Surrogate Tumor Antigen Vaccination Induces Tumor-Specific Immunity and the Rejection of Spontaneous Metastases

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Abstract

The nonimmunogenic 4T1 murine mammary carcinoma model and a model surrogate tumor antigen (sTA) were employed to explore the possibility of inducing tumor-specific immunity through active immunization in the absence of defined tumor-associated antigens. Immunization of naive mice with protein-based sTA resulted in protection from s.c. challenge, with 4T1 modified to express the sTA (4T1.sTA), or from a sTA-expressing unrelated tumor cell line (mKSA). Immunization had no effect on parental 4T1 tumor growth or the formation of parental 4T1 spontaneous lung metastases. Mice that were sTA immunized and successfully rejected 4T1.sTA challenge also rejected a subsequent challenge in the contralateral flank with parental 4T1 and strikingly prevented the formation of spontaneous parental 4T1 lung metastases. The rejection of parental 4T1 seemed to be specific for and associated with unknown 4T1 tumor-associated antigens, because rejection of mKSA did not induce cross-protection against a challenge with parental 4T1. To evaluate the effect of this vaccine approach on established disease, mice were simultaneously challenged on day 0 with 4T1.sTA and parental 4T1 in contralateral flanks and then immunized on days 3, 10, 17, and 24 with sTA protein. Tumor growth and metastasis were delayed in four of five animals, and 20% (2 of 5) of the animals were tumor free at the completion of the experiment. Together, these data suggest that prior vaccination with a sTA followed by inoculation with poorly immunogenic tumor cells modified to express the sTA activates determinant spreading and the induction of systemic tumor immunity resulting in indigenous tumor rejection. (Cancer Res 2005; 65(7): 2938–46)

Introduction

The field of cancer vaccine development is growing rapidly and has become the focus of much intense investigation. Cancer vaccines have evolved from crude tumor cell lysates given with an adjuvant to the sophisticated application of ex vivo engineered dendritic cells. Indeed, proteins, peptides, DNA, viral vectors, heat shock proteins, and dendritic cells as well as various combinations and permutations of these modalities have been and are being explored and refined as vaccines against cancer (1, 2). In the midst of this intense study, one thing is becoming increasingly clear: the most effective vaccines may be those composed of a defined tumor-associated antigen(s) (TAA). Thus, the need to discover, define, and validate additional TAA for cancers to include prostate, breast, and lung cancers remains an obstacle for effective vaccine development (3). To this end, new vaccine methods that circumvent the need for in hand, defined TAA could be extremely valuable.

The BALB/c-derived 4T1 mammary carcinoma model is a unique nontransgenic model of metastatic breast cancer based on explantable, syngeneic, clonal tumor cells (4). The growth characteristics, lack of immunogenicity, and aggressive, spontaneous metastatic properties of this model closely resemble those characteristics observed for human breast cancer (5). 4T1 tumor cells are relatively easy to culture and form tumors in healthy, immunocompetent BALB/c mice following s.c. inoculation with as few as 5,000 to 7,000 cells (6, 7), making this a useful in vivo model to study the effects of active immunization against aggressive, nonimmunogenic cancers for which no TAs have been defined.

Epitope or determinant spreading as it relates to specific immune responses against a given antigen was first described as the dispersion of immunologic specificities from a single dominant epitope to subdominant epitopes (8–10) within the original antigenic target (intramolecular determinant spreading) or to epitopes carried by distinct antigens (intermolecular determinant spreading; ref. 11). Early studies showed that clones of specific T cells were responsible for driving determinant spreading in models of autoimmunity (12). Driver T-cell clones as the mechanism of determinant spreading initiation in autoimmunity were later supported by murine models of tumor vaccination. A vaccine composed of a single peptide epitope from chicken ovalbumin delivered on irradiated RMAS cells initiated a CTL response that resulted in the rejection of ovalbumin-expressing tumors and the generation of CTL with specificities for additional ovalbumin epitopes (intramolecular determinant spreading) as well as the generation of CTL with specificities for epitopes on distinct antigens (intermolecular determinant spreading; ref. 13). In a separate study, a single peptide, P1A, induced CTL that rejected P1A’ tumor challenge and subsequently yielded CTL activity and tumor protection against challenge with a P1A’ variant, which resulted from determinant spreading to a second defined epitope (P1E; ref. 14). These studies showed that peptide vaccines directed at the induction of CTL responses are capable of inducing tumor immunity and that determinant spreading occurs, resulting in the expansion of the immune response to additional epitopes in murine tumor models of tumor protection.

