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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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 nonpreconditioned 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 SN7 (IgG1) 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 x 10^6 of SN7-dgRA or 4 x 2 x 10^6 of SN7; the total dose (96 pg) of SN7-dgRA corresponded to 14% of the LD50 dose. SN7-dgRA showed a strong antitumor efficacy in all groups of treated mice. All of the day-2 group mice (n = 7) and six (66.7%) of the day-4 group mice (n = 9) survived healthily for as long as followed (240 days), whereas four (57.1%) of the day-6 group mice (n = 7) survived healthily for as long as followed (200 days). 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 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 SCID4 mouse model consisting of highly disseminated human B-cell leukemia/lymphoma by systemic inoculation of BALL-1a into nonpreconditioned 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.
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/scid mice (SCID mice) were obtained from Taconic (Germantown, NY). Unless otherwise stated, 6-8-week-old male mice 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.

Abs 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, and the VDJ-reacting liver, small bowel, and testis. In the same assay, SN7 reacted with normal spleen. Control murine IgG (MOPC 195 variant; IgG1κ) was prepared in our laboratory (13). dgRA was obtained from Inland Laboratories (Austin, TX). SMPT was purchased from Pierce Chemical Co. (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) cell lines were used for transplantation into nonconditioned SCID mice. The in vivo adaptation was described previously (3). The transplantability of the resultant BALL-1a was not affected by culturing in vitro for over 6 weeks.

In the transplantation experiments, mice were injected i.v. with BALL-1 or BALL-1a suspended in 0.2 ml of PBS via the tail vein. The inoculated mice were monitored daily 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 mouse after laparotomy immediately after sacrifice or within 24 h after the death of the mouse. Then, various tissues were removed, fixed in 10% buffered formalin, paraffin-embedded, sectioned, and stained with H&E (13). The stained tissues were examined by light microscopy.

Nucleotide Sequence Analysis of the IgH-VDJ Gene of BALL-1. The sequence was analyzed as described previously (11). Briefly, high molecular weight DNA was isolated from BALL-1 cells by the SDS/proteinase K method, purified by phenol/chloroform extraction, precipitated in absolute ethanol, and resuspended in 10 mM Tris-EDTA (pH 8.3). Oligonucleotide primers and probes were synthesized by the phosphoramidite method on an automated 391 DNA synthesizer (Applied Biosystems, Foster City, CA), and 5’ trityl-retained oligonucleotides were purified by chromatography. The oligonucleotide primers and probes were labeled with [-y-32P]dATP and [γ-32P]dCTP (3000 Ci/mmol; Amersham, Arlington Heights, IL) as described previously (11). The purified oligonucleotides were directly ligated into pCR 1000 plasmid (Invitrogen) and were directly sequenced from the purified plasmid by the dideoxy chain termination method (16). The positive-strand primers were directed specific amplification of the IgH-VDJ region of the tumor cells among DNA obtained from the animal organs infiltrated with the tumor cells. The IgH-V, family-specific primer used was 5’-TCCCTGAGACTCTCTGTGACAGCTT-3’. The negative-strand primer sequence was 5’-GGTTCAGCGGCCCCTTTCATCAGGAGCTCCC-3’. The positive-strand primer sequence was 5’-AGACCCCGGATATCATGTTAGGTCCTCC-3’. The negative-strand primer sequence was 5’-ATGGGTGTTATGACGAGCTTCC-3’. Specific primers were designed to amplify both human and mouse actin genes with the same efficiency. The positive-strand primer sequence is 5’-ATGGGTGTTATGACGAGCTTCC-3’, and the negative-strand primer sequence is 5’-AGACCCCGGATATCATGTTAGGTCCTCC-3’. The positive-strand primer sequence was 5’-TCCCTGAGACTCTCTGTGACAGCTT-3’. The negative-strand primer sequence was 5’-GGTTCAGCGGCCCCTTTCATCAGGAGCTCCC-3’. The positive-strand primer sequence was 5’-AGACCCCGGATATCATGTTAGGTCCTCC-3’.

