Antitumor Efficacy of a Human Major Histocompatibility Complex Nonrestricted Cytotoxic T-Cell Line (TALL-104) in Immunocompetent Mice Bearing Syngeneic Leukemia

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ABSTRACT

The human MHC nonrestricted cytotoxic T-cell line TALL-104 displays potent tumoricidal activity both in vitro and in animals bearing either spontaneous or induced tumors. In the present study, we used B6D2F1 mice engrafted with the syngeneic pre-B leukemia cell line 7OZ as a model system to investigate the mechanisms by which TALL-104 cells display antitumor activity in an immunocompetent host. In vitro studies indicated that 7OZ cells are resistant to TALL-104 cell-induced necrotic death, as measured in 51Cr release assays, but can be killed by the xenogeneic effectors via apoptotic mechanisms. Adoptive transfer experiments showed that 70% of cyclosporin A-treated mice that received multiple i.p. injections of γ-irradiated (nonproliferating) TALL-104 cells at an early or intermediate stage of disease did not develop any clinical or molecular signs of leukemia. If the same treatment was initiated at an advanced disease stage, it doubled the survival of the animals. Importantly, 100% of the treated mice that remained disease-free in a 4-month follow-up period rejected a further challenge of high-dose 7OZ cells; these mice had developed tumor-specific humoral and cellular responses. In another set of experiments, marrows from leukemic mice containing ≤10% 7OZ cells were purged ex vivo with irradiated TALL-104 cells and engrafted into sublethally irradiated B6D2F1 mice up to 80% of the recipients failed to develop leukemia, and 40% of them were protected against a 7OZ rechallenge 4 months later. TALL-104 purging efficacy was lower if the marrows were infiltrated by ≤30%. Ex vivo purging with a human lymphokine-activated killer cell preparation in the same conditions proved to be less effective. The overall data indicate the potent antileukemic effects of TALL-104 cells in vivo and provide evidence that xenogeneic effectors can induce tumor regression via a dual mechanism of direct tumoricidal activity and recruitment of endogenous antitumor immunity.

INTRODUCTION

Adaptive therapy with autologous LAK1 cells or tumor-infiltrating lymphocytes in association with high-dose rhIL-2 have resulted in significant antitumor responses in some types of cancer, such as melanoma and renal cell carcinoma (1–3). However, severe systemic toxicity and difficulty in generating sufficient numbers of effector cells with potent tumoricidal activity have represented two major limiting factors in these forms of treatment (4).

We have developed a new experimental approach to adaptive therapy of cancer that might overcome the above limitations. Our strategy uses a potent MHC nonrestricted human leukemic T cell line (CD3/TCRaβ+, CD4-, CD8+, CD56+, CD16-), designated TALL-104 (5–8), which lyases tumors across several species yet spares cells from normal tissues. Although strictly dependent on rhIL-2 for long-term maintenance in vitro and expression of tumoricidal activity, TALL-104 cells appear to display strong antitumor effects in vivo in the absence of exogenously added IL-2 (9, 10), thus eliminating the adverse effects associated with LAK/rhIL-2 therapy. In addition, TALL-104 cells can be continuously expanded in culture, providing an unlimited and reliable source of clonal effector cells with stable cytotoxic activity for cell therapy approaches. The CD3/TCR complex does not seem to play a crucial role in the direct tumor cell killing by TALL-104 cells, as shown in experiments using blocking antibodies. However, treatment with OKT3 monoclonal antibody augments significantly the tumoricidal activity of these cells against natural killer-susceptible and -resistant tumor targets and fresh tumor specimens (6). TALL-104 cells induce necrotic tumor cell death via secretory pathways involving perforin and serine esterases or kill targets through the release of cytostatic/cytotoxic mediators, such as TNF-α, TNF-β, IFN-γ, or transforming growth factor-β (6–8). The tumor specificity of TALL-104 cells is under investigation as compared to that of IL-2-expanded LAK cells from healthy donors. The observation that TALL-104 cells kill tumors across MHC barriers indicates that they recognize phylogenetically well-conserved membrane structures on the target cells.

