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Combination Immunotherapy of Primary Prostate Cancer in a Transgenic Mouse Model Using CTLA-4 Blockade[1]

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ABSTRACT

We have previously shown that antibodies to CTLA-4, an inhibitory receptor on T cells, can be effective at inducing regression of transplantable murine tumors. In this study, we demonstrate that an effective immune response against primary prostate tumors in transgenic (TRAMP) mice can be elicited using a strategy that combines CTLA-4 blockade and an irradiated tumor cell vaccine. Treatment of TRAMP mice at 14 weeks of age resulted in a significant reduction in tumor incidence (15% *versus* control, 75%), as assessed 2 months after treatment. Histopathological analysis revealed that treated mice had a lower tumor grade with significant accumulation of inflammatory cells in interductal spaces when treated with anti-CTLA-4 and a granulocyte-macrophage colony-stimulating factor-expressing vaccine. Vaccination of nontransgenic mice with this regimen resulted in marked prostatitis accompanied by destruction of epithelium, indicating that the immune response was, at least in part, directed against normal prostate antigens. These findings demonstrate that this combinatorial treatment can elicit a potent antiprostate response and suggest potential of this approach for treatment of prostate cancer.

INTRODUCTION

Recent advances in our understanding of the mechanisms regulating T-cell activation have allowed the development of better strategies for the immunotherapy of cancer. It has become clear, for example, that full activation of naïve T cells requires not only stimulation of the antigen receptor by peptide/MHC complexes, but also costimulatory signals mediated by engagement of CD28 by B7 (CD80 or CD86; Ref. 1). B7 expression is limited to "professional" APCs[4], such as dendritic cells, macrophages, and activated lymphocytes (1). One of the reasons for the poor immunogenicity of tumors may be their lack of expression of these costimulatory ligands (2, 3). Induction of B7 expression on murine tumor cells by genetic modification has been shown to greatly enhance the effectiveness of tumor cell vaccines in providing protection against tumor challenge. However, B7+ tumor cells have not been found to be particularly effective in treating established tumors (reviewed in Ref. 4). Other strategies in tumor immunotherapy rely, at least in part, on enhancing costimulation. These include the use of tumor cells transduced to express GM-CSF to enhance cross-priming of T cells by professional APCs (5), dendritic cells pulsed with peptides (6, 7) or RNA (8) to provide immunization in the context of a potent APC, anti-CD40 antibodies to enhance expression of costimulatory ligands on APCs (9–11), and interleukin-2 to bypass the need for costimulation (12, 13).

More recently, costimulation has been shown to be more complex than previously thought; CTLA-4 is a second T-cell counter-receptor for B7 (14) that plays a critical role in attenuating T-cell responses. CTLA-4 engagement may inhibit the initiation of T-cell responses by raising the threshold of signals needed for full activation, or may also play a role in terminating ongoing T-cell responses (15, 16). Anti-CTLA-4 antibodies that block CTLA-4/B7 interactions enhance *in vivo* T-cell responses to peptides, superantigens, and parasites, and can exacerbate experimental autoimmune encephalomyelitis (for review, see Ref. 15). Administration of anti-CTLA-4 antibodies is sufficient to induce the rejection of newly implanted, and in some cases, well established tumors in several transplantable murine tumor systems (17–20). The effectiveness of CTLA-4 blockade in these systems seems to be dependent on the inherent immunogenicity of the tumor. Whereas CTLA-4 blockade by itself is not effective in the treatment of poorly immunogenic transplantable tumors such as the mammary carcinoma SM1 (21) or the melanoma B16 (22), eradication of these tumors can be achieved when anti-CTLA-4 is administered together with an irradiated tumor cell vaccine expressing GM-CSF. In the case of the B16 melanoma, tumor rejection is regularly accompanied by a progressive depigmentation that resembles the vitiligo accompanying immunotherapy in many human melanoma patients (23–25). This result suggests that in mice, as in man, the antimelanoma response is, in part, directed to normal melanocyte-specific antigens (22, 23).

In contrast to the considerable literature documenting immunological responses to melanoma in humans and in mouse models, there is a paucity of data concerning immunological responses to prostate tumors. We have shown that CTLA-4 blockade is sufficient to obtain partial or complete regression of s.c. implants of tumor cell lines (26) derived from the TRAMP mice in syngeneic, nontransgenic C57BL/6 male mice (18). In the current study, we examined the potential of CTLA-4 blockade in the treatment of primary cancer in TRAMP mice. We found that CTLA-4 blockade in combination with irradiated tumor cell vaccines was effective at reducing tumor incidence and the severity of prostatic lesions. We also noted significant accumulation of inflammatory cells in the prostates of some TRAMP mice that received vaccinations. Finally, we show that the antitumor response is directed, in part, against antigens expressed by normal prostate, because immunization of nontransgenic mice with GM-CSF-expressing tumor cell vaccines under conditions of CTLA-4 blockade can result in marked prostatitis. This work demonstrates for the first time the effectiveness of this immunotherapeutic regimen in primary cancer and indicates that prostatic tumors may express tissue-specific antigens that provide targets for immunotherapy.

