Cell Therapy of a Highly Invasive Human Breast Carcinoma Implanted in Immunodeficient (SCID) Mice

Sophie Visonneau, Alessandra Cesano, Michael H. Torosian, and Daniela Santoli

The Wistar Institute [S. V., A. C., D. S.], and The Hospital of the University of Pennsylvania [M. H. T.], Philadelphia, Pennsylvania 19104

ABSTRACT

Although enormous progress has been made in the detection and treatment of localized (nonmetastatic) breast cancer, there has been relatively moderate progress toward the effective treatment of advanced disease. This study investigates the antitumor efficacy of a potent MHC nonrestricted cytotoxic human T cell line (TALL-104) upon transfer into a clinically relevant mouse model of metastatic breast cancer. Fragments from a surgical specimen of a patient with infiltrating ductal carcinoma were implanted s.c. in the flank region of severe combined immunodeficient (SCID) mice. One hundred % of the animals developed a local tumor mass that metastasized to subaxillary and inguinal lymph nodes, bones, lungs, liver, kidneys, ovaries, and brain, very closely mimicking the human disease. Multiple i.p. transfers of γ-irradiated (nonproliferating) TALL-104 cells into mice bearing low tumor burden (the primary tumor mass weighed only 150 mg) completely arrested local tumor growth and prevented systemic spread into local lymph nodes and distant organs. Remarkably, cell therapy administered in an advanced disease stage (when the tumor weighed 2 g) induced a significant or total regression of established metastasis with no obvious effects on the primary tumor mass. Profound antitumor effects against both local and systemic disease were instead seen in mice that received cell therapy after surgical excision of the primary tumor. The implications of these data in adjuvant breast cancer therapy are discussed.

INTRODUCTION

The systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy has been shown to increase disease-free survival and decrease mortality (1). However, analysis of the effects of systemic adjuvant therapy on the first sites of breast cancer relapse indicates the still-limited impact of currently used treatments on breast cancer mortality (2). These data point to the need for more effective adjuvant therapies against metastatic breast cancer. A variety of therapeutic approaches have recently been suggested: better use of existing chemotherapeutic agents (i.e., new schedules of administration and/or drug combinations), the continued development of naturally occurring cytotoxic agents (e.g., taxol), biological agents (cytokines), and immunological approaches (3). Although each of the suggested approaches has merit, immunological approaches can offer the significant benefit of killing tumor cells that express multiple drug resistance, a phenomenon often encountered in the use of chemotherapy. Among possible immunological approaches, therapeutic strategies based on NK3 and lymphokine-activated killer cells have certain distinct advantages, one of the most important being that their cytotoxic activity against tumors does not require prior effective immunization with any specific tumor-derived antigen.

This laboratory has developed a novel and potent cell therapy approach that holds promise in the future management of cancer patients in remission after treatment with conventional therapeutic modalities. Our strategy is based on the use of the IL-2-dependent leukemic T cell line TALL-104 (CD3/TcRoβ⁺ CD8⁺ CD4⁻ CD56⁺ CD16⁻; Refs. 4, 5), which displays uniquely potent MHC nonrestricted cytotoxic and cytostatic activities against tumors across species without affecting cells from normal tissues (4–9). TALL-104 cells seem to display strong antitumor effects in vivo in the absence of exogenously added IL-2 (8–11), thus eliminating the adverse effects associated with lymphokine-activated killer/IL-2 therapy (12, 13). In addition, TALL-104 cells can be expanded continuously in culture, thus providing an unlimited source of effector cells with stable tumoricidal activity for cell-based adoptive therapy approaches. The nature of the target antigen(s) recognized by TALL-104 cells is still under investigation. Although the CD3/TcR complex does not seem to play a direct role in tumor cell killing by TALL-104 cells, treatment with OKT3 antibody potentiates their cytotoxic and cytostatic functions against NK-susceptible and NK-resistant tumors (5). TALL-104 cells induce necrotic cell death via a perforin-dependent secretory pathway and can also kill targets through the release of cytotoxic mediators, such as tumor necrosis factor (TNF)-α, TNF-β, IFN-γ, or transforming growth factor (TGF)-β (5–7). In previous years, we have provided evidence on the tumoricidal effects of γ-irradiated (nonproliferating) TALL-104 cells in immunodeficient mice.

