Adjuvant Treatment of Canine Osteosarcoma with the Human Cytotoxic T-Cell Line TALL-104

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ABSTRACT

The human cytotoxic T-cell line TALL-104 has been used successfully to treat cancer in experimental mouse models with implanted tumors and in dogs with spontaneously occurring malignancies. This study investigated the efficacy of TALL-104 cells given in an adjuvant setting to dogs with appendicular osteosarcoma after surgery and chemotherapy. Of the 23 dogs enrolled in the study, 20 had undergone amputation of the affected limb, and 3 had undergone limb salvage surgery. After surgery, all dogs but one received cisplatin (CDDP) chemotherapy (60 mg/m2 i.v. every 21 days × 1–4 cycles). Four dogs also received one to six cycles of CDDP before limb amputation. After CDDP therapy, dogs without overt metastasis received γ-irradiated (40 Gy) TALL-104 cells systematically (107/kg) for 5 consecutive days, followed by 2-day monthly boosts (at the same dose) for a total of 9 months. Of the 23 dogs treated, 9 survive disease-free at 12.1–29.5 months after surgery, 11 died of metastatic disease between 5 and 21.5 months, 1 experienced a relapse in the lung 9.5 months after surgery but is still alive without further treatment at 13 months, 1 developed progressive neuropathy at 4 months after surgery, and 1 developed progressive neuropathy at 5.9 months after surgery. The overall median survival time is 11.5 months, and the median disease-free interval is 9.8 months. Our cell therapy results compare favorably with historical median survival times (up to 9 months) and disease-free intervals (up to 7.5 months) of dogs with osteosarcoma receiving standard therapy (surgery and chemotherapy) and support the effectiveness of adjuvant TALL-104 cell administration in preventing or delaying disease recurrence in these dogs.

INTRODUCTION

The biological behavior of canine osteosarcoma is close to the one seen in humans; therefore, the dog has been suggested as a model for the human disease (1). Canine osteosarcoma is a highly metastatic cancer commonly seen in large-breed dogs (2). Approximately 98% of dogs have micrometastases at the time of diagnosis (3–6). Amputation alone results in a median ST4 of 3–6 months (7, 8); the 1-year survival rate is 21%, and all dogs are dead at 16 months (7). Amputation followed by chemotherapy (CDDP and/or doxorubicin) increases median STs up to 9 months (7, 9, 10), with a 1-year survival rate of 37% (7). However, adjuvant chemotherapy is unlikely to be curative. Immunotherapy is an attractive adjunction to standard chemotherapy because it offers the prospect of enhancing antitumor effects through nonoverlapping mechanisms and generally also without overlapping toxicities (11). In this respect, Kurzman et al. (12) showed that the administration of liposome-encapsulated muramyl tripeptide after surgery and chemotherapy resulted in a median DFI of 11.2 months, the longest described in the literature for dogs with limb osteosarcoma treated by amputation and other forms of adjuvant therapy.

Our laboratory has developed a new immunotherapeutic approach to cancer based on the use of the clonal human T-cell line TALL-104 (CD3+, CD8+, CD56+, and CD16+) that is endowed with MHC nonrestricted killer activity against a broad range of tumors across several species, sparing cells from normal tissues (13–16). Adoptive transfer of γ-irradiated (40 Gy) TALL-104 cells into immunodeficient mice has induced regression of transplanted human hematopoietic and nonhematopoietic tumors (17–21). The same results were obtained in immunocompetent mice bearing syngeneic leukemia (22) and in pet dogs with spontaneous advanced tumors (23, 24) in which TALL-104 cell transfer was followed by the development of endogenous antitumor immunity (22–24). These results prompted us to test the ability of TALL-104 cells to induce regression of established micrometastasis or to prevent metastatic spread in dogs with osteosarcoma after amputation and chemotherapy. As described in this report, TALL-104 cells given as a single agent after surgery and CDDP chemotherapy resulted in a significant antitumor activity in dogs with osteosarcoma (median ST = 11.5 months).

MATERIALS AND METHODS

Case Selection. Twenty-three dogs diagnosed with histologically confirmed primary appendicular osteosarcoma
were enrolled in this clinical trial conducted at the Veterinary Oncology Services and Research Center (West Chester, PA) starting in July 1996. Complete work-up, including complete blood counts, kidney and liver functions, and chest X-rays, was performed before enrollment in the trial: only dogs without clinical evidence of overt metastases were considered eligible for the study. The clinical characteristics of these dogs at the beginning of the cell therapy are summarized in Table 1. The last dog was enrolled in March 1998. Written consent for entry into the trial was obtained from each pet’s owner before treatment.