We have documented the ability of γ-irradiated, nonproliferating TALL-104 cells to reduce or abrogate the growth of human tumors implanted in SCID mice (9, 10) and to effectively purge human marrows from lysis-sensitive or -resistant leukemic blasts while sparing normal hematopoietic precursors (11). In addition, a preclinical Phase I study recently conducted in canine cancer patients with refractory malignancies has indicated both the safety and antitumor efficacy of TALL-104 cells in an immunocompetent host bearing spontaneous tumors (12). In the present study, we have used B6D2F1 mice engrafted with the syngeneic pre-B cell leukemia cell line 7OZ (13) as a model to evaluate the mechanisms by which xenogeneic effectors display antitumor efficacy in vivo against a lysis-resistant tumor.

The overall findings demonstrated that, in appropriate experimental conditions, multiple transfers of irradiated TALL-104 cells arrested the spread of 7OZ cells in these mice, leading to complete abrogation of leukemia, and resulted in induction of endogenous antitumor immunity. Moreover, ex vivo purging experiments showed the ability of irradiated TALL-104 cells to effectively purge 7OZ cell-infiltrated marrows. Taken together, these data in a xenogeneic model system indicate the ability of cytokine-activated, nonproliferating effectors to display direct antitumor activity and to elicit strong host responses against the tumor.

MATERIALS AND METHODS

Cell Lines. TALL-104 cells were maintained at 37°C in 10% CO2 in IMDM (Life Technologies, Inc., Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (Sigma Chemical Co., St. Louis, MO) and rhIL-2 (100 units/ml; specific activity, 1 × 105 units/mg), a gift from Dr. Maurice Gately (Hoffmann-La Roche Inc., Nutley, NJ). TALL-104 cells grown in these conditions and displaying high killer activity in vitro were
designated as TALL-104 K⁺ in this study to distinguish them from parallel cultures of TALL-104 cells that were purposely expanded in the absence of IL-2 and were, therefore, devoid of killer activity (TALL-104 K⁻). The murine leukemia 7OZ cell line [originally induced with methyl-nitrosourea in thymo-
tomized (C57BL/6xDBA/2)F₁ mice; Ref. 13] was purchased from American Type Culture Collection (Rockville, MD) and maintained at 37°C in 5% CO₂ in RPMI 1640 (Life Technologies, Inc.) containing 10% heat-inactivated fetal bovine serum, glutamine, antibiotics, and 5 × 10⁻³ M 2-mercaptoethanol. The U937 human myelomonocytic leukemia, cultured in the same conditions as 7OZ cells but without 2-mercaptoethanol, was used as standard lysis-susceptible target in cytotoxicity assays (see below). All cell lines were Mφroplasma-free by repeated PCR testing, using an American Type Culture Collection kit, and by electron microscopy.

LAK Cell Preparations. PB mononuclear cells from healthy donors were separated by centrifugation on Accu-Prep lymphocyte gradient (Accurate Chemical, Westbury, NY), depleted of monocytes by double adherence to plastic for 1 h at 37°C, and cultured for 3 days in complete IMDM and 100 units/ml rIL-2 to generate LAK cells.

Mice. Four-week-old (C57BL/6xDBA/2)F₁ mice (hereafter designated B6D2F₁) were purchased from The Jackson Laboratory (Bar Harbor, ME) and maintained in the Wistar Institute’s Animal Facility in a pathogen-free environ-

Histopathological Analysis of 7OZ Cell-Engrafted Mice. Indicated numbers of 7OZ cells were inoculated i.p. in saline. Mice were checked daily for signs of disease and sacrificed when severely ill. Autopic analysis was performed on all animals, including those that were found dead. Long-term survivors were also sacrificed to confirm their disease-free status at the molecular level in the BM and PB (see below). All organs were routinely collected regardless of their autopic appearance, fixed in 10% buffered formalin, and paraffin embedded; 4-μm sections were cut and stained with H&E.