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2 To whom requests for reprints should be addressed, at The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104. Phone: (215) 898-3978; Fax: (215) 573-7919.

3 The abbreviations used are: NK, natural killer; IL-2, interleukin 2; BM, bone marrow; PBL, peripheral blood lymphocyte; IMDM, Iscove’s modified Dulbecco’s medium; MGG, May-Grunwald-Giemsa; mAb, monoclonal antibody; SCID, severe combined immunodeficient.
mouse models with transplantable human tumors (8, 9). The present study demonstrates the efficacy of TALL-104 cell therapy against metastatic breast cancer xenografts.

MATERIALS AND METHODS

Tumor Specimen. A tumor biopsy was obtained at mastectomy from a female 88 years of age diagnosed with infiltrating ductal carcinoma of the breast. Metastases were present in 3 of 10 axillary lymph nodes. Immunohistochemical analysis demonstrated 0% nuclear staining for estrogen and progesterone receptors. A biochemical hormone receptor assay indicated no detectable estrogen receptors and the presence of only 24 femtomol/mg protein progesterone receptors.

Engraftment of the Tumor Biopsy in SCID Mice. Six-week-old female CB-17 scid/scid (SCID) mice were purchased from Charles River Laboratories (Wilmington, MA) and maintained in a pathogen-free environment at the Wistar Institute Animal Facility. IgM nonproducer mice were treated i.p. with 30 mg/kg etoposide (Vepesid, Bristol Laboratories, Princeton, NJ) to inhibit their innate immunity, and 4 days later, were implanted s.c. in the flank region with small fragments (5 × 5 mm) from the biopsy specimen. Mice were anesthetized with metofane inhalation before surgery, which consisted of a 5-mm incision to form a pocket between skin and muscle in which the tumor fragment was placed. The wound was then closed with a surgical clip. The local tumor masses that developed in these animals were excised and subpassaged as fragments in four generations of SCID mice. Upon each transfer, the tumor displayed progressively more aggressive growth and metastatic behavior.

Gross Pathology and Histopathology. Mice were sacrificed when showing signs of heavy tumor burden (i.e., > 10% body weight), ulceration of skin with bleeding, and/or signs of severe respiratory distress, weakness, and lethargy. Complete necropsy was performed in all mice; regardless of appearance, most organs (including spleen, liver, kidneys, ovaries, lungs, heart, thymus, peripheral lymph nodes, brain, spinal cord, ribs, and legs) were removed, fixed in 10% formalin and paraffinembedded, and 4-μm sections were cut and stained with H&E. BM samples obtained from mouse femoral bones and PBLs isolated on Accu-Prep lymphocytes gradients (Accurate Chemical, Westbury, NY) from blood drawn by cardiac puncture were concentrated on slides by cytocentrifugation and stained with MGG. Aliquots of BM cells and PBLs were ultracentrifuged (10,000 rpm for 15 min) and kept as dry pellets at −20°C for PCR analysis (see below). In all experiments, primary tumor masses and lymph nodes were weighed using a precision balance before fixation.

Surgery. In some experiments, mice whose primary tumors were not adherent to the skin nor to the abdominal muscles were anesthetized by metofane inhalation. After sterilization of the surgical region, a small incision was made along the tumor mass, and the tumor was removed by gentle disassociation from the surrounding tissues using round smooth-blade scissors. The surgical wounds were then closed with silk sutures.

Recovery of Human Breast Tumor Cells. Part of the tumor mass and lymph nodes collected at necropsy were minced through metal grids and centrifuged on Accu-Prep lymphocyte gradients to remove dead cells and erythrocytes. Breast tumor cells recovered at the interface were resuspended in IMDM (Life Technologies, Inc., Grand Island, NY), supplemented with 10% heat-inactivated fetal bovine serum (Sigma Chemical Co., St. Louis, MO) and incubated in a 37°C humidified 5% CO2 incubator for short-term cytotoxicity and proliferation assays (see below). Dry pellets were also prepared and kept at −20°C for DNA extraction and PCR analysis.

