Development of a Severe Combined Immunodeficiency (SCID) Mouse Model Consisting of Highly Disseminated Human B-Cell Leukemia/Lymphoma, Cure of the Tumors by Systemic Administration of Immunotoxin, and Development/Application of a Clonotype-specific Polymerase Chain Reaction-based Assay

ABSTRACT

A new severe combined immunodeficiency (SCID) mouse model consisting of highly disseminated human B-cell leukemia/lymphoma was developed by i.v. inoculation of BALL-1a, an in vivo adapted malignant B-cell line. A 100% transplantability was achieved in non preconditioned SCID mice using various BALL-1a doses between 2.5 x 10^6 and 6 x 10^6 cells. Hind-leg paralysis preceded the death of the mice. Utility of the developed tumor model for the therapeutic studies was investigated by i.v. administration of an anti-B-cell monoclonal antibody (mAb) SN7 (IgGl) and its conjugate with deglycosylated ricin A chain (dgRA). The therapy was initiated 2, 4, or 6 days after tumor inoculation using 4 x 24 μg of SN7-dgRA or 4 x 20 μg of SN7; the total dose (96 μg) of SN7 (IgGl) and its conjugate with deglycosylated ricin A chain. Unconjugated SN7 showed a significant antitumor efficacy but was less effective than SN7-dgRA. A PCR-based assay specific for the clonogenic BALL-1a tumor was developed and applied to determine tumors in various organs of BALL-1a-bearing SCID mice. The assay was highly sensitive in screening for trace quantities of residual tumors in various organs of SCID mice, and it could detect 1 malignant cell/2.5 x 10^6 tissue cells. The PCR-based assay was shown to be much more powerful than the conventional histological analysis in detecting residual tumors. Furthermore, we could estimate quantities of the detected tumors by the PCR-based assay. It is remarkable to find that all examined organs of some of the SN7-dgRA-treated mice were tumor-free as determined by the clonotype-specific PCR-based assay. The present results show the usefulness of the newly developed SCID mouse model, SN7-dgRA, and the clonotype-specific PCR-based molecular assay for the study of therapy of human B-cell leukemia/lymphoma.

INTRODUCTION

Antibody-guided specific delivery of cytotoxic agents to tumor targets is a highly attractive complement/alternative to conventional chemo-radiotherapy of cancer. Neither conventional chemotherapy nor radiotherapy is capable of properly distinguishing tumor cells from normal cells. In contrast, antibody-guided therapy using appropriate antibodies may be capable of distinguishing tumor cells from many normal cells and tissues. However, a number of problems remain to be solved or minimized before immunoconjugates can be effectively applied for therapy of many cancer patients (1, 2). Development of appropriate animal models of human tumors will be valuable to study these problems.

In the present study, we developed a new SCID mouse model consisting of highly disseminated human B-cell leukemia/lymphoma by systemic inoculation of BALL-1a into non preconditioned SCID mice. BALL-1a is an in vivo adapted cell line of BALL-1, a B ALL cell line (3). No significant difference was detected between BALL-1 and BALL-1a when 11 B-cell markers including immunoglobulins were compared (3). Previous studies (3–6) 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 most cases of B lymphoma. B ALL is associated with a poor prognosis and is closely related to B lymphoma in its clinical and immunological features (7–9). B ALL in children is probably a leukemia phase of non-Hodgkin’s lymphoma or Burkitt’s lymphoma (9). Although chemotherapy and/or radiotherapy is capable of inducing initial remission in the majority of B ALL and 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 ALL and B lymphoma.

In this study, we used the developed SCID mouse model to investigate its utility for evaluating the therapeutic potential of mAb and IT targeted to B-cell leukemia/lymphoma. The IT was prepared by conjugating mAb SN7, an anti-B-cell mAb, to dgRA using SMPT, which generates a relatively stable disulfide linker in vivo (10). The therapy was initiated at different times after tumor inoculation by systemic administration of IT or mAb. SN7-dgRA showed highly effective therapeutic efficacy. In addition, unconjugated SN7 was effective in the therapy, although less effective than SN7-dgRA.

Nucleotide sequence analysis of the gene coding for the IgH V region of BALL-1 revealed that BALL-1 IgH belongs to the IgH-V3 subgroup and possesses a unique IgH-VDJ gene sequence. A family-specific primer derived from the first framework region of the IgH-V3 gene segment and a clonotype-specific primer derived from the unique IgH-VDJ sequence were used to detect the clonogenic BALL-1a tumor specifically by adapting the procedure of Greenberg et al. (11). The assay was found to be highly sensitive in screening for trace quantities of BALL-1a tumors in various organs of SCID mice.