Detection of Humoral Responses against 7OZ Cells. Sera were collected from B6D2F₁ mice by retroorbital puncture both before 7OZ cell engraftment and after TALL-104 cell therapy. Sera were diluted (1:2 to 10⁻⁴) in FACS buffer (PBS without Ca²⁺ and Mg²⁺, 0.1% NaCl, and 2% IgG-free horse serum). 7OZ or TALL-104 cells (1 × 10⁵/well) were cultured for 1 h at room temperature with 50 μl of the sera dilutions, washed twice, and incubated for 1 h at 4°C with FITC-conjugated F(ab)₂ fragment goat antimouse IgG (heavy and light chain specific) (Organon Teknika-Cappel, Durham, NC) diluted 1:200. After three more washes, the wells were resuspended in FACS buffer and analyzed by flow cytomtery using an Ortho cytofluorograph cell sorter.

Detection of Cellular Responses against 7OZ Cells. 7OZ-specific cellular immune responses were evaluated in survivor mice by assessing lymphocyte proliferation and ml-3 production in response to irradiated 7OZ cells in a MLR. Age-matched B6D2F₁ mice never injected with 7OZ/TALL-104 cells were used as controls. Spleen cells from different mice were obtained by gently pressing the freshly harvested organs with a glass stopper on a Petri dish. Erythrocyes, granulocytes, and debris were removed by Accu-Prep lymphocyte gradient centrifugation. 7OZ stimulator cells were irradiated (6000 rads) and plated (2 × 10⁵) with spleen cells (2 × 10⁵) in triplicates in a total volume of 200 μl in the presence and absence of ml-3 (100 units/ml; Endogen Inc., Boston, MA). Seventy-two hours after stimulation, the cells were resuspended in FACS buffer and analyzed by flow cytomtery using an Ortho cytofluorograph cell sorter.

RESULTS

TALL-104 Cells Induce Apoptotic Death in 7OZ Cells. Long-term (18-h) ⁵¹Cr-release assays demonstrated the total resistance of
7OZ cells to TALL-104 K⁺ and LAK cell killing (Fig. 1, top). The U937 leukemic target, which is highly susceptible to lysis by these effectors (but not to TALL-104 K⁻ cells; Fig. 1, top) was used as positive control in these experiments. Proliferation assays revealed the strong cytostatic activity (close to 99% inhibition of [³H]thymidine incorporation) of TALL-104 cells against both U937 and 7OZ targets, even at the low E:T ratio of 1:1 (Fig. 1, bottom). Interestingly, the other two effector cell populations, i.e., TALL-104 K⁺ cells and LAK cells from normal donors, did not have cytostatic activity on 7OZ cells (Fig. 1). Kinetics experiments (data not shown) indicated that the inhibitory effects of TALL-104 K⁺ cells on 7OZ cell proliferation were already evident at 4 h, reached the peak (99% inhibition) at 8 h, and remained stable until 72 h (last time point tested). Neutralizing antibodies against IFN-γ, TNF-α, and TNF-β (alone or in association) did not reduce the antiproliferative activity of TALL-104 cells on 7OZ cells (data not shown). Different tests were performed to determine whether cytostatic activity was associated with the induction of apoptotic death in 7OZ cells. As shown in Fig. 2A, DNA fragmentation assays failed to demonstrate the formation of the typical DNA ladder in 7OZ cells incubated for 4 h with TALL-104 cells. By contrast, the JAM test (Fig. 2B), morphological criteria (Fig. 2C), and the TUNEL assay (Fig. 2D), performed on the same cell mixtures, all provided evidence for TALL-104 cell-induced 7OZ cell apoptosis.

In Vivo Model of 7OZ Leukemia. Eight groups of 4-week-old B6D2F₁ mice (n = 3) were injected i.p. with different numbers of 7OZ cells, as indicated in Fig. 3. At the lowest dose (1 or 10 cells), most mice died between 50 and 65 days. At the highest dose (10⁷), 7OZ cells induced imminently fatal leukemia in 100% of the animals within 10–16 days. Death resulted from acute respiratory failure with pulmonary edema, although mice were asymptomatic until 4–6 h before death. At autopsy, all mice presented marked splenomegaly. Neither ascites nor tumor masses were detectable in the peritoneal and pleural cavities. Microscopic analysis (Fig. 4) showed marked leukemic infiltration in the PB and BM (Fig. 4, A and B, respectively). A massive leukemic embolization of the vessels (small and medium sized) was present in the lungs of these mice (Fig. 4, C and D), explaining the sudden onset of acute and fatal respiratory failure. The same type of emboli was also present in the brain (Fig. 4F). The splenic parenchyma was totally replaced by leukemic cell infiltrates (Fig. 4E).

