Regression of bone metastases following adoptive transfer of anti-CD3-activated and IL-2-expanded tumor vaccine draining lymph node cells

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Received 15 November 2003; accepted in revised form 20 May 2004

Key words: adoptive immunotherapy, bone metastasis, melanoma, micrometastasis, T lymphocytes

Abstract

As many as 80% of patients with breast, prostate, or lung cancer develop bone metastases during the course of their illness. However, thus far, no attempts have been made to explore the potential value of adoptive immunotherapy with antigen-specific T lymphocytes specifically for the treatment of skeletal metastases. Here, we demonstrate tumor regression in a preclinical model of bone metastases from the murine B16BL6 melanoma following adoptive transfer of effector T lymphocytes obtained from tumor vaccine draining lymph nodes. The antitumor effect required transfer of high number of effector cells, which was dependent on CD8⁺ cells as demonstrated by vivo depletion of different T cell subsets, and was magnified if effector cells were administered to the arterial supply of the bone/bone marrow. Using flow cytometric analysis, CFSE-labelled Thy1.1⁺ donor T cells were isolated from the bone marrow of tumor-bearing mice at 24 h and 6 days following adoptive transfer. At the latter time point cell division of the transferred effector cells was detectable. Currently, no curative treatment is known for skeletal metastases in clinical practice. Considering the promising early findings in the present study, further studies exploring the therapeutic potential of adoptive immunotherapy for metastatic disease to the skeleton are warranted.

Abbreviations: AIT – adoptive immunotherapy; β-ME – beta-mercaptoethanol; CFSE – carboxylfluorescein diacetate; CM – complete medium; FACS – fluorescence activated cell sorting; FBS – fetal bovine serum; GM-CSF granulocyte-macrophage-colon-stimulating factor; HBSS – Hank’s balanced salt solution; IL-2 – interleukin 2; NEAA – non-essential amino acids; PerCP – peridin chlorophyll protein; SIA – systemic intra-arterial tumor cell injection; Tₑ – effector T cells; TIL – tumor infiltrating lymphocytes; TVDLN – tumor vaccine draining lymph node

Introduction

The skeleton is one of the most common target organs of metastases in human cancer [1]. Depending on the site of origin of the primary cancer, bone metastases are diagnosed in 23% to 84% of patients [2]. Although breast, prostate and lung cancers most frequently metastasize to the bone/bone marrow, up to 45% of melanoma patients also develop skeletal metastases [1]. Bone metastases often result in complications such as several pain, hypercalcemia, pathologic fracture, and spinal cord or nerve root compression. Currently, no curative treatment is known; bisphosphonates, chemotherapy, local radiation, surgery and analgesics only provide palliation [3]. To date, the therapeutic potential of adoptive immunotherapy (AIT) has not been explored specifically for the treatment of bone metastases. The adoptive transfer of in vitro activated and expanded T lymphocytes has, however, demonstrated significant antitumor activity in various animal models using different murine cancer cell lines [4–6]. Recently, Dudley et al. [7], though not studying bone metastases, also reported very promising findings in the clinical setting using adoptive transfer of selected tumor-reactive T cells in patients with metastatic melanoma following a nonmyeloablative chemotherapy regimen. In our laboratory, we previously evaluated a preclinical model of bone metastases for the study of AIT [8]. Injection of tumor cells into the left cardiac ventricle of mice, a model first described by Arguello et al. [9], led to a high incidence of bone metastases and was found to be suitable for the study of AIT. Several properties of bone contribute to its uniqueness as a metastatic target. Bones constitute a well-vascularized, physically defined microcompartment. Within bone, metastases are most frequent at sites of red bone marrow, where vascular sinusoids lined by endothelial cells have fenestrae and lack a basement membrane [10].