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The clonotype-specific PCR-based assay clearly showed effective killing of tumor cells by SN7-dgRA and SN7.

MATERIALS AND METHODS

Mice. Male and female C.B-17/ScTac-scid mice (SCID mice) were obtained from Taconic (Germantown, NY). Unless otherwise stated, 6–8-week-old male mice in 10–12% body weight were used in the present study. The mice were maintained in a protected environment in cages with filter bonnets in a laminar flow unit (Lab Products, Maywood, NJ) as described previously (12). Animals were given autoclaved food and water ad libitum, and all manipulations were performed in a laminar flow hood.

Human Cell Lines. BALL-1 and BALL-1a were cultured in RPMI 1640 supplemented with 8% heat-inactivated fetal bovine serum, 100 units/ml penicillin, and 50 μg/ml streptomycin.

mAbs and Reagents. mAb SN7 (IgG1κ) was generated in our laboratory (3). SN7 reacts with most of B leukemia-lymphoma samples and defines a 30-kDa glycoprotein antigen. Characterization of the chemical properties of the antigen is under way. SN7 reacted with all 15 B-cell non-Hodgkin’s lymphoma specimens tested, all 27 B-cell chronic lymphocytic leukemia specimens tested, and all 6 B-cell prolymphocytic leukemia specimens tested. Colony-forming unit assays using normal human bone marrows and SN7 IT suggested that the SN7 antigen was not expressed on hematopoietic progenitors. In immunohistochemical analysis, SN7 did not react with several normal human tissues tested, which included brain, heart, kidney, pancreas, thyroid, trachea, bronchus, lung, liver, small bowel, and bone marrow tissues.

Preparation of BALL-1a Tumor in Different Mice by the PCR-based Assay. Individual organ specimens were minced and then homogenized in 5 ml of PBS using a sterile homogenizer. The homogenized solution was transferred to a 15-ml tube and centrifuged. After decanting the supernate, the pellet was subjected to high molecular weight DNA extraction, and the clonotype-specific BALL-1 IgH-VDJ gene was amplified by PCR as described above. To avoid carry-over contamination in the PCR procedure, the preparation of the oligonucleotide primers and analysis of the amplified products was carried out in different rooms, and barrier pipette tips were used in all procedures. In addition, a reagent control without DNA was included in all of the PCR tests. Contamination of the reagent control was never observed during the present experiments of the PCR-based assay.

Autoradiographs were prepared and scanned densitometrically using transillumination, converted into a computer Scanner film by Microtek B&W, and quantitated by Scan Analysis. The infiltration by the BALL-1a tumor cells into each organ was represented by the ratio of the IgH-VDJ/actin signal intensity, and the level of a tumor burden was estimated by extrapolating the ratio onto the standard curve.

Preparation of IT. The purified IgGs of mAb SN7 and an isotype-matched control murine IgG (MOPC 195 variant; IgG1κ) were individually conjugated with dgRA using SMPT as described previously (3, 10). The resulting IgG-dgRA was separated from the free (unconjugated) dgRA by gel filtration on a Sepharose S-300 column (13). The contaminating free IgG was removed from the conjugates by chromatography on a Blue Sepharose column (6, 19). The conjugates were characterized as described previously (13). The major component (i.e. 76%) of the isolated IT preparation was IgGdgRA2. The remaining 24% consisted of IgGdgRA1, IgGdgRA3, and IgGdgRA4. The preparation contained a small amount (approximately 7%) of free antibody.

In Vitro Cytotoxic Activity of IT. The cytotoxic activity of SN7-dgRA was determined by a protein synthesis inhibition assay as described previously (20). Briefly, BALL-1a and MOLT-4, a control cell line, were incubated in triplicate with varying concentrations of SN7-dgRA or SN7 for 24 h. The incubated cells were centrifuged, washed, and resuspended in leuine-free medium containing 1 μCi of [3H]leucine. The cell suspensions were incubated for 4 h and centrifuged, and the pelleted cells were washed. Cells were harvested on glass fiber filters using a multiple semiautomated cell harvester (type 7010; Skatron, Inc., Sterling, VA), and the 3H radioactivity was determined in a liquid scintillation spectrometer. Protein synthesis in the conjugate- or antibody-treated cells was expressed as the percentage of [3H]leucine incorporated into control cells not exposed to conjugate or antibody.

