Successful Treatment of Disseminated Human Hodgkin's Disease in SCID Mice With Deglycosylated Ricin A-Chain Immunotoxins

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To evaluate the effects of deglycosylated ricin A-chain (dgA) immunotoxins against disseminated Hodgkin's lymphoma, we used RFT5.dgA (CD25) and IRac.dgA (70 kD) to treat L540Cy Hodgkin cells in severely immunodeficient SCID mice. In this model, more than 90% of the animals developed multiple lymphomas in various organs such as the lymph nodes, liver, bone marrow, and extranodal sites that killed untreated animals after a mean survival time (MST) of 36.3 days. A single intraperitoneal injection of 8 μg of either immunotoxin rendered 95% (RFT5.dgA) and 93% (IRac.dgA), respectively, of mice tumor-free when applied 1 day after tumor challenge. The MST of the RFT5.dgA-treated group was extended by more than 90 days (P < .00001). SCID mice treated 12 days after tumor challenge had lower remission rates (46%), suggesting that the antitumor effect of the immunotoxins depends on the number of tumor cells present. We conclude that ricin A-chain immunotoxins have potent antitumor effects against disseminated Hodgkin's tumors in SCID mice and that this model is ideally suited for the evaluation of different immunotoxin treatment modalities.

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MATERIALS AND METHODS

Materials. Tissue culture medium RPMI 1640 and fetal calf serum were purchased from GIBCO-Biocult Ltd (Paisley, UK). Sepharose G25 (fine grade), Sephaeryl S200 HR, and blue Sepharose CL-6B were obtained from Pharmacia Ltd (Uppsala, Sweden). Falcon tissue culture flasks were purchased from Becton Dickinson (Oxnard, CA). Human Cot-1 DNA was from GIBCO BRL, Life Technologies, Inc (Gaithersburg, MD). 5[α[32P]-dATP, L-[4,5-3H]-Leucine, and Hybrid-N-Transfer membranes were obtained from Amersham International (Braunschweig, Germany). Proteinase K and Denhardt's solution were from Sigma (St Louis, MO). The Cellfree IL-2R Test Kit was purchased from T Cell Diagnostics, Inc (Cambridge, MA). Ber-H2 antibody, biotinylated rabbit antimouse MoAb (E 413), and a streptavidin-biotin-complex labeled with alkaline phosphatase (K 391) were from Dako Diagnostics (Hamburg, Germany). New Fuchsin was obtained from Serra Feinbiochemica (Heidelberg, Germany).

Cells. The cell line L540Cy was maintained in RPMI 1640 supplemented with 10% (vol/vol) heat-inactivated fetal calf serum, 2 mmol/L L-glutamine, 50 IU/mL penicillin, and 50 μg/mL streptomycin. Cells were cultured in an atmosphere of 5% CO2 and humidified air. The L540Cy is a mutant of the L540 cell line, which has been shown to have superior take rates in mice as compared with the parental L540.

Chinese hamster ovary (CHO) cells grew as adherent cells in the same medium. For the preparation of cell suspensions they had to be detached by adding trypsin-EDTA solution (10%).

Antibodies. The MoAbs used in this study were RFT5 (IgG1), IRac (IgG1), and Ber-H2 (IgG1). RFT5 recognizes the CD25 antigen, IRac has been reported to recognize a 70-kD antigen on Hodgkin and Reed-Sternberg cells, and Ber-H2 binds to the CD30 antigen. The mouse MoAb OX7 (IgG1) recognizing the mouse Thy 1.1 antigen was used as nonspecific isotype control.