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[4] The abbreviations used are: APC, antigen-presenting cell; GM-CSF, granulocyte-macrophage colony-stimulating factor; TRAMP, transgenic adenocarcinoma of mouse prostate; GM-TRAMPC, GM-CSF-transduced prostatic carcinoma cells derived from TRAMP mice.

MATERIALS AND METHODS

Mice. All animal procedures were performed according to NIH guidelines under protocols approved by the University of California Animal Care and Use Committee. TRAMP mice were bred within our colony on a pure C57BL/6 background. In TRAMP mice, the SV40 T antigen transgene expression is under the transcriptional control of the rat probasin promoter that directs expression to prostatic epithelium in an androgen-regulated manner. Pathogenesis of neoplasia in TRAMP mice mirrors that in man. When transgene expression begins at puberty, male TRAMP mice develop hyperplasia (5–8 weeks of age), frank neoplasia (8–12 weeks of age), and eventually invasive adenocarcinoma with metastasis to the lungs, lymph nodes, and bone (15–20 weeks of age; Ref. 29). For these experiments, TRAMP mice were backcrossed one time with FVB/N mice and screened for the presence of the transgene by PCR, as described previously (27).

Mice received s.c. vaccinations of 1×10^6 cells each of irradiated (12,000 rads) TRAMP-C1 and TRAMP-C2 or their GM-CSF-transduced derivatives, GMTRAMP-C1/C2. To maximize antigenic challenge, this treatment was repeated two additional times, 3 days apart. Seven days after the initiation of vaccination, mice received i.p. injections of 100 μg of anti-CTLA-4 (clone 9H10; Ref. 28) or with purified hamster IgG (Jackson Immunoresearch Corp., West Grove, PA). Additional doses of antibody were administered 3 and 6 days after the first treatment. Mice were euthanized at the indicated age, and the prostatic complex was microdissected under a stereomicroscope. Tumor incidence was initially assessed at necropsy and confirmed by histopathological examination, using a score of 4.0 (see below, invasive adenocarcinoma) as the defining criterion.

Histopathological Analyses. The prostatic complex was microdissected into the individual lobes and fixed in 10% neutral buffered formalin. Tissues were processed and stained with H&E for routine histopathological analyses. TRAMP tissues were graded blindly by two individuals using the following criteria (29): (*a*) normal epithelium was assigned a score of 1.0; (*b*) early signs of prostatic intraepithelial neoplasia with tufting of the epithelium and increased nucleus:cytoplasm ratio were scored as 2.0; (*c*) more advanced prostatic intraepithelial neoplasia with noted cribiform structures and an increase in mitotic and/or apoptotic figures was scored as 3.0; (*d*) the loss of interductal spaces and the invasion of basement membranes was scored as 4.0; (*e*) total loss of ductal lumens with evidence of adenocarcinoma was scored as 5.0; and (*f*) sheets of anaplastic cells were scored as 6.0. Each arbitrarily numbered sample was scanned for the peak severity at $\times$4 and graded at a magnification of $\times$10. To generate a mean peak score, the maximum histological score for the ventral, dorsal, or lateral prostate lobes for each animal was used to calculate a mean for the treatment group. The predominant peak score for all TRAMP animals was 4.0, with few histological scores below 3.0.

Cell Culture. TRAMP-C cells are early passage (10–15 passages *in vitro*), nonclonal epithelioid tumor cells independently derived from a TRAMP mouse and were propagated as described (26). To obtain GM-CSF-expressing lines, cells were infected with a retrovirus containing the mouse *gm-csf* gene driven by the Maloney murine leukemia virus LTR, using the ψCRIP producer line (Somatix, Inc., Alameda, CA), as described (21). GM-CSF production was assayed by ELISA (PharMingen, San Diego, CA). Both GMTRAMP-C1 and GMTRAMP-C2 secreted GM-CSF at 150–200 ng/ml/1×10^6 cells/24 h. Cells used for injection were released from tissue culture dishes with trypsin (BioWhittaker) and washed three times in HBSS (BioWhittaker). Cells were resuspended at a density of 1×10^7 cells/ml, irradiated with 12,000 rads using a Cs-source irradiator, and injected s.c. in a volume of 0.1 ml.