To determine the kinetics of 7OZ cell infiltration of BM and PB after an i.p. transfer, six mice were injected with 10⁷ cells and sacrificed at the time points indicated in Fig. 5. PCR amplification of 7OZ-specific CDR-III region was performed on DNA extracted from their BM and PB. 7OZ cell PCR-specific products were detectable as early as day 2 in their BM and day 4 in their PB (Fig. 5).

Antileukemic Effects of γ-irradiated TALL-104 Cells in the 7OZ/B6D2F₁ Model. In vivo experiments were designed to test the antitumor efficacy of TALL-104 killer cells in immunocompetent B6D2F₁ mice bearing 7OZ leukemia. Fig. 6 shows the survival of mice that received 10⁷ 7OZ cells i.p. on day 0 and irradiated TALL-104 cells (2 × 10⁷ i.p.) five times on alternate days, starting at different times after tumor challenge. Both control and experimental mice received daily i.p. injections of CsA (10 mg/kg) starting the day before the first TALL-104 cell transfer and continued throughout the therapy. All mice that received either 7OZ cells alone, 7OZ cells in

![Fig. 1. Cytotoxic and cytostatic activities of γ-irradiated K⁺ and K⁻ TALL-104 cells and LAK cells against U937 and 7OZ targets. Results of 18-h [⁵¹Cr]-release assays are shown in the top panels and of 18-h [³H]thymidine uptake inhibition assays in the bottom panels; bars, SD. Abscissas indicate the E:T ratios used.](image-url)
region of 7OZ cells confirmed the disappearance of leukemic cells from the PB of the survivor mice (Fig. 6).

Remarkably, 100% of the mice that survived after TALL-104 therapy and were rechallenged with 7OZ cells (2 × 10^7) did not

combination with daily administration of CsA, or 7OZ and irradiated TALL-104 cells without CsA died between days 10 and 15, as expected from the survival data in Fig. 3. Adoptive transfer of TALL-104 cells in a more advanced disease stage (i.e., starting on day 4 after tumor challenge) doubled the survival of the animals, as compared to the control mice. By contrast, 70% of the 7OZ-injected mice that received CsA and were treated with irradiated TALL-104 cells from the day of tumor challenge (day 0) or starting on day 2 remained disease free. At 4 months, PCR amplification of the CDR-III-specific

Fig. 2. Induction of apoptotic death in 7OZ cells by TALL-104 cells. A. gel analysis of DNA extracted from TALL-104 cells (Lane 2), 7OZ cells (Lane 3), 7OZ cells grown in the absence of 2β-mercaptoethanol (positive control for apoptosis; Lane 4), and TALL-104/7OZ cell mixture (E:T, 5:1) incubated 4 h at 37°C (Lane 5). B. JAM test. TALL-104 cells were incubated with [3H]thymidine-labeled 7OZ cells for 1, 4, or 18 h at the indicated E:T ratios. C. May-Grünewald-Giemsa staining of cytospin preparations of TALL-104/7OZ cell mixtures (after incubation at 37°C for 4 h) showing conjugate formation between effectors and targets (i). Arrow: the apoptotic nucleus of a 7OZ target cell surrounded by TALL-104 cells. D. histochemical detection of apoptosis in the same preparations by the TUNEL assay. Arrows: two apoptotic nuclei of 7OZ cells.

Fig. 3. Survival curve of B6D2F1 mice (n = 3) injected i.p. with the indicated numbers of 7OZ cells.