In the present study, we employed the aggressive, tumorigenic, and nonimmunogenic 4T1 murine mammary carcinoma model and SV40 large tumor antigen (Tag) as a model surrogate tumor antigen (sTA) to explore the possibility of inducing tumor-specific immunity through active immunization in the absence of a defined TAA and to address whether vaccination with a soluble whole protein would activate determinant spreading and tumor rejection in vivo. Mice immunized with sTA rejected challenge with 4T1
tumor cells modified to express the sTA, which led to subsequent rejection of parental 4T1 tumor cells. Rejection of parental 4T1 tumor formation seemed to be specific for and associated with unknown 4T1 TAA, because rejection of sTA-expressing unrelated mKSA kidney-derived tumor cells did not induce cross-protection against a subsequent contralateral challenge with parental 4T1 tumor cells. Moreover, in a tumor treatment experiment where mice were simultaneously inoculated in contralateral flanks with parental 4T1 tumor cells and sTA-expressing 4T1 tumor cells followed by sTA vaccination, 20% of the animals were tumor free at the completion of the experiment. Taken together, these data suggest that immunization with a whole, soluble protein-based sTA induces intermolecular determinant spreading, resulting in the induction of systemic tumor immunity and the subsequent rejection of s.c. tumor growth and prevention of the formation of lethal, spontaneous metastases.

Materials and Methods

Surrogate tumor antigen. Recombinant SV40 Tag served as a model sTA and was generated in the S99 insect cell line using the baculovirus AcNPV expression system. Recombinant Tag was extracted from insect cells and immunoaffinity purified by methods described previously (15). Purity was assessed by SDS-PAGE and silver staining (16) and the concentration was determined using an absorption coefficient at 280 nm for a 1% solution.

Mice and cell lines. Female, 6- to 8-week-old female BALB/c mice were purchased from the NIH (Frederick, MD). All animals were cared for and treated according to Institutional Animal Use and Care Committee guidelines at Texas Tech University Health Sciences Center (Lubbock, TX). The tumorigenic murine mammary carcinoma cell line 4T1 was a kind gift from Fred Miller (Karmanos Cancer Institute, Detroit, MI). The tumorigenic SV40-transformed BALB/c murine kidney cell line designated mKSA (17) was used as a control for tumor growth and immunity following sTA immunization (18). Cells were cultured in RPMI 1640 (Fisher Scientific, Pittsburgh, PA) supplemented with 10% heat-inactivated fetal bovine serum, 2 mM/l L-glutamine, 250 ng/mL fungizone, 50 IU/mL penicillin, 50 μg/mL streptomycin, 50 μg/mL gentamicin, and 10 mM/L HEPES. The 4T1.sTA cell line was generated by transfecting parental 4T1 tumor cells with a plasmid encoding SV40 Tag as a model sTA using LipofectAMINE Plus reagent (Invitrogen Corp., Carlsbad, CA) and methods described previously (19).

RNA isolation and reverse transcription-PCR. Briefly, total RNA was isolated from mKSA (sTA positive), 4T1.sTA (sTA transfected), P815 (sTA negative), and 4T1 (sTA negative) using Trizol reagent (BioWhittaker, Walkersville, MD) and 1 μg was reverse transcribed to obtain cDNA. The primers and PCR conditions were described previously (19). PCR results were visualized on a 2% agarose gel containing ethidium bromide; assessment of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression served as an internal reference control.

