Anti-B4-blocked Ricin Immunotoxin Shows Therapeutic Efficacy in Four Different SCID Mouse Tumor Models

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ABSTRACT

Anti-CD19 monoclonal antibody anti-B4 (IgG1) conjugated to the novel toxin-blocked ricin forms a potent immunotoxin, anti-B4-blocked ricin, that kills greater than 4.5 logs of CD19-positive cells in vitro after a 24-h exposure to a conjugate concentration of 5 × 10^{-9} m (1.11 µg/ml). The efficacy of anti-B4-blocked ricin in vivo was assessed in survival models of SCID mice bearing either a human B-cell lymphoma (Namalwa), a human non-T and non-B acute lymphoblastic leukemia (Nalm-6), or a murine B-cell lymphoma transfected with the human CD19 gene (300B4). In one model, 5 × 10^6 tumor cells were injected i.p., and 1 h later the mice were treated with one bolus injection of anti-B4-blocked ricin at 100 µg/kg/day for 5 days. Controls included similar treatment with anti-B4 antibody (72 µg/kg/day or 2 mg/kg/day for 5 days) alone or with the isotype-matched nonspecific immunotoxin, N901-blocked ricin (100 µg/kg/day). In a second model, 4 × 10^6 tumor cells were injected i.v., and 7 days later mice were treated i.v. as above. Anti-B4-blocked ricin showed efficacy by killing in vivo up to 3 logs of tumor cells. Only very limited or no effects were observed in animals treated with either anti-B4 antibody alone or N901-blocked ricin control conjugate. The concentration of anti-B4-blocked ricin in the blood of animals was 150 ng/ml after the first i.v. injection and about 800 ng/ml following the fifth injection of conjugate. This increase may be due to damage to the reticuloendothelial system by anti-B4-blocked ricin, since the rate of clearance of carbon from blood also decreased 5-fold after five injections as compared to the rate after only one injection. These studies indicate that anti-B4-blocked ricin has the potential to increase survival times of hosts with malignant disease.

INTRODUCTION

Monoclonal antibodies carrying toxins offer a unique possibility of specifically killing tumor cells (1–3). We previously reported the preparation of anti-B4-bR2 (4), a conjugate between the anti-CD19 monoclonal antibody anti-B4 and a modified form of the plant toxin ricin that has its sugar-binding sites blocked by covalently cross-linked affinity ligands (5). Anti-B4-bR was shown to specifically kill antigen-positive cells in vitro (4), and its in vivo nonspecific toxicity and pharmacokinetics were assessed in mice and nonhuman primates.3 In this paper we present data showing the preclinical efficacy of anti-B4-bR in survival models of SCID mice bearing human and murine lymphomas and leukemias that express the CD19 antigen. Survival models of the tumor measurement models were used after carefully considering advantages and disadvantages of both types of animal models (6, 7).

MATERIALS AND METHODS

Immunotoxins. anti-B4-bR was prepared by conjugating blocked ricin (5) to the anti-CD19 antibody anti-B4 (8) as described previously (4). N901-bR was prepared analogously using the anti-CD56 antibody N901 (9). Both antibodies belong to the subclass IgG1. The immunotoxins were formulated in phosphate-buffered saline containing 1 mg/ml of human serum albumin.

Human and Murine Tumor Cell Lines. The CD19-positive human tumor cell lines used were the Burkitt’s lymphoma line Namalwa (ATCC CRL 1432) and the non-B non-T acute lymphoblastic leukemia line, Nalm-6 (a kind gift of Dr. Jerome Ritz, Dana-Farber Cancer Institute, Boston, MA). 300B4 is a murine B-cell lymphoma line (10) that was transfected with the human CD19 gene and was generously given to us by Dr. T. Tedder (Dana-Farber Cancer Institute). Cell lines were maintained in RPMI 1640 supplemented with 10% heat-inactivated (56°C, 30 min) fetal bovine serum (JRH Bioscience, Kansas City, MO), 4 mM L-glutamine, penicillin G (10 units/ml), and streptomycin (10 µg/ml). The culture medium, amino acids, and antibiotics were purchased from Whittaker Bioproducts, Inc. (Walkersville, MD). Cells were maintained at 37°C in a humidified atmosphere containing 6% CO_2.