Fig. 4. Histopathological analysis of tissues from B6D2F1 mice injected with 7OZ cells. A. PB smear with high numbers of circulating leukemic cells. Arrow, a mitotic figure. B. BM cytospin showing a complete replacement of normal murine hematopoiesis by leukemic cells. C and D. massive leukemic embolization of lung vessels (v) of small and medium size indicated by arrows. E. complete replacement of normal spleen parenchyma by leukemic cell infiltrates. F. brain section (b, cerebral parenchyma); arrows, embolized vessels. A and B. May-Grünewald-Giemsa staining. ×400; C–F. HE stain. ×200.
develop leukemia and were shown to be disease free, both clinically and at the molecular level, 4 months later. This finding indicated the presence of active antitumor immunity in the mice that had undergone adoptive therapy with irradiated TALL-104 cells. Noteworthy in this respect is the finding that most of the mice (80%) that had been initially injected with lethally irradiated (8000 rads) 7OZ cells and, 3 months later, challenged with nonirradiated 7OZ cells (2 × 10^7 i.p.) were not protected against the rechallenge and died of leukemia. The two TALL-104-treated mice that died on days 65 and 75 after 7OZ cell inoculation (Fig. 6) presented a completely different disease as compared to the clinical and anatomopathological picture described in Fig. 4. Specifically, during the 2 weeks preceding death, these animals developed progressive subascillar and submandibular lymph nodes and neurological symptoms (motor weakness and lethargy), suggesting a slower and more localized disease. Histopathological analysis of tissues from these animals confirmed the presence of 7OZ infiltrates in the enlarged lymph nodes (Fig. 7, A and B) and marked accumulation of 7OZ cells in the meningeal spaces (Fig. 7, C and D) but showed a low number of leukemic cells in their PB (Fig. 7E). These findings might reflect the growth of 7OZ cells that escaped TALL-104 cell killing in immunologically privileged sites, such as the central nervous system. PCR analysis of brain, lymph node, and PB confirmed the identity of 7OZ cells in these tissues (data not shown).

Ex Vivo BM Purging Efficacy of Irradiated TALL-104. In a first set of experiments, the survival of sublethally irradiated B6D2F1 mice engrafted i.p. with various doses of 7OZ cells was analyzed. Eight groups (n = 3) of 4-week-old B6D2F1 mice were sublethally irradiated and injected i.p. with the indicated doses of 7OZ cells (Fig. 8). Irradiation prior to 7OZ cell engraftment markedly reduced the length of 7OZ-induced disease, especially when low numbers of leukemic cells were injected (see Fig. 3 for comparison); this finding increased drastically the sensitivity of this model for MRD detection.

In the subsequent BM purging experiments, donor mice were

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Fig. 5. Kinetics of 7OZ cell infiltration of BM and PB in B6D2F1 mice. Four-week-old mice were injected i.p. with 10^7 7OZ cells and sacrificed at the indicated time points. PCR amplification of the 7OZ cell CDR-III-specific region was performed on DNA extracted from their BM and PB.

Fig. 6. Survival of B6D2F1 mice engrafted with 7OZ cells and treated with γ-irradiated TALL-104 cells. Mice received 10^7 7OZ cells i.p. on day 0 and irradiated TALL-104 cells (2 × 10^7 i.p.) five times on alternate days following the outlined schedule. Both control and experimental mice received daily i.p. injections of CsA (10 mg/kg) starting the day before the first TALL-104 cell transfer and continued throughout the therapy. Mice surviving on day 120 were rechallenged with 2 × 10^7 7OZ cells i.p.

Fig. 7. Histopathological analysis of tissues from two TALL-104-treated B6D2F1 mice that died on days 65 and 75 after 7OZ cell inoculation. A. section of an enlarged lymph node in the frontal leg of one of these mice. Note the leukemic infiltration of muscles and soft tissues. b, bone; m, muscle; t, tumor cells. B. section of a subascillar enlarged lymph node. Also in this case, leukemic infiltration of the local muscles was present. m, muscle; s, salivary gland; t, tumor cells. C and D, brain sections with marked leukemic infiltrates in the meningeal spaces (arrows). h, cerebral parenchyma; t, tumor cells. The PB (E) of this animal had a relatively low infiltration (arrows). A-D, H&E staining; E, May-Grünwald-Giemsa staining. A-E, ×200.