Western analysis. The cell lines mKSA, P815, 4T1.sTA, and 4T1 were grown to confluency in RPMI supplemented as described above. Cells were harvested and counted and whole cell protein lysates were prepared for 4T1, 4T1.sTA, P815, and mKSA at a concentration of 1 × 10⁶ cells equivalents/mL using methods described previously (20). Briefly, samples were loaded on a standard reducing SDS-PAGE (4% stacking, 7% resolving) and run at 200 V for 1 hour. Next, the gel-separated samples were electrophoretically transferred to nitrocellulose and the membrane was blocked in PBS containing 1% normal goat serum, 1% milk, and 0.02% Tween for 2 hours at room temperature. To visualize the expression of the model sTA (SV40 Tag), the membrane was probed with the anti-SV40 Tag monoclonal antibody (mAb) Pab 405 overnight at 4 °C (19) and then washed thrice with PBS-0.02% Tween, following which goat anti-mouse IgG-specific horseradish peroxidase reagent was added at a 1:2,000 dilution for 2 hours at room temperature. Finally, the membrane was washed and developed with 3,3'-diaminobenzidine reagent as a substrate (Sigma, St. Louis, MO).

Intracellular detection of surrogate tumor antigen in tumor cell lines. Intracellular detection of sTA in viable cell lines was done using a commercially available Cytofix/Cytoperm kit (BD Biosciences, San Diego, CA) with the following modifications. Briefly, actively growing tumor cell lines were harvested, adjusted to 500,000 cells/mL, washed twice with 1 mL ice-cold PBS, pelleted, and then resuspended in 875 μL ice-cold PBS. Next, ice-cold fixation solution (125 μL) was added to the cells followed by 1-hour incubation at 4 °C. Following incubation, cells were pelleted, the supernatant was aspirated, and 1× permeabilization/wash solution (1 mL) was added for 20 minutes at 37 °C in the dark. Permeabilization/wash buffer (1×) was used in all subsequent washes and dilutions to maintain permeabilization of cells. Following permeabilization, cells were washed once and pelleted, the supernatant was aspirated, and FITC-conjugated SV40 Tag–specific mAb (Pab 405, IgG1; 100 μL; 1 μg final concentration) or irrelevant FITC-conjugated antibody (IgG1) was added with incubation for 30 minutes at 4 °C in the dark. Finally, cells were washed, resuspended in 1× permeabilization/wash buffer (1 mL), and stored in the dark. All assays were analyzed by flow cytometry (FACSscan, Becton Dickinson, Franklin Lakes, NJ) within 2 hours of staining.

Immunization and tumor protection. Mice were immunized four times i.p. with sTA in alum at 1-week intervals at a concentration of 5 μg animal for the first three injections and a concentration of 15 μg animal for the final injection. Control groups of mice were immunized with either PBS or alum alone using identical methods and schedules (18). Mice in all groups were bled from the dorsal tail vein before immunization and 7 days following each immunization. Three tumor cell lines were used for s.c. tumor challenge at the following doses: mKSA (1 × 10⁶), 4T1.Tag (1 × 10³), and 4T1 (1 × 10⁴). Following harvest and counting, tumor cells were resuspended in versene (PBS-EDTA, Fisher Scientific) to prevent aggregation and injected s.c. in 100 μL. Primary challenge was given in the right flank with either mKSA (sTA positive) or 4T1.sTA followed 2 weeks later by a secondary challenge in the left flank of all animals with parental 4T1 tumor cells. Tumor size was recorded by measuring perpendicular angles using calipers every 2 to 3 days. Tumor volume (mm³) was calculated using the equation: \((a \times b^2)/2\), where \(b\) is the smaller of the two measurements. To evaluate metastasis, lungs were harvested and injected with an India ink solution and tumor nodules were enumerated as described below.