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Ricin A-Chain Immunotoxins Against HD

**Immunotoxins.** Two different immunotoxins, RFT5.dgA and IRac.dgA, were prepared as described elsewhere. In brief, the MoAbs were treated with the heterobifunctional linker, 4-succinimidyl-oxycarbonyl-o-methyl-o-(2- pyridyldithio)toluene (SMPT) to introduce an average of 1.7 activated disulfide groups per molecule of antibody. The derivatized protein was separated from unreacted material by gel chromatography on a Sephadex G-25 column and mixed with freshly reduced deglycosylated ricin A-chain. After 72 hours, residual thiol groups were inactivated with 0.2 mmol/L cysteine. The immunotoxin preparation was then purified from unreacted ricin A-chain, cysteine, and high molecular weight aggregates on a Sephacryl S200 HR column. Finally, free antibody was removed by chromatography on a Blue Sepharose CL-6B column.

**Cytotoxicity assays.** L540Cy Hodgkin cells in complete medium were distributed into 96-well plates with each well containing 2 x 10⁴ cells in 100 µL. Immunotoxins were added to give final concentrations ranging from 1 x 10⁻⁸ to 1 x 10⁻¹³ mol/L. The plates were incubated for 24 hours at 37°C in an atmosphere of 5% CO₂ in humidified air, pulsed with 1 µCi/well [³H]-leucine, and incubated for another 24 hours. The cells were then harvested onto glass fiber filters using a Titrtek cell harvester and the incorporated [³H]-leucine was measured by liquid scintillation (Beckmann, LS/801). The concentration (IC₅₀) required to achieve a 50% reduction of protein synthesis relative to untreated control cultures was calculated.

**Animals.** The SCID mice were obtained from our own colony and maintained under sterile conditions. Antibiotic prophylaxis was not provided.

**Establishment of the tumor model and antitumor experiments.** Adult 4- to 6-week-old SCID mice were injected intravenously via the tail vein with 1 x 10⁷ L540Cy Hodgkin cells suspended in 400 µL of phosphate-buffered saline (PBS) containing 0.2% bovine serum albumin (BSA). Vital signs were recorded daily, and weight was measured once a week. Four to 6 weeks after inoculation of the tumor cells, untreated animals developed signs of progressive disease including weight loss, ruffled fur, and inactivity. The SCID mice were then killed. After macroscopic examination, different organs, including lymph nodes, liver, spleen, lung, kidneys, thymus, intestine, caecum, muscle, and extranodal tumors, were either fixed in 10% buffered formalin or snap-frozen in liquid nitrogen. Cyto-centrifuge slides were prepared from bone marrow extracted from the tibiae. Mouse serum was obtained by heart puncture and centrifugation of the blood.

SCID mice with elevated Ig levels were identified using an enzyme-linked immunosorbent assay (ELISA) and excluded from further studies. For treatment experiments, the mice were randomly divided into groups of five or six. One day after inoculation of L540Cy Hodgkin cells, the SCID mice received a single intraperitoneal (IP) injection of immunotoxin or antibody in a volume of 400 µL PBS containing 0.2% BSA. The dose of immunotoxin (48 µg of total protein corresponding to 8 µg of ricin A-chain) represented the same proportion of the LD₅₀ (50% lethal dose) of RFT5.dgA and IRac.dgA in nude mice. The doses of unconjugated MoAbs (40 µg) matched those in the immunotoxins.

All animals were killed after 6 weeks and examined for signs of tumor growth by gross macroscopy, immunohistopathology, and dot blotting of extracted DNA from various organs. To evaluate the effects of immunotoxin treatment on the mean survival time (MST), groups of 10 SCID mice were treated with RFT5.dgA as indicated above. RFT5- and PBS-treated animals served as controls.

**Immunohistopathology.** Organs were fixed in 10% buffered formalin, paraffin-embedded, sectioned, and then stained with hematoxylin and eosin or Giemsa's solution for microscopic examination. Immunohistochemistry was performed using the streptavidin-biotin-complex method (ABC method) described previously. In brief, the sections were digested with trypsin followed by incubation with the CD30 MoAb Ber-H2 (dilution 1:50) for 30 minutes at room temperature. After brief washes in Tris-buffered saline, the slides were incubated with a biotinylated rabbit antimouse MoAb (F(ab) fragments, E 413, dilution 1:400, 30 minutes). After washing, the slides were incubated with streptavidin-biotin-complex labeled with alkaline phosphatase (K 391) for 30 minutes. After another wash, the alkaline phosphatase reaction was developed with the New Fuchsin method. The slides were counterstained with Haemalaun and mounted.