In Vitro Cytotoxicity of anti-B4-bR. Namalwa, Nalm-6, and 300B4 cells were exposed to various concentrations of anti-B4-bR, and the surviving fraction of cells were determined using the growth back-extrapolation assay as described previously (11).

Efficacy Models. Female SCID mice, 18 to 20 g in size, were purchased from Immune, Inc. (San Diego, CA). Four survival models using Namalwa, Nalm-6, and 300B4 cells were established to assess the therapeutic efficacy of anti-B4-bR. In one survival model, 5 × 10^6 Namalwa or 300B4 cells were injected i.p. in growth medium (0.1 ml). One h later, the animals were given the first i.v. injection of anti-B4-bR, which was followed by four more injections on successive days. Each animal received thus in total 5 × 100 µg/kg of conjugate. In the second survival model, 4 × 10^6 Namalwa or Nalm-6 cells in growth medium (0.2 ml) were injected via the tail vein. Seven days later, treatment with anti-B4-bR (5 × 100 µg/kg on consecutive days) was commenced. Control groups of tumor-bearing mice were treated analogously with either anti-B4 antibody alone (72 µg/kg/day or 2 mg/kg/day for 5 days) or the nonspecific (does not bind to tumor cells) conjugate N901-bR (100 µg/kg/day for 5 days). Each treatment group consisted of 9–12 mice. For the determination of drug serum levels, six additional tumor-bearing animals were treated as above with anti-B4-bR, N901-bR, and anti-B4 antibody, respectively. Blood samples were collected from the retroorbital plexus at 1 and 24 h after each drug injection, and sera were prepared and then kept frozen at −80°C until analyzed. Serum levels of anti-B4-bR, N901-bR, and anti-B4 antibody were plotted as the mean ± SD of three measurements.

Histopathology. Tumor growth in mice was assessed on day 21 or 23 following i.v. injections of 4 × 10^6 Namalwa or Nalm-6 cells, respectively. Mice were sacrificed and then preserved in 10% buffered formalin. Tissue sections were prepared and stained with hematoxylin and eosin. The extent of tumor present in tissues was graded from +1 to +7 in order of increasing tumor burden. Histopathology was performed by Dr. Norval W. King (New England Regional Primate Center, Southborough, MA).

Determination of Serum Content of anti-B4-bR. Two independent ELISA protocols were used to determine the concentrations of anti-B4-bR, N901-bR, and anti-B4 in the serum of animals. The ELISA methods were identical to those that will be reported elsewhere,3 except that the microtiter plates were coated with a monoclonal anti-ricin antibody (anti-BR12; Immu-
Therapeutic Efficacy of Anti-B4-Blocked Ricin

Concentration of Anti-B4-bR (M)

Fig. 1. In vitro cytotoxicity of anti-B4-bR to antigen-positive Namalwa (○), Nalm-6 (▲), and 300B4 (□) cells. Cells were exposed to different concentrations of anti-B4-bR for 24 h, and the surviving fractions were determined as described previously (11).

Measurement of Phagocytic Activity. The phagocytic activity in mice was monitored by measuring the rate of clearance of carbon black (Farber-Castell Corp., Newark, NJ) from blood. Mice were given i.v. injections of anti-B4-bR (100 µg/kg) once or 5 times on consecutive days. Twenty-four h after the first or the fifth injection of the conjugate, a 10% solution of carbon black (0.1 ml/mouse) was injected i.v., and the rate of clearance of the carbon from blood was determined over a 32-min period as described previously (12).

