Establishment of New SCID and Nude Mouse Models of Human B Leukemia/Lymphoma and Effective Therapy of the Tumors with Immunotoxin and Monoclonal Antibody: Marked Difference between the SCID and Nude Mouse Models in the Antitumor Efficacy of Monoclonal Antibody

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ABSTRACT

BALL-1, a human B leukemia/lymphoma cell line, was transplanted into nude and SCID mice under various conditions. The transplantation was substantially improved by preadaptation of BALL-1 by serial passages in newborn and young nude mice. We were able to establish the desirable conditions where 100% of SCID and nude mice that were inoculated i.p. with various doses of the adapted BALL-1 (termed BALL-1a) developed tumors. Tumors in SCID mice were disseminated to various tissues in a manner analogous to tumors in patients with B leukemia/lymphoma, whereas tumors in nude mice were not as widely disseminated and grew mainly as ascites. Flow cytometric analyses showed that all of the 11 tested cell surface markers of the parental BALL-1a were well maintained on the tumor cells recovered from the SCID and nude mice.

The utility of the developed tumor models for the therapeutic studies was investigated by i.p. or i.v. administration of an anti-B leukemia/lymphoma monoclonal antibody, termed SN7 (IgG1k), and SN7 immunotoxin (IT) that was prepared by conjugating SN7 to ricin A chain (RA) or deglycosylated RA (dgRA). In the nude mouse model study, SN7-RA that had been administered i.p. suppressed the tumor growth completely in all of the treated mice (n = 5) without any sign of tumor or undesirable side effects for as long as followed (i.e., 350 days), whereas unconjugated SN7 showed only a slight therapeutic effect. A control RA conjugate was not effective. In the SCID mouse model studies, several sets of experiments were carried out by i.p. or i.v. administration of IT, monoclonal antibody, or control IT. In the first three sets of experiments, SCID mice inoculated with 1.1 x 10^6 BALL-1a cells received an i.p. administration of phosphate-buffered saline or three different doses (i.e., 4 x 10 μg, 4 x 20 μg, and 4 x 30 μg) of therapeutic agents (SN7-RA and SN7). Virtually an identical result was obtained from the three experiments. All of the phosphate-buffered saline control group mice (n = 15) died within 35 days post tumor inoculation. In contrast, all of the mice that were treated with SN7-RA (n = 19) or with SN7 (n = 15) survived for as long as followed (i.e., 250 days). However, the unconjugated SN7 was less effective than SN7 IT for tumor suppression in SCID mice that were inoculated with a larger tumor burden (i.e., 4 x 10^6 BALL-1a cells). The efficacy of SN7 and SN7 IT in the SCID mouse model was further studied by the systemic i.v. administration of the therapeutic agents. The i.v. therapy experiments were carried out using SN7 IT and control IT containing dgRA. In the first two sets of the experiments, therapy of SCID mice that were inoculated i.p. with 1.1 x 10^6 tumor cells was initiated one day post tumor inoculation by i.v. administration of different doses (i.e., a total of 120 and 80 μg, respectively) of the therapeutic agents. In each experiment, all of the mice (n = 6 and 7) that were treated with SN7-dgRA survived for as long as followed (i.e., 200 and 140 days, respectively). Of the mice that were treated with 120 and 80 μg, respectively, unconjugated SN7, 66.7% (n = 6) and 42.9% (n = 7) survived. In the third i.v. therapy experiment, the dose of SN7-dgRA was reduced to a total of 60 μg (i.e., 3 x 20 μg), and the initiation of the therapy with SN7-dgRA was delayed until 2 days or 3 days post tumor inoculation. All of the IT-treated mice (n = 8) survived for 110 days. Thus, the i.v. administered SN7-dgRA induced complete tumor suppression in all of the treated mice for as long as followed. In addition, the i.v.-injected unconjugated SN7 was effective for tumor suppression, although it is less effective than SN7-dgRA.

The present results demonstrate that SN7 IT is highly effective for tumor suppression in both animal models by i.p. or i.v. administration. Unconjugated SN7 was only marginally effective for tumor suppression in nude mice but strongly effective in SCID mice.

