Improved Tumor Immunity Using Anti-Tyrosinase Related Protein-1 Monoclonal Antibody Combined with DNA Vaccines in Murine Melanoma

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Abstract

Passive immunization with monoclonal antibody TA99 targeting melanoma differentiation antigen tyrosinase-related protein-1 (Tyrp1; gp75) and active immunization with plasmid DNA encoding altered Tyrp1 both mediate tumor immunity in the B16 murine melanoma model. We report here that TA99 enhances Tyrp1 DNA vaccination in the treatment of B16 lung metastases, an effect mediated by immunologic mechanisms as Tyrp1 has no known role in regulating tumor growth. TA99 is shown to increase induction of anti-Tyrp1 CD8+ T-cell responses to DNA vaccination against Tyrp1 as assessed by IFN-γ ELISPOT assays. Immunohistochemistry studies reveal that TA99 localizes rapidly and specifically to B16 lung nodules. Augmentation of T-cell responses is dependent on the presence of tumor as well as on activating Fc receptors. Furthermore, TA99 enhances DNA vaccination against a distinct melanoma antigen, gp100(pmel17/silver locus), improving antitumor efficacy, augmenting systemic CD8+ T-cell responses to gp100, and increasing CD8+ T-cell infiltration at the tumor site. Epitope spreading was observed, with CD8+ T-cell responses generated to Tyrp1 peptide in mice receiving gp100 DNA vaccination in the presence of TA99. Finally, we show that TA99 improves therapeutic efficacy of DNA vaccination combined with adoptive T-cell transfer in treatment of established subcutaneous B16 melanoma. In conclusion, TA99 enhances DNA vaccination against both the target antigen Tyrp1 and a distinct melanoma antigen gp100 in an Fc receptor–dependent mechanism, consistent with enhanced cross-presentation of tumor-derived antigen. Monoclonal antibodies should be tested as vaccine adjuvants in the treatment of cancer. [Cancer Res 2008;68(23):9884–91]

Introduction

Cancer immunotherapy is a difficult challenge both because of the "self" nature of antigens found on tumors and the ability of cancer to actively evade protective immune responses through mechanisms such as regulatory T-cell recruitment, MHC antigen down-regulation, and production of immunosuppressive cytokines (1, 2). Much effort has been concentrated on achieving high circulating frequencies of antitumor CD8+ T cells. There is substantial evidence, however, that T cells recognizing cancer antigens, even when present in high numbers, are insufficient to reject established tumors (3). One strategy to improve T cell–based immunotherapy is to combine it with antibodies targeting antigens relevant to a specific tumor type (4, 5).

Antibodies modulate T-cell responses in infectious disease, autoimmune, and cancer through Fc domain interactions with surface receptors on antigen presenting cells (6). Dendritic cells (DC) pulsed with antigen-antibody complexes (immune complexes, IC) containing ovalbumin are a more effective vaccine against ovalbumin expressing B16 than are DCs pulsed with ovalbumin alone (7). Anti–her-2/neu monoclonal antibody (mAb) therapy has moreover been shown, in vivo, to improve uptake of cellular vaccines, and subsequent antigen presentation by DCs to CD8+ T cells (8, 9).

In this study, we define a new role for mAb TA99 as an adjuvant for therapeutic DNA vaccination against B16 melanoma, a spontaneously arising, poorly immunogenic transplantable tumor. TA99 is an IgG2a murine antibody directed against tyrosinase-related protein-1 (Tyrp1), of identical specificity to an antibody isolated from the serum of a melanoma patient (10). TA99 protects mice from synchronous administration of B16 melanoma in an Fc receptor–dependent fashion but loses efficacy against established tumors (11). In this study, TA99 is combined with DNA vaccines against the target antigen Tyrp1 and against distinct melanosomal antigen gp100 (12–14). These vaccines abrogate tolerance by inducing T cells reactive against altered antigen that then cross-react with native epitopes. Vaccination protects against subsequent tumor inoculation but is less effective in the treatment of established B16 lung metastasis (Fig. 1B). We chose to test combination therapy in a therapeutic model because it is more relevant to human cancer and because recent studies have shown that antitumor mAb can stimulate CD8+ T-cell responses against the target antigen in some patients (15).