Quantitation of In Vivo Tumor Cell Kill. Survival times in days for 50% of animals that were treated with anti-B4-bR were compared with the respective survival times of animals that had been inoculated with a different number of tumor cells and were not treated. The number of tumor cells inoculated in the treatment group was then divided by the number of tumor cells inoculated in the control group that had the most similar survival time for 50% of animals. This ratio is reported as the experimentally determined in vivo tumor cell log kill.

Statistical Analysis. The product limit method was used to estimate the survival function (13). The survival function (also called the survival curve) is the probability that, for any specified survival time, a mouse will survive at least that long or longer. The comparison of survival curves to determine whether two random samples could have arisen from identical survival functions was carried out by the log-rank test and Wilcoxon test (14) at the 5% significance level (two-tailed). The statistical analysis was performed by Dr. Young Park (ImmuNoGen Inc., Cambridge, MA).

RESULTS

In Vitro Cytotoxicity of anti-B4-bR against Various Tumor Cell Lines. We have shown previously (4) that anti-B4-bR is cytotoxic for the B4-positive Burkitt's lymphoma cell line Namalwa with an IC50 of about 1.3 × 10^{-12} M (24-h exposure) and that the surviving fraction of Namalwa cells is less than 1 × 10^{-4} at a conjugate concentration of 5 × 10^{-9} M. We had also shown that the B4-negative Molt-4 cells were 200-fold less sensitive and were only killed with an IC50 of 2.5 × 10^{-9} M upon 24 h of exposure to the conjugate (4). Fig. 1 shows that the acute lymphoblastic leukemia cell line Nalm-6 and the Burkitt's lymphoma cell line Namalwa are similarly sensitive in vitro to anti-B4-bR. The IC50 values for Namalwa, Nalm-6, and 300B4 cells were
about 16, 17, and 5 pm, respectively. The cytotoxicity of anti-B4-bR was completely abolished by the addition of an excess (1 \times 10^{-8} \text{ M}) of anti-B4 antibody (data not shown). The CD19-transfected murine 300B4 cells showed about 3-fold more sensitivity to the conjugate (at a surviving fraction of 0.1), which is probably due to the greater B4 antigen density on the cell surface (data not shown).

**Characterization of in Vivo Tumors by Histopathology.** Groups of four SCID mice were given injections of $4 \times 10^{6}$ Namalwa or Nalm-6 cells via the tail vein. Twenty-one to 23 days after the tumor...
injection, the animals were sacrificed and subjected to a histological
analysis for tumor growth. Both tumor cell lines showed tropism for
the ovaries (+6), leptomeninges of the brain (+1 to +3), bone mar-
row of the vertebra (+4), dura mater of the spinal cord (+4), and the
muscles surrounding the spinal column (+4). Namalwa cells addi-
tionally showed tropism for the axillary and/or periaortic lymph nodes
(+3 to +4) in three of the four mice.

**Antitumor Efficacy of anti-B4-bR in i.p. Survival Models.** For
the 300B4 and the Namalwa i.p. tumor models, treatments started 1 h
after tumor cell injection and consisted of one daily i.v. injection on 5
consecutive days. The effects of anti-B4-bR and anti-B4 antibody on
the life span of mice bearing i.p. tumors of murine 300B4 cells are
shown in Fig. 2. Fig. 2A demonstrates the effect of the size of the
tumor inoculum on the length of the survival of mice. The time of
survival of 50% of inoculated mice decreases with increasing numbers
of injected tumor cells, the time being 9 days for inoculation with 5 X
10^7 cells, 11 days with 5 X 10^6 cells, 13 days with 5 X 10^5 cells, and
18 days with 5 X 10^4 cells.