INTRODUCTION

B ALL is associated with poor prognosis and closely related to B lymphoma in its clinical and immunological features (1–3). B ALL in children is probably a leukemia phase of non-Hodgkin’s lymphoma or Burkitt’s lymphoma (3). B ALL is rare, but B lymphoma is a major group of the human hematological malignancies. Although conventional chemotherapy and/or radiotherapy is capable of inducing initial remission in the majority of B lymphoma patients, most of these patients relapse and eventually succumb to the disease. Therefore, there is a definite need for developing nonconventional alternative therapeutic modalities for B leukemia and lymphoma. mAb-based therapies of leukemia and lymphoma have been a focus of interest of many researchers for the past decade (reviewed in Ref. 4). Although substantial progress has been made in understanding various factors involved with the mAb-based therapies (4, 5), many problems and factors associated with the therapies remain to be studied and defined.

In this study, we developed new SCID and nude mouse models of B leukemia/lymphoma and investigated their utility for evaluating the therapeutic potential of mAb and IT. In developing the animal models, we used BALL-1, a B ALL cell line (6). The present and previous studies (6–8) on the cell surface phenotype of BALL-1 and the in vivo adapted BALL-1 (BALL-1a) showed that they represent relatively mature B malignant cells and share many cell surface markers with the majority of cases of B lymphoma. The results presented here indicate the usefulness of the animal models of BALL-1a for evaluating anti-B leukemia/lymphoma agents. Detailed comparative studies between nude and SCID mouse models bearing BALL-1a tumor showed definite advantages of the SCID mouse model over the nude mouse model. In addition, the results showed that our anti-B leukemia/lymphoma IT was capable of inducing complete and prolonged suppression of tumor growth in the animal models by being administered either i.p. or systemic i.v. Furthermore, the present study unexpectedly revealed a marked difference between nude and SCID mouse models in the antitumor efficacy of mAb.

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3 The abbreviations used are: ALL, acute lymphoblastic leukemia; mAb, monoclonal antibody; SCID, severe combined immunodeficiency; IT, immunotoxin; PBS, fetal bovine serum; RA, ricin A chain; dgRA, chemically deglycosylated RA; FACS, fluorescence-activated cell sorter; SMPT, 4-succinimidyl-6-oxo-carbonyl-α-methyl-e-(2-pyrindylidithio)-toluene; PBS, phosphate-buffered saline; RIA, radioimmunoassay; MST, mean survival time.

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MATERIALS AND METHODS

Mice. Female and male C.B-17/scid mice (SCID mice) were obtained from Taconic (Germantown, NY). Female athymic Ncr-nu (mnu/mnu) mice (nude mice) were obtained from the Frederick Cancer Research Facility of the National Cancer Institute. Newborn nude mice were obtained from the purchased pregnant nude mice. Unless otherwise stated, 6–9-week-old female mice were used in the present study. These mice were bred and maintained in a protected environment in cages with filter bonnets in a laminar flow unit (Lab Products, Maywood, NJ) as described previously (9). Animals were given autoclaved food and water ad libitum, and all manipulations were performed in a laminar flow hood.

Human Cell Lines. BALL-1, BALL-1a, and MOLT-4, an established T cell line, were cultured in RPMI 1640 supplemented with 8% (for BALL-1 and BALL-1a) or 4% (for MOLT-4) heat-inactivated FBS, 100 units/ml penicillin, and 50 μg/ml streptomycin. HT-1080, a human fibrosarcoma cell line, was grown in Dulbecco’s modified Eagle’s medium (GIBCO) supplemented with 10% FBS and 50 μg/ml gentamicin as described previously (9).

Monoclonal Antibodies and Reagents. Anti-HLA-DR (nonpolyclonal) and Leu 16 (anti-CD20) mAbs were purchased from Becton Dickinson (San Jose, CA). mAbs specific for each of γ, δ, ε, and δ chains of human immunoglobulins were obtained from AMAC, Inc. (Westbrook, ME). mAbs SN3, anti-CD24; Refs. 10 and 11), G4–3A7 (anti-HLA-DR; Ref. 12), and SN8 defining a unique extracellular epitope of the B29 protein component of the B-cell antigen receptor complex (8, 13, 14) were generated in our laboratory and reported previously. Three new mAbs, SN7, SN9, and SN11, were recently generated in our laboratory, and details of these mAbs will be reported elsewhere. Briefly, these mAbs react with the majority of B leukemia-lymphoma samples but are directed toward individually different cell surface antigens. SN7, SN9, and SN11 define a M, 30,000 glycoprotein, an HLA-DQ-like antigen and a M, 42,000 glycoprotein, respectively. Control murine IgG (MOPC 195 variant; IgG1k) was prepared in our laboratory (15). RA and dgRA were obtained from Inland Laboratories (Austin, TX). SMPT was purchased from Pierce (Rockford, IL).