Cyto-centrifuge slides of bone marrow were fixed in 100% acetic and stained with CD25, CD30, and IRac MoAbs using the APAAP-technique that has been described in detail elsewhere.

**DNA isolation and dot-blots.** For DNA isolation, various mouse organs were grounded in a cold mortar. The solution was resuspended in 5 mL of lysis buffer (1% sodium dodecyl sulfate [SDS], 100 mmol/L EDTA) and incubated with 400 µg/mL proteinase-K (20 mmol/L Tris-HCl, pH 7.5) for 16 hours. After incubation with 200 µg/mL RNase A (10 mmol/L Tris-HCl, pH 7.5) for another 30 minutes, the sample was extracted first with equilibrated phenol (pH > 7.8), then three times with a mixture of phenol/chloroform/isooamylalcohol (25:24:1), and finally with chloroform/isoamylalcohol (24:1). Eventually, the DNA was ethanol-precipitated, dried overnight, and resuspended in 300 µL TE buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 7.4). The concentration of the genomic DNA was determined by fluorescence-electrophoresis (ethidium bromide labeling) on a 1% agarose gel.

One hundred nanograms of each sample was transferred to the Hybond-N-Transfer membrane. The samples were air-dried overnight and then prehybridized for 24 hours at 42°C in a solution containing 50% formamide, 1% SDS, SSPE (0.75 mol/L NaCl, 50 mmol/L NaH₂PO₄, H₂O, 5 mmol/L Na₂EDTA, pH 7.4), Denhardt’s solution (0.1% BSA, 0.1% ficoll, 0.1% polyvinylpyrrolidone), 50% dextran sulfate, and 50 µg/mL salmon sperm DNA. Hybridization was performed with the prehybridization buffer and 100 ng of a [³²P]-labeled cDNA probe overnight at 42°C. Cot-1 DNA was obtained from human placental DNA and enriched for repetitive DNA sequences such as the Alu I and Kpn I family members. The transfer membrane was washed at high stringency and autoradiographed.

The percentage of the human DNA present in the mice organs was estimated by computerized densitometry. The following human-hamster DNA mixtures were used as controls: 50% human-50% hamster, 25% human-75% hamster, 5% human-95% hamster, 0.5% human-99.5% hamster, 0.05% human-99.95% hamster, and 0.005% human-99.995% hamster.

**Establishment and characterization of cultures.** Tumors were removed under sterile conditions, rinsed in complete medium, and finely minced with a scalpel. Tumor cell containing medium was then transferred into Falcon tissue flasks with a syringe and incubated in complete medium. Cyto-centrifuge slides were prepared from the recultured cells and stained with IRac, RFT5, and Ber-H2 MoAbs, respectively, using the APAAP-technique (see above). When the cultures appeared to be homogeneous for L540Cy cells (about 2 weeks after tumor excision), the cells were checked for antigen expression by FACS analysis using standard techniques described elsewhere and retreated with immunotoxins in vitro (see Cytotoxicity Assays).

**IL-2R ELISA.** Serum of tumor-bearing SCID mice was examined for circulating IL-2R complexes using a standard IL-2R ELISA. Serum from healthy animals served as controls.
Statistical analysis. To compare different treatment modalities, the animals were killed 6 weeks after tumor cell inoculation and staged according to the extent of tumor burden. Mice with tumor growth restricted to the lymph nodes were scored as stage I. Liver involvement was scored as stage II and additional extranodal disease as stage III. Stage IV was defined as extended disease with the involvement of lymph nodes, liver, and/or extranodal disease plus additional bone marrow involvement. The different scores per group were added and divided by the number of animals per group, resulting in an average staging index per group. The statistical significance was determined by the Wilcoxon-Mann-Whitney U-test. The survival data were tested for statistical significance using the Cox-Mantel test.