For the different treatment courses, groups of mice (9 or 10 animals/
treatment group) were given i.p. injections of the highest number of
tumor cells (5 X 10^7 cells). One group of mice was then treated with
single injections of anti-B4-bR at 100 μg/kg/day on 5 consecutive
days. Control groups of animals were treated similarly with N901-bR
(5 X 100 μg/kg/day) and equimolar amounts of anti-B4 antibody (5 X
72 μg/kg/day). A third control group was treated with a 28-fold greater
molar amount of free anti-B4 antibody (5 X 2 mg/kg/day). Fig. 2
shows that only animals which were treated with the tumor-specific
conjugate anti-B4-bR showed a significantly increased life span over
untreated or anti-B4 antibody-treated animals (P = 0.002, log-rank
test; P = 0.0005, Wilcoxon test). The time of survival of 50% of the
animals was prolonged from 9 days in the untreated group to 19 days
in the conjugate-treated groups (Fig. 2, A and B). Treatment with the
non-specific isotype-matched conjugate N901-bR did not result in any
prolongation of survival time (50% survival on day 9; Fig. 2C), and
treatment with the equivalent dose or high dose of free anti-B4 antibo-
body shifted the survival curve only minimally (1.2-fold) from 9 days
in the untreated group to 11 days in the treated group (Fig. 2, B and
C). Treatment of tumor-bearing mice with anti-B4-bR led to a loss in
body weight during the first week that reached 30% on day 8 after
initiation of treatment and was followed by an increase in body
weight. The initial decrease is probably due not only to toxicity of the
conjugate but also to the inhibition of tumor growth.

Serum concentrations of anti-B4-bR, N901-bR, and anti-B4 antibo-
dy of the above treated animals were measured by ELISA 1 and 24
h after each injection. Concentrations of anti-B4-bR (Fig. 3A) and
N901-bR (Fig. 3B) in serum were similar, each being greater at 1 h
than at 24 h after each injection due to rapid clearance of the conju-
gates. Interestingly, 1-h serum levels of both immunotoxins increased
with each of the five daily injections, for anti-B4-bR from about 150
ng/ml after the first injection to about 800 ng/ml after the fifth injec-
tion (Fig. 3A) and for N901-bR from about 150 ng/ml after the first
injection to about 300 ng/ml after the fifth injection (Fig. 3B). A very
small trend if any was observed for injections of anti-B4 antibody
alone. Five injections of anti-B4 at doses as high as 2 mg/kg/day gave
serum concentrations of about 40–50 μg/ml after each injection (Fig. 3
C).

A second i.p. tumor model was established with the human Burkitt’s
lymphoma cell line Namalwa. Again, 5 X 10^7 cells were injected i.p.,
and groups of 12 animals were treated with anti-B4-bR (100 μg/kg/
day) or anti-B4 antibody (72 μg/kg/day) for 5 consecutive days start-
ing 1 h after tumor inoculation. Survival times of 50% of mice treated
with anti-B4 antibody and anti-B4-bR were 36 days and 46 days,
respectively (Fig. 4A) (P = 0.02, log-rank test; P = 0.01, Wilcoxon
test). Mice treated with anti-B4 antibody increased in body weight
during the entire life span, whereas anti-B4-bR-treated mice again
showed a decrease in body weight with a maximal loss of 17% on day
9 after starting the treatment (Fig. 4B). The increase in body weight
after about 10 days, which was also observed in the first model, is
probably partially due to tumor recurrence. Serum concentrations of
anti-B4-bR 1 h after each injection increased again as above from
about 150 ng/ml after the first injection to about 600 ng/ml after the
fifth injection.