RESULTS

Reduction of Primary Tumor Incidence in TRAMP Mice following Treatment with Cell-based Vaccines and Anti-CTLA-4. Given the potency of CTLA-4 blockade combined with cell-based vaccines in poorly immunogenic transplantable tumor models, we examined the effectiveness of this strategy in the treatment of primary prostatic cancer in TRAMP mice (27). A cohort of 180 male TRAMP mice received vaccinations of a combination of irradiated TRAMP-C1 and TRAMP-C2 (TRAMP-C1/C2) or TRAMP-C1/C2 transduced to express the murine *gm-csf* gene (GMTRAMP-C1/C2) at about 3.5 months of age. Antibody treatment was begun 7 days after vaccination. To obtain an early indication of the effectiveness of the treatments, four mice from each group were euthanized 3 weeks after treatment and examined for tumor incidence at gross necropsy and conformed at the microscopic level. Although there were no significant differences in mean animal or urogenital tract weight between the treatment groups, there was a striking difference in tumor incidence. Irrespective of vaccine, 11 of 12 mice (92%) in the treatment groups receiving control antibody had detectable tumor. In contrast, only 3 of 12 (25%) mice receiving anti-CTLA-4 had detectable tumor.

At 3 weeks after treatment, the tumors in the control antibody-treated mice were sufficiently large to warrant concern about survival of the remaining mice. Therefore, the remaining 25 mice in each group were euthanized 5 weeks later to allow assessment of tumor incidence and tumor grade. Similar to the analysis at 3 weeks after treatment, there was no significant difference in animal weight or prostate weight between any of the treatment groups. However, there were significant differences in tumor incidence (Fig. 1*A*). A significantly lower tumor incidence was observed in mice treated with anti-CTLA-4 and either the TRAMP-C1/C2 vaccine (43%, $P = 0.05$) or the GMTRAMP-C1/C2 vaccine (33%, $P = 0.009$) than in mice treated with control antibody alone (69%). Treatment with anti-CTLA-4 alone had no significant effect on tumor incidence (64%), and there was no significant reduction in tumor incidence in mice receiving the control antibody treatment and either vaccine (55%-TRAMP-C1/C2 and 75%-GMTRAMPC1/C2). Thus, neither CTLA-4 blockade nor vaccination alone was effective at treating primary tumors in TRAMP mice. However, the combination of anti-CTLA-4 and either vaccine synergized to decrease tumor incidence. The expression of GM-CSF by the vaccine may further enhance the antitumor response because the tumor incidence was slightly lower in mice that received vaccinations of GMTRAMP-C1/C2 (33% anti-CTLA-4+GMTRAMP-C1/C2 *versus* 43%-anti-CTLA-4+TRAMP-C1/C2).

Because each group contained mice from litters with birth dates 2 weeks apart, tumor incidence was reassessed as a function of age at the initiation of treatment. As shown in Fig. 1*B*, for mice that received vaccinations of GMTRAMP-C1/C2 there was significant reduction in tumor incidence in the mice treated at 14 weeks of age ($P = 0.003$), but not in the group treated at 16 weeks of age ($P = 0.1$). This suggests that the stage of tumor development at the time of immunotherapy of TRAMP mice influenced the efficacy of treatment. Tumor incidence in mice treated with TRAMP-C1/C2 and anti-CTLA-4 at 14 and 16 weeks of age was equivalent.

Reduction of Tumor Grade in TRAMP Mice Treated with Combination Immunotherapy. To assess the severity of prostate lesions in TRAMP mice, the individual lobes of the prostate were prepared for routine histopathological analysis and scored as described in "Materials and Methods." As shown in Fig. 2*A*, there was a significant reduction in the severity of lesions in mice treated with anti-CTLA-4 and either vaccine. Specifically, TRAMP mice treated with TRAMP-C1/C2 and anti-CTLA-4 had a significantly lower score (mean peak score, 4.6) than control immunoglobulin-treated mice (mean peak score, 5.5; $P = 0.03$). Even more striking was the finding that mice treated with GMTRAMP-C1/C2 and anti-CTLA-4 had a significantly lower tumor grade (mean peak score, 3.9) than all three control groups: control immunoglobulin/no vaccine (mean peak score, 5.5; $P = 0.0009$), control immunoglobulin/GMTRAMP-C1/C2 (mean peak score, 5.5; $P = 0.0002$), and anti-CTLA-4 treatment alone (mean peak score, 4.8; $P = 0.04$). Treatment with anti-CTLA-4 alone or either vaccine without CTLA-4 blockade had no significant effect on tumor grade. These findings demonstrate that in addition to reducing the incidence of primary tumors, vaccination reduced the severity of prostatic lesions in TRAMP mice.