Toxicity of IT in Mice. The LD₅₀ of IT in female BALB/c and SCID mice was determined as described by Ghetie et al. (21). In this test, groups of four mice were given i.p. 0.2, 0.4, or 0.6 mg of IT. In an additional test, groups of two mice were given i.p. 0.075, 0.15, 0.30, 0.60, or 1.2 mg of IT. The mice
were weighed before IT injection and daily thereafter and were observed for 7 days. LD50 values were determined by plotting the percentage of mortality versus the injected dose. The same LD50 value was obtained by the two tests.

Therapy of Tumor-bearing Mice. SCID mice that were inoculated i.v. with \(2 \times 10^5\) BALL-la cells were untreated (control) or treated by i.v. administration of an isotype-matched control IgG (20 \(\mu\)g), unconjugated mAb SN7 (IgG1κ; 20 \(\mu\)g), control conjugate (24 \(\mu\)g) containing an isotype-matched murine IgG, or SN7 IT (24 \(\mu\)g) via the tail vein. The treatment was initiated 2, 4, or 6 days after the tumor inoculation and was repeated three times by daily administration on successive days.

Treatment Efficacy. During the treatment, the mice were monitored daily for morbidity and mortality as described previously (22). Paralysis of the mice was carefully monitored and recorded when complete HLP was observed. The weight of the mice was measured twice a week using an electronic balance (OHAUS Model GT210). Statistical analysis of the data for the comparison of different groups of mice was carried out using the log-rank test as described previously (23). A gross examination of various tissues was performed on each sacrificed or dead mouse after laparotomy. Then, various tissues of selected mice were removed and subjected to histological analysis and PCR-based assay (see above). The examined tissues include brain, femoral bone marrow, heart, kidney, liver, lung, and spleen.

RESULTS

Transplantation of BALL-1 and BALL-1a into SCID Mice. SCID mice were used without any preconditionings such as X-irradiation. Mice were given tumor cells via the tail vein. In the initial experiments, the in vitro maintained parental BALL-1 cell line was used to establish tumors in SCID mice. Only 33.3–66.7% of the mice developed tumors when the mice were inoculated with \(2.5 \times 10^5\) to \(6.6 \times 10^5\) BALL-1 cells (Table 1). Tumors developed in all of the mice that were given increased doses of BALL-1, i.e. \(2.0 \times 10^5\) to \(180 \times 10^5\) BALL-1 cells; mean survival time was 77.7–81.2 days. The results indicate that nonpreconditioned SCID mice bearing BALL-1 i.v. tumors are not an ideal animal model for studying the therapy of human B-cell leukemia/lymphoma. This is because large tumor doses are necessary for achieving a 100% transplantability of tumor and because tumors in the transplanted mice grew very slowly.

Therefore, BALL-1a, an in vivo adapted BALL-1 cell line (3), was used in the subsequent transplantation experiments, and the results are summarized in Table 1. Tumors developed in all of the mice inoculated with \(2.5 \times 10^5\) to \(60 \times 10^5\) BALL-1a cells; mean survival time was 26.2–36.2 days. The results show that BALL-1a is readily transplantable in nonpreconditioned SCID mice at relatively low tumor doses, and it grows much faster than the parental BALL-1 cell line in SCID mice. In addition, HLP preceded the death of mice by a relatively narrow range of time in all cases in the present studies (see Table 1); the result allows us to use the paralysis instead of the death of the mice as the end point of the experiments (see below and “Discussion”).

Patterns of Dissemination of BALL-1a Tumors in SCID Mice. Histological examination revealed the extensive tumor involvement in various organs of the SCID mice. Some of the results are shown in Fig. 1. The tumor-involved tissues included bone marrow, lymph nodes (abdominal, mediastinal, periaortic, pararenal, and so forth), spleen, liver, lung, parathyroid gland, mediastinal brown fat, and skeletal muscle. In addition, diffuse infiltration by the tumor was seen in the leptomeninges, but the parenchyma of the examined brains was uninvolved by the tumor (Fig. 1D). The cardiac and renal parenchyma were uninvolved by the tumor, but there was infiltration of the pericardium and renal capsule and surrounding soft tissues by the tumor.