We report here that TA99 synergizes with DNA vaccination against target antigen Tyrp1 in the treatment of established B16 lung metastases and also enhances CD8+ T-cell responses in an Fc receptor–dependent fashion. We find that TA99 binds rapidly and specifically to B16 tumor cells in vivo, and augmentation of CD8+ T-cell responses depends on presence of tumor, suggesting that cross-presentation of antigen by TA99 is important. Furthermore, TA99 improves DNA vaccination against a distinct melanoma antigen gp100, improving CD8+ T-cell responses against gp100 and inducing epitope spreading from gp100 to Tyrp1. Finally, TA99 enhances adoptive T-cell transfer combined with DNA vaccination in the treatment of subcutaneous B16. These findings implicate a broad role for mAbs as adjuvants for vaccination against cancer.
control antibody W6/32 (18) was affinity-purified from hybridoma supernatant by the same facility. In some experiments, TA99 and W6/32 were labeled with Alexa Fluor 488 Protein Labeling kit (Invitrogen/Molecular Probes). Pooled IgG from rodent serum was not used as a control, due to the presence of a minority sialylated species in serum, which binds inhibitory Fc receptors with high affinity and can suppress immune responses (19, 20).

Plasmid constructs. The gp100 DNA vaccine is a human gp100 (hgp100) expression vector consisting of full-length hgp100 cDNA cloned into the WRG/BEN vector, as previously described (13). The Tyrp1 DNA vaccine is epitope enhanced Tyrp1 fused to the VP22 construct as described (21).

DNA immunization. DNA was administered using a gene gun as previously reported (22). Briefly, 1-μm gold particles (Alfa Aesar) were coated with plasmid DNA and precipitated onto Teflon tubing. Tubing containing 1 μg of DNA were loaded into a gene gun (Accell; PowderMed) and administered to each abdominal quadrant at 400 pounds/inch².

Peptides and ELISPOT. Peptides analyzed, including gp100/pmel 17 peptide gp10025–33, Tyrp1455–462, and Ova257–264 (SIINFEKL), were synthesized by Genemed Synthesis at >80% purity, confirmed by high performance liquid chromatography. Spleens harvested 4 to 5 d after the third DNA injection were mechanically disrupted, and RBCs were lysed. CD8+ T cells were positively selected by incubation with magnetic anti-CD8 beads (Miltenyi Biotec), and IFN-γ production was determined by standard ELISPOT assay (12) after 20 to 36 h of incubation of CD8+ T cells (10⁵ per well) with EL4-targeted cells pulsed with 1 μg/ml of peptide. Plates were analyzed using an automated ELISPOT reader system with KS 4.3 software (Carl Zeiss).

Immunohistochemistry. Dissected lungs were snap frozen in optimum cutting temperature. Three-micrometer sections were either visualized fresh or fixed in acetone and stained with the following antibodies from BD Pharmingen: anti-CD4 FITC (RM4-5), anti-CD8 FITC (53–67), or, from Caltag/Invitrogen, anti-F/480 APC (BM8). All sections were counterstained with 4',6-diamidino-2-phenylindole (DAPI) nuclear stain. For quantification of infiltrating CD8+ or CD4+ T cells, tumor nodules were located using H&E staining, and labeled cells were counted in fields located at the center of the tumor. Samples were visualized using a Carl Zeiss Axioplan 2 imaging upright fluorescence microscope.

Adoptive transfer experiments. Animals were inoculated s.c. with 12,500 B16 cells and, 7 d later, irradiated (600 Gy whole body irradiation from a 137Cs source) followed by adoptive transfer of 30 million splenocytes with or without 50,000 CD8+ cells isolated from spleen and lymph nodes of pmel-1 TCR transgenic animals (17) by negative selection according to the manufacturer’s instructions (Easysep). Mice received three weekly treatments with TA99, gp100 DNA vaccine, or both beginning on day 8, and tumor growth was monitored by biweekly measurement.