The histological data were also reanalyzed for tumor grade as a function of age at time of treatment. As was the case for tumor

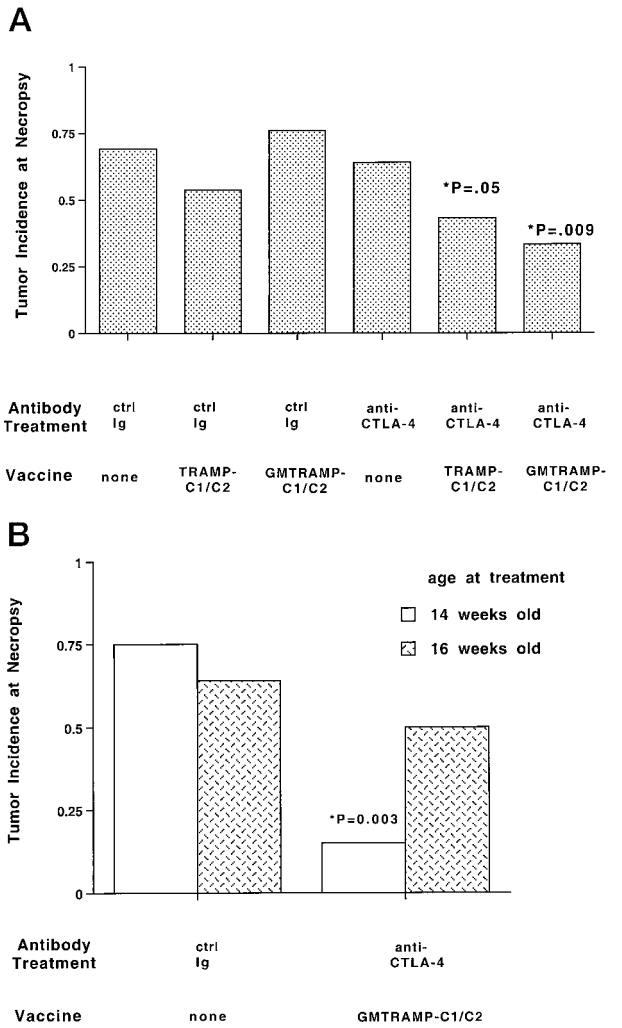

Fig. 1. TRAMP mice treated with TRAMP-C cells and anti-CTLA-4 have a lower tumor incidence than control-treated animals. Mice were vaccinated at 14–16 weeks of age and received i.p. injections of either hamster anti-CTLA-4 or a control hamster IgG fraction (*ctrl Ig*). *A*, mice treated with a TRAMP-C vaccine and anti-CTLA-4 had a significantly lower tumor incidence (43%) than ctrl Ig-treated mice (63%, $P = 0.05$). Mice treated with GMTRAMP-C1/C2 and anti-CTLA-4 had an even lower tumor incidence (33%, $P = 0.01$) than ctrl Ig-treated mice; there was no significant difference between mice treated with the two different vaccines. *, χ^2 *versus* ctrl Ig/no vaccine. *B*, reanalysis of data presented in *A* for tumor incidence as a function of age at the time of treatment revealed that the significant reduction in tumor incidence was from the mice treated at 14 weeks of age. In contrast, there was no significant reduction in tumor incidence in mice treated at 16 weeks of age. *, χ^2 *versus* ctrl Ig/no vaccine, treated at same age (14 weeks).

incidence, the greatest effect on severity of lesions was in mice treated at 14 weeks of age. Mice treated with GMTRAMP-C1/C2 and anti-CTLA-4 (mean peak score, 3.5) had a lower tumor grade than mice treated with GMTRAMP-C1/C2 and control immunoglobulin (mean peak score, 5.3; $P = 0.0002$) or mice treated with control immunoglobulin alone (mean peak score, 4.7; $P = 0.0002$; Fig. 2*B*). Interestingly, when treated at 16 weeks of age, TRAMP mice receiving the GMTRAMP-C1/C2 vaccine and anti-CTLA-4 (mean peak score, 4.5) only had a slightly lower mean peak score than mice treated with GMTRAMP-C1/C2 and control immunoglobulin (mean peak score, 5.6; $P = 0.03$). Tumor grade in mice treated TRAMP-C1/C2 at 14 and 16 weeks of age was equivalent.