Immunofluorescence Staining of the Breast Xenografts. Tumor cell suspensions recovered at necropsy were plated at 105/well in 96-well round-bottomed plates (Falcon), washed twice in PBS without Ca2+ and Mg2+, fixed with 3.7% paraformaldehyde-1% BSA (Sigma) solution at 4°C, washed three more times with fluorescence-activated cell-sorter buffer (PBS without Ca2+ and Mg2+, 0.1% NaN3, and 2% IgG-free horse serum), and permeabilized with 0.1% Triton X-100 (Sigma) at 4°C. Fifty μl of the FITC-conjugated mAb anti-Pan cytokeratin clone C11 (1:50 final dilution; Sigma; Ref. 14) were added to each well for 45 min at 4°C. After three washes, the cells were resuspended in fluorescence-activated cell-sorter buffer and analyzed by flow cytometry as described previously (7). The following mAbs were used at 10−2 dilution for the detection of specific T and B lymphoid markers: OKT3 (anti-CD3), B67.1 (anti-CD2; provided by Dr. Perussia, Thomas Jefferson University, Philadelphia, PA), and Leu-M1 (anti-CD19; Becton Dickinson, San Jose, CA). The Leu-12 mAb (anti-CD15; Becton Dickinson) was used to detect myeloid markers.

TALL-104 Cells. TALL-104 cells were maintained in humidified incubators at 37°C with 10% CO2 in endotoxin-free IMDM supplemented with 10% heat-inactivated fetal bovine serum and 100 units/ml human recombinant interleukin (rhIL)-2 (a gift from Dr. Maurice Gately, Hoffman-La Roche, Nutley, NJ). Cells were monitored weekly for mycoplasma contamination using a commercial PCR kit (American Type Culture Collection, Rockville, MD). TALL-104 cells were γ-irradiated (40 Gy) prior to use in vitro and in adoptive therapy.

Cytotoxicity Assays. Irradiated TALL-104 cells were tested at four concentrations in 18-h 51Cr-release assays (5, 7) against a fixed number (104/well) of breast tumor cells in suspension. Cytotoxic activity was evaluated in the presence or absence of 1 μg/ml murine mAb OKT3, which was added to the test just before the addition of the 51Cr-labeled targets. Percentage-specific 51Cr-release was calculated from the mean of two replicates.

Proliferation Assays. Irradiated TALL-104 cells were incubated at four different concentrations with a fixed number (105/well) of human breast tumor cells. After 16 h, the cultures were pulsed with 1 μCi/well [3H]TdR (2 Ci/mmol; Amersham, Arlington Heights, IL) for 18 h and harvested on fiberglass paper. Radioactivity was measured in a β-counter (Packard Instruments, Downers Grove, IL; Refs. 5, 7). Percentage of [3H]TdR uptake inhibition was calculated according to the formula: % inhibition = M-E/M, where M is the mean cpm of human breast cancer cells incubated alone, and E is the mean cpm of cocultured cells.

Cytokine Production. Irradiated TALL-104 cells (2 × 105/ml) were incubated overnight at 37°C with or without 1 μg/ml OKT3 and/or breast cancer cells at an E:T ratio of 10:1.
Fig. 1  Macroscopic (A and B) and microscopic (C-I) analysis of the growth of a primary human breast tumor (p.t.) in a SCID mouse. Arrows indicate metastatic axillary and inguinal lymph nodes in A and a lumbo-aortic tumor mass (invading both kidneys) in B. C, ascites fluid filled with tumor cells; metastatic nodules (m) are present in the liver (D; l, normal parenchyma), kidneys (E), and lungs (F; br, bronchus). Metastases (m) are also observed in the meningeal space infiltrating the brain parenchyma (br; G) and in the spinal cord (s.c.; H). Arrows in I indicate breast cancer cells in the PB. C-H, H&E staining; I, MGG staining. C-I, ×200.