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In association with selective attachment of cancer cells to adhesive substrates in bone, this may facilitate localization and subsequent growth of metastatic tumor cells in the skeleton. In addition, spreading cancer cells might also profit from a rich supply of bonederived and hematopoietic growth factors [11]. Paradoxically, the bone marrow is also a prominent organ involved in antigen presentation and immune response [12], and seems to be a rich source of protective immune cells in immunized animals [13]. Müller et al. nicely demonstrated that the bone marrow and lymph nodes are also privileged sites where potentially metastatic tumor cells can be controlled in a dormant state by the immune system in mice inoculated with the lacZ gene tagged DBA/2 lymphoma (EblacZ) [14]. Taken together, the unique microenvironment of bone offers favorable conditions for the establishment of micrometastases, yet at the same time it is a rich source of protective immune cells. In patients, the detection of bone marrow micrometastases has been described as an independent predictor for subsequent clinical relapse in distant organs [15]. Several other clinical observations suggest that the bone marrow may be an important site of persistence of dormant tumor cells in patients treated with curative intent [16, 17]. This balance between tumor cell number and immune response might eventually break down and result in the development of secondary tumors at distant sites many years after successful treatment of the primary tumor. Potential explanations include impaired immunosurveillance or the development of tolerance. Based on this biology, it seemed promising to explore the potential of adoptive T cell immunotherapy specifically for the treatment of metastatic disease to bone/bone marrow. To our knowledge, only very limited data are available on trafficking and function of adoptively transferred T cells in the bone microenvironment. However, tumor infiltrating lymphocytes (TIL) have been observed to migrate to metastases throughout the body including the skeleton following adoptive transfer via intravenous infusion [18, 19]. Here, we report on the regression of bone metastases in a murine melanoma model mediated by the adoptive transfer of anti-CD3-activated and IL-2-expanded tumor vaccine-draining lymph node (TVDLN) cells.

Materials and methods

Mice and tumor cell lines

Female C57BL/6J (B6) and B6.PL-Thy1a/Cy mice, 8 to 10 weeks old, were purchased from The Jackson Laboratory (Bar Harbor, Maine). Recognized principles of laboratory animal care were followed (Guide for the Care and Use of Laboratory Animals, National Research Council, 1996). D5 is a poorly immunogenic subclone of the spontaneously arising B16BL6 melanoma. D5-G6 is a stable clone of D5 that was transduced with a murine GM-CSF retroviral MFG vector [20]. D5-G6 cells secrete ~200 ng GM-CSF/ml/10^6 cells/24 h. All tumor cells were cultured as described previously [8, 21]. In brief, we used complete medium (CM), which consisted of RPMI 1640 (BioWhittaker, Walkersville, Maryland) supplemented with β-ME (Aldrich, Milwaukee, Wisconsin), 10% FBS (Life Technologies, Grand Island, New York), NEAA, sodium pyruvate and l-glutamine. Cell lines were maintained in T-75 or T-150 culture flasks in a 5% CO2 incubator at 37 °C.

Experimental model of bone metastases

After brief trypsinization in the culture flasks, 10^6 tumor cells were washed and resuspended in 0.1 ml Hank’s balanced salt solution (HBSS) (BioWhittaker, Walkersville, Maryland). Animals were anesthetized with pentobarbital (Nembutal) 0.05 mg/g i.p. For systemic intra-arterial administration (SIA), tumor cells were inoculated into the left cardiac ventricle of the animal using a 27-gauge needle as described by Arguello et al. in 1988 [9] and evaluated in our lab [8]. The animals were sacrificed 15 to 18 days after tumor cell injection and a necropsy was performed. To simplify assessment of the tumor burden in bone/bone marrow, we considered bones with the slightest sign of pigmented tumor colonies as positive for bone metastases. Tumor colonies of pigmented melanoma cells to the bone can readily be seen on various parts of the skeleton but particularly on well vascularized portions of long bones, such as the femur (Figure 1B).

Histological examination

In brief, forelimbs or hindlimbs from animals were fixed in 10% phosphate-buffered formalin (pH 7.2) and decalcified in 14% EDTA solution. Standard techniques were used for paraffin embedding. For detection of bone metastases the embedded samples were cut into 5 μm sections and stained using haematoxylin and eosin (H&E). Slides were visualized using using a phase contrast microscope with an original magnification of ×20.

Flow cytometric analysis (FACS)

For the purpose of T cell tracking, samples of the bone marrow and spleen were prepared for flow cytometric analysis as described in the ‘Laboratory Protocols for the Immunotherapy of Cancer’ (Surgery Branch, NCI). PE-conjugated anti-CD8 and PerCP-conjugated anti-CD90.1 (Thy1.1) mAbs were purchased from BD Pharmingen (San Diego, California). To assess cell division, effector T cells were labeled using CFSE as described earlier [22]. Flow cytometry was performed on a FACScan (Becton Dickinson, Mountain View, California), and data were analyzed using CellQuest software (Becton Dickinson).

Generation of T_E and adoptive immunotherapy (AIT)

For adoptive T cell transfer we used a standard protocol as described earlier [4, 8, 21]. Briefly, one million D5-G6 tumor cells were injected s.c. into both the hind and fore flanks of wt B6 mice. Eight days following vaccination, the draining superficial axillary and inguinal lymph nodes were harvested. TVDLN were resuspended and activated in CM