Transplantation of BALL-1 and BALL-1a into Mice. The in vitro-maintained BALL-1 and the in vivo-adapted BALL-1 (termed BALL-1a) were used for transplantation into nude and SCID mice. The in vivo adaptation was carried out using slight modifications of our previously reported procedure (12). Briefly, newborn (1-week-old) nude mice were inoculated i.p. with 2 x 10^7 parental BALL-1 cells per mouse. Three of the three inoculated mice developed tumors. The tumor cells were recovered, and 7 x 10^6 tumor cells mixed with 1 x 10^8 cells of X-irradiated (6,000 rad) HT-1080 were injected i.p. into X-irradiated (3 x 200 rad) young (3- to 4-week-old) nude mice. Three of the three inoculated mice developed tumors. This transplantation was repeated twice. Then, 3 x 10^7 tumor cells were transplanted i.p. without HT-1080 into X-irradiated (3 x 200 rad) young adult (6- to 9-week-old) nude mice, and the transplantation was repeated until a total of 7 serial passages. The transplantability of the resultant BALL-1a was not affected by culturing in vitro for over 6 weeks.

In the initial transplantation experiments, the parental BALL-1 was injected i.p. into X-irradiated nude mice and nonconditioned SCID mice. However, more extensive transplantation experiments were carried out using BALL-1a. BALL-1a was injected i.p. into nonconditioned nude mice as well as X-irradiated nude mice in addition to nonconditioned SCID mice. Briefly, mice were injected i.p. with BALL-1 suspended in 0.5 ml PBS. X-irradiation of nude mice was carried out by irradiating weekly the mice with 200 rad for 3 weeks (15). The inoculated mice were monitored for morbidity and mortality and weighed twice a week using an electronic balance (OHAUS Model GT210).

Tissue Histology. A gross examination of various tissues was performed on each tumor-inoculated dead mouse after laparotomy within 24 h after the death. Then, various tissues were removed, fixed in 10% buffered formalin, paraffin embedded, sectioned, and stained with hematoxylin and eosin (15). The stained tissues were examined by light microscopy.

Detection of Tumor by Cellular RIA. Leukemia cells in the lymph node, ascites, bone marrow, and mediastinum of some of the mice that were inoculated with the BALL-1a tumor cells were tested by using a cellular RIA with 125I-labeled mAb in a manner similar to that described previously (9). As controls, bone marrow cells of healthy mice, the parental BALL-1, and MOLT-4 were included in the assay.

Flow Cytometric Analysis. The cell surface antigens were examined by a standard procedure as described recently (16). Briefly, 1 x 10^6 cells were incubated with an appropriate amount of mAb at 4°C for 60 min. After three washings with PBS containing 0.1% NaN3 and 1% bovine serum albumin, the cells were incubated at 4°C for 60 min with fluorescein isothiocyanate-conjugated F(ab')2 of sheep anti-mouse IgG (Sigma, St. Louis, MO). The cells were washed three times and analyzed using a Becton Dickinson FACScan. Tumor cells recovered from ascites or lymph nodes of the tumor-bearing mice were cultured in vitro for 2 weeks before the analysis to remove contaminating mouse cells.

Preparation of IT. The purified IgGs of mAb SN7 and an isotype-matched control murine IgG (MOPC 195 variant; IgG1k) were individually conjugated with RA as described previously (15) but with a modification: ionic strength in the conjugation buffer was increased to minimize protein precipitation during conjugation. To this end, PBS (pH 7.4) containing 1 mM EDTA was replaced with 20 mM sodium phosphate buffer (pH 7.4) containing 0.3 M NaCl and 1 mM EDTA. SN7 IT and control conjugate were also prepared by conjugating the purified IgGs with dgRA; in this conjugation, SMPT was used as described by Thorpe et al. (17). The resulting IgG-RA or IgG-dgRA was separated from the free (unconjugated) RA or dgRA by gel filtration on a Sepharose S-300 column (15). The contaminating free IgG was removed from the conjugates by chromatography on a Blue Sepharose column (18). The conjugates were characterized as described previously (15).