Toxicity of IT in Mice. The LD50 dose of SN7-dgRA was determined to be 33.3 \(\mu\)g/g body weight in both normal BALB/c mice and SCID mice. This result was obtained by using two test procedures (see “Materials and Methods”). The result indicates that SN7-dgRA is as safe as IgG-RFB4-dgRA, an anti-CD22 IT that was used for clinical trials by Amlot et al. (24) and Saussville et al. (25); the LD50 of IgG-RFB4-dgRA in mice was 14 \(\mu\)g/g body weight (21). It should be noted that both SN7 and IgG-RFB4 are IgG1 mAbs and that both SN7-dgRA and IgG-RFB4-dgRA were generated by using SMPT as the coupling agent.

Therapy of SCID Mice Bearing BALL-1a Tumor. In the therapeutic studies of SCID mice, mice were inoculated i.v. with \(2 \times 10^5\) BALL-1a cells based on the earlier transplantation experiments (see Table 1). In a set of experiments, SCID mice that were inoculated i.v. with tumor cells were divided into five groups. Each of the first four groups \((n = 7\) for each group) of mice was treated by i.v. administration of: (a) an isotype-matched control murine IgG (MOPC 195 variant; IgG1κ; \(4 \times 20 \mu\)g); (b) dgRA conjugate of the control IgG (termed MOPC-dgRA; \(4 \times 24 \mu\)g); (c) unconjugated SN7 (\(4 \times 20 \mu\)g); or (d) SN7-dgRA (\(4 \times 24 \mu\)g). The total dose of SN7-dgRA (96 \(\mu\)g) corresponded to 14% of the LD50 dose of SN7-dgRA. The therapy in the first four groups of mice was initiated 2 days after tumor inoculation, and the IT or IgG was given in four equal doses on days 2, 3, 4, and 5. The fifth group of the mice \((n = 9)\) was given SN7-dgRA, as were the group 4 mice, but the therapy was initiated 4 days after tumor inoculation, and the IT was given in four equal doses on days 4, 5, 6, and 7. Results are shown in Fig. 2. All of the two control groups \((n = 14)\) showed complete HLP within 40 days after tumor inoculation. The MPT of group 1 and group 2 mice was 25.6 ± 6.8 days and 21.4 ± 0.9 days, respectively. One of the group 3 mice \((n = 7)\), which were treated with free SN7 2 days after tumor inoculation, survived for as long as followed (230 days); the MPT of the six paralyzed mice was 104.7 ± 40.3 days. In contrast, all of the group 4 mice \((n = 7)\), which were treated with SN7-dgRA 2 days after tumor inoculation, survived for as long as followed. Among the group 5 mice \((n = 9)\), which were treated with SN7-dgRA 4 days after tumor inoculation, six mice survived for as long as followed. The MPT of the three paralyzed mice was 100.3 ± 23.8 days.

The antitumor efficacy of SN7-dgRA is statistically significant compared with controls when the therapy was initiated either 2 or 4 days after tumor inoculation \((P < 0.0001)\). The antitumor efficacy of SN7 is also statistically significant \((P < 0.0001)\). However, SN7-dgRA is significantly more effective for the tumor suppression than SN7 \((P = 0.0013)\).

In an extension of the studies, initiation of the therapy was further delayed until day 6 after tumor inoculation. The result is shown in Fig. 2.

Table 1 Transplantation of BALL-1 and the in vivo adapted BALL-1a into SCID mice

<table>
<thead>
<tr>
<th>Cells</th>
<th>No. of cells (10^5 x)</th>
<th>Transplantability</th>
<th>Mean survival time ± SD (days)</th>
<th>MPT ± SD (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALL-1</td>
<td>0.25</td>
<td>2/6</td>
<td>78.0 ± 5.2</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>0.74</td>
<td>4/6</td>
<td>81.2 ± 18.4</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>4/6</td>
<td>77.7 ± 22.6</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>6.6</td>
<td>4/6</td>
<td>36.2 ± 4.2</td>
<td>29.8 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>5/5</td>
<td>30.4 ± 3.2</td>
<td>23.2 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>60.0</td>
<td>6/6</td>
<td>28.2 ± 2.5</td>
<td>22.5 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>180.0</td>
<td>6/6</td>
<td>26.6 ± 2.3</td>
<td>21.4 ± 1.6</td>
</tr>
<tr>
<td>BALL-1a</td>
<td>0.25</td>
<td>5/5</td>
<td>32.2 ± 9.5</td>
<td>26.6 ± 8.7</td>
</tr>
<tr>
<td></td>
<td>0.74</td>
<td>5/5</td>
<td>26.6 ± 5.6</td>
<td>20.0 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>5/5</td>
<td>26.6 ± 3.6</td>
<td>20.0 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>6.7</td>
<td>5/5</td>
<td>26.6 ± 2.3</td>
<td>21.4 ± 1.6</td>
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<tr>
<td></td>
<td>20.0</td>
<td>5/5</td>
<td>26.6 ± 2.3</td>
<td>21.4 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>60.0</td>
<td>5/5</td>
<td>26.6 ± 5.6</td>
<td>20.0 ± 2.6</td>
</tr>
</tbody>
</table>