![Graph](image-url)

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1363

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Antitumor Efficacy of anti-B4-bR in i.v. Survival Models. Treatment in the i.v. tumor models using Namalwa and Nalm-6 cells was initiated 7 days after tumor cell inoculation and consisted of one daily i.v. injection on 5 consecutive days. The first i.v. tumor model in SCID mice was established by inoculating animals (10 or 12 animals/group) with increasing numbers of human Namalwa cells via tail vein injections and then determining survival curves. Intravenous injections of $4 \times 10^5$, $4 \times 10^6$, or $4 \times 10^7$ Namalwa cells showed survival times for 50% of inoculated animals of 39, 35, 31, and 25 days, respectively (Fig. 5A). Mice were then given injections of the highest number of Namalwa cells ($4 \times 10^6$ cells) and treated after 7 days with five i.v. injections of anti-B4-bR (100 µg/kg/day) or equimolar amounts of anti-B4 antibody (5 × 72 µg/kg/day) on 5 consecutive days (Fig. 5B). Treatment increased the life span of animals from 27 days (50% survival of animals treated with anti-B4 antibody) to 41 days (50% survival of animals treated with anti-B4-bR). This difference was highly significant; the values were $P = 0.001$ by the log-rank test and $P = 0.0001$ by the Wilcoxon test. Treatment with equimolar amounts of anti-B4 antibody (5 × 72 µg/kg/day) or with a 28-fold molar excess of anti-B4 antibody (5 × 2 mg/kg/day) showed identical 50% survival (27 days). Five daily injections of N901-bR at 100 µg/kg/day gave no increase in survival over untreated animals (Fig. 5C). Thus, the increased survival of mice treated with anti-B4-bR can be attributed to specific elimination of tumor cells in an antigen-dependent manner. Changes in body weights of tumor-bearing mice following treatment with anti-B4-bR or anti-B4 antibody (72 µg/kg/day for 5 days) are shown in Fig. 5D. Interestingly, mice given anti-B4 antibody alone showed a continuous rapid decrease in body weight, probably due to effects caused by rapid tumor progression. Tumor-bearing mice treated with anti-B4-bR lost weight during the treatment phase, possibly due to drug toxicity and decrease in tumor burden, and started to regain weight 4 days after treatment had stopped. This suggested that anti-B4-bR treatment could control tumor growth.

Serum concentrations of anti-B4-bR, N901-bR, and anti-B4 antibody were also assayed in this tumor model (Fig. 6). Again, as expected, the serum concentrations of the conjugates anti-B4-bR and N901-bR were lower 24 h after each treatment than 1 h after injection of the drugs, and the 1-h serum levels increased from about 150 ng/ml after the first injection to about 600 ng/ml after the fifth injection (Fig. 6A) for anti-B4-bR and from about 150 ng/ml to about 400 ng/ml for N901-bR (Fig. 6B). Again, serum concentrations of anti-B4 antibody in mice given 2 mg/kg/day for 5 days did not show an increasing trend of similar magnitude and ranged from 40 to 60 µg/ml (Fig. 6C).

A second i.v. tumor model in SCID mice was established with the human leukemia cell line Nalm-6. Fig. 7A shows the survival curves for animals (10 or 12 animals/group) inoculated with different amounts of Nalm-6 cells. Fifty 5% survival times ranged from 38 to 25 days for animals inoculated with $4 \times 10^3$ cells and $4 \times 10^6$ cells, respectively. Animals were then given injections of the highest number of tumor cells ($4 \times 10^6$) and treated 7 days later using the same protocol as in the first i.v. model. Treatment of mice with anti-B4-bR at 100 µg/kg/day for 5 days increased the 50% survival time to 30 days, as compared to 22 days for the mice treated with equimolar amounts of anti-B4 antibody (Fig. 7B). This difference was highly significant; the $P$ values were 0.0001 by the log-rank test and 0.0001 by the Wilcoxon test. Serum levels of conjugate followed the same trend as in the other tumor models, i.e., maximum measured levels increased from about 150 ng/ml after the first injection to about 700 ng/ml after the fifth injection (Fig. 7C). In addition, the losses in body weight were similar (Fig 7D), i.e., untreated mice rapidly lost weight, while the weight loss in treated mice was temporarily slowed down.