RESULTS

Growth of L540Cy Hodgkin cells in SCID mice. Intravenous inoculation of $1 \times 10^7$ L540Cy cells via the tail vein induced tumor growth in 91.9% of untreated SCID mice (34 of 37). Signs of progressive disease became visible after ap-
proximately 4 weeks in untreated animals. Animals were killed after 6 weeks. Lymphomas were observed in lymphatic organs like lymph nodes (92%; Fig 1A), liver (57%; Fig 1B), and bone marrow (8%; Fig 1C). Lymph nodes involved included axillary, inguinal, cervical, paraaortic, caecal, mesenteric, iliac, gluteal, renal, and portal nodes. The human Hodgkin's lymphomas grew as distinct white nodules of solid consistency and variable size. In addition to the nodal tumor formation, extranodal tumor growth was observed in bones (8 animals), kidneys (6 animals), testes (2 animals), lungs (2 animals), and muscle (1 animal). Hindleg paresis caused by enlarged gluteal lymph nodes adjacent to the sciatic nerve was observed in 3 of 37 animals. In contrast to the growth pattern of Hodgkin's lymphomas in humans, there was no evidence of spleen involvement. Average spleen size (12 X 3 X 1 mm) of tumor-bearing animals was similar to the spleen size of healthy SCID mice.

Histologically, the tumors appeared as large cell anaplas-
Fig 2. Dot-blot analysis of DNA extracted from SCID mouse organs and cell suspensions of L540Cy HD and CHO cells. DNA probes were hybridized with $^{32}$P-labeled human cot-1 DNA. An intense positive signal can be seen in the autoradiograph when the probe contains DNA from more than $1 \times 10^4$ human cells. (A) DNA from organs of an untreated animal (testes [4], tumor of the testis [5], tumor of the liver [6], liver [7], gluteal lymph node [8], and axial/inguinal lymph nodes [9]). Screening of DNA extracted from liver, spleen, and axial/inguinal lymph nodes of four mice treated with $8 \mu g$ IRac.dgA 1 day after tumor cell inoculation (B, 4 through 9; C, 4 through 9), three animals treated with $8 \mu g$ RFT5.dgA on day 1 (D, 4 through 9; E, 7 through 9), and six animals treated with an immunotoxin cocktail ($4 \mu g$ RFT5.dgA + $4 \mu g$ IRac.dgA) on day 1 (F, 1 through 9; G, 1 through 9). DNA extracted from caecum (4), lung (5), testes (6), liver (7), spleen (8), and axial/inguinal lymph nodes (9) of a healthy SCID mouse (H) and salmon-sperm DNA (100 ng, 10 ng, and 1 pg) (J, 1 through 3) served as negative controls. Mixtures of L540Cy and CHO ($1 \times 10^5$ L540Cy + $2 \times 10^7$ CHO [3], $1 \times 10^4$ L540Cy + $1.99 \times 10^7$ CHO [4], $1 \times 10^7$ L540Cy + $1.9 \times 10^7$ CHO [5], $1 \times 10^6$ L540Cy + $1.9 \times 10^7$ CHO [6], $5 \times 10^4$ L540Cy + $1.5 \times 10^7$ CHO [7], $1 \times 10^7$ L540Cy + $1 \times 10^7$ CHO [8], $2 \times 10^7$ L540Cy [9]) (l) were used for calibration. Different concentrations (10 ng, 100 ng, and 1 $\mu g$) of cot-1 DNA served as positive controls (J, 7 through 9).