Statistics. Statistical analysis was performed using Prism Software (GraphPad Software, Inc.). Survival analysis was performed using the log-rank (Mantel-Cox) test. Differences were considered statistically significant at P < 0.05, using a two tailed Student’s t test.

Results
TA99 improves vaccination with optimized Tyrp1 DNA. We have previously shown that tolerance to Tyrp1 can be overcome by rational optimization of peptide anchor residues, favoring MHC Class I binding. The Tyrp1 vaccine used in this study consists of a DNA plasmid encoding epitope-enhanced Tyrp1 mutated at 10 residues, including mutation A463M, creating the immunodominant 455 to 463 H2-Dk restricted epitope (12). The full-length construct is fused to VP22, a herpes simplex virus protein known to enhance DNA vaccination through intercellular spreading or other mechanisms (21). Immunization protects mice from subsequent inoculation with B16 melanoma but has limited efficacy against established tumors (Fig. 1B). MAbs enhance CD8+ T-cell responses in autoimmunity and cancer, and our optimized Tyrp1 DNA vaccine is CD8+ T cell dependent (12). We therefore tested the
hypothesis that TA99 (anti-Tyrp1) improves DNA vaccination against Tyrp1 in the treatment of B16 melanoma. Mice were injected via the tail vein with B16 melanoma, and therapy was subsequently initiated with DNA vaccine, TA99, or a combination, as shown in the schema in Fig. 1A, followed by quantification of lung metastases and T-cell responses against the immunodominant Tyrp1_{455-463} D^b restricted epitope.

Quantitation of surface lung metastases revealed a significant reduction in tumor burden in mice receiving both TA99 and Tyrp1 DNA vaccine compared with mice given either treatment alone (Fig. 1B). As measured by IFN-γ ELISPOT assay, there was a 2-fold increase in the CD8+ T-cell response against Tyrp1_{455-463} in the spleens of animals receiving combination therapy (Fig. 1C). No significant anti-Tyrp1 CD8+ T-cell responses were induced by antibody alone. These data show that TA99 significantly improves DNA vaccination against the target antigen Tyrp1 in the treatment of B16 melanoma lung metastases, and that antitumor efficacy correlates with an enhanced Tyrp1 CD8+ T-cell response.

TA99 binds specifically to B16 tumors, and the CD8+ T-cell response mediated by TA99 requires presence of the tumor. To investigate localization of TA99 in vivo, Alexa Fluor 488–labeled TA99 (TA99-a488) was injected into tumor-bearing mice. Twenty-four hours later, animals were sacrificed and frozen sections of lung tissue examined using fluorescence microscopy. Antibody TA99, but not isotype control W6/32 (W6/32-a488), localized rapidly and specifically to tumor nodules (Fig. 2A). Surprisingly, TA99 was visualized within the cytoplasm of tumor cells (Fig. 2B). Antibody also accumulated within tumor-infiltrating F4/80+ macrophages (Fig. 2C). We hypothesized that the observed antibody binding to the tumor is required for enhancement of CD8+ T-cell responses to the target antigen Tyrp1. To test this, tumor-bearing and nontumor-bearing mice were treated as in Fig. 1A and T-cell responses measured using an IFNγ ELISPOT assay (Fig. 2D). No significant difference in CD8+ T-cell responses to the vaccine were found in nontumor-bearing mice, based on whether or not they received TA99. There was, however, a doubling of CD8+ T cells in positive controls bearing B16 tumor. Baseline responses to the vaccine were depressed in tumor-bearing animals relative to nontumor bearers. These results show that TA99 binds rapidly and specifically to B16 tumor nodules, and that the antibody is present within tumor-infiltrating myeloid cells. Furthermore, we show a requirement for the presence of tumor for enhancement of anti-Tyrp1 CD8+ T cells by TA99.