[a] Cells were suspended in PBS to predetermined concentrations, and 0.2 ml of PBS containing the appropriate number of the cells was inoculated into individual mice via the tail vein. Mice were not subjected to any preconditionings such as X-irradiation.

[b] Results are expressed as the number of mice with tumors/total number of mice inoculated with BALL-1 or BALL-1a. Tumor-free mice were monitored for 160 days.

[c] ND, not determined.
Fig. 1. Histological appearance of leukemic infiltrates in SCID mice after i.v. injection of BALL-1a. In A (femoral bone marrow), there is complete obliteration of the marrow by the leukemia cells (H&E, ×50). In B (spleen), the red pulp is diffusely infiltrated by the leukemic blast cells; the large cells represent residual extramedullary megakaryopoiesis (H&E, ×50). In C (liver), there are prominent portal and periportal leukemic infiltrates (H&E, ×50). In D (brain), the leukemic infiltrate is seen in the leptomeninges but spares the cortex (H&E, ×50).

Fig. 2. Systemic therapy of SCID mice bearing BALL-1a i.v. tumors. The therapy was initiated 2 days or 4 days after tumor inoculation by injecting i.v. SN7 (A) or SN7-dgRA (B and C). The control IgG (●) and control IgG-dgRA (X) were given 2 days after tumor inoculation. Before use, mAb SN7, control IgG, SN7-dgRA, and control IgG-dgRA were individually diluted in PBS containing mouse serum albumin (0.05% final concentration) and sterilized by filtering through PBS-prewashed Millex GV filters (Millipore) in a laminar flow hood. The total dose of SN7-dgRA corresponded to 14% of the LD_{50} dose.

3. Mice of three control groups [i.e., untreated group (n = 7), control IgG-treated group (n = 6), and control IT-treated group (n = 6)] showed paralysis with similar MPTs to the two control groups in Fig. 2, except that one of the seven mice in group 1 (untreated) survived for a relatively long time without paralysis until 75 days after tumor inoculation. Although we do not know the cause of the longer survival of this control mouse, histological analysis and a PCR-based assay showed that various tissues (i.e., bone marrow, heart, liver, lung, and spleen) were heavily infiltrated with tumors when it died (see below). Six of the seven mice in group 4 that were treated with unconjugated SN7 showed paralysis with a MPT of 45.5 ± 7.5 days, but the one remaining mouse survived for as long as followed, i.e., 200 days. Three of the seven mice in group 5 that were treated with SN7-dgRA showed paralysis with a MPT of 59.3 ± 24.6 days, but the four remaining mice (57%) survived healthily for as long as followed. The antitumor efficacy of SN7-dgRA is statistically significant compared to control IT (P = 0.0002). SN7 is also significantly effective compared to control IgG (P = 0.0009).

The test results showed that SN7-dgRA is effective for suppressing tumor growth in SCID mice bearing BALL-1a i.v. tumors by systemic administration of a relatively small dose (i.e., a total dose of 14% of