In Vitro Cytotoxicity Test of IT. The in vitro cytotoxic activity of IT was determined by a protein synthesis inhibition assay and a clonogenic assay. The protein synthesis inhibition assay was carried out as described recently (16). The clonogenic assay was carried out as described below. BALL-1a was suspended to 1 x 10^5 cells/ml in RPMI 1640 containing 8% FBS, 100 units/ml penicillin, and 50 μg/ml streptomycin (medium A), and IT was diluted to appropriate concentrations in medium A. The BALL-1a suspension was incubated with appropriate concentrations of IT at 37°C for 24 h in a humidified atmosphere with 5% CO2 in air. The incubated cells were washed three times with medium A and once with a clonogenic medium which consisted of RPMI 1640 containing 10% FBS, 12 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 0.28% methycellulose (EM Science), 2 mM l-glutamine, 100 units/ml penicillin, and 50 μg/ml streptomycin. The washed cells were plated to 1 x 10^2 cells/ml in the clonogenic medium, and a series of 5-fold serial dilutions were prepared. From each dilution, 6 aliquots (100 μl each) were plated in 96-well flat-bottomed microtiter plates that had already been seeded with 2 x 10^3 irradiated (5,000 rad) normal human peripheral blood mononuclear cells in 100 μl of the clonogenic medium as feeder. Plates were incubated for 14 days at 37°C in a humidified atmosphere with 5% CO2 in air. Growth of colonies was scored visually using an inverted phase microscope. Statistical analysis of the data was carried out as described by Bast et al. (19) and Uckun et al. (20) using a Spearman estimate (21).

Therapy of Tumor-bearing Mice. Nude and SCID mice that were inoculated i.p. with BALL-1a were treated by i.p. or i.v. administration of PBS, unconjugated mAb SN7 (IgG1k), control conjugate containing an isotype-matched murine IgG, or SN7 IT. The treatment was initiated 1, 2, or 3 days after the final inoculation and repeated twice or three times by daily administration on successive days. In the systemic i.v. therapy, IT or control IT that had been prepared by conjugating mAb or control IgG to dgRA using SMPT was administered i.v. via the tail vein of the mice.

Treatment Efficacy. During the treatment, the weight of the mice was measured twice a week using an electronic balance (OHAUS Model GT210), and the mice were monitored daily for morbidity and mortality (22). A gross examination of various tissues was performed on each dead or sacrificed mouse after laparotomy. Then, various tissues of selected mice were removed, and the histology of the tissues was examined as described above (see “Tissue Histology”). The examined tissues included brain, femoral bone marrow, kidney, liver, lung, and spleen.

RESULTS

Transplantation of BALL-1 and BALL-1a into Nude Mice. In the initial attempt to transplant the parental BALL-1 into nude mice, the mice were X-irradiated, and the tumor cells were inoculated i.p.
with BALL-la cells. On the other hand, ascitic tumor developed in all of the tumors. The results are summarized in Table 1. No tumor of the X-irradiated mice that were inoculated with 1 × 10⁷ or 3 × 10⁷ BALL-la cells. The survival times of the mice that were inoculated with 4 × 10⁷ cells fell within a relatively narrow range, whereas those of the mice inoculated with 1 × 10⁷ cells were scattered over a wide range. Therefore, mice inoculated with 4 × 10⁷ BALL-la cells were used for therapeutic studies in nude mice (see below). All nude mice with the growing tumor showed prominent abdominal distention with a large volume of ascites and died within 10 days after they developed the abdominal distention.

**Transplantation of BALL-1 and BALL-1a into SCID Mice.** In the present study, SCID mice were used without any preconditionings such as X-irradiation. Tumors developed in all of the SCID mice that were inoculated i.p. with 1.8 × 10⁷ or 5.4 × 10⁷ cells of the parental BALL-1; MST at the two tumor doses was 62.5 and 50.8 days, respectively (Table 1). The transplantability of BALL-1a was 100% among SCID mice inoculated i.p. with tumor doses between 6.7 × 10⁶ and 5.4 × 10⁷ cells, and there was an inverse relationship between survival time of mice and the number of inoculated tumor cells (Fig. 1). In addition, the results show that BALL-1a grows much faster in SCID mice than the parental BALL-1 (Table 1). The fatal symptoms of the mice bearing BALL-1 and BALL-1a tumors became apparent about 5 or 6 days before their death; they exhibited lethargy, slight abdominal distention, ruffled fur, and respiratory distress.

**Patterns of Dissemination of BALL-1a Tumors in Nude and SCID Mice.** In nude mice, tumors that were inoculated i.p. grew mainly as ascites. Tumor involvement was observed in abdominal lymph node, pancreas, mediastinum, and occasionally peribronchial lymph node and spleen.

Gross examination at autopsy of the i.p. tumor-bearing SCID mice revealed that they had less ascites than the corresponding nude mice but showed massive swelling of paraaortic lymph nodes and mesenterium. Histological examination revealed the extensive tumor involvement in various tissues of the SCID mice. Some of the results are shown in Fig. 2. The tumor-involved tissues included liver, spleen, pancreas, fallopian tube, pericardium, peribronchial and abdominal lymph node, bone marrow, mediastinum, and mesentry.