TA99-mediated enhancement of therapeutic Tyrp1 vaccination is Fc receptor dependent. The antitumor effect of TA99 in the prophylactic setting is abrogated in mice deficient in Fc receptors (23–25). Antibodies also exert immunomodulatory effects through ligation of complement receptors and other mechanisms (26). We therefore tested whether the immunomodulatory properties of TA99 are dependent on Fc receptors. Mice deficient in the FcR common γ chain (FcRγ−/−), and therefore unable to express activating Fc receptors I, III, and IV, were treated as per protocol (Fig. 1A). FcRγ−/− animals did not benefit from therapy with TA99 in combination with DNA vaccination (Fig. 3A). Surprisingly, FcRγ−/− animals developed a higher tumor burden at the same dose of tumor cells than did wild-type controls.

Figure 2. TA99 localizes to B16 lung metastasis in vivo, and presence of the tumor is required for TA99-mediated enhancement of T-cell responses. A, distribution of TA99 in lungs of tumor-bearing mice. Alexa Fluor 488–labeled TA99 (green) was injected i.p. into animals bearing day 19 B16 lung metastases. Twenty-four hours later, fresh-frozen sections of lung tissue were counterstained with DAPI nuclear stain (blue) and examined by fluorescence microscopy (× 10). Distribution of isotype control mAb W6/32 (green) is shown for comparison. B, accumulation of TA99 (green) injected i.p. in vivo within the cytoplasm of tumor cells (×100). C, colocalization of Alexa Fluor 488–labeled TA99 (green) with macrophage marker F4/80 APC (pink; ×40). Staining with isotype control rat IgG2a was negative (data not shown). D, presence of tumor is required for TA99-mediated enhancement of CD8+ T-cell responses. Tumor-bearing and nontumor-bearing animals (5–10 per group) were treated as in schema Fig. 1A with control animals receiving PBS injection or empty vector DNA, and an IFNγ ELISPOT assay was performed on pooled CD8+ splenocytes. TA99 significantly enhanced response to the vaccine in tumor-bearing animals (P = 0.016) but not in nontumor bearers (P = 0.15). Nontumor-bearing animals had significantly higher baseline responses to the vaccine. This result is representative of two experiments.
sugestng a role for Fc receptors in cancer immune surveillace. T-cell responses were assessed by ELISPOT in each individual animal (Fig. 3B) and TA99 did not enhance T-cell responses in FcRγ−/− animals, in contrast to the effect observed in wild-type controls. These data show that Fc receptors are required for mAb TA99-mediated enhancement of CD8+ T-cell responses.