Fig. 3. Delayed systemic therapy of SCID mice bearing BALL-1a i.v. tumors. The therapy was initiated 6 days after tumor inoculation by administering i.v. control IgG (X), control IgG-dgRA (A), SN7 (B), and SN7-dgRA (C). Another group was untreated (●) as a control. Doses of unconjugated IgG and IT are the same as those in Fig. 2, i.e., 4 × 20 µg and 4 × 24 µg, respectively, for IgGs and dgRA conjugates. The mAb SN7, control IgG, SN7-dgRA, and control IgG-dgRA were treated as described in the legend to Fig. 2 before use.
Thirteen nucleotides between the V and J segments did not match any known D gene primer and BALL-I clonotype-specific primer were used to specifically amplify the BERD transferase. A BALL-I clonotype-specific primer was designed to include all N segments and were designated as N sequences incorporated by the terminal deoxynucleimmunoglobulin heavy chain, an IgH-V3 segment was recombined to an IgH-J segment. In the variable region of the BALL-I to include the N sequences as well as a 5' portion of the J sequence (bold type). The IgH-V3 family-specific primer and IgH-J family-specific primer sequences, which were used to amplify the BALL-I gene. On the other hand, generic primer and IgH-J family-specific primer sequences (26–28) were used to construct a clonotype-specific primer for BALL-I, i.e. 5'-AGACCCGATATCATAGTAATGGGTCCC-3'. This primer was used together with a 5' positive-strand IgH-V3-family-specific primer, 5'-TCCCTGAGACTCTCCTGTGAGCTGCACCTGCTTCTGTC-3', to selectively amplify the BALL-I-specific IgH gene. The other hand, generic primers were developed to amplify both human and mouse actin genes with equal efficiency (see "Materials and Methods" for the primers). The actin amplification served as an internal control for multiple DNA preparations from different tissue specimens. A standard curve was generated using genomic DNA of the BALL-I cells serially diluted onto 1 µg of background DNA that was obtained from BALB/c mouse spleen. Examples of such a standard curve are shown in Figs. 5 and 6. Consistent signal intensities of amplified actin gene reflected equivalent total DNA input. The signal intensity of the IgH-VDJ gene was correlated with the amount of BALL-1 DNA and yielded a level of sensitivity in detection of 10 pg of BALL-1 DNA among 1 µg of background DNA (Figs. 5 and 6). Standard curves were generated by plotting the ratios of IgH-VDJ/actin signal intensities against known amounts of BALL-1 DNA (29). To extend the linear range in the standard curve for quantitative purposes, two different autoradiographic exposures, 1 h (Fig. 5) and overnight (Fig. 6), were used. In this manner, it was possible to estimate the number of BALL-1/BALL-1a cells in each specimen examined.

**Determination of Tumors in Tissues of SCID Mice by Histological Analysis and PCR-based Assay.** Tissues of SCID mice that were inoculated with BALL-1a and treated with mAb or IT were examined by histological analysis and PCR-based assay. Histological examination showed heavy tumor involvement in various tissues of all mice in control groups but no detectable tumor in tissues of mice that were treated with SN7 or SN7-dgRA and survived. Tissues of selected mice were further analyzed by a clonotype-specific PCR-based assay. Results of the PCR-based assay are summarized in Table 2. Tissues from mice that were either treated with a control dgRA conjugate (MOPC-dgRA) or untreated were heavily loaded with tumor cells as determined by the PCR-based assay. Bone marrow and liver harbored...
Tissues from SN7- or SN7-dgRA-treated mice were either tumor-free (Lanes 6—11, a variety of organs from a BALL-la-inoculated mouse that was treated with exposure was used instead of 1-h exposure. Lanes 1-5, serially diluted BALL-la DNA, evaluated as described in the legend to Fig. 5 except that overnight autoradiographic exposure was used instead of 1-h exposure. Lanes 6—11, a variety of organs from a BALL-la-inoculated mouse that was treated with SN7-dgRA: bone marrow, heart, kidney, lung, liver, and spleen, respectively.

![Image](image_url)

**Fig. 6.** Quantitative evaluation of leukemic infiltration and minimal residual disease. Minimal residual disease among the mice treated with SN7 or SN7-dgRA was evaluated as described in the legend to Fig. 5 except that overnight autoradiographic exposure was used instead of 1-h exposure. Lanes 1—5, serially diluted BALL-la DNA, from 10 pg to 100 ng, onto background DNA obtained from a BALB/c mouse spleen. Lanes 6—11, a variety of organs from a BALL-la-inoculated mouse that was treated with SN7-dgRA: bone marrow, heart, kidney, lung, liver, and spleen, respectively.

