Immunotherapy with vaccines combining MHC class II/CD80+ tumor cells with interleukin-12 reduces established metastatic disease and stimulates immune effectors and monokine induced by interferon γ

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Abstract Because they are difficult to treat, animal models of widespread, established metastatic cancer are rarely used to test novel immunotherapies. Two such mouse models are used in this report to demonstrate the therapeutic efficacy and to probe the mechanisms of a novel combination immunotherapy consisting of the cytokine interleukin-12 (IL-12) combined with a previously described vaccine based on MHC class II, CD80-expressing cells. BALB/c mice with 3-week established primary 4T1 mammary carcinomas up to 6 mm in diameter and with extensive, spontaneous lung metastases show a significant reduction in lung metastases following a 3-week course of immunotherapy consisting of weekly injections of the cell-based vaccine plus injections of IL-12 three times per week. C57BL/6 mice with 7-day established intravenous B16 melF10 lung metastases show a similar response following immunotherapy with IL-12 plus a vaccine based on B16 MHC class II, CD80-expressing cells. In both systems the combination therapy of cells plus IL-12 is more effective than IL-12 or the cellular vaccine alone, although, in the 4T1 system, optimal activity does not require MHC class II and CD80 expression in the vaccine cells. The cell-based vaccines were originally designed to activate tumor-specific CD4+ T lymphocytes specifically and thereby provide helper activity to tumor-cytotoxic CD8+ T cells, and IL-12 was added to the therapy to facilitate T helper type 1 lymphocyte (Th1) differentiation. In vivo depletion experiments for CD4+ and CD8+ T cells and natural killer (NK) cells and tumor challenge experiments in beige/nude/XID immunodeficient mice demonstrate that the therapeutic effect is not exclusively dependent on a single cell population, suggesting that T and NK cells are acting together to optimize the response. IL-12 may also be enhancing the immunotherapy via induction of the chemokine Mig (monokine induced by interferon γ), because reverse PCR experiments demonstrate that Mig is present in the lungs of mice receiving therapy and is most likely synthesized by the tumor cells. These results demonstrate that the combination therapy of systemic IL-12 and a cell-based vaccine is an effective agent for the treatment of advanced, disseminated metastatic cancers in experimental mouse models and that multiple effector cell populations and anti-angiostatic factors are likely to mediate the effect.

Key words Immunotherapy · Metastatic mammary carcinoma · IL-12 · Angiogenesis · CD80 · MHC class II · CD4+ T cells

Introduction

Many of the recently explored immunotherapy strategies for the treatment of cancer have focused on the improved activation of tumor-specific immunity. For example, administration of interleukin-12 (IL-12), a cytokine that favors T helper type 1 lymphocytes (Th1) and natural killer (NK) cell development and stimulates anti-angiogenic chemokines [12, 20], reduces tumor burden in numerous mouse tumor systems [4, 38, 39]. Likewise, the treatment of mice with established primary and/or metastatic tumor with irradiated immunogenic tumor cells, constitutively expressing MHC class I molecules and transfected/transduced with the costimulatory molecule CD80, reduces primary tumor mass and/or small metastatic tumor load [6, 41]. This latter approach is based on the premise that the genetically engineered tumor cells present both antigen-specific and costimulatory T cell activation signals to the relevant CD8+ T lymphocytes.
Another immunotherapeutic strategy aimed at specifically improving the generation of tumor-specific CD4+ T lymphocytes uses autologous tumor cells transfected with syngeneic MHC class II genes plus CD80 costimulatory molecule genes as cell-based vaccines for the treatment of mice with established primary and metastatic cancer. This therapy is based on the hypothesis that enhanced generation of CD4+ tumor-specific T helper lymphocytes facilitates CD8+ T cell activation and promotes stable, long-term immune memory against recurrence of primary tumor and/or outgrowth of micrometastases [27, 28]. Treatment with MHC-class-II-transfected-cell-based vaccines has yielded significant reductions in solid tumor mass [1, 2] and in established, spontaneous metastatic disease [32].