**Treatment Protocol.** Primary treatment was based on amputation of the affected limb or digit in 20 dogs and limb salvage surgery in the other 3 dogs. Amputations were performed either at the Veterinary Hospital in West Chester (65% of the patients) or in other hospitals by referring veterinarians, in a routine manner, i.e., complete forequarter amputation or hip disarticulation. Within 24 h after surgery, all of the dogs but one were started on CDDP chemotherapy (60 mg/m² i.v.) following standardized saline diuresis: these dogs and serum samples were obtained before study, immediately after each cell boost and at bimonthly intervals thereafter. Blood counts, kidney and liver functions, and chest X-rays, were properly instructed to report on the well-being of their pets during therapy. Chest X-rays were taken monthly before each cell boost and at bimonthly intervals thereafter. Blood and serum samples were obtained before study, immediately before each TALL-104 cell administration, and monthly throughout the follow-up period. Patient evaluation was continued to determine DFI and ST for each dog. DFI was defined as time from surgery to evidence of detectable metastases. ST was defined as time from surgery to death or euthanasia due to metastatic disease. Euthanasia was performed when requested by the pet’s owner.

**Large-Scale Expansion of TALL-104 Cells for Therapy.** TALL-104 cells were grown in endotoxin-free Iscove’s modified Dulbecco’s medium (Life Technologies, Inc., Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (Atlanta Biologicals, Norcross, GA) and 100 units/ml recombinant human IL-2 (Chiron Therapeutics, Emeryville, CA) in humidified incubators at 37°C with 10% CO₂ in T-175 vented cap flasks (Falcon, Franklin Lakes, NJ). Mycoplasma contamination was monitored weekly on cell samples taken from at least two flasks using a commercial PCR kit (American Type Culture Collection, Rockville, MD). Three times a week, cells were harvested by centrifugation in 250-ml conical tubes (Corning, New York, NY), washed twice in saline (Abbott Laboratories, King of Prussia, PA), resuspended in freezing medium (Iscove’s modified Dulbecco’s medium containing 50% fetal bovine serum and 10% DMSO; Sigma, St. Louis, MO), transferred to blood transfer packs (Baxter Diagnostics, Inc. Glendale, PA), and stored at −70°C for up to 18 months. When needed for injections, frozen bags were thawed in a 37°C water bath; the cells were then washed three times in saline, resuspended in 100 ml of saline, γ-irradiated (40 Gy) using a 137Cs source, and trans-
ferred to a new blood transfer pack for systemic administration within 2–4 h of irradiation. Cell aliquots were removed from each bag for the determination of cytotoxic activity against the human leukemic NK-sensitive K562 cell line (14), sterility, and endotoxin levels (quality control assays). Endotoxin levels were determined using the *Limulus Amoebocyte Lysate Endochrome-K* test kit (Charles River Endosafe, Charleston, SC) as recommended by the manufacturer.

**Cytotoxicity Assays.** PBMCs were isolated from heparinized blood samples by Accu-Prep (specific gravity, 1077 g/ml; Accurate Chemical, Westbury, NY) lymphocyte gradient centrifugation and tested as effectors in an 18-h 51 Cr release assay against K562 cells, as described previously (14). Whenever tumor biopsy samples from the dogs entered in the study were available, cell suspensions prepared by mechanical separation of the tumor tissues were used as targets in 18-h 51 Cr release assays to assess their *in vitro* sensitivity to the lytic activity of TALL-104 cells and autologous PBMCs.

**Cytokine Assays.** The presence of human IFN-γ, TNF-α, GM-CSF, and TNF-β in the dogs’ serum collected pre-study and throughout TALL-104 cell therapy was tested using human cytokine-specific ELISA kits [Endogen (Boston, MA) and R&D (Minneapolis, MN)] according to the manufacturers’ instructions (15). The sensitivity of the assays was 2 pg/ml for IFN-γ and GM-CSF, 5 pg/ml for TNF-α (Endogen), and 7 pg/ml for TNF-β (R & D).