The largest number of tumor cells, representing up to 25% of total cells in the tissue. A heavy tumor load was also detected in lung and spleen. Tissues from SN7- or SN7-dgRA-treated mice were either tumor-free or retained only a small number of the residual tumor cells (<30 tumor cells/µg of tissue DNA corresponding to 2.5 × 10⁵ tissue cells). Although there is a good correlation between the results of the PCR-based assay and histological evaluation, the PCR-based assay is much more sensitive than the histological analysis. By the PCR-based assay, none or only very small amounts of the tumor (<20 tumor cells/2.5 × 10⁵ tissue cells) were detected in various tissues of mice that were treated with SN7 or SN7-dgRA and survived for a long time (>200 days or >230 days). It is remarkable that no tumor was detected in any tissues from mice 7 and 10, which were treated with SN7-dgRA. The untreated mouse 3 survived for an exceptionally long time [paralysis time was 75 days (see Fig. 2); tissues were collected 81 days after tumor inoculation]. However, all examined tissues were heavily loaded with tumor cells. Mouse 5 treated with SN7 died earlier than mouse 3. However, much less tumor was detected in all examined tissues of mouse 5 compared with the tissues of mouse 3. Some mechanisms other than tumor growth may have contributed to the death of this mouse. Despite this unexpected result, the data clearly demonstrate that the presented clonotype-specific PCR-based assay is an extremely sensitive and specific assay that is very useful for quantitatively detecting the clonotypic tumor, particularly for determining residual tumors. In addition, data show that SN7-dgRA is highly potent for suppressing/killing the tumor in vivo.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>BALL-la tumor cells in tissues of SCID mice as determined by a clonotype-specific PCR-based assay</th>
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</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Treatment</td>
</tr>
<tr>
<td>1</td>
<td>MOPC-dgRA</td>
</tr>
<tr>
<td>2</td>
<td>MOPC-dgRA</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
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<td>SN7-dgRA</td>
</tr>
<tr>
<td>12</td>
<td>SN7-dgRA</td>
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</table>

a Post tumor inoculation. Mouse 3 was an exceptionally long-lived untreated mouse. Except for mouse 5, all of the No. 4—12 mice survived healthily at the end of the experiments.

1 µg of tissue DNA corresponds to approximately 2.5 × 10⁵ tissue cells (14). The specimens in which tumor cells were detected by histological examination are marked with.

b Post tumor inoculation. Mouse 3 was an exceptionally long-lived untreated mouse. Except for mouse 5, all of the No. 4—12 mice survived healthily at the end of the experiments.

DISCUSSION

In this study, we developed a new SCID mouse model of human B-cell tumors to study immunotherapy of B-cell leukemia/lymphoma. This tumor model displayed various properties that are desirable for animal models of human B-cell leukemia/lymphoma: (a) all of the mice inoculated i.v. with varying doses of BALL-la (between 2.5 × 10⁵ and 6 × 10⁶ cells) developed tumors without any preconditionings (such as X-irradiation) of the mice, and the transplanted tumor grew relatively rapidly. It should be noted that BALL-la tumor cells display a phenotype that is typical of many relatively mature B-cell leukemia/lymphomas (3); (b) the tumor was highly disseminated into various organs in a manner analogous to many B-cell tumors in patients; and (c) HLP preceded the death of mice in all cases, which allowed us to use the paralysis time rather than the death of the animals as the end point in the therapeutic experiments. Previously, SCID mice were inoculated i.p. with BALL-la (3). However, the present tumor model is different from the previous BALL-la i.p. tumor model in that the tumor is more extensively disseminated, and HLP is consistently observed in the present i.v. tumor model compared with the earlier model. HLP did not precede the death in most mice that were inoculated i.p. with BALL-la. The consistent appearance of HLP in the present tumor model is similar to the i.v. inoculated SCID mouse model of Daudi, a Burkitt’s lymphoma cell line, reported by Ghetie et al. (30). Schmidt-Wolf et al. (31) and Shah et al. (32) also reported that SCID mice inoculated i.v. with B lymphoma cell lines SU-DHL-4, OCI-Ly8, and Namalwa often developed HLP.
However, SCID mice that were inoculated i.p. with Daudi (30), SU-DHL-4 (31), OCI-Ly8 (31), Namalwa (32), and MO1043 (a chronic lymphocytic leukemia cell line; Ref. 33) were not reported to develop HLP. SCID mice inoculated i.p. with BALL-1a (3) developed HLP only occasionally. Probably, HLP is a reflection of the degree of tumor infiltration into bone marrow. For instance, unlike other B leukemia/lymphoma cell lines (e.g., Daudi), BALL-1a infiltrates into bone marrow after it is inoculated i.p. into SCID mice. However, the degree of the bone marrow infiltration of the i.p. inoculated BALL-1a is substantially less compared with the i.v. inoculated BALL-1a. Ghetie et al. (30) reported that compression of the spinal canal by tumors is responsible for HLP.