We also carried out cellular RIA with the cells harvested from lymph nodes, ascites, bone marrow, and mediastina of nude and SCID mice. Freshly harvested cells from these tissues reacted strongly with 125I-labeled SN7, which indicated that these tissues were involved with BALL-1a tumor (data not shown). These results are consistent with the results of gross and histological examination.

**Phenotype Analysis.** Cell surface markers of BALL-1a tumors recovered from nude and SCID mice were compared to those of the parental BALL-1, the parental BALL-1a, and the adapted BALL-1a by FACS analysis using 11 mAbs. The test results are presented in Table 2. The cell surface markers on the parental BALL-1 that were detected by the 11 mAbs were well maintained on the BALL-1a tumor. Thus, the in vivo grown BALL-1a tumor cells, either in nude or SCID mice, retained well the phenotype of the parental BALL-1.

**Therapy of Nude Mice Bearing BALL-1a Tumor.** The utility of the nude mouse model bearing BALL-1a tumor for therapeutic studies was investigated in the present study. Nude mice bearing BALL-1a tumor were generated as described above by inoculating individual X-irradiated mice with 4 × 10⁷ BALL-1a cells. Each group (n = 5 except for the PBS control group where n = 4) of mice was treated i.p. with PBS, 20 μg of RA conjugate of an isotype-matched control murine plasmacytoma IgG (MOPC 195 variant; IgGl-K), unconjugated mAb SN7, and SN7 IT (Fig. 3). In this experiment, the injection was initiated 24 h post tumor inoculation and repeated three times on successive days. All of the PBS control group mice and the control IT group mice died within 28 days after tumor inoculation. MST values were 21 ± 6.1 and 21.0 ± 3.6 days, respectively; these values are close to that (23.0 ± 2.2 days) found in an earlier transplantation experiment where the same tumor dose was used for transplantation into X-irradiated nude mice (Table 1). Treatment of tumor-bearing mice with unconjugated SN7 extended their MST only slightly to 34.2 ± 2.0 days, and all mice died within 37 days posttumor inoculation. In contrast, all mice that were treated with SN7 IT survived for as long as followed, i.e., for 350 days, and these mice showed no sign of tumor or undesirable side effects. Thus, SN7-RA was able to induce complete and prolonged tumor suppression in the nude mouse model, whereas mice of each control group died early and within a narrow range of survival time.

### Table 1 Transplantability of the parental BALL-1 and the adapted BALL-1a in immunodeficient mice

<table>
<thead>
<tr>
<th>Mice</th>
<th>X-irradiation of mice</th>
<th>No. of cells</th>
<th>Transplantability</th>
<th>MST ± SD (days)</th>
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</thead>
<tbody>
<tr>
<td>BALL-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nude</td>
<td>+</td>
<td>1 × 10⁷</td>
<td>2/5</td>
<td>(63.0, n = 2)</td>
</tr>
<tr>
<td>Nude</td>
<td>+</td>
<td>3 × 10⁷</td>
<td>2/5</td>
<td>(60.5, n = 2)</td>
</tr>
<tr>
<td>SCID</td>
<td>−</td>
<td>1.8 × 10⁷</td>
<td>5/5</td>
<td>62.5 ± 10.0</td>
</tr>
<tr>
<td>SCID</td>
<td>−</td>
<td>5.4 × 10⁷</td>
<td>5/5</td>
<td>50.8 ± 5.5</td>
</tr>
<tr>
<td>BALL-1a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nude</td>
<td>−</td>
<td>3 × 10⁷</td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td>Nude</td>
<td>−</td>
<td>6 × 10⁷</td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td>Nude</td>
<td>+</td>
<td>1 × 10⁷</td>
<td>5/5</td>
<td>47.8 ± 27.1</td>
</tr>
<tr>
<td>Nude</td>
<td>+</td>
<td>3 × 10⁷</td>
<td>5/5</td>
<td>ND</td>
</tr>
<tr>
<td>Nude</td>
<td>+</td>
<td>4 × 10⁷</td>
<td>5/5</td>
<td>23.0 ± 2.2</td>
</tr>
<tr>
<td>SCID</td>
<td>−</td>
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<td>5/5</td>
<td>40.8 ± 3.0</td>
</tr>
<tr>
<td>SCID</td>
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<td>2 × 10⁷</td>
<td>5/5</td>
<td>28.6 ± 6.1</td>
</tr>
<tr>
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<td>6 × 10⁷</td>
<td>5/5</td>
<td>25.6 ± 5.7</td>
</tr>
<tr>
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<td>−</td>
<td>1.8 × 10⁷</td>
<td>5/5</td>
<td>22.4 ± 4.1</td>
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<tr>
<td>SCID</td>
<td>−</td>
<td>5.4 × 10⁷</td>
<td>5/5</td>
<td>20.6 ± 2.8</td>
</tr>
</tbody>
</table>