Immunoassay for detection of surrogate tumor antigen–specific antibodies. An ELISA was employed to detect the presence of sTA (Tag)–specific antibodies in the sera of mice following immunization using methods described previously (21, 22). Briefly, 96-well microtiter plates (Nunc Maxisorp, Fisher Scientific) were coated overnight at 4 °C with 50 μL of the model sTA, recombinant SV40 Tag, in PBS at a final concentration of 200 ng/well. Plates were blocked with 10% normal goat serum/PBS for 1 hour at 37 °C to prevent nonspecific binding. Next, 2-fold dilutions (beginning dilution,
and whether immunization with sTA protein in alum would result in rejection of 4T1.sTA tumor cells compared with parental 4T1 tumor cells. In brief, groups of five mice were immunized i.p. with 5 µg purified recombinant sTA in alum in a total volume of 100 µL once every 7 days for 3 weeks followed by 15 µg sTA in alum on the fourth week. Following immunization with sTA, mice were monitored for the induction of sTA-specific immunity by collecting serum samples and measuring anti-sTA antibody titers using methods and an ELISA generated in and reported by our laboratory (22). All three groups of mice (total of 15 mice) immunized with sTA in alum generated detectable IgG antibody titers specific for the sTA 2 weeks following the final immunization with near-identical end point titers of 1:2,000. In contrast, mice immunized with PBS or alum alone showed no detectable IgG antibody titers to the sTA when sera were analyzed by ELISA with the sTA on the solid phase.

Following immunization with sTA in alum, alum alone, or PBS, mice were challenged with either mKSA, parental 4T1, or 4T1.sTA at an inoculation dose of 5 x 10⁶, 1 x 10⁷, or 1 x 10⁸, respectively, and tumor growth was measured using calipers as described in Materials and Methods. Mice challenged with mKSA tumor cells following immunization with sTA in alum did not develop tumors (Fig. 2A), whereas mice that were immunized with alum alone or PBS developed tumors at approximately the same rate (Fig. 2A-C). All the mice challenged with parental 4T1 tumor cells following immunization with either sTA in alum, alum alone, or PBS developed tumors at similar rates regardless of the immunization they were given (Fig. 2B). Importantly, mice challenged with 4T1.sTA tumor cells following immunization with sTA in alum did not develop tumors compared with mice immunized with alum alone or PBS, which developed tumors at similar rates (Fig. 2C). These data establish that immunization with a soluble, whole protein sTA induces immune responses capable of rejecting lethal challenge with sTA-positive tumor cells as well as show that transfection of parental 4T1 tumor cells with the sTA had no effect on in vivo tumor growth of the 4T1 cell in immunized mice.

4T1 tumor cells spontaneously metastasize to the lungs forming lethal tumor burdens. To evaluate metastasis, lungs were harvested and stained with an India ink solution and tumor nodules were enumerated. S.c. 4T1.sTA tumor challenge following immunization with sTA in alum not only resulted in rejection of the s.c. tumor but also prevented the development of spontaneous lung metastases (Table 1). In contrast, if mice were immunized with sTA in alum and challenged with parental 4T1 tumor cells, lung tumor nodules were present ranging from 10 to 18 large visible tumor nodules (Table 1; mice had to be sacrificed by day 42 following primary challenge with parental 4T1 tumor cells due to morbidity associated with the tumor burden). Therefore, immunization with sTA in alum induced sTA-specific immunity that was capable of preventing tumor formation and metastasis by nonimmunogenic 4T1 tumor cells modified to express the model sTA.

Tumor protection as a result of surrogate tumor antigen–induced determinant spreading. To determine whether a sTA-based vaccine could be used to induce determinant spreading and rejection of indigenous tumors in the absence of known, defined TAA, groups of mice were immunized with sTA in alum and challenged with either mKSA tumor cells or 4T1.sTA tumor cells (as described for Fig. 2). Mice that were immunized with sTA in alum and rejected a primary s.c. challenge with the control sTA-positive, kidney-derived mKSA tumor cell line failed to reject a subsequent s.c. challenge with parental 4T1 tumor cells given 2 weeks later in...
the opposite flank (Fig. 3A). However, for the group of mice immunized with sTA in alum that rejected a primary s.c. challenge with 4T1.sTA tumor cells, subsequent rejection of a secondary s.c. challenge with parental 4T1 tumor cells given 2 weeks later in the opposite flank was observed (Fig. 3B). These data suggest that mice were protected from mKSA and 4T1.sTA tumor growth in the primary challenge due to the specific immune response that was generated following immunization with sTA in alum as seen in Fig. 2A and C and that protection from secondary challenge with parental 4T1 mammary tumor cells (Fig. 3B) was due to cross-presentation of unknown TAA shared by 4T1.sTA and parental 4T1 tumor cells resulting in determinant spreading and rejection of parental 4T1 tumor cells. This was supported by the observation that rejection of primary challenge with the unrelated kidney-derived mKSA tumor cells, although sTA-based vaccine driven, had no effect on 4T1 mammary-derived tumor cell growth (Fig. 3A). Thus, the initial immune response induced by immunization with sTA in alum was specific for the model sTA and enabled mice to reject 4T1.sTA tumor challenge resulting in the initiation of determinant spreading and cross-priming to unknown TAA in 4T1 tumor cells, providing protection against secondary s.c. challenge with parental 4T1 tumor cells.