Cell-free conditioned medium was harvested, filtered, and tested for the presence of IFN-γ, TNF-α, TNF-β, and granulocyte-macrophage colony stimulating factor using commercial ELISA kits (Endogen, R&D, Boston, MA), as described (7, 10). Breast tumor cell cultures incubated alone for 10 days were also tested for cytokine production.

E:T Conjugate Formation. Conjugate formation was measured upon mixing TALL-104 cells with human breast tumor cells at a 5:1 ratio for 2 h in a 37°C humidified 10% CO2 incubator. The cell mixture was then resuspended gently, concentrated on slides by cytocentrifugation, and stained with MGG.

Adoptive Transfer Therapy. Irradiated TALL-104 cells (2 × 10^7 in 500 μl of PBS) were administered i.p. either alone or in combination with 1 μg/10^6 cells OKT3 to tumor-bearing SCID mice. The schedule of TALL-104 cell administrations was adapted to the disease stage of the mice (metastatic disease, minimal disease, and surgery settings), as detailed in the “Results” section. Control animals received i.p. injections of PBS alone or PBS + OKT3.

Biodistribution of TALL-104 Cells in Tumor-bearing SCID Mice. TALL-104 cells (2 × 10^7/mouse) were labeled overnight with 0.25 mCi of Na₂[^32]CrO₄ (DuPont NEN, Boston, MA) in a 37°C humidified 10% CO₂ incubator and were γ-irradiated (40 Gy). After three washes in IMDM, cells were resuspended in 500 μl of PBS, and a 50-μl aliquot was assessed for isotope incorporation in a gamma counter. Labeled cells were injected i.p. into SCID mice with metastatic disease. After 24 h, the mice were sacrificed, all their major organs were collected and weighed, and their radioactivity was measured in a gamma counter. Results were expressed as cpm/g of organ collected.

PCR Assays. The PB and BM samples of the tumor transplanted mice (treated or not treated with TALL-104 cells) were subjected to PCR analysis using two primers specific for the human YNZ.22 mini-satellite region (10, 11, 15). The samples were harvested 4 days after the last TALL-104 injection (at the time of necropsy in the experimental group). A ^32P-labeled oligonucleotide probe recognizing 25 nucleotides in the middle of the amplified sequences was used to demonstrate the specificity of the PCR products (15).

Statistical Analysis. Mean ± SD values were analyzed for statistical significance using the Student’s t-test for unpaired data.

RESULTS

Growth and Dissemination of Human Breast Tumor Fragments in SCID Mice. Tumor fragments from a patient with infiltrating ductal carcinoma were implanted s.c. in the flanks of etoposide-pretreated SCID mice. The locally growing tumor masses displayed a progressively more aggressive growth with passages, killing the animals within 15–30 days at the fourth engraftment. Clinically, the tumor-bearing mice showed progressive lethargy with respiratory distress, gait disturbance secondary to paralysis of the hind legs, hemorrhagic ascites, and weight loss. Macroscopic examination at necropsy revealed the presence of a large primary tumor mass, enlarged superficial (axillary and inguinal) lymph nodes (Fig. 1A), kidney metastases (Fig. 1B), intrathoracic and abdominal lymph nodes, hepatic nodular metastasis with hemorrhagic ascites, splenomegaly, and lung metastases (data not shown). Histological analysis confirmed the heavy infiltration of all of these organs (Fig. 1, D–F), and revealed the presence of cancer cells in the brain and in the vertebrae with infiltration of the meningeal spaces leading to

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spinal cord compression (Fig. 1, G and H). In some areas, the tumor cells were organized in differentiated rosette-like patterns, a typical feature of human adenocarcinomas (Fig. 2, A and B); cytologically, pseudopodes of the membrane were found, another typical feature of cells from epithelial origin (Fig. 2C). In the terminal stages, breast cancer cells could always be demonstrated in the ascites (Fig. 1C), PB (Fig. 1H), and BM (data not shown). These findings were confirmed at the molecular level by PCR analysis (data not shown).