**TA99 enhances antitumor therapeutic efficacy of gp100 DNA vaccination in an Fc receptor--dependent manner.** Based on our results from Fig. 2, we concluded that TA99 enhances DNA vaccination through ligation of Tyrp1 on tumor cells rather than by binding exclusively to the protein product of the vaccine. B16 melanoma expresses other antigens besides Tyrp1, and we hypothesized that TA99 improves presentation of these antigens through enhanced uptake of antibody-bound tumor debris. We and others have previously shown that xenogeneic immunization with hgp100 DNA induces reactivity against the native mouse peptide, gp10025-33, and protects animals from subsequent B16 tumor inoculation (13, 14). To determine whether TA99 improves DNA vaccination against gp100 as it does against Tyrp1, mice were treated as in Fig. 1A except that they were vaccinated against gp100 instead of Tyrp1. Mice receiving combination therapy had a significant improvement in tumor burden, whereas animals treated with either agent alone did not (Fig. 4A). To assess the role of Fc receptors, mice deficient in activating Fc receptors were vaccinated with gp100 in the presence or absence of TA99. Wild-type animals showed significant enhancement of the antitumor efficacy of gp100 against Tyrp1455-463 and Ova257-264 was measured by IFNγ ELISPOT assay. Mice (three per group) were treated as in Fig. 1A but with gp100 DNA instead of Tyrp1 vaccine. Control animals received PBS injection or empty vector DNA. At 23 d, surface lung metastases were quantified. Only the combination group showed a statistically significant decrease in tumor burden relative to untreated controls (P = 0.040), whereas FcRγ−/− animals did not (P = 0.89). C, CD8+ T-cell responses measured by ELISPOT assay. Mice (three per group) were treated as in A, and an IFNγ ELISPOT was performed on CD8+ cells from pooled splenocytes using targets pulsed with murine gp10025-33, Tyrp1455-463, or Ova257-264. TA99 enhanced T-cell responses to gp100, and T-cell reactivity against Tyrp1 was also observed in the combination group but not in any of the other groups. Results in C are representative of two experiments. D, assessment of epitope spreading in individual mice. Mice (n = 5) were treated with gp100 DNA vaccination and TA99 or gp100 DNA vaccination and isotype control mAb W6/32 and reactivity against Tyrp1455-463, and Ova257-264 was measured by IFNγ ELISPOT. Two of five animals in the group receiving TA99 and gp100 vaccine displayed reactivity against Tyrp1, whereas no animals receiving vaccine alone did, consistent with pooled data in C. No significant reactivity was detected against Ova257-264 in any group (data not shown).

**Figure 3.** TA99-mediated enhancement of DNA vaccination against target antigen Tyrp1 is Fc receptor dependent. A, quantification of lung metastases. Mice (n = 8–9), deficient in the FcRγ−/− and wild-type animals, were treated as per schema in Fig. 1A, with control animals receiving isotype control W6/32 or empty vector DNA. Combination therapy with antibody and vaccine yielded benefit in wild-type animals (P = 0.0028) but not in Fc receptor–deficient animals (P = 0.91). FcRγ−/− animals developed significantly more lung metastases than wild-type animals, regardless of treatment group. B, CD8+ T-cell responses. Splenocytes from individual mice from A were harvested, and IFNγ ELISPOT assay was performed on CD8+ cells as in Fig. 1. TA99 significantly enhanced T-cell responses to Tyrp1 in wild-type animals (P = 0.012) but not in FcRγ−/− mice (P = 0.70). CD8+ splenocytes from animals treated with TA99 alone were pooled based on gender and ELISPOT assay revealed no significant reactivity to Tyrp1.

**Figure 4.** TA99 enhances gp100 DNA vaccination in an Fc receptor--dependent manner and mediates epitope spreading to Tyrp1. A, quantification of lung metastases in wild-type animals treated with gp100 DNA vaccination, TA99, or both. Mice (n = 12–14) were treated as per schema Fig. 1A but with gp100 vaccine instead of Tyrp1 vaccine. Control animals received PBS injection or empty vector DNA. At 23 d, surface lung metastases were quantified. Only the combination group showed a statistically significant decrease in tumor burden relative to untreated controls (P = 0.040). B, quantitation of lung metastases in FcRγ−/− mice. Mice, (n = 5–6) were treated with either gp100 DNA or gp100 DNA plus TA99 combination therapy. Wild-type mice had a significant (P = 0.016) decrease in surface lung metastases with combination therapy, whereas FcRγ−/− animals did not (P = 0.89). C, CD8+ T-cell responses measured by ELISPOT assay. Mice (three per group) were treated as in A, and an IFNγ ELISPOT was performed on CD8+ cells from pooled splenocytes using targets pulsed with murine gp10025-33, Tyrp1455-463, or Ova257-264. TA99 enhanced T-cell responses to gp100, and T-cell reactivity against Tyrp1 was also observed in the combination group but not in any of the other groups. Results in C are representative of two experiments. D, assessment of epitope spreading in individual mice. Mice (n = 5) were treated with gp100 DNA vaccination and TA99 or gp100 DNA vaccination and isotype control mAb W6/32 and reactivity against Tyrp1455-463, and Ova257-264 was measured by IFNγ ELISPOT. Two of five animals in the group receiving TA99 and gp100 vaccine displayed reactivity against Tyrp1, whereas no animals receiving vaccine alone did, consistent with pooled data in C. No significant reactivity was detected against Ova257-264 in any group (data not shown).
DNA vaccination in the presence of TA99, whereas mice deficient in the FcR common γ chain did not (Fig. 4B). These experiments show that TA99 enhances the therapeutic efficacy of DNA vaccination against gp100, in an Fc receptor–dependent fashion.