In addition to sTA-based, vaccine-induced protection against s.c. challenge with parental 4T1 tumor cells as a result of apparent determinant spreading, immunization with sTA in alum prevented the formation of spontaneous 4T1 lung metastases in mice that rejected a primary s.c. challenge with 4T1.sTA tumor cells. Mice immunized with sTA in alum that rejected a primary s.c. challenge with mKSA tumor cells did not reject the formation of parental 4T1 spontaneous lung metastases following the secondary s.c. challenge with parental 4T1 tumor cells (Table 2; Fig. 3C, c). However, when mice were immunized with sTA in alum and rejected a primary s.c. challenge with 4T1.sTA, a secondary s.c. challenge with parental 4T1 tumor cells was rejected and the formation of spontaneous metastases was prevented.

Figure 1. Expression of the model sTA in murine tumor cells. A, RT-PCR demonstrating expression of the model sTA (SV40 Tag) in 4T1 murine tumor cells following transfection with a plasmid encoding the full-length cDNA for SV40 Tag. mKSA is a SV40-transformed, SV40 Tag–positive murine kidney-derived tumor of BALB/c origin and served as a positive control. P815 is a Tag-negative murine tumor cell line. GAPDH served as an internal reference control. B, Western blot analysis of murine tumor cell lysates generated from 1 × 10⁸ cell equivalents demonstrating protein expression of sTA using a mAb (Pab 405) with specificity for the model sTA (SV40 Tag). The model sTA typically blots as two bands ~90 kDa. mKSA is a sTA-positive murine kidney-derived tumor of BALB/c origin and served as a positive control. The sTA-negative tumor cell line P815 served as a negative control. GAPDH served as a control for protein loading. C, expression of sTA protein by flow cytometry. Intracellular sTA detection was accomplished using a Cytofix/Cytoperm reagent and a mAb (Pab 405) with specificity for SV40 Tag. Left, staining of mKSA tumor cells as a positive control; center, lack of staining of parental 4T1 tumor cells; right, staining of 4T1 tumor cells modified to express the model sTA (SV40 Tag). Solid, fluorescence of cells alone; solid line, staining with an isotype-matched control antibody (IgG); dashed line, staining with the FITC-conjugated sTA (SV40 Tag)–specific mAb (Pab 405).
parental 4T1 lung metastases was prevented (Table 2; Fig. 3C, d). Strikingly, no 4T1 tumor nodules were visible in the lungs of mice immunized with sTA in alum followed by rejection of a primary s.c. challenge with 4T1.sTA tumor cells (Fig. 3C, d). In contrast, parental 4T1 lung tumor nodules ranged from 2 to 17 in mice that rejected a primary s.c. challenge with parental 4T1 tumor cells (Table 2). These results suggest that unknown TAA present in parental 4T1 tumor cells play an important role in rejecting parental 4T1 tumor cell challenge via recognition of unknown TAA expressed by parental as well as sTA-modified 4T1 tumor cells.