Expression of Epithelial-like Markers and Hormone Receptors by the Tumor Xenografts. The monoclonal anti-Pan cytokeratin conjugate mAb C11 used in this study (14) recognizes an epitope present in most human epithelial tissues and helps discriminate between carcinomas on one hand and nonepithelial tumors, such as lymphomas and gliomas, on the other. The tumor xenografts were found to express this marker (45.4 ± 1.2% staining; Fig. 2D) and to completely lack surface T and B lymphoid (OKT3/CD3, B67.1/CD2, Leu12/CD19) and myeloid (LeuM1/CD15) markers (data not shown), thus excluding the outgrowth of a tumor with hematopoietic features. Like the original tumor, the xenografts were immunohistochemically estrogen and progesterone receptor-negative.

In Vitro Susceptibility of Human Breast Tumor Cells to TALL-104 Cell Lysis. Irradiated TALL-104 cells displayed high levels of cytotoxic and cytostatic activities on single-cell suspensions isolated from the breast tumor biopsy, as measured in 18-h 51Cr-release and [3H]TdR uptake assays, respectively (Fig. 3, A and B). Fig. 3C and 3D show the formation of a TALL-104/breast tumor cell conjugate followed by the delivery of the lethal hit. Both cytotoxic and cytostatic activities were slightly increased upon the addition of OKT3 mAb (Fig. 3, A and B). This antibody also induced the production of high levels of IFN-γ, TNF-α, TNF-β, and granulocyte-macrophage colony stimulating factor in TALL-104 cells (Fig. 3E) as measured by ELISA. As reported previously (6, 7), OKT3 induced moderate levels of cytokine production in TALL-104 cells. No cytokines were detected in the conditioned medium of either breast cancer cells or TALL-104 cells cultured alone. These in vitro data prompted us to test the antitumor effects of TALL-104 cells in the SCID mouse model of metastatic breast cancer described above.

Efficacy of Adoptively Transferred γ-irradiated TALL-104 Cells against Metastatic Disease. In the first set of experiments, TALL-104 cell therapy was initiated at an advanced disease stage, when the s.c. tumor mass weighed about 2 g and the axillary and/or inguinal lymph nodes were palpable. Irradiated TALL-104 cells were injected i.p. every day for 2 weeks with or without OKT3. Four of six control mice (two given PBS and two given PBS + OKT3) died during the 2 weeks of daily cell therapy with the very aggressive systemic disease described above. At completion of cell therapy (i.e., 4 days after the last TALL-104 cell injection), the remaining two control mice and all 10 experimental mice treated with TALL-104 cells ± OKT3 were sacrificed, and necropsy and histopathological analysis were performed. No significant difference in weight between the primary tumor mass of the treated mice and that of the control survivor mice was detected (Fig. 4A). However, the necrotic and histopathological appearances of
Fig. 3 Cytotoxic (A) and cytostatic (B) activities of γ-irradiated TALL-104 cells against breast cancer cell suspensions isolated from a tumor fragment. Eighteen-h 51Cr-release (A) and 3H-TdR incorporation (B) assays were carried out in the presence or absence of 1 μg/ml OKT3 mAb at the indicated E:T ratios. In B, the total count of 3H-TdR taken up by tumor cells was 11,780 ± 1,050 cpm. C and D, the sequence of events occurring during breast cancer cell lysis induced by TALL-104 cells (e, effector cell; t, target cell). C, the binding of a TALL-104 cell (e) to a breast tumor cell (t); arrows, azurophilic granules present in the effector cell. D, a breast cancer cell death induced by TALL-104 cells with a mechanism suggesting apoptosis (MGG staining), ×1000. E, the levels of cytokines produced by TALL-104 cells following an 18-h incubation with human breast cancer cells (B, C.) at the ratio of 10:1 in the presence and absence of 1 μg/ml OKT3. SD values < 2.0 (B) and < 16.0 (C) not shown.