*a* Mice were X-irradiated weekly with 200 rads (1 rad = 0.01 Gy) for 3 weeks before transplantation.

*b* Results are expressed as the number of mice with tumors/total number of mice inoculated i.p. with BALL-1 or BALL-1a.

*c* Not determined. Mice were sacrificed to harvest tumor on day 20.

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**Fig. 1.** Dose-dependent survival time of SCID mice that were inoculated i.p. with BALL-1a. The SCID mice had not been subjected to any preconditionings prior to the transplantation. Individual mice of each group (n = 5) were inoculated with 6.7 × 10⁶, 2 × 10⁷, 6 × 10⁷, 1.8 × 10⁷, or 5.4 × 10⁷ cells of BALL-1a.

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Table 2 Phenotype of BALL-la tumor cells that were recovered from the tumor-bearing SCID and nude mice

The in vitro-maintained parental BALL-i cell line was included in the flow cytometry test to compare its cell surface marker profile with those of the BALL-la tumor samples from the SCID and nude mice. The values given are percentage of positive cells.

<table>
<thead>
<tr>
<th>mAb</th>
<th>Tumor cells from mice</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SCID</td>
<td>Nude</td>
</tr>
<tr>
<td>Anti-ig λ chain</td>
<td>98.8</td>
<td>98.6</td>
</tr>
<tr>
<td>Anti-ig μ chain</td>
<td>91.1</td>
<td>92.8</td>
</tr>
<tr>
<td>Anti-ig δ chain</td>
<td>84.5</td>
<td>43.9</td>
</tr>
<tr>
<td>Anti-HLA-DRf</td>
<td>96.7</td>
<td>97.8</td>
</tr>
<tr>
<td>G4-3A7g</td>
<td>99.8</td>
<td>99.5</td>
</tr>
<tr>
<td>Leu 16 (anti-CD20)</td>
<td>97.3</td>
<td>97.8</td>
</tr>
<tr>
<td>SN3 (anti-CD24)</td>
<td>99.8</td>
<td>99.9</td>
</tr>
<tr>
<td>SN7f</td>
<td>99.2</td>
<td>98.3</td>
</tr>
<tr>
<td>SN8 (anti-B29)</td>
<td>97.9</td>
<td>99.6</td>
</tr>
<tr>
<td>SN11d</td>
<td>98.7</td>
<td>96.9</td>
</tr>
<tr>
<td>MOPC control IgG</td>
<td>0.2</td>
<td>0.1</td>
</tr>
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</table>

a The in vitro-maintained parental BALL-1 cell line.
b A control T ALL cell line.
c A mAb defining a nonpolymorphic determinant of HLA-DR molecules (Becton Dickinson).
d See "Materials and Methods."

Fig. 2. Photomicrographs of hematoxylin and eosin-stained organs of SCID mice engrafted with BALL-la. Bone marrow (A, × 50) is densely infiltrated with human leukemia cells. The liver shows perivascular and sinusoidal infiltration (B, × 25). The leukemic infiltration is also found in peribronchial lung parenchyma (C, × 25) and peribronchial lymph node (D, × 200).

Therapy of SCID Mice Bearing BALL-la Tumor. In the initial therapeutic studies of SCID mice, mice were inoculated with $1.1 \times 10^6$ BALL-la cells based on the earlier transplantation experiments (Table 1). Three sets of experiments were carried out. In the first experiment, SCID mice bearing BALL-la tumor were treated with PBS and $4 \times 10$ μg (the initial dose of 10 μg followed by three daily injections on successive days) of unconjugated SN7 and SN7 IT. The therapy was initiated 24 h post tumor inoculation. In the second and third experiments, the dose of the therapeutic agent was increased to $4 \times 20$ μg and $4 \times 30$ μg, respectively. An identical result was obtained from the three experiments. The result obtained using $4 \times 20$ μg of SN7 and SN7 IT is illustrated in Fig. 4. All of the PBS control group mice (total n = 15) died within 35 days after tumor inoculation. In contrast, all mice that were treated with unconjugated SN7 (total n = 15) or SN7 IT (total n = 19) survived without any sign of tumor for as long as followed, i.e., for 250 days. It was unexpected that unconjugated SN7 was as effective as SN7 IT for tumor suppression in the above studies. This finding is in contrast to the result obtained using nude mice bearing BALL-la tumor (Fig. 3). The antitumor efficacy of SN7 and SN7 IT was further studied using mice with a larger tumor burden as described below.