Characterization of immunotoxins. The immunotoxins used in the present study were generated by linking MoAbs of the IgG1 subclass to deglycosylated ricin A-chain. Both RFT5.dgA (CD25) and IRac.dgA (unclustered 70 kD) had little cross-reactivity with normal human tissues when tested on cryostat sections: RFT5.dgA binds to a few activated lymphocytes and macrophages in lymphoid organs, IRac.dgA had only very minor crossreactivity. The two immunotoxins were extremely powerful against L540Cy Hodgkin cells in vitro (Fig 3). The concentration at which the protein synthesis of L540Cy cells is inhibited by 50% ($IC_{50}$) is $6 \times 10^{-12}$ mol/L for RFT5.dgA, which is identical to that of whole ricin under the same experimental conditions. RFT5.dgA is the most potent immunotoxin against HD described so far. IRac.dgA is the second most potent immunotoxin, with an $IC_{50}$ of $1 \times 10^{-11}$ mol/L.

Effects of immunotoxin treatment in SCID mice. To compare different treatment modalities, the extent of tumor infiltration was scored according to the pathologic staging. The data of the treatment experiments are summarized in Table 2. In the untreated control group (n = 37), there were
Table 1. Different Methods for Detection of Human L540Cy Hodgkin Cells in SCID Mice

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Abbreviations: M, gross macroscopy (-, no visible tumors; +, macroscopical stage I; ++, macroscopical stage II; ++++, macroscopical stages III/IV); I, immunohistopathology (-, no detection of human tumor [CD30+, CD25+, or IRac+] cells; +, detection of tumor cells infiltrating 10% or less of the cut organ; ++, detection of tumor cells infiltrating more than 40% of the cut organ; ++++, detection of less than 10,000 ng human DNA; ++, detection of 10,000 to 20,000 ng DNA; +++, detection of 20,000 ng human DNA or more).

34 animals with lymph node infiltration, 21 animals with liver metastases, 14 animals with extranodal lymphomas, and 3 animals with bone marrow infiltration, resulting in an average staging index of 1.95. In contrast, treatment with 8 pg of RFT5.dgA 1 day after inoculation of the tumor cells rendered 22 of 23 animals tumor-free when examined on day 42, resulting in a complete remission rate of 95%. One animal had a single L540Cy tumor at the lower jaw. The staging index in this group was 0.16. This effect was highly statistically significant as compared with the untreated control group (P < .00001).

The results for SCID mice treated with IRac.dgA were similar. After treatment on day 1, only 1 of 15 mice had bone marrow infiltration with L540Cy cells, resulting in 93% complete remissions and a staging index of 0.26 (P < .00001).

Effect of immunotoxin treatment on MST of tumor-bearing SCID mice. Groups of 10 SCID mice were treated with RFT5.dgA, RFT5, and PBS, respectively, 1 day after tumor challenge with L540Cy Hodgkin cells. As illustrated in Fig 4, the MST of the PBS-treated control group was 36.3 (±1.6) days. Upon macroscopical examination, all animals in this group had extended tumor masses. Treatment with the RFT5 antibody alone extended the MST by 11.3 (±5.5) days (P = .0002) without inducing complete remissions. In contrast, 7 of 10 mice treated with 8 pg of RFT5.dgA 1 day after the inoculation of L540Cy cells were in continuous complete remission more than 180 days after treatment. This result is highly statistically significant (P < .00001).

One animal treated died for unknown reasons on day 68 without exhibiting evidence of tumor growth. The other two animals treated with RFT5.dgA died from progressive lymphoma growth in lymph nodes, liver, kidney, and muscle on days 82 and 93, respectively. Recultures of these tumors showed that most malignant cells expressed the CD30 and the IRac (70 kD) antigen similar to the parental L540Cy Hodgkin cells. In contrast, more than 70% of the recultured cells were CD25-. In cytotoxicity assays, these sublines were less sensitive to RFT5.dgA than the original L540Cy line. Similar results have been observed in a previous study, suggesting that CD25- mutants survived the treatment with RFT5.dgA causing late relapses.