Treatment of established 4T1 tumors as a result of surrogate tumor antigen–induced determinant spreading. Finally, we evaluated whether vaccinating with a model sTA would have an effect on established tumors in a treatment scenario. Mice were inoculated s.c. simultaneously with 4T1 and 4T1.sTA tumor cells in contralateral flanks. Three days after tumor inoculation, animals in each group were immunized i.p. with sTA in alum or alum alone on days 3, 10, 17, and 24. Growth of the tumors on each flank was monitored and measurements were taken with calipers as described in Materials and Methods. Given that it

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NOTE: Mice were immunized i.p. with sTA in alum four times at 1-week intervals and challenged s.c. with tumor cells 14 days following the last immunization. All values represent s.c. tumor growth (mm³) and number of lung metastases for individual mice.

* S.c. tumor size was determined with calipers, measuring perpendicular angles.

† Lungs were harvested when s.c. tumor size reached ~1 × 1 cm or by the day indicated. Tissue was stained with India ink solution and destained with Fekete's solution and tumor nodules were counted and recorded.
takes ~10 to 14 days to induce immunity following immunization, this represents a 13- to 17-day established tumor, albeit minimal disease. Immunization with sTA in alum resulted in the cure of 4T1.sTA tumors in five of five mice (Fig. 4C), whereas alum had no significant effect on 4T1.sTA s.c. tumor growth (data not shown). Mice that were immunized with alum alone also failed to regress 4T1 parental tumors in the opposite flank (Fig. 4D). Strikingly, two of five mice were cured and two additional mice had a significant reduction in tumor growth with delayed onset of their parental 4T1 tumors when immunized with the sTA and simultaneously inoculated with 4T1.sTA and 4T1 tumor cells (Fig. 4B). All five mice in this group were cured of their right flank sTA-expressing 4T1 tumors (Fig. 4C) and 80% (4 of 5) had a complete or partial response against their parental 4T1.sTA-negative left flank tumors (Fig. 4B), demonstrating that it may be possible to treat established disease with sTA-based vaccination to induce determinant spreading to unknown TAA expressed by indigenous tumors, represented herein by parental 4T1 tumor cells.

**Discussion**

No other form of medical intervention has had a greater impact on world health than vaccination. The identification of tumor rejection antigens and the genes that encode them, with the development of relevant animal tumor models involving the very same antigens, should provide the information and opportunity necessary to bring strategies for active immunization against many...
forms of human cancer into the clinic. In the past 5 to 7 years alone, great strides have been made toward the definition of relevant molecular targets on human tumor cells that may serve as TAAs for immunotherapy and vaccine development. Active vaccination against cancer is a treatment approach that has proven effective in preclinical murine models. However, vaccines have been difficult to exploit for highly tumorigenic and poorly immunogenic tumors, such as the 4T1 mammary carcinoma model for which no TAAs have been reported. We hypothesized that in the absence of known TAA it is possible to induce tumor-specific immunity using a vaccine that is composed of a sTA to induce cross-presentation of unknown TAA and tumor-specific immunity as a result of intermolecular determinant spreading. To address this, we used the 4T1 murine mammary carcinoma model and SV40 Tag as a sTA to induce tumor-specific immunity and subsequent cross-presentation of unknown mammary carcinoma-associated antigens.

The observation that T cells could be primed against antigens carried by cells lacking the proper MHC necessary to be recognized by T cells was first reported by Bevan (24). Since the first reports, the phenomenon has been extensively studied in models of autoimmunity, infectious disease, and, to a lesser degree, cancer (25). It has been shown that cross-presentation is mediated by professional, bone marrow–derived antigen-presenting cells and most potently by dendritic cells (26). For example, as tumor cells die or are actively destroyed, they release their complement of antigens into the microenvironment. These antigens are captured and processed by dendritic cells, which then migrate to regional lymph nodes to present the processed antigens to naive T cells. Once primed and activated, these T cells are capable of finding intact tumor cells that share the antigen and the appropriate MHC, recognize the tumor cells, and destroy them. The proper maturation and activation of the dendritic cells is required before they are capable of priming naive T cells. The microenvironment and cytokines necessary and capable of dendritic cell activation are best elicited by infectious agents or danger signals induced by infectious agents. Our model sTA is a viral protein and as such

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NOTE: Mice were immunized i.p. with sTA in alum four times at 1-week intervals and challenged s.c. with tumor cells following the last immunization. All values represent the number of lung metastases for individual mice. *Mice challenged with mKSA following sTA immunization had no effect on subsequent 4T1 tumor growth or the formation of spontaneous 4T1 lung metastases. †Mice challenged with 4T1.sTA following immunization rejected parental 4T1 tumor growth and prevented the formation of spontaneous 4T1 lung.