injected i.p. with $10^7$ 7OZ cells. Two days later, mice were sacrificed, and their marrows (containing $\leq 10\%$ blasts) were harvested, pooled, and purged overnight with either of the following irradiated effectors at the E:T of 1:1: cytotoxic (K$^+$) TALL-104 cells, IL-2-deprived (K$^-$) TALL-104 cells, LAK cells, or no effectors. The cell mixtures were then transferred into sublethally irradiated B6D2F, recipients, and the survival of the different groups of mice was compared. Prior to transfer, an aliquot of each group of marrows was subjected to DNA extraction and PCR amplification of the 7OZ CDR-III-specific region to test the purging efficacy of each effector cell preparation. Fig. 9 shows representative results from such an analysis; despite the presence of DNase (10 µg/ml) during the overnight purging mixture, no difference was observed, at the molecular level, between TALL-104 K$^+$ purged and unpurged marrows. Similar results were obtained when TALL-104 K$^-$ and LAK cells were used as purging agents (data not shown). Nevertheless, close to 80% of the sublethally irradiated mice that received BM purged with irradiated TALL-104 K$^+$ cells survived (Fig. 10) and were shown to be disease free by PCR analysis 4 months later (day 120). By contrast, all mice that received either unpurged BM or marrows incubated with TALL-104 K$^-$ cells died between days 20 and 25 after BM transfer. BM purging with LAK cells resulted in a significant prolongation of survival; however, only one mouse of five (20%) was clinically and molecularly disease free at 4 months.

After 7OZ rechallenge ($2 \times 10^7$ i.p.) on day 120, both this mouse and one of the five mice that had survived the engraftment of TALL-104-purged marrows developed leukemia and died within 15 days of acute respiratory distress. By contrast, two mice in the latter group were partially protected, as indicated by their slowly progressive and localized disease, clinically similar to the one shown in Fig. 7 within the adoptive immunotherapy experiments. Finally, the last two mice of the same group did not display any clinical or molecular signs of disease during a 6-month follow-up period.

To evaluate the purging efficacy of TALL-104 cells in an advanced disease stage, B6D2F, mice were injected with $10^7$ 7OZ cells and sacrificed 4 days later, when their marrows were highly infiltrated (contained $\geq 30\%$ blasts). Marrows were harvested, pooled, and incubated overnight with irradiated TALL-104 K$^+$ cells, LAK cells, or no effectors (E:T, 1:1). The cell mixtures were then transferred into sublethally irradiated B6D2F, recipients, and the survival of the three groups of mice was compared (Fig. 11). Although mice receiving TALL-104-purged marrows displayed a double survival time as compared to mice receiving LAK cell-purged or unpurged marrows, they all died within 28 days. This finding indicates that the marrow purging efficacy of irradiated TALL-104 cells is inversely proportional to the tumor load at the time of treatment, and that these effectors function optimally in a situation mimicking MRD rather than in advanced disease.

**Development of Humoral and Cellular Immune Responses against 7OZ Cells.** Mice that survived the rechallenge with 7OZ cells, either as a consequence of TALL-104 cell therapy or upon engraftment of TALL-104-purged marrows, were evaluated for the development of humoral responses specific to 7OZ cells. Sera were collected 10 days after the rechallenge and subjected to immunofluorescence analysis. As shown in Table 1, sera from all three animals that had survived after TALL-104 cell treatment were reactive with 7OZ cells (Table 1, mice nos. 1–3). The specificity of this reactivity is demonstrated by the fact that sera collected from the same mice at the time of initiation of TALL-104 therapy (mice nos. 1–3, Table 1) and sera from control mice that had never been injected with 7OZ cells (control mice nos. 6–8, Table 1) were all negative. Moreover, none of the sera showed reactivity with TALL-104 cells (data not shown).
XENOGENEIC EFFECTORS FOR CANCER THERAPY

Fig. 10. Survival of B6D2F1 mice engrafted with unpurged and purged leukemic marrows. B6D2F1 mice were injected i.p. with $10^7$ 7OZ cells and sacrificed 2 days later. Marrows were harvested, pooled, and purged overnight with irradiated TALL-104 K⁺, TALL-104 K⁻, or LAK cells. The cell mixtures were transferred into sublethally irradiated (400 rads) B6D2F1 recipients, and the survival of the different groups of mice was compared. Mice surviving on day 120 were rechallenged with $2 \times 10^7$ 7OZ cells i.p.

shown). Mice nos. 4 and 5, used as recipients of TALL-104 purged marrow grafts, also developed antibodies against 7OZ cells, although their serum titers were lower than those seen in mice nos. 1-3 (Table 1).