Effects of immunotoxin cocktails. To test the effects of a combination of two immunotoxins against different anti-
gens (cocktail) on L540Cy cells, two groups of a total of 11 SCID mice were treated with a mixture of 4 μg IRac.dgA and 4 μg RFT5.dgA 1 day after the inoculation of L540Cy cells. The treatment with the immunotoxin cocktail induced lasting complete remissions in all SCID mice resulting in a staging index of 0. No L540Cy Hodgkin cells were detected by means of gross examination, immunohistopathology, and DNA dot-blotting (Table 2). The effect of the immunotoxin cocktail was highly statistically significant as compared with the control group (P < .00001) but not superior to single immunotoxin treatment.

Effect of immunotoxin treatment on mice with larger tumor burden. A group of 11 SCID mice was treated with RFT5.dgA 12 days after the inoculation of L540Cy cells to test the antitumor potency of RFT5.dgA on larger tumor burdens. Upon death on day 42, 5 of 11 mice had no detectable L540Cy tumors, resulting in a complete remission rate of 46% (Table 2) and a staging index of 1.09. These results were statistically significant (P = .0307), but less pronounced compared with the earlier schedule in which the animals were treated 1 day after tumor challenge.

Effect of treatment with the RFT5 and IRac antibodies. Treatment of SCID mice with the RFT5 or IRac MoAbs alone did not result in statistically significant antitumor effects. There were no complete remissions in the IRac-treated group (n = 5; staging index = 1.4). In the RFT5-treated group, 8 of 9 animals developed lymphomas, resulting in a staging index of 1.56. The effects of the antibodies alone against L540Cy cells in SCID mice might be related to the activity of natural killer (NK) cells. Pretreatment of SCID mice with anti-asialo GM1 antibodies has been reported to induce an effective NK-cell depletion in SCID mice, which abrogates the antitumor effects of antibody treatment in this model.

Soluble CD25 levels in SCID mice with disseminated Hodgkin’s lymphoma. Serum samples of SCID mice with disseminated L540Cy Hodgkin’s tumors and healthy controls were evaluated for circulating IL-2 receptor (sCD25) using a standard ELISA method. As shown in Fig 5, mice examined 1 day after the injection of 1 × 10^7 L540Cy Hodgkin cells had detectable sCD25 levels averaging 105 U/mL (range, 54 to 155 U/mL; n = 10). When measured 42 days after injection of tumor cells, animals with Hodgkin’s lymphomas limited to the lymph nodes (stage I) had average sCD25 levels of 403 U/mL (range, 175 to 515 U/mL; n = 8). By contrast, animals with extended disease involving liver and extranodal sites had average sCD25 levels of 1,095 U/mL (range, 305 to 2,125 U/mL; n = 10). These results suggest that the amount of sCD25 shed from the L540Cy Hodgkin cells is correlated with the tumor burden.

DISCUSSION

The major finding to emerge from this study is that immunotoxins constructed with deglycosylated ricin A-chain and the SMPT linker have potent antitumor effects against disseminated Hodgkin’s lymphomas in SCID mice. A single injection of either immunotoxin, RFT5.dgA (CD25) or
IRac.dgA (70 kD, unclustered), 1 day after challenge with 1 × 10⁷ L540Cy cells rendered more than 90% of animals tumor-free when examined 42 days after treatment. The MST of mice treated with RFT5.dgA was extended by more than 120 days as compared with 36 days in a PBS-treated control group (Table 2). RFT5.dgA induced complete remissions in 95% of animals (22 of 23) and IRac.dgA resulted in 93% complete remissions (14 of 15). The MST of SCID mice treated in additional experiments with RFT5.dgA 1 day after tumor challenge was greater than 150 days as compared with 36 days in a PBS-treated control group.