Table 2. Effect of 4T1.sTA and mKSA rejection following sTA immunization on the formation of spontaneous 4T1 lung metastases

Figure 4. sTA-induced rejection of established 4T1 tumors. Mice were simultaneously challenged s.c. in contralateral flanks with 4T1.sTA and 4T1 tumor cells and immunized on days 3, 10, 17, and 24 with either sTA in alum or alum alone. Tumor development was monitored and recorded. A, treatment of 4T1 tumor cells as a result of immunization with alum alone (n = 4). B, treatment of 4T1 tumor cells as a result of immunization with sTA in alum (n = 5). C, treatment of 4T1.sTA tumor cells as a result of immunization with sTA in alum (n = 5). Representative of two separate experiments.
possesses the immunogenicity required to induce an immunologic environment that would be optimal for antigen cross-presentation and subsequent determinant spreading. Previous studies by other investigators employing the 4T1 tumor model showed reduction in metastases of 4T1 (30–32), prolong survival (30, 32, 33), and a slowing in growth of primary tumors (6, 34); however, few reported cure of the primary tumor or complete protection from spontaneous lung metastasis. Using the sTA-based vaccine, we were able to elicit a potent immune response that resulted in complete protection from parental 4T1 tumor challenge and rejection of the formation of lethal, spontaneous lung metastases. Moreover, we were able to cure 4T1 tumor cells and further evaluate the role of antibody as a mechanism in rejecting sTA-expressing tumors following sTA protein immunization.

Others have approached the idea of vaccinating against cancer by using hapten-coated tumor cells or allo-MHC molecules as the foreign antigen (35, 36) called xenogenization (37, 38). Haptens are not as immunogenic as whole foreign proteins and the mechanisms of immunity may be different for allo-MHC than for foreign protein antigens. These approaches largely centered on administration of the modified or xenogenized tumor cells as the vaccine and were minimally effective. Whole cell xenogenized tumor vaccines may not elicit the proper immune response necessary to induce potent tumor immunity via cross-presentation and determinant spreading, which is likely why these approaches have largely been abandoned. A variation on this approach was to mutagenize the nonimmunogenic tumor cells, immunize with the mutant cells, and challenge with parental tumor cells. Van Pel and Boon showed that rejection of a nonimmunogenic thymic leukemia cell line could be accomplished if the challenge followed immunization with the same cell line that was treated with the mutagen N-methyl-N-nitro-N-nitrosoguanidine (39); this approach led to the discovery of the first human melanoma-associated antigen.

Cross-presentation in human cancer vaccine studies has been reported and is believed to be important in rejection of cancer in patients following immunotherapy. Thomas et al. showed the first direct evidence that T-cell responses can be generated via cross-presentation in pancreatic cancer patients vaccinated with pancreatic cancer cell lines engineered to secrete granulocyte macrophage colony-stimulating factor. Three patients generated MHC-I-restricted T-cell responses against epitopes in the TAA mesothelin carried by the vaccine cancer cell lines, although none of the patients possessed the MHC-I of the vaccine cancer cell line (40). In addition, human monocyte-derived dendritic cells were shown to be more effective at cross-presentation in association with patients with head and neck cancer when loaded with apoptotic tumor cells if proinflammatory cytokines or CD40L was included in the preparation (41).

Our approach is unique in that it employs the use of a defined, foreign protein as a sTA-based vaccine to drive determinant spreading to unknown TAA. By vaccinating with a nonsensf sTA, an immune response is induced that focuses the proper immunologic mechanism(s) and environment on the target tumor cells, enabling the potent cross-priming of immune cells for the subsequent rejection of the indigenous/parental tumor.
References


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