the tumors were consistently and strikingly different in the control and treated mice, with tumor necrosis present only in the latter group (Fig. 4B). Specifically, macroscopically notable necrosis in the primary tumor masses was observed within 7–10 days from the beginning of cell therapy in all of the five mice treated with TALL-104 cells + OKT3 and in three of five animals that received TALL-104 cells alone (Fig. 4C). In one mouse treated with TALL-104 cells + OKT3, necrosis progressed until the tumor mass disappeared, leaving behind only a necrotic scar (Fig. 4C, quadrant 4). Histological analysis of tissues from this mouse failed to reveal any sign of metastatic disease in all organs examined. Unlike the primary tumor
masses, the total weight of superficial metastatic lymph nodes (axillary and inguinal) from the cell-treated animals (±OKT3) was significantly lower than that of the PBS (±OKT3)-injected mice (Fig. 4D). At necropsy, no treated mice showed macroscopic signs of metastatic disease. Histological analysis revealed the presence of micrometastases in the lungs, liver, and kidneys of 50% of these animals, whereas the other 50% were virtually free of systemic disease. In the group of mice treated with TALL-104 cells + OKT3, one animal had micrometastatic involvement of the lungs, another animal had lung and kidney micrometastases, and the third had a more systemic spread with metastatic micronodules in lungs, liver, kidneys, and brain.

**Efficacy of TALL-104 Cells in Mice with Small Tumor Burden.** The following experiments were designed to test the antitumor efficacy of TALL-104 cells in a setting of minimal disease. Twelve mice bearing small breast tumor grafts (average mass of the primary tumor at the beginning of therapy was 150 mg) were injected with irradiated TALL-104 cells (±OKT3) or PBS (±OKT3) daily for 2 weeks followed by two weekly injections. Four days later, all mice were sacrificed, and necropsy was performed. As shown in Fig. 5A and 5B, control animals treated with PBS ± OKT3 displayed large primary tumor masses (0.934 ± 0.033 g), as well as axillary/inguinal lymph nodes (0.2213 ± 0.0187 g). Histological analysis revealed lung micrometastases in...
and primary mass (A)

Fig. 5 A, antitumor effects of irradiated TALL-104 cells (±OKT3) on the primary mass (A) and lymph nodes (sum of axillary and inguinal; B) of SCID mice treated in a stage of minimal disease (n = 3 in the experimental groups; n = 2 in the control groups). SD < 0.005 (in the case of the tumor mass) and SD < 0.002 (in the case of lymph nodes) not shown. C, PCR amplification of the human minisatellite region YNZ.22 in PB and BM of mice treated with TALL-104 cells in a minimal disease stage. Breast tumor cells (Lane 1), mice injected with PBS (Lanes 2 and 3), OKT3 (Lanes 4 and 5), TALL-104 cells alone (Lanes 6–8), and TALL-104 cells + OKT3 (Lanes 9–11) are shown. Lane 12, distilled water.

75% of these animals. By contrast, neither primary tumor masses nor metastatic lymph nodes were found in the cell-treated mice (±OKT3); histological analysis confirmed the absence of malignant cells both in the tissues surrounding the initial area of engraftment and in lymph node regions. Furthermore, no lung metastases could be detected in any of the treated mice even after careful analysis of 10 serial sections in each lung preparation. PCR analysis showed the presence of breast cancer cells in the PB and BM of all control animals but not in the treated ones (Fig. 5C).