**TAA99 increases CD8+ T-cell responses to gp100 and mediates epitope spreading to Tyrp1.** TA99 binds tumor in vivo, and we reasoned that the antibody would also enhance presentation of gp100 to CD8+ T cells. Mice were treated as in Fig. 1A and an ELISPOT assay was performed using peptides gp10025-33 and Tyrp1455-63 (Fig. 4C). CD8+ T-cell responses to gp100 were heightened by TA99, and, surprisingly, reactivity to Tyrp1 was observed in the combination group, despite the fact that these animals had not been vaccinated against Tyrp1. Reactivity against Tyrp1455-63 was present in the pooled animals at lower levels than reactivity against gp100, either because all animals reacted less vigorously to Tyrp1, or because only some animals in the group displayed reactivity against this antigen. An experiment performed on individual mice (Fig. 4D) revealed that two of five animals treated with TA99 and gp100 DNA had strong CD8+ T-cell responses against Tyrp1455-63, compared with zero of five in animals receiving gp100 vaccination alone. These data show that TA99 improves CD8+ T-cell responses to gp100 and mediates epitope spreading to Tyrp1.

**Combination therapy with TA99 and gp100 vaccination enhances tumor infiltration by CD8+ T cells but not CD4+ T cells.** We next sought to determine whether the enhancement of systemic reactivity against tumor antigens correlates with alterations in patterns of T-cell infiltration into tumor. In these studies, CD8+ and CD4+ cells in high power fields (×20) within tumor nodules were counted. Results, shown in Fig. 5A, show a doubling in the density of tumor infiltrating CD8+ lymphocytes in the combination therapy group compared with either antibody or vaccine alone, whereas the number of CD4+ infiltrating lymphocytes was similar in all treated mice. These results show that enhanced therapeutic efficacy of combination therapy with TA99 and gp100 vaccination correlates not only with higher levels of systemic reactivity to antigens expressed by B16 but also with higher levels of CD8+ T-cell infiltration at the tumor site. We conclude that, in the context of vaccination, mAb TA99 enhances infiltration of the tumor by CD8+ T cells, further supporting an immunomodulatory function for TA99 in the generation of an effective antitumor CD8+ T-cell response.

**TAA99 improves the efficacy of DNA vaccination combined with adoptive T-cell transfer in the treatment of established subcutaneous B16 melanoma.** Human melanomas generally arise first in skin, and we therefore studied whether TA99 could enhance DNA vaccination in the treatment of subcutaneous B16. B16 grows very rapidly in subcutaneous tissues, producing large tumors as early as 10 d after injection. The treatment schema described in Fig. 1A is of 25-d duration and was therefore ineffective in the treatment of subcutaneous B16 as animals developed large tumors necessitating sacrifice before the vaccinations were complete (data not shown). A more effective vaccination strategy to treat established subcutaneous tumors using adoptively transferred gp100-specific CD8+ T cells has been developed in our laboratory, and the protocol and mechanism is detailed in a separate publication.5 We tested whether TA99 could enhance efficacy of this treatment regimen. Animals bearing day 7 tumors were treated with a combination of TA99 and adoptive transfer of splenocytes mixed with gp100-specific CD8+ T cells derived from pmel-1 TCR transgenic animals (17) followed by 3 cycles of DNA vaccination against gp100. As shown in Fig. 6, TA99 significantly enhances the therapeutic efficacy of vaccination after irradiation and adoptive T-cell transfer. Control animals receiving TA99 in the absence of vaccine were infused with splenocytes to account for the transfer of naïve cells into a lymphopenic host. Intriguingly, the adoptive transfer significantly enhanced the therapeutic potency of TA99, which is generally not very effective in treating established subcutaneous tumors. This finding may be attributed to the 600 cGy of irradiation included in the adoptive transfer protocol, and is consistent with known synergy between mAbs and cytotoxic therapies (27). In summary, data presented here shows that TA99 enhances T-cell–based immunotherapy of subcutaneous B16 murine melanoma.