**Immunological Monitoring.** Immunological studies were performed on serum samples and PBMCs (before study, before each TALL-104 cell injection, and during the follow-up period) to monitor the development of TALL-104-specific humoral and cellular immunity, respectively. To monitor the development of the TALL-104-specific cellular immune response, PBMC samples were tested against 51 Cr-labeled TALL-104 cells in an 18-h cytotoxic assay, as described above. Monitoring of anti-TALL-104 cell antibodies in the dogs’ sera was performed by immunofluorescence, as described previously (23, 24).

**PCR Analysis.** PBMC samples were obtained immediately before each TALL-104 cell infusion and at various intervals during and after cell therapy. DNA was extracted from the PBMCs and frozen. The presence of circulating TALL-104 cells in each cell extract was evaluated by PCR analysis using two primers specific for the human minisatellite region YNZ.22. An oligonucleotide probe recognizing 24 nucleotides in the middle of the amplified sequence was used to demonstrate the specificity of the PCR products by Southern blot hybridization, as described previously (23).

**Statistical Analysis.** Median and mean DFI and ST were calculated for all 23 dogs enrolled. The results of *in vitro* tumor susceptibility to TALL-104 cell lysis were correlated with me-
dian DFI and ST. The Cox proportional hazards model was used to adjust for dogs still alive. Mean ± SD values were analyzed for statistical significance using Student’s t test for unpaired data. P, 0.05 was considered significant.

RESULTS

Patient Population. Between July 1996 and March 1998, a total of 23 dogs with appendicular osteosarcoma were entered in this study at the Veterinary Oncology Services and Research Center. The diagnosis of osteosarcoma was based on clinical, radiographic, and morphological criteria (i.e., histological reports from veterinary pathologists). A total of 35% of the dogs enrolled in the trial were referred to the Veterinary Oncology Services and Research Center by other veterinarians.

Table 1 summarizes the clinical characteristics of the canine population enrolled: 52% of the dogs were males, and 48% were females; 14 dogs (60%) were 7–8 years old at diagnosis (age range, 1–11 years). The most represented breed was the golden retriever (one-third of the study population) followed by Doberman, rottweiler, and mixed breed (each represented about 10% of the study population). The most common sites of primary tumor lesions were the distal radius and/or ulna (48%), followed by the distal tibia (22%), proximal humerus (13%), metatarsus (8.5%), and proximal tibia (8.5%). Twenty dogs had complete amputation of the affected limb or digit, whereas three underwent limb salvage surgery. Two of the limb salvage procedures and six of the limb amputations were performed out of the Veterinary Oncology Services and Research Center. All dogs but one received adjuvant chemotherapy, consisting in the vast majority of cases (78%) of four cycles of CDDP (60 mg/m² each 3 weeks), which was started perioperatively (the day after surgery) in 65% of the animals (Table 2). The intervals between the last cycle of chemotherapy, and the first cycle of cells were quite varied, ranging from 1–45 days, though in the most dogs was 7 days (Table 3).

Toxicity. Toxicities were recorded during and after treatment. Six of 23 dogs (26%) receiving TALL-104 cell treatment developed grade 1 and 2 toxicities during and/or within 24 h after cell administrations. Toxicity was mostly limited to the GI tract (vomiting and diarrhea), usually appearing within the first 24 h after cell injection and then receding rapidly. Symptoms were easily preventable by premedication with antihistaminic drugs. One dog had a hypotensive episode during the first cell injection, likely related to the fast infusion rate. The symptoms promptly receded when the cell administration was halted. The same dog did not experience further toxicity during the subsequent cell injections, which were administered at a slower rate and without premedication.

DFI and Overall ST. A total of 11 of the 23 dogs (47.8%) in the study developed metastatic disease between 4.1 and 21.5 months after surgery (10 were euthanized, and 1 died of advanced disease; Table 3); 2 of 23 dogs (8.7%) developed severe discopathy (dog 5) and progressive neuropathy (dog 7) at 4 and 5.9 months, respectively, after surgery and were euthanized at the owner’s request; although radiographic exams showed no evidence of bone metastases, necropsy was not performed to rule out cancer spread. Ten of 23 dogs but one received adjuvant chemotherapy, consisting in the vast majority of cases (78%) of four cycles of CDDP (60 mg/m² each 3 weeks), which was started perioperatively (the day after surgery) in 65% of the animals (Table 2). The intervals between the last cycle of chemotherapy, and the first cycle of cells were quite varied, ranging from 1–45 days, though in the most dogs was 7 days (Table 3).