PCR-based Clonotype-specific Assay. The genetic information from the BALL-1 IgH-VDJ juxtaposed region was used to construct a clonotype-specific primer for BALL-1, 5’-AGACCCCGGATATCATGTTAGGTCCTCC-3’. The BALL-1 clonotype-specific primer, directed specific amplification of the IgH-VDJ region of the tumor cells among DNA obtained from the animal organs infiltrated with the tumor cells. The IgH-V, family-specific primer used was 5’-TCCCTGAGACTCTCTGTGACAGCTT-3’. The positive-strand primer sequence is 5’-ATGGGTGTTATGACGAGCTTCC-3’, and the negative-strand primer sequence is 5’-AGACCCCGGATATCATGTTAGGTCCTCC-3’. The positive-strand primer sequence was 5’-TCCCTGAGACTCTCTGTGACAGCTT-3’. The negative-strand primer sequence was 5’-GGTTCAGCGGCCCCTTTCATCAGGAGCTCCC-3’. The positive-strand primer sequence was 5’-AGACCCCGGATATCATGTTAGGTCCTCC-3’. The negative-strand primer sequence was 5’-ATGGGTGTTATGACGAGCTTCC-3’. Specific primers were designed to amplify both human and mouse actin genes with the same efficiency. The positive-strand primer sequence is 5’-ATGGGTGTTATGACGAGCTTCC-3’, and the negative-strand primer sequence is 5’-AGACCCCGGATATCATGTTAGGTCCTCC-3’. The positive-strand primer sequence was 5’-TCCCTGAGACTCTCTGTGACAGCTT-3’. The negative-strand primer sequence was 5’-GGTTCAGCGGCCCCTTTCATCAGGAGCTCCC-3’.
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 x 10^5 BALL-1a cells were untreated (control) or treated by i.v. administration of an isotype-matched control IgG (20 μg), unconjugated mAb SN7 (IgG1κ; 20 μg), control conjugate (24 μg) containing an isotype-matched murine IgG, or SN7 IT (24 μg) 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 0.25 x 10^5 to 6.6 x 10^5 BALL-1 cells (Table 1). Tumors developed in all of the mice that were given increased doses of BALL-1, i.e. 20 x 10^5 to 180 x 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 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 0.25 x 10^5 to 60 x 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 unininvolved by the tumor (Fig. 1D). The cardiac and renal parenchyma were unininvolved 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 μg/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 Sauvialle et al. (25); the LD50 of IgG-RFB4-dgRA in mice was 14 μg/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 x 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 x 20 μg); (b) dgRA conjugate of the control IgG (termed MOPC-dgRA; 4 x 24 μg); (c) unconjugated SN7 (4 x 20 μg); or (d) SN7-dgRA (4 x 24 μg). The total dose of SN7-dgRA (96 μg) 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.

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×)</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>7/16</td>
<td>78.0 ± 5.2 ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>0.74</td>
<td>4/6</td>
<td>81.2 ± 18.4 ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>4/6</td>
<td>77.7 ± 22.6 ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>6.6</td>
<td>5/5</td>
<td>32.2 ± 4.2 29.8 ± 1.7</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>5/5</td>
<td>30.4 ± 3.2 23.2 ± 1.3</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>60.0</td>
<td>6/6</td>
<td>26.2 ± 2.5 22.5 ± 1.8</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>180.0</td>
<td>6/6</td>
<td>26.2 ± 2.3 21.4 ± 6.6</td>
<td>ND</td>
</tr>
<tr>
<td>BALL-1a</td>
<td>0.25</td>
<td>5/5</td>
<td>32.2 ± 9.5 26.6 ± 8.7</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>0.74</td>
<td>5/5</td>
<td>32.2 ± 9.5 26.6 ± 8.7</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>10/10</td>
<td>26.6 ± 5.6 20.0 ± 2.6</td>
<td>ND</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.

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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).

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

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).

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Thirteen nucleotides between the V and J segments did not match any known D gene primer and BALL-l clonotype-specific primer were used to specifically amplify the terminal deoxynucleotidyl transferase. A BALL-i clonotype-specific primer was designed to include all N segments and were designated as N sequences incorporated by the terminal deoxynucleotidyl transferase. A BALL-ia clonotype-specific primer was designed to include all N sequences as well as a 5' portion of the J1 segment (bold type). The IgH-V3 family-specific primer and IgH-J1@,5-specific primer sequences, which were used to amplify the IgH-VDJ segment, are represented in low cases. In the variable region of the BALL-l clonotype, a DNA sequence unique for BALL-l and specific for tumor suppression, although it was less effective than SN7 IT.