To study the recruitment of 7OZ-specific cellular immunity in the TALL-104-treated survivor mice, MLR cultures were set up using splenocytes from three mice as responders and irradiated 7OZ cells as stimulators. As shown in Table 2, a significant increase in in vitro proliferation ($P < 0.05$) and a modest but consistent increase in mIL-3 production ($P < 0.2$, n.s.) were present in the splenocyte cultures of all three surviving mice upon stimulation with irradiated 7OZ cells and mIL-2. By contrast, no differences were found in splenocyte cultures from the same mice incubated without stimulators or in MLR cultures from two control mice (Table 2).

The above data suggest that both branches of the immune system were recruited during TALL-104 cell treatment.

**DISCUSSION**

The CD3⁺/TCRαβ⁺ CD8⁺ CD16⁻ TALL-104 cell line represents a MHC nonrestricted homogeneous T-LAK cell population endowed with potent tumoricidal activity across several species. Our in vivo studies in SCID mice have shown that adoptively transferred TALL-104 cells can, in the absence of exogenous human IL-2, induce regression of metastatic disease arising from implanted human tumors (9, 10). More recently, we have investigated the feasibility of using this approach in dogs bearing spontaneous tumors to obtain information relative to the possible clinical applicability of TALL-104 cells in immunocompetent recipients (12). The results of this Phase I preclinical investigation demonstrated not only the absence of toxic effects of TALL-104 cells in dogs with advanced, refractory malignancies but also the induction of remarkable and unexpected clinical responses, including a long-lasting complete remission (12).

**Fig. 11. TALL-104 purging efficacy of leukemic marrows in advanced disease.** B6D2F1 mice were injected with $10^7$ 7OZ cells and sacrificed 4 days later. Their marrows were harvested, pooled, and incubated overnight with irradiated TALL-104 K⁺ cells. LAK cells, or no effectors. The cell mixtures were then transferred into sublethally irradiated B6D2F1 recipients, and the survival of the three groups of mice was compared.
In the present study, mice engrafted with the syngeneic leukemia cell line 70Z were used as a model system to investigate the mechanisms by which TALL-104 cells display antitumor effects in an immunocompetent host. The choice of this model lies upon three reasons: (a) the aggressiveness of 70Z-induced leukemia, which enabled us to evaluate the antitumor efficacy of TALL-104 cells in a short period of time; (b) the high reproducibility of the clinical and pathological manifestations and of the kinetics of disease development in these mice, which allowed us to obtain significant differences between control (untreated) and TALL-104-treated animals using a relatively low number of mice (4-10/group); and (c) the availability of molecular markers (CDR-I/II region) for PCR detection of leukemia upon in vivo and ex vivo purging with TALL-104 cells. Previous experiments in murine tumor models have demonstrated that adoptively transferred autologous LAK cells display antitumor activity in vivo only if administered in conjunction with rIL-2 (19-21). The observed synergy between LAK cells and IL-2 was attributed to the ability of this cytokine to induce the in vivo proliferation of the transferred effectors in preferential sites, such as lungs, liver, kidneys, mesenteric lymph nodes, and spleen. The fact that irradiated (nonproliferating) LAK cells had proved ineffective in these murine tumor models indicated that the in vivo proliferation of adoptively transferred effectors was critical to their mechanism of action (21).