Adjuvant Cell Therapy after Surgical Excision of the Primary Tumor Mass. The antitumor efficacy of TALL-104 cells was evaluated in some mice subjected to surgical removal of the primary tumor to create a clinical setting similar to that of breast cancer patients at diagnosis. For this purpose, six mice bearing s.c. breast tumor grafts that appeared highly mobile and nonadherent to the skin or to the abdominal wall underwent surgical excision of the tumor followed by TALL-104 cell treatment according to the schedule used for treatment of minimal disease (see above). At surgery, the tumors weighed 0.583 ± 0.191 g. Because tumor removal reduced breast cancer cell dissemination, none of the control mice receiving PBS died before completion of cell treatment in the experimental group. Four days after the last weekly injection of TALL-104 cells, all mice were sacrificed and necropsied. As shown in Fig. 6A, TALL-104 cell treatment reduced the regrowth of breast tumor cells at the primary site and the metastatic spread to lymph nodes to 15 and 4.5%, respectively, as compared to the weights of the tumor and lymph nodes in the control animals (P < 0.001). At necropsy, the untreated mice presented heavy tumor involvement of most organs, including brain and spinal cord (Fig. 6B, top), whereas no macroscopic metastases were detected in any of the TALL-104-treated mice (Fig. 6B, bottom), and only a few micrometastases were seen histologically in the lungs (50% of the mice), kidneys (25% of the mice), and spinal cord (25% of the mice). PCR analysis showed infiltration of breast tumor cells in the PB and BM of 100% of the control mice and in the PB of 50% of the treated mice; no tumor cells were detectable in the BM of the treated animals (Fig. 6C). It is noteworthy that in one of the TALL-104 cell-treated mice, the primary tumor mass did not grow back after surgery and, at necropsy (4 weeks after cell therapy initiation), this mouse was found to be disease-free both clinically and histologically.

Biodistribution of TALL-104 Cells in Tumor-bearing SCID Mice. Effector cell accumulation at the tumor site is crucial for the efficacy of adoptive immunotherapy. Experiments in healthy (nontumor-bearing) SCID mice showed that 51Cr-labeled γ-irradiated TALL-104 cells injected i.p. migrated mainly to the spleen within 2 h after transfer and were found in the kidneys, ovaries, and liver 24 h later (data not shown). The distribution of TALL-104 cells remained unchanged 24–72 h after transfer. Table 1 presents data relative to the 24 h biodistribution of 51Cr-labeled TALL-104 cells in nine SCID mice with metastatic disease 1 month after implantation of the breast tumor fragments. The distribution of 51Cr-labeled TALL-104 cells in major thoracic and abdominal organs was similar to that observed in healthy mice (data not shown). Interestingly, levels of radioactivity observed in metastatic lymph nodes were consistently 2–6 times higher than those compared in the primary tumor masses. Moreover, in mice (nos. 2–5 and 9) that showed...
Fig. 6 Effects of TALL-104 cells on tumor cell growth and dissemination after the surgical removal of the primary mass. A, the significant tumor regrowth and lymph node spread in mice treated with TALL-104 cells (n = 4) as compared to mice injected with PBS (n = 2). B, histopathological findings in tissues from mice that received TALL-104 cell therapy or PBS injections after excision of the primary tumor mass. Control mice displayed a heavy tumor burden in all organs examined, including the brain (arrows, tumor infiltration of the ventricular brain space). C, PCR analysis of PB and BM in mice treated with PBS (Lanes 1, 2 and 7, 8, respectively) or with TALL-104 cells (Lanes 3-6 and 9-12, respectively). Lanes 13 and 14, breast tumor cells (Lane 14 contains twice the amount of cellular DNA as Lane 13); Lane 15, distilled water.

an imbalanced tumor load in a kidney and adjacent ovary, radioactivity levels were 2–3 times higher in the organs with more tumor involvement than in the less affected contralateral organs, suggesting a preferential accumulation of the effector cells to tumor sites.

DISCUSSION

Invasive breast cancer most commonly disseminates to the lungs, liver, bone, and brain (16, 17). For preclinical investigations of a new experimental therapy, in vivo tumor models that can harbor breast cancer metastases within at least one of these host organs should be sought as appropriate targets for assessing the efficacy of the therapeutic approach. Breast carcinoma xenografts are notoriously difficult to grow in immunodeficient rodents; only ~60% of cell lines and less than 10% of fresh tissue explants produce local tumors that are transplantable or able to metastasize (18–21). However, Sakakibara et al. (22) have shown recently that the placement of 48 primary breast tumors within the gonadal fat pad of SCID mice resulted in 25% of the tumors growing rapidly and having metastatic properties, 52% having a slow, nonmetastatic growth, and 23% failing to grow at all.