Although conventional chemotherapy and/or radiotherapy is capable of inducing initial remission in the majority of B ALL and B lymphoma patients, most of these patients relapse and eventually succumb to the disease. Until now, only a few ITs seem to be promising for the therapy of B-cell leukemia/lymphoma (reviewed in Refs. 1 and 2). In this regard, the development of a new promising anti-B leukemia/lymphoma IT will be valuable. The present IT, SN7-dgRA, seems to be promising. SN7-dgRA shows an excellent antitumor efficacy by systemic therapy using a relatively low dose, i.e. 14% of the LD_{50} dose, and no overt side effects were detected under the therapeutic conditions. Its toxicity in mice is relatively low, i.e. its LD_{50} value is 33.3 mg/kg body weight. SN7-dgRA showed an exceedingly strong in vitro cytotoxic activity against malignant B cells but showed no significant cytotoxicity against control cells. Furthermore, SN7-dgRA, at 4 nm, was able to eliminate over 99.9999% (over 5.5 logs) of clonogenic BALL-1a tumor cells in a clonogenic assay (3).

Recently, several investigators including ourselves showed that dgRA ITs could be effective for suppression of human tumors in immunodeficient mice when dgRA was conjugated to appropriate mAbs (e.g., Refs. 3, 21, 23, and 34–37). The conjugation was carried out using either N-succinimidyl 3-(2-pyridyldithio)propionate (SPMT) or SMPT. SMPT generates a sterically hindered disulfide bond, whereas N-succinimidyl 3-(2-pyridyldithio)propionato generates an unhindered disulfide bond. The hindered disulfide bond was shown to be more stable than the unhindered disulfide bond in vivo (10). Furthermore, it was shown that dgRA ITs prepared by using SMPT are safe and potentially effective in clinical trials (24, 25). Therefore, the present SN7-dgRA was prepared by using SMPT.

In tumor therapy, the degree of eradication of residual tumors in the host will be an important prognostic factor. In this regard, we developed a highly sensitive clonotype-specific PCR assay to determine the degree of tumor eradication in the IT- and mAb-treated mice. This assay is highly sensitive and allows us to detect a single malignant cell among 2 × 10^{5} normal cells. Application of the clonotype-specific PCR assay demonstrated that SN7-dgRA and SN7 are highly effective for decreasing the tumor burden or eradicating the tumor in the BALL-1a-bearing mice. No tumor was detected in any examined tissues in two of the SN7-dgRA-treated mice (Table 2). In other SN7-dgRA-treated mice, the tumor burden was markedly decreased. For instance, the amount of tumors in the bone marrow samples of SN7-dgRA-treated mice was less than 0.1% of the tumors in the bone marrow samples of the control mice that were not treated or were treated with control IT. Schmidt-Wolf et al. (31) and Uckun et al. (38) applied a PCR-based assay specific for t(14;18) chromosomal translocation and an assay specific for human β-globin, respectively, to detect human tumors in the tissues of SCID mice. However, they did not report the quantities of tumors in the tissues of the mice as determined by the PCR-based assay. Therefore, a comparison of the present PCR-based assay with the above assays is difficult. Nevertheless, the present result and the results of Schmidt-Wolf et al. (31) and Uckun et al. (38) demonstrate the usefulness of the PCR-based assay to detect residual human tumors that we would be unable to detect in the animals using conventional assays. In addition, an appropriately designed PCR-based assay will allow us to estimate quantities of the residual tumors.

Unconjugated mAb SN7 (IgG1k) showed significant antitumor efficacy in SCID mice, although it was less effective than SN7-dgRA (Figs. 2 and 3). The mechanisms by which mAb SN7 exerts an antitumor effect in vivo are not known. Murine IgG1 antibodies do not fix complement effectively and are generally ineffective in directing antibody-dependent cell-mediated cytotoxicity (e.g., Refs. 39 and 40). A possible mechanism by which mAb SN7 exerts antitumor effect could be negative signaling (41).

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REFERENCES


Development of a Severe Combined Immunodeficiency (SCID) Mouse Model Consisting of Highly Disseminated Human B-Cell Leukemia/Lymphoma, Cure of the Tumors by Systemic Administration of Immunotoxin, and Development/Application of a Clonotype-specific Polymerase Chain Reaction-based Assay

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