Differentiation between mAb SN7 and SN7 IT for Their Antitumor Efficacy. In an attempt to differentiate antitumor efficacy of SN7 IT from that of unconjugated SN7, we carried out therapeutic experiments using SCID mice with a larger tumor burden, i.e., SCID mice inoculated with $4 \times 10^7$ BALL-la cells. The result of the initial

Fig. 3. Complete and prolonged suppression of tumor growth in nude mice by SN7-RA. The therapy of the nude mice bearing BALL-la tumor was carried out by i.p. injection of PBS, control RA conjugate containing an isotype-matched murine control IgG, unconjugated SN7, or SN7-RA. All of the mice treated with SN7 IT survived for as long as followed and did not show any sign of tumor or undesirable side effects. The unconjugated SN7 was only marginally effective for tumor suppression.
was untreated (group 1) or treated by i.v. injection of 40 μg of SN7-RA. Three of the seven mice that were treated with 4 × 20 μg of SN7 IT survived for as long as followed. The result in the second experiment, the dose of the therapeutic agents was reduced to 20 μg, and the treatment was repeated with the same dose using a total of 80 μg (4 × 20 μg). The result is shown in Fig. 6B. All of the group 1 mice (n = 5) that were treated with control dgRA IT died with a MST of 15.0 ± 2.8 days. Three of the group 2 mice (n = 7) that were treated with unconjugated SN7 survived for as long as followed (140 days), while four remaining mice died with a MST of 60.5 ± 15.9 days. None of the group 3 mice (n = 7) that were treated with SN7-dgRA developed tumors, and all seven mice survived without any sign of tumor for as long as followed. In the third i.v. therapy experiment, the total dose of the SN7-dgRA was further reduced to a total of 60 μg (3 × 20 μg), and the initiation of the therapy was delayed until 2 or 3 days post tumor inoculation. The result is shown in Fig. 6C. All of the untreated control mice (n = 5) developed tumor and died with a MST of 29.4 ± 2.9 days. In contrast, none of the group 2 (n = 4) and group 3 (n = 4) mice that were treated with SN7-dgRA starting on days 2 and 3, respectively, developed tumors for as long as followed (110 days). Thus, SN7-dgRA could effectively suppress tumor growth in SCID mice by systemic i.v. administration when therapy was initiated either 1, 2, or 3 days post tumor inoculation.

In Vitro Cytotoxicity. The in vitro cytotoxic activity of IT against BALL-1a and MOLT-4 (control) was determined by a protein synthesis inhibition assay. SN7-RA and SN7-dgRA showed 50% inhibitory concentration values of 3.2 and 1.9 μM, respectively, against BALL-1a in the absence of any potentiator. These ITs did not show any significant cytotoxicity against MOLT-4 control cells at the IT concentrations tested, i.e., between 10 nM and 0.01 μM. Unconjugated SN7 did not show any significant cytotoxicity against either BALL-1a or MOLT-4. Thus, SN7 IT showed a highly potent and specific cytotoxicity against BALL-1a.

The in vitro cytotoxic activity of SN7 IT was further tested by a clonogenic assay (see “Materials and Methods” for details). SN7-dgRA, at either 0.16 μM or 4 μM concentration, was able to eliminate over 99.996% (over 5.5 logs) of clonogenic BALL-1a tumor cells, which represents the limit of the sensitivity of the assay.

DISCUSSION

Since Rygaard and Povlsen (23) reported a successful transplantation of human malignant tumor into athymic nude mice over 20 years
were further used for therapeutic studies by i.p. or i.v. administration compared the nude and SCID mouse models of human B leukemia/lymphoid blood cells including monocytes, granulocytes, megakaryocytes, erythrocytes, and natural killer cells. Thus, a SCID mouse model of human tumors may provide a useful in vivo model for studying the biology of human tumors and therapeutic efficacy of various anti-human tumor agents, despite the deficient T- and B-cell functions.