Quantitation of in Vivo Tumor Cell Kill. Comparison of the 50% survival times in the groups of animals treated with anti-B4-bR with the 50% survival times in untreated animals inoculated with different numbers of tumor cells was used to estimate the tumor cell kill in vivo (Table 1). The immunoconjugate treatment eliminated more than 3 logs (99.9%) of murine 300B4 cells and human Namalwa cells and
1–2 logs (90–99%) of human Nalm-6 tumor cells. This difference in cell kill did not correlate with the in vivo proliferation rates of these three cell lines. The in vivo tumor doubling times for 300B4, Namalwa, and Nalm-6 tumors were 0.75, 1.7, and 1.5 days, respectively, as estimated from semilogarithmic plots, where the number of cells in the inoculum was plotted on the ordinate in logarithmic scale versus the survival times in days for 50% of the animals on a linear abscissa. Estimates made for cell kill as determined experimentally (i.e., from the cell titration curves) were similar to those calculated from the formula (15) described Table 1.

**Effect of anti-B4-bR on Phagocytosis in Mice.** In every tumor model we tested, we observed that the serum levels attained 1 h after each injection of conjugate steadily increased with increasing numbers of injections. Because most foreign proteins are cleared from circulation by the reticuloendothelial system, we speculated that this observation might be indicative of damage to phagocytic cells. We

<table>
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* Estimates made from the formula (15)

\[
\text{Log}_{10} \text{cell kill} = \frac{T - C}{3.32} \times T_d
\]

where \( T \) and \( C \) are the survival times for 50% of the animals in days for the anti-B4-bR treated and the control groups, respectively; 3.32 is the number of cell doublings per log of cell growth; and \( T_d \) = doubling time of tumor cells in mice (300B4 = 0.75 days, Namalwa = 1.7 days, Nalm-6 = 1.5 days).
This may be due in part to the lower activity of the macrophages effects in relation to toxicity to the host and therefore therapeutic rapidly lethal to the untreated animals; (b) survival models quantitate producible growth for most human tumor xenografts in SCID mice.4 Therefore set out to study the effect of one and five daily injections of anti-B4-bR at 100 μg/kg on phagocytic activity by measuring the clearance of carbon black from the blood of these mice. Fig. 8 shows that mice given one injection of anti-B4-bR cleared the carbon particles from blood efficiently with less than 1% of the injected amount remaining in circulation 30 min after administration. However, animals that had received five immunconjugate treatments had a strongly reduced capacity for clearing the carbon particles, with more than 50% of the injected dose left in circulation after 30 min.

DISCUSSION

The objective of this investigation was to develop survival models of human lymphoma and leukemia in SCID mice for evaluating the therapeutic efficacy of anti-B4-bR, an immunconjugate containing the anti-CD19 monoclonal antibody anti-B4 and the toxin-blocked ricin. SCID mice were preferred over conventional nude mice or triply deficient NIH-3 nude mice (deficient in T- and B-lymphocyte and the anti-CD 19 monoclonal antibody anti-B4 and the toxin-blocked ricin. SCID mice were preferred over conventional nude mice or triply deficient NIH-3 nude mice (deficient in T- and B-lymphocyte and natural killer cells) because we observed a more consistent and reproducible growth for most human tumor xenografts in SCID mice. This may be due in part to the lower activity of the macrophages present in the SCID mice.4

Two types of efficacy models have been used routinely for assessing anticancer agents for their therapeutic potency, i.e., survival models and tumor measurement models, and we have previously reviewed the advantages and disadvantages of these models (6, 7). Survival models were used in the present study since the ultimate efficacy of anti-B4-bR will be assessed in the clinic by its ability to prolong the life of patients with disseminated lymphoma. The major advantages of using survival models are: (a) the tumor can be highly metastatic and rapidly lethal to the untreated animals; (b) survival models quantitate the full effect of an anticancer agent because they measure therapeutic effects in relation to toxicity to the host and therefore therapeutic efficacy; (c) survival models allow the study of effects of therapy on minimal residual tumor burden.