In an attempt to generate a more potent antitumor effect, IL-12 and CD80 therapies have been combined to target the activation of CD8+ T cells. Because in vitro studies have shown that IL-12 plus CD80 produces optimal T cell proliferation and interferon γ (IFN-γ) production [23, 25] as well as stimulating a primary antitumor response in vitro [17], it is not surprising that IL-12 and CD80 synergize to bring about significant regression of established primary tumor as well as inducing immunological memory against recurrence of primary tumor [11, 33]. Although a principal function of IL-12 is its ability to promote CD4+ Th1 differentiation, surprisingly, IL-12 therapy has not previously been combined with other therapies that specifically target the activation of tumor-specific CD4+ T cells. To test the potential effect of targeting with IL-12 plus CD4+, we have combined systemic IL-12 therapy with immunization using MHC class II/CD80 genetically modified tumor cells for the treatment of established (induced i.v. and spontaneous) metastatic disease. Previous in vivo studies testing IL-12 therapy have used mouse tumor models consisting of either solid, subcutaneous primary tumors, or very early metastases induced by intravenous injection of malignant cells [4, 7, 9, 10, 11, 31, 33]. Although these model systems provide some insight into the potential role of therapeutic agents in the treatment of cancer, they are not realistic clinical situations in which larger metastatic tumor loads are likely to be encountered and for which more effective treatments are needed.

To test potential immunotherapies more rigorously against larger metastatic loads, we have used two mouse tumor systems. The 4T1 mammary carcinoma tumor is a very poorly immunogenic and highly malignant tumor that rapidly and spontaneously metastasizes to lymph nodes, lung, liver, brain, and blood following growth of the primary tumor in the mammary gland [24, 32]. This disease progression closely parallels human breast cancer and makes the 4T1 tumor an excellent model for human disease [32] and a rigorous animal model of advanced spontaneous metastatic disease.

As a second model system, we have used the B16-derived melf10 melanoma [16]. This tumor is also very poorly immunogenic and highly malignant, and metastasizes immediately to the lung when inoculated intravenously. Similar to approximately 15% of human cancers, melf10 has markedly reduced levels of MHC class I molecules. This phenotype probably contributes to its reduced immunogenicity and heightened tumorigenicity. Many investigators have used the melf10 tumor as an experimental model; however, most studies use lung metastases very early after they are established (e.g. 3 days or less after i.v. inoculation). We have used longer-established melf10 lung metastases (therapy begins on day 7 after i.v. inoculation) to test the combination vaccine more rigorously. For both tumors, the combined therapy is more effective than either therapy alone, and appears to be mediated by multiple independent effector mechanisms including T lymphocytes, NK cells, and possibly chemokine production that has been linked to anti-angiogenesis.

Materials and methods

Cells and transfectants

Melf10 is a high metastatic variant of the C57BL/6-derived B16 melanoma [16]. 4T1 is a spontaneously metastatic, poorly immunogenic BALB/c-derived mammary carcinoma [24]. Culture conditions for both tumors have been previously described [29, 32]. Generation and characterization of 4T1 transfectants expressing I-A^d and CD80 and B16melF10 transfectants expressing I-A^d and CD80 have been previously described [29, 32].

Mice

Mice were purchased from The Jackson Laboratory (Bar Harbor, Me.) or bred in the UMBC Animal Facility from breeding pairs purchased from The Jackson Laboratory. Experiments using the 4T1 mammary carcinoma or melf10 melanoma were performed in female BALB/c mice and C57BL/6 male or female mice, respectively. All mice were between 6 weeks and 6 months in age.

Tumor challenges and metastases assays

Tumorigenesis and metatstasis formation by the melf10 [29] and 4T1 [32] tumors were performed as previously described. Briefly, for experimental metastases, 10^5 melf10 cells/100 μl for each mouse were inoculated intravenously (i.v.) into the tail vein of C57BL/6 mice on day 0; the mice were sacrificed 3–4 weeks later and their lungs observed and weighed. For spontaneous metastases 7 x 10^3 4T1 cells/50 μl for each mouse were inoculated into the abdominal mammary gland of BALB/c female mice on day 0; the mice were sacrificed 6 weeks later and the number of clonogenic metastatic cells in the lungs assessed by growth in medium supplemented with 6-thioguanine [32]. Mice carrying tumors were closely followed for symptoms of pain and distress and were sacrificed when they became moribund. On the basis of previous studies [32], 4T1-bearing mice with up to 10 000 clonogenic metastatic 4T1 cells in their lungs are considered responder mice. All animal procedures followed the Principles of laboratory animal care (NIH publication 85-23, revised 1985) and were approved by the UMBC Institutional Animal Care and Use Committee.

Antibodies

Protein A or protein G purification of MHC-class-II-specific mAb 3JP (I-A^d) [19], MKD6 (I-A^d) [21], MHC-class-I-specific mAb 20-8-4 (H-2 K^b ) [30], 28-14-8 (H-2D^d) [30], CD4 (GK1.5 [42]), and CD8 (2.43 [35]) was as previously described [29, 32]. Fluorescently coupled CD3, CD4, CD8, NK.1.1, and B220 mAb were purchased from Pharmingen.