In Vitro Cytotoxic Activity. The 50% inhibitory concentration of SN7-dgRA against BALL-1a cells was 1.9 pm in the absence of any potentiators. SN7-dgRA did not show any significant cytotoxicity against control MOLT-4 cells at the IT concentrations between 10 nm and 0.01 pm. Unconjugated SN7 did not show any significant cytotoxicity against either BALL-1a or MOLT-4 under the assay conditions.

Determination of the Nucleotide Sequence of the IgH-VDJ Gene of BALL-1. We determined the IgH-VDJ gene sequence of BALL-1 to identify a DNA sequence unique for BALL-1 and BALL-1a tumors. The sequence is shown in Fig. 4. The IgH-VDJ sequence of BALL-1 contained a gene segment corresponding to a member of the IgH-V5 family and an IgH-J1 segment. Thirteen nucleotide sequences at the V-J junction did not match any known D gene segments and were designated as N sequences incorporated by the terminal deoxynucleotidyl transferase. A BALL-1 clonotype-specific primer was designed to include all N sequences and the 5' portion of the J1 segment (bold type). The IgH-V5 family-specific primer and BALL-1 clonotype-specific primer were used to specifically amplify the BALL-1a DNA among various tissue specimens.

The LD50 dose) when therapy was initiated either 2, 4, or 6 days after tumor inoculation. Unconjugated SN7 (IgG1) was significantly effective for tumor suppression, although it was less effective than SN7 IT.

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Determination of the Nucleotide Sequence of the IgH-VDJ Gene of BALL-1. We determined the IgH-VDJ gene sequence of BALL-1 to identify a DNA sequence unique for BALL-1 and BALL-1a tumors. The sequence is shown in Fig. 4. The IgH-VDJ sequence of BALL-1A contained a gene segment corresponding to a member of the IgH-V5 family and an IgH-J1 segment. Thirteen nucleotide sequences at the V-J junction did not match any published IgH-HD gene segments and were designated as N sequences and the 5' portion of the J1 segment (bold type). The IgH-V5 family-specific primer and BALL-1 clonotype-specific primer were used to specifically amplify the BALL-1a DNA among various tissue specimens.
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/2.5 × 10^5 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 and histological evaluation, the PCR-based assay is highly potent for suppressing/killing the tumor in vivo.

### 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-1a (between 2.5 × 10^3 and 6 × 10^6 cells) developed tumors without any preconditions (such as X-irradiation) of the mice, and the transplanted tumor grew relatively rapidly. It should be noted that BALL-1a 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-1a (3). However, the present tumor model is different from the previous BALL-1a 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-1a. 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.

### Table 2

**BALL-1a tumor cells in tissues of SCID mice as determined by a clonotype-specific PCR-based assay**

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Treatment</th>
<th>Initiation of therapy (day)</th>
<th>Tissue Collection (day)</th>
<th>Bone marrow</th>
<th>Spleen</th>
<th>Liver</th>
<th>Heart</th>
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*Post tumor inoculation.

1 μg of tissue DNA corresponds to approximately 2.5 × 10^5 tissue cells (14). The specimens in which tumor cells were detected by histological examination are marked with *

For mouse 1 and 2, brain was also tested for tumor infiltration; 5120 and 3148 tumor cells, respectively, were detected per 2.5 × 10^6 tissue cells.

Capsular and perirenal soft tissue involvement by tumor was histologically seen in the absence of renal parenchymal infiltration.

ND, not determined.
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 LD50 dose, and no overt side effects were detected under the therapeutic conditions. Its toxicity in mice is relatively low, i.e. its LD50 value is 33.3 μg/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 99.9996% (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 or SMPT. SMPT generates a sterically hindered disulfide bond, whereas N-succinimidyl 3-(2-pyridyldithio)propionate 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(4;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 (IgG1κ) 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).

ACKNOWLEDGMENTS

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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

Minoru Yoshida, Rachel J. Rybak, Youngnim Choi, et al.


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