Table 3 Clinical follow-up and outcome after cell therapy

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Time between last chemotherapy and first cell administration</th>
<th>DFIa (mo)</th>
<th>Relapse site</th>
<th>STb (mo)</th>
<th>Reason for euthanasia</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>1 day</td>
<td>5.4</td>
<td>Pelvis</td>
<td>6</td>
<td>Metastatic disease</td>
</tr>
<tr>
<td>2</td>
<td>1 day</td>
<td>8</td>
<td>Lung</td>
<td>9.4</td>
<td>Metastatic disease</td>
</tr>
<tr>
<td>3</td>
<td>7 days</td>
<td>5.5</td>
<td>Femur</td>
<td>6</td>
<td>Metastatic disease</td>
</tr>
<tr>
<td>4</td>
<td>7 days</td>
<td>29.5</td>
<td>NAa</td>
<td>29.5</td>
<td>NA (alive)</td>
</tr>
<tr>
<td>5</td>
<td>7 days</td>
<td>4</td>
<td>NA</td>
<td>4</td>
<td>Disc disease</td>
</tr>
<tr>
<td>6</td>
<td>7 days</td>
<td>28</td>
<td>NA</td>
<td>28</td>
<td>NA (alive)</td>
</tr>
<tr>
<td>7</td>
<td>7 days</td>
<td>5.9</td>
<td>NA</td>
<td>5.9</td>
<td>Neurologic disease</td>
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<tr>
<td>8</td>
<td>7 days</td>
<td>21.5</td>
<td>Bone</td>
<td>21.5</td>
<td>Metastatic disease</td>
</tr>
<tr>
<td>9</td>
<td>5 days</td>
<td>5</td>
<td>Lung</td>
<td>5.3</td>
<td>Metastatic disease</td>
</tr>
<tr>
<td>10</td>
<td>7 days</td>
<td>22</td>
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<td>NA (alive)</td>
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<td>4 days</td>
<td>6.9</td>
<td>Lung</td>
<td>9.1</td>
<td>Metastatic disease</td>
</tr>
<tr>
<td>12</td>
<td>45 days</td>
<td>6.1</td>
<td>Lung</td>
<td>6.1</td>
<td>Metastatic disease</td>
</tr>
<tr>
<td>13</td>
<td>10 days</td>
<td>20.8</td>
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<td>20.8</td>
<td>NA (alive)</td>
</tr>
<tr>
<td>14</td>
<td>7 days</td>
<td>11.8</td>
<td>Lung</td>
<td>19.5</td>
<td>Metastatic disease</td>
</tr>
<tr>
<td>15</td>
<td>7 days</td>
<td>19.5</td>
<td>NA</td>
<td>19.5</td>
<td>NA (alive)</td>
</tr>
<tr>
<td>16</td>
<td>30 days</td>
<td>10.1</td>
<td>Bone/lung</td>
<td>11</td>
<td>Metastatic diseaseb</td>
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<tr>
<td>17</td>
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<td>6.1</td>
<td>Lung</td>
<td>6.1</td>
<td>Metastatic disease</td>
</tr>
<tr>
<td>18</td>
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</tr>
<tr>
<td>19</td>
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<td>14.1</td>
<td>NA</td>
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<td>NA (alive)</td>
</tr>
<tr>
<td>20</td>
<td>7 days</td>
<td>4.1</td>
<td>Rib</td>
<td>5</td>
<td>Metastatic disease</td>
</tr>
<tr>
<td>21</td>
<td>21 days</td>
<td>13</td>
<td>NA</td>
<td>13</td>
<td>NA (alive)</td>
</tr>
<tr>
<td>22</td>
<td>7 days</td>
<td>12.1</td>
<td>NA</td>
<td>12.1</td>
<td>NA (alive)</td>
</tr>
<tr>
<td>23</td>
<td>7 days</td>
<td>9.5</td>
<td>Lung</td>
<td>13</td>
<td>NA (alive with disease)</td>
</tr>
</tbody>
</table>

a DFI is defined as the time from surgery to evidence of clinically detectable metastasis.
b ST is defined as the time from surgery to death or euthanasia due to advanced disease.
c NA, not applicable.
d This dog was not euthanized because it died of advanced disease.
dogs (43.5%) are still alive at the time of this writing, with disease at 13 months after surgery (relapse occurred in the lung at 9.5 months; Table 3), and 9 without disease at 12.1–29.5 months after surgery. One of these dogs (dog 6) is still disease-free at 28 months after surgery, despite the fact that it was withdrawn from the study by the owner in an early stage, e.g., after the 5-day cycle of cell injections. The median ST for the overall patient population was 11.5 months, and the median progression-free survival duration was 9.8 months (Fig. 1, A and B). The mean ST and DFI were 13.1 and 12.3 months, respectively.

