results of this study suggest that H65-RTA is an effective immunosuppressive agent. Used as secondary treatment of AGVHD, H65-RTA is safe and yields results comparable with or better than those seen with ATG. Therefore, H65-RTA can provide an acceptable alternative to ATG in this critically ill patient population.

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Use of an anti-pan T-lymphocyte ricin a chain immunotoxin in steroid-resistant acute graft-versus-host disease

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