On the basis of previous results, which show a more successful engraftment of human malignancies upon pretreatment of SCID mice with etoposide (23, 24), we used the same approach to optimize the engraftment of breast tumor biopsies in this study. Etoposide suppresses the natural immunity of the host (NK cells) and myelopoiesis (granulocytes and macrophages; Ref. 25). By using this immunosuppressant and the engraftment protocol described here, we had success in grafting 10 of 16 (~60%) breast tumor biopsies of various histological types, including infiltrating ductal and/or lobular, and invasive inflammatory carcinomas.4 The primary tumor masses that formed in the flanks of these animals often metastasized to the same organs that are affected in the human disease,4 thus providing a clinically relevant system for evaluating the antitumor
efficacy of our TALL-104 cell therapy approach in situations of both low and heavy tumor burden. The present study shows that, similar to our previous studies in murine models with xenogenic or syngeneic tumors (7, 8, 11), the antitumor effects of TALL-104 cell therapy were almost complete when the primary tumors were administered at an early stage of disease or after the removal of the primary tumor mass (minimal disease); remarkably, profound effects were also observed when cell therapy was given after the establishment of metastasis in regional nodes and distant organs.

IL-2-cultured TALL-104 cells were lethally irradiated prior to their administration to the animals to inhibit their proliferation and leukemogenic properties in an immunodeficient host (26, 27). Because this treatment diminishes effector cell persistence in the host tissues (8, 10) and, therefore, their long-lasting and effective interaction with the tumor target, we chose to treat the tumor-bearing animals with an aggressive treatment schedule based on daily delivery of high-dose TALL-104 cells for 2 weeks followed by weekly maintenance boosts. OKT3 was given to enhance the tumoricidal activity of TALL-104 cells via direct triggering of their CD3/TCR complex (5, 7, 10). When therapy was administered in a setting of highly advanced disease, remarkable regression of metastasis was seen; no lesions were detectable macroscopically, and micrometastases were present in only 50% of the animals. Although no significant effects were seen on the growth of the primary tumor mass as compared to that of the control groups, this comparison likely underestimates the effect of cell therapy on the size and weight of the primary tumor mass because 80% of the control mice actually died 8–17 days before the experimental mice were sacrificed. Biodistribution analysis of $^{51}$Cr-labeled TALL-104 cells in tumor-bearing mice during advanced disease confirmed the high migratory ability of these effectors and suggested their preferential accumulation in organs bearing the highest tumor load. When cell therapy was initiated at an earlier stage, i.e., when the primary tumor mass was only 0.15 g, the results were maximal with total prevention of metastatic spread and growth of the local tumor. Moreover, in a clinical setting of first-line treatment, i.e., after surgical removal of the primary breast tumor, it was clear that residual cancer cells of mice that did not receive cell therapy grew back in the original site of engraftment and metastasized aggressively into local nodes and distant organs. In this setting, TALL-104 cell therapy had profound effects both on the regrowth of the primary tumor mass and on metastatic spread. PCR analysis indicated that only 50% of the cell-treated animals harbored cancer cells in their PB and none in their BM; these results could be due to sampling because the BM is harvested from the femoral bones and represents less than one-third of the total mouse BM, whereas the PB represents cells circulating in the whole body.

The therapeutic efficacy of TALL-104 cells in an immunocompetent host might be greater than that observed in an immunodeficient model system. In fact, our recent studies in immunocompetent mice bearing syngeneic leukemia (11) have demonstrated that TALL-104 cells can induce specific antitumor immunity in the host, thus protecting from further leukemic cell challenges. Recently published (10) and ongoing studies in dogs with metastatic breast cancer are validating the feasibility of using these cells for treating patients with an intact immune system. However, it is also clear from these studies and the present investigation that this type of therapy might not effectively eliminate 100% of occult metastases. Only well-controlled, randomized clinical trials in women at high risk of relapse after remission induction will provide definite evidence for the efficacy of this novel cell therapy approach in preventing relapses and prolonging disease-free survival.

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Cell therapy of a highly invasive human breast carcinoma implanted in immunodeficient (SCID) mice.


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