Two mice treated with RFT5.dgA died of progressive lymphoma growth on days 82 and 93, respectively (Fig 4). Macroscopic and immunohistologic examinations showed L540Cy tumor growth in lymph nodes, liver, kidney, and muscle. Analyses of recultures showed that most of the Hodgkin cells recultured lacked the CD25 antigen. Obviously, antigen-negative mutants had survived the initial treatment and caused relapses. Similar observations have been made in an AKR mouse model in which Thy 1.1-negative mutants induced death of 20% of mice treated with an immunotoxin against the Thy 1.1 antigen.¹⁷ Treatment with a combination of two immunotoxins (“cocktail”) should reduce the likelihood of mutant cell escape. In SCID mice, an immunotoxin cocktail consisting of RFT5.dgA and IRac.dgA rendered 100% of SCID mice tumor-free when examined 42 days after treatment (1/1 of 11; Table 2). Because these results did not show statistical significance when compared with single immunotoxin treatment, we are currently determining the long-term survival data of SCID mice for both immunotoxins and immunotoxin cocktails. Preliminary results based on calibration experiments suggest that 4 logs of L540Cy cells can be killed with 8 µg (A-chain) of RFT5.dgA when administered 1 day after tumor challenge (Engert et al., unpublished observations). Based on the extension of the mean survival from 45.9 to 87.2 days of SCID mice challenged with Daudi cells, others have calculated in vivo killing of 4 logs of tumor cells using similar ricin A-chain immunotoxins against CD22.¹ In a different survival model, an anti-B4–blocked ricin immunotoxin (CD19) showed antitumor efficacy by extending the mean survival of human B-cell lymphoma-bearing SCID mice from 25 to 41 days, which represented 3 logs of cell kill.²⁶ The results obtained with RFT5.dgA applied 12 days after tumor challenge were inferior to the treatment on day 1 (46% vs 95% complete remissions), suggesting that the antitumor effect of immunotoxin treatment might, at least in part, depend on the number of L540Cy cells present.

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Fig 5. Level of sCD25 in SCID mice blood serum, as measured with a standard ELISA. Mice were inoculated with 1 × 10⁷ L540Cy cells on day 0 and killed either 1 (n = 5) or 42 days later, suffering from limited (stage I; n = 10) or from extended disease (stages II–IV; n = 11). Blood was obtained by heart puncture. Results are given with error bars.
other factor is probably the formation of solid L540Cy tumors in the animals 12 days after the initial injection, which are less accessible to the bloodstream of intravenously administered drugs.27

The impressive antitumor effects observed against disseminated human L540Cy tumors in this study parallel those observed against solid Hodgkin's tumors in nude mice.3 Possible reasons are the high sensitivity of Hodgkin's lymphomas to immunotoxin treatment and the use of second generation immunotoxins that, in contrast to their predecessors, have potent antitumor effects in vivo.28,29 In SCID mouse models, similar constructs have produced pronounced effects against disseminated human B-cell lymphoma1 and T-cell leukemia.2 Taken together, these results point towards a possible clinical use of immunotoxins against lymphomas in humans. Although cures in patients with massive tumor burdens are unlikely to occur, immunotoxins might be useful as an additional treatment modality in high-risk patients to kill residual tumor cells. This view is supported by the results of a recent study in which 3 of 4 heavily pretreated patients who had residual tumor cells after autologous bone marrow transplantation were rendered tumor-free by treatment with an anti-CD19 ricin A-chain immunotoxin.40 Importantly, these results were obtained with a sensitive PCR method in which as few as 1 lymphoma cell in 106 normal cells can be detected.31

One of the possible problems using immunotoxins against antigens that are being shed from the cell surface is the binding of immunotoxin to the circulating antigen resulting in a reduced antitumor effect. Our results suggest that shedding of scD25 in SCID mice is dependent on the tumor cell mass (Fig 5). This has been described for Hodgkin's lymphoma in humans,32 although it might be impossible to differentiate between scD25 shed from H-RS cells and scD25 shed from activated lymphocytes. Because the major strength of immunotoxins in humans will be to treat residual tumor cells after standard chemotherapy rather than bulky disease, the amount of scD25 is expected to be rather small in these patients.

In summary, ricin A-chain immunotoxins have potent antitumor effects against disseminated Hodgkin's lymphomas in SCID mice. Currently, we are using this model to further optimize the immunotoxin treatment strategies including scheduling and dosing. These experiments parallel the ongoing clinical trial of RFT5.dgA in patients with resistant HD.

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