**Discussion**

Vaccination is generally not potent enough to treat patients with established cancer. A first step toward generating an efficacious
vaccine in humans is to develop one that is beneficial in animals bearing established aggressive tumors such as B16 melanoma. A vaccine that is an effective prophylactic generally fails in a tumor-bearing host because the tumor itself alters the immunologic milieu, crippling nascent antitumor immune responses. We have combined passive administration of mAb TA99 with DNA vaccination against the same antigen, Tyrp1, and found this combination to be highly effective therapy for B16 lung metastasis (Fig. 1B). Tyrp1, the target of TA99, is a differentiation antigen without known oncogenic function expressed by human and murine melanomas, and also by normal melanocytes (10, 28). The therapeutic efficacy of TA99, therefore, unlike that of anti-her2 antibody (9) or anti–epidermal growth factor receptor antibody (29, 30), cannot be attributed to interruption of oncogenic signaling but rather exclusively to immunologic activity. Thus, we show that mAb against a tumor antigen is an effective vaccine adjuvant in treatment of a poorly immunogenic tumor.

The observed therapeutic interaction between TA99 and vaccine can be explained most simply by a combination of antibody-dependent cytotoxicity mediated by the antibody and T-cell responses generated by the vaccine (23). T-cell assays, however, revealed that CD8+ T-cell responses in the spleen against Tyrp1 were elevated in the combination therapy group compared with vaccination alone. This finding is consistent with prior data showing that antibodies can enhance T-cell responses to irradiated cellular vaccines (7, 9, 31, 32). In these studies, the hypothesized mechanism of vaccine enhancement is improved antigen presentation due to direct binding of the antibody to the vaccine cells (8).

In the context of DNA vaccination in this study, there are three sources of Tyrp1 antigen to which TA99 presumably binds: the protein product of the DNA vaccine, normal melanocytes, and the B16 tumor. Although Tyrp1 was initially defined as an intracellular antigen, cell surface expression on B16 cells was confirmed by mixed hemadsorption rosetting assay (33). Although we were unable to detect accumulation of TA99 in the skin, perhaps because of lower levels of surface expression, or because of background autofluorescence, TA99 was shown to localize rapidly and specifically to B16 lung nodules (Fig. 2). ELISPOT assays, meanwhile, revealed no significant enhancement of the CD8+ T-cell response by TA99 in nontumor-bearing animals.

Tumor therefore is required for the immunomodulatory effect of TA99. Baseline responses, however, were higher in nontumor-bearing animals and we cannot exclude the possibility that enhancement did not occur because baseline T-cell responses were higher in these nontumor-bearing animals. In this regard, we note that TA99 enhances the immunogenicity of gp100 DNA, a vaccine to which TA99 does not bind (Fig. 4). This strongly suggests that the primary mechanism of immune enhancement is not ligation of the protein product of the DNA vaccine but rather ligation of the tumor with subsequent cross-presentation of antigen. However, the antibody alone is insufficient to induce T-cell responses in the absence of vaccination. Thus, we show requirement for a three-way interaction between antibody, tumor, and vaccine.