Correlation between Laboratory Findings and Clinical Outcome. Serum samples obtained from the dogs at various times before, during, and after therapy were evaluated for the presence of human cytokines (TNF-α, IFN-γ, TNF-β, and GM-CSF) possibly released by TALL-104 cells upon tumor cell interaction in vivo. No correlation was found between the serum levels of human cytokines and clinical outcome and/or toxicity (data not shown).

As measured in 51Cr release assays using the human NK-sensitive K562 cell line as a target, none of the dogs displayed baseline peripheral blood NK activity before TALL-104 cell treatment; however, some of them developed significant levels of NK activity during or at different times after the end of cell therapy (data not shown). It is noteworthy that when all 23 dogs were analyzed and separated into two groups depending on their clinical outcome (disease-free survivors versus relapsed dogs), the development of NK activity was a statistically significant ($P < 0.01$) indicator of disease recurrence (Fig. 2).

Tumor biopsies from 12 dogs were obtained and tested for in vitro sensitivity to TALL-104 cell lysis. Eight tumor samples showed significant sensitivity to TALL-104 cell killing, whereas the other four biopsies were resistant (Fig. 3A). Interestingly, the median DFI and ST were higher in the group of dogs whose tumors were sensitive to in vitro killing by TALL-104 cells (14.1 and 15.4 months, respectively) compared to the group of dogs that had lysis-resistant tumors (8.6 and 12.7 months, respectively). However, because of the small sample size within the two groups, no statistical correlation could be made between both DFI and ST and the in vitro sensitivity of the tumors to TALL-104 cell killing (Fig. 3, B and C).

Immune Responses against TALL-104 Cells. All dogs developed antibodies against TALL-104 cells between 10 and 15 days after the first TALL-104 cell injection. This humoral immune response reached a plateau at day 30 and was still present 2 years after beginning cell therapy (data not shown). Cellular immune responses against TALL-104 cells could be demonstrated in about 80% of the PBMC samples at different intervals during and after cell therapy. However, these responses were generally short-lasting and became undetectable within a few months from the last cell injection (data not shown).

Detection of Circulating TALL-104 Cells. PCR amplification of the human minisatellite region YNZ.22 performed on the PBMCs of the treated dogs documented the disappearance of TALL-104 cells from their blood 24 h after cell infusions. The lack of long-term persistence of the cells in the circulation was confirmed by repeating PCR analysis in each dog several months after the beginning of cell therapy (data not shown).
In vivo models that are relevant to human cancer are greatly needed, particularly for evaluating new anticancer strategies (1). Osteosarcoma is the most common naturally occurring skeletal neoplasm in canines (approximately 10,000 new cases/year in the United States alone) and has remarkable similarities to the human disease with regard to metaphyseal location, histopathological features, biological behavior (including the propensity to metastasize and the organ distribution of metastases), and response to treatment (1–6). In this respect, canine osteosarcoma has been shown to be an excellent model for the same histological disease in man (1, 3, 6). Adjuvant chemotherapy after radical surgery has been shown to improve survival in both humans and dogs (6). Unfortunately, despite this treatment, >90% of dogs and >35% of humans relapse at distant sites, particularly in the lungs; in fact, lung metastases are the most common cause of cancer-related mortality in dogs and children with osteosarcoma (1). Based on these biological similarities, it is reasonable to expect that an evaluation of new adjuvant chemotherapy or immunotherapy in dogs may have a direct application to the management of human osteosarcoma.

Our group has developed a new cell therapy approach to cancer based on the use of a lethally irradiated clonal human T-cell line (TALL-104) that is endowed with a uniquely potent tumoricidal activity across MHC barriers yet spares cells from normal tissues (13–16). Although dependent on recombinant human IL-2 for growth and expression of cytotoxicity in vitro, TALL-104 cells do not need concomitant administration of exogenous cytokines to exert antitumoral activity in vivo (17–24). We have previously shown that adoptive transfer of γ-irradiated (40 Gy) TALL-104 cells in tumor-bearing animals resulted in significant antitumoral effects not associated with severe toxicity (17–24). In particular, two previous studies conducted in canine patients with advanced tumors have revealed important information on the possible use of TALL-104 cells as anticancer agent, specifically, their ability to antagonize tumor growth even in an advanced disease setting of refractory malignancies (23, 24). The major objective of the present trial was to evaluate the potential antitumor activity of TALL-104 cells used as an adjuvant agent after standard treatment (surgery + chemotherapy) in dogs with spontaneously occurring osteosarcoma.