Four survival models of SCID mice were developed: two i.p. models using the human Burkitt’s lymphoma line Namalwa or the murine B-cell line 300B4, which had been transfected with the human CD19 gene; and two i.v. models using the Namalwa cell line or the human leukemia cell line Nalm-6. In all four models, untreated tumor-bearing mice died in a reproducible manner. In the i.v. models, these survival time of the animals correlated with the number of tumor cells injected.

When injected i.v. the two human tumor cell lines Namalwa and Nalm-6 showed tropism for the ovaries, leptomeninges of the brain, bone marrow of the vertebra, dura mater of the spinal cord, and the muscles surrounding the spinal column. The Nalm-6 cells further showed tropism for the axillary and/or periarteric lymph nodes. These animals suffered from hindleg paralysis, probably as a result of extensive tumor growth in the vertebra, spinal meninges, and spinal nerves, which made them atrophied and weak and left them with no desire or ability to eat or drink. The extensive pulmonary edema and hemorrhage observed on autopsy may also have contributed to their death. The edema and hemorrhage may have resulted from the occlusion of capillary vessels in the lungs by the accumulation of neoplastic cells within their lumens, therefore causing vascular leakage by mechanical obstruction, or by stimulating the release of cytokines. Interestingly, while there were tumor cells present in the lungs, these cells failed to form neoplastic nodules. Animals given tumor cells i.p. gave rise to large multiple abdominal tumors but did not develop paralysis. No further histopathological studies were undertaken in these animals. Other investigators have used SCID mice to study the homing and spread of human leukemia cells (16, 17). Jansen et al. (17) examined the spread of a human t(4;11) leukemia injected into SCID mice by i.v. and i.p. routes and found a pattern of tumor spread that compares well with our findings with i.v. injected Namalwa and Nalm-6 tumor cells.

Anti-B4-bR was efficacious in all four survival models. Treatment with anti-B4-bR caused significant prolongation of the survival of mice inoculated i.p. with 300B4 or Namalwa tumor cells and of mice bearing established intravenous Namalwa or Nalm-6 tumors. This effect was not observed in animals treated with a nonspecific antibody conjugate of blocked ricin, thereby demonstrating that the antitumor effect in mice caused by anti-B4-bR is antigen specific. Similar mice treated with anti-B4 antibody alone at doses up to 28 times greater than the anti-B4-bR dose showed only an insignificant prolongation of survival time.

This appears to be the first report showing the therapeutic efficacy of a whole blocked ricin antibody conjugate against human lymphoma and leukemia in survival models of SCID mice. Most investigators in the past have used antibody ricin A chain and other toxin conjugates (18, 19).

Multiple injections of antibody conjugates of blocked ricin increased the retention of the conjugates in the bloodstream of animals. This effect did not depend on the site of the tumor in the animal or on whether the immunotoxin did target the tumor, nor did it depend on animals having a xenograft tumor. Experiments measuring the clearance of carbon black provided evidence that repeated injections of blocked ricin conjugates damage the reticuloendothelial system. This finding was not unexpected since toxic effects of ricin to Kupffer cells in mice (20) and in rats (21) had been described before.

Further studies with the animal models described in this report are under way to evaluate the therapeutic effects of combined treatments with anti-B4-bR and conventional chemotherapeutic agents. A Phase I clinical trial with anti-B4-bR has shown that this immunotoxin can
be administered as a daily bolus infusion for 5 days with tolerable, reversible toxicity (22). Phase II/III trials of anti-B4-bR are currently in progress.

ACKNOWLEDGMENTS

We are grateful to Drs. Norval King and Young Park for performing the histopathology and the statistical analysis, respectively.

REFERENCES

Anti-B4-blocked Ricin Immunotoxin Shows Therapeutic Efficacy in Four Different SCID Mouse Tumor Models

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