Recently, a number of investigators reported SCID mouse models of human leukemia and lymphoma (26–32). However, a direct comparison between the nude and the SCID mouse models of the same tumor has been very rare in these reports. This is particularly true for the therapeutic studies. In the present study, we established and compared the nude and SCID mouse models of human B leukemia/lymphoma. Our result demonstrated that the SCID mouse model has advantages over the nude mouse model in the following aspects: (a) preconditioning (X-irradiation) was necessary for the transplantation in the case of nude mice but was not necessary in the case of SCID mice; (b) 100% transplantation was achieved with lower tumor doses in SCID mice compared to nude mice; and (c) tumors were more disseminated in SCID mice than in nude mice. These results are consistent with our recent findings using nude and SCID mouse models of human B chronic lymphocytic leukemia (33).

In the present study, the developed nude and SCID mouse models were further used for therapeutic studies by i.p. or i.v. administration of a mAb and its RA and dgRA conjugates. The IT was highly potent for tumor suppression in both nude and SCID mice, and they show nearly normal differentiation and functions of nonlymphoid blood cells including monocytes, granulocytes, megakaryocytes, erythrocytes, and natural killer cells. Thus, a SCID mouse model of human tumors may provide a useful in vivo model for studying the biology of human tumors and therapeutic efficacy of various anti-human tumor agents, despite the deficient T- and B-cell functions.

The SCID mouse models in the antitumor efficacy of mAb SN7 (IgG1κ). Unconjugated SN7 was only marginally effective for the tumor suppression in nude mice, whereas it was strongly effective in SCID mice. The poor antitumor efficacy of the unconjugated SN7 in the present nude mouse model is consistent with our recent finding that the same mAb was also not effective for tumor suppression in nonirradiated nude mice bearing tumor of NALM-6, a pre-B ALL cell line.4 Previously, we tested five other IgG1κ mAbs (i.e., SN1, SN2, SN5, SN5d, and SN6) for their antitumor efficacy in nude mice (X-irradiated and nonirradiated) bearing human T leukemia or pre-B leukemia (9, 15, 22, 34, 35). None of the unconjugated mAbs showed effective antitumor activity. These results are consistent with the present finding.

Because of the unexpected antitumor efficacy of unconjugated SN7 in SCID mice, we carried out several additional therapeutic studies of SN7 and SN7 IT in SCID mice. The strong antitumor efficacy of SN7 was observed by both i.p. and i.v. administration (Figs. 4 and 6). It also showed substantial efficacy in SCID mice bearing a large tumor burden (Fig. 5). However, the antitumor efficacy of SN7 was not as strong as SN7 IT (Figs. 5 and 6). Therefore, the antitumor efficacy of SN7 IT is not entirely due to the mAb part of the IT. The in vitro tests showed that SN7 IT is strongly cytotoxic to target tumor cells (BALL-1a) but not cytotoxic against control cells, which indicates the specific nature of the cytotoxicity of SN7 IT. The unconjugated SN7 is not cytotoxic against either BALL-1a or control cells in vitro. At the present time, we do not know the mechanisms by which the unconjugated SN7 exerts antitumor efficacy in SCID mice.

Recently, Ghetie et al. (36) reported that HD37, an IgG1 anti-CD19 mAb, was as effective as HD37-dgRA for tumor suppression in SCID mice bearing Daudi lymphoma cells. We are not aware of any reports that describe the antitumor efficacy of HD37 in a nude mouse model. It would be interesting to know the antitumor efficacy of HD37 in nude mice bearing Daudi tumor. The strong antitumor efficacy of an unconjugated IgG1 mAb in SCID mice is apparently not universal. For instance, Jansen et al. (30) and Shah et al. (32) reported that their IgG1 anti-CD19 mAbs were not effective for tumor suppression in SCID mice. The mechanisms by which SN7 exerts antitumor efficacy in SCID mice remain to be solved.

In conclusion, the nude and SCID mouse models of human B leukemia/lymphoma that were developed in the present study appear to be very useful for evaluating the therapeutic efficacy of antitumor agents. SN7 IT used in the present study is highly potent for tumor suppression, and the results warrant further studies of SN7 IT. It will

be interesting to define the mechanisms by which the unconjugated SN7 exerts effective antitumor efficacy in SCID mice but not in nude mice.

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Establishment of New SCID and Nude Mouse Models of Human B Leukemia/Lymphoma and Effective Therapy of the Tumors with Immunotoxin and Monoclonal Antibody: Marked Difference between the SCID and Nude Mouse Models in the Antitumor Efficacy of Monoclonal Antibody

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