Although we calculated and presented both DFI and ST in our cohort of cell-treated dogs for sake of completeness, we believe DFI to be the most meaningful parameter for comparison to veterinary trials reported in the literature because it strictly reflects the success or failure of a given experimental treatment in preventing relapses. By contrast, ST values are highly influenced by the postmetastatic management of the dogs (euthanasia or palliative therapy) that can vary substantially from one veterinary center to another.

The results of this study compare favorably to previous studies evaluating DFI after standard treatment (amputation combined with CDDP chemotherapy; Refs. 7–11). In those trials, the dogs had a median DFI of 7–7.5 months. In the present study, the addition of adoptive TALL-104 cell therapy after CDDP chemotherapy increased the median DFI to 9.8 months (mean DFI, 12.3 months). A median DFI of 11.2 months was reported in a study by Kurzman et al. (12) in which a different form of immunotherapy, liposome-encapsulated muramyl tripeptide, was evaluated as an adjuvant agent after CDDP chemotherapy in a smaller group of dogs with osteosarcoma. In our study with TALL-104 cells, the median DFI and ST values would have been 11 and 12.5 months, respectively, if the analysis had included only dogs that died or were euthanized because of metastatic disease. Thus, the lower values of 9.8 (DFI) and 11.5 (ST) months reflect the inclusion in the analysis of the two dogs (dog 5 and dog 7) that developed severe discopathy and progressive neuropathy without evidence of metastases in early stages. These symptoms are often encountered in canine osteosarcoma after limb amputation and were unlikely to be related to TALL-104 cell injections. The fact that dog 6 is still alive without evidence of disease 28 months after surgery after completion of only one 5-day cycle of TALL-104 cell therapy suggests the ability of these cells to rapidly activate tumor-specific immune responses in the host.
ber of cell injections required in the induction course and the need for monthly maintenance boosts are under investigation in this laboratory; preliminary trafficking and cell clearance studies in healthy canines (25) suggest that a single high dose of effector cells might be a better therapeutic approach than multiple daily injections.

No severe toxicity (grade 3 or 4) was observed in association with multiple i.v. injections of TALL-104 cells, except for one dog that experienced a hypotensive crisis during the first 15 min of the first injection; however, a too quick rate of cell administration was likely the cause of that isolated and brief episode of hypotension because the symptoms promptly receded when the infusion was interrupted and never recurred in the subsequent cell injections, which were administered at a slower rate. Mild (grade 1–2) and transient GI toxicity (vomiting and diarrhea) was observed in 26% of the treated dogs but was easily controlled with premedications. GI toxicity has also been reported to occur in 80% of the cases during lymphokine-activated killer/IL-2 therapy in humans (26). Moreover, because some of the cell-treated dogs have now survived disease free for more than 20 months after surgery (dogs 4, 6, 10, and 13), we could exclude the induction of chronic or late side effects including TALL-104-induced leukemia, based on the lack of persistence of these cells in the circulation, as demonstrated by PCR.

An important observation in this study was the appearance of in vitro MHC nonrestricted cytotoxicity against K562 cells in some of the dogs after TALL-104 cell administration; the development of peripheral blood NK activity in these dogs, either during the course of TALL-104 treatment or during the follow-up period, was a statistically significant indicator (P < 0.01) of disease recurrence. Interestingly, high levels of NK activity were observed before the signs of relapse could be detected clinically by standard techniques (e.g., X-rays), thus giving an important prognostic value to the in vitro cytotoxic assay. At the moment, the interpretation of this observation is merely speculative; however, because the same pattern of immunological response was observed in our previous two canine studies (23, 24) in which it was proven to strictly correlate with the ability of the dogs’ PBMCs to kill and recognize their own tumor in vitro, we believe that the high levels of NK activity in relapsing dogs might be explained as the result of a bystanding activation of NK cells, secondary to the induction of endogenous antitumor immunity after adoptive transfer of TALL-104 cells.

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REFERENCES


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