This mechanism whereby both tumor and vaccine are required for antibody mediated immunomodulation differs from prior models where antibody binds and opsonizes the vaccine itself (9, 29, 31), and this difference has implications for clinical trial design because it allows antibodies to be combined with diverse vaccination strategies. Intriguingly, in the breast cancer model used by Kim and colleagues (8), therapeutic benefit was seen when antibody and tumor were injected synchronously, although the vaccine had been given 14 days prior. Therefore, it is theoretically possible that observed enhanced therapeutic efficacy and CD8+ T-cell responses with antibody administration in this other experimental model may also be mediated, in part, by direct binding of the antibody to the tumor.

Antibodies modulate immune responses via Fc domain interactions with cell surface receptors, including complement receptors and/or Fc receptors (6). When administered synchronously with tumor, TA99 protects mice in a mechanism independent of CD8+ T cells, but dependent on activating Fc receptors, most likely by ligating FeRIV or FeR I on macrophages (25, 34). Meanwhile, the hypopigmentation produced by the antibody can be mediated by either Fc receptors or the complement system (24). In this context, we show that the immunomodulatory effect of TA99 on CD8+
T cells is FcR dependent. This finding is consistent with the known affinity of IgG2a antibodies for activating Fc receptors (35), and our results complement data from Kim and colleagues (8) that cleaved antibody is insufficient to mediate enhancement of CD8+ T-cell responses to a whole cell vaccine. Therapeutic agents modulating Fc receptor signaling would therefore have clinical application in trials combining antibodies with vaccines.

FcRγ−/− mice inoculated with B16 melanoma by tail vein develop B16 lung nodules more rapidly than do wild-type animals. Theoretically, this may be due to abrogation of the protection normally provided by naturally occurring antibodies against B16. However, B16 is a very poorly immunogenic tumor and the serum of untreated wild-type animals does not contain detectable levels of antibodies against melanoma differentiation antigens (36, 37) or B16 cell lysates. Neither can the enhanced tumor growth in FcRγ−/− mice be attributed to a deficiency in the natural antitumor activity of natural killer cells as this was previously shown to be unimpaired (16). Nonetheless, the data suggests that activating Fc receptors do play a role in antitumor immunosurveillance, possibly by transmitting activating signals induced by tonic binding of nonspecific ICs.

Furthermore, in the treatment of subcutaneous B16 melanoma, TA99 heightens the therapeutic effect of gp100 DNA vaccination combined with adoptive T-cell transfer of a T-cell clone specific for gp100. These data show that TA99 has the potential to enhance T-cell immunity in both pulmonary and skin disease in mice. A human anti-Tyrp1 antibody is currently under clinical development (38), and our data highlights potential advantages of combining this human antibody with T-cell–based therapies in clinical trials.

It is somewhat surprising that TA99, in combination with gp100 DNA vaccination, yields enhanced CD8+ T-cell responses to gp100 and mediates reactivity against Tyrp1. The presence of epitope spreading to Tyrp1 in some animals and not others may reflect differences in the repertoire between genetically identical animals due to thymic selection. In addition, it remains possible that TA99 also mediates spreading to other as of yet unidentified epitopes besides Tyrp1. In any case, as gp100 and Tyrp1 are both localized in the melanosome, TA99 potentially facilitates entry of both proteins into antigen presenting cells. From a clinical perspective, it is noteworthy that antibody TA99 can enhance CD8+ T-cell responses against two distinct melanosomal antigens. This finding shows that antibodies can be used in conjunction with vaccines targeting tumor antigens to broaden the immune response against cancer.

From an immunologic perspective, the adjuvant potency of TA99 can be explained by cross-presentation of tumor antigen, whereby tumor cells serve as a source of antigen for bone marrow–derived cells (39). These antigen-presenting cells express Fc receptors and are susceptible to modulation by antibody-bound tumor antigen. Combination therapy with antibodies and vaccines should be further explored in patients with cancer. Antibodies can be combined with all types of vaccines and should not be restricted to use with whole cell vaccines. Clinical investigators may also consider the option of combining antibodies with vaccines targeting antigens on the same tumor cell, particularly if vaccine and antibody targets are in the same subcellular compartment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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