Our approach to adoptive therapy with TALL-104 cells is innovative as it intentionally excludes the use of IL-2 to avoid the dose-limiting toxicities observed in cancer patients receiving systemic IL-2 therapy (22). In addition, although these effectors would be eventually rejected by a host with an intact immune system, as confirmed in the present study using CsA nontreated mice, we chose to lethally irradiate TALL-104 cells immediately prior to adoptive transfer to add an extra safety precaution to the therapy and exclude the extreme possibility of a long-term persistence of this leukemic killer clone in the host tissues. According to the murine studies described above (21), both the lack of addition of adjuvant IL-2 and lethal irradiation should have reduced the antitumor efficacy of TALL-104 cells via a direct inhibition of their proliferative response. However, our recent trafficking studies have shown that γ-irradiation does not impair the ability of TALL-104 cells, injected either i.v. or i.p., to quickly migrate to distant organs, such as lung, liver, spleen, bone marrow, kidneys, and ovaries.3 5

As also demonstrated in SCID mouse models bearing human tumors (9, 10), the efficacy of adoptively transferred TALL-104 cells in the B6D2F1/70Z model was maximal when the treatment was started at an early disease stage (low tumor burden). Similarly, ex vivo TALL-104 purification of leukemic marrows was more efficient when the marrows were moderately infiltrated. The lack of TALL-104 cell toxicity against early hematopoietic precursors was demonstrated by the finding that transfer of the purified marrows (containing irradiated TALL-104 cells) into sublethally irradiated B6D2F1 recipients did not impair their hematopoietic reconstitution. The fact that engraftment of TALL-104-purged marrows in these animals did not induce leukemia, despite being PCR positive, confirms our previous findings with human TALL-104-resistant leukemias (11) that a PCR-positive result after purging does not necessarily indicate a failure of the purging procedure but rather the PCR detection of cells that are either growth arrested or undergoing apoptotic death. Importantly, such PCR+ cells appear to be devoid of tumorigenic activity when transferred into healthy mice (11).


Irradiated TALL-104 cells were more effective than human LAK cells in purging leukemic marrows ex vivo. A similar comparison between these two types of human effectors and between TALL-104 cells and murine (syngeneic) LAK cells could not be done in the adoptive therapy experiments because it is not possible to generate in vitro a high number of LAK cells that would maintain a stable phenotype and cytotoxic function within the time frame (about 2 weeks) established in our experimental design. At difference with LAK cells, the clonal TALL-104 cell line not only can be expanded to very large numbers but also retains a steady cytotoxic activity for 6–8 months, thus providing a continuous source of stable effectors for protocols based on multiple delivery of cytotoxic agents.

A surprising finding in the present investigation was that 100% of the tumor-bearing mice that remained disease free upon TALL-104 cell therapy and 40% of sublethally irradiated recipients that survived the engrafment of TALL-104-purged marrows were protected against leukemia when rechallenged with high-dose 70Z cells. The recruitment of tumor-specific humoral and cellular immunity in these animals was possibly a consequence of increased antigen presentation by macrophages upon phagocytic clearance of apoptotic 70Z cells. This hypothesis might explain the difference in percentage of surviving animals following TALL-104 adoptive therapy (100%) versus transfer of TALL-104-purged marrows (40%), because in the latter situation, antigen release should have been lower. However, the finding that primary challenge of a group of mice with γ-irradiated (apoptotic) 70Z cells, followed by rechallenge with nonirradiated 70Z cells 3 months later, did not protect from leukemia demonstrates that apoptotic tumor cell death alone does not provide a sufficient activation signal for tumor cell recognition by the immune system. The release of chemotactic activating factors, such as granulocyte/macrophage colony-stimulating factor, tumor necrosis factor-α and interferon-γ in two human cytotoxic T-cell lines. In Vitro Cell Dev. Biol., 28A: 648–656, 1992.


The dual ability of lethally irradiated TALL-104 cells to abrogate tumor cell growth directly and through the induction of host immunity indicates the high therapeutic potential of TALL-104 cells in adoptive transfer and marrow purging approaches in an adjuvant setting.

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Cancer Research

Antitumor Efficacy of a Human Major Histocompatibility Complex Nonrestricted Cytotoxic T-Cell Line (TALL-104) in Immunocompetent Mice Bearing Syngeneic Leukemia

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