Perhaps the most striking histological feature of these analyses was observed in mice treated with GMTRAMP-C1/C2 and anti-CTLA-4, where there was an accumulation of inflammatory cells in the interductal spaces (Fig. 3, *C* and *D*). In these mice, inflammatory cells were closely associated with the vasculature of the stroma. In contrast, there was no significant accumulation of inflammatory cells in any of the control immunoglobulin-treated mice (Fig. 3*B*). In TRAMP mice treated with a GM-CSF-expressing vaccine alone, there were occasional areas where inflammatory cells were detected, but these sites were not nearly as extensive as those observed in mice also treated with anti-CTLA-4 (data not shown). The morphological features of the infiltrating cells suggested that the perivascular inflammation was comprised of lymphoid and myeloid cells.

Induction of Prostatitis in Nontransgenic Mice by Vaccination and CTLA-4 Blockade. The reduction in incidence and severity of tumors, together with the inflammatory infiltrates of the prostate observed after immunization, were indicative of a potent immune response. The fact that tumorigenesis in these mice is driven by prostate-specific expression of SV40 Tag raised the possibility that the antitumor response was directed against epitopes derived from products of the viral oncogenes. We considered this to be unlikely because TAg expression could not be detected in the vaccine tumor cells by reverse

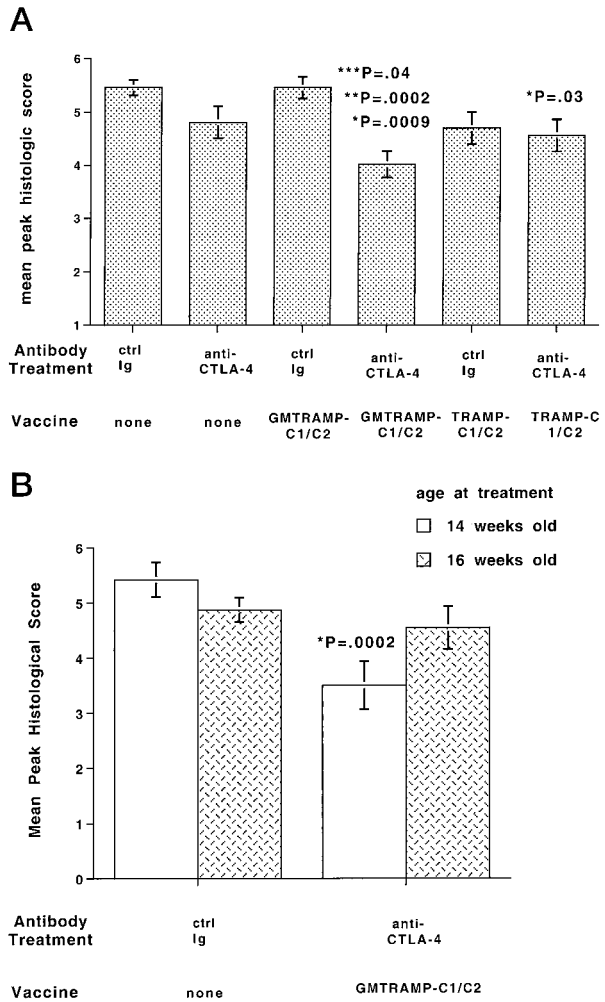

Fig. 2. TRAMP mice treated with TRAMP-C vaccines and anti-CTLA-4 exhibit a reduction in the severity of prostatic lesions. Tissues were scored blindly for the progression of disease, as described in "Materials and Methods." *A*, in mice treated with unmodified TRAMP-C vaccines and anti-CTLA-4 (mean peak score, 4.6) there was a significant reduction in severity of lesions compared with sham-treated mice (ctrl Ig/no vaccine; mean peak score, 5.5). In mice treated with both the GMTRAMP-C1/C2 vaccine and anti-CTLA-4 (mean peak score, 3.9) there was a significant reduction in histological severity of disease compared with sham-treated or both single-treatment groups. *, *versus* ctrl Ig/no vaccine; **, *versus* ctrl Ig/GMTRAMP-C1/C2; ***, *versus* anti-CTLA-4/no vaccine; statistically significant using Fisher, Scheffe, and Boneferroni/Dunn tests. *B*, reanalysis of data in *A* demonstrates that similar to tumor incidence, TRAMP mice treated with GMTRAMP-C1/C2 and anti-CTLA-4 had a reduction in tumor grade when treated at 14 weeks of age, but not when treated at 16 weeks of age. *, *versus* ctrl Ig/no vaccine and ctrl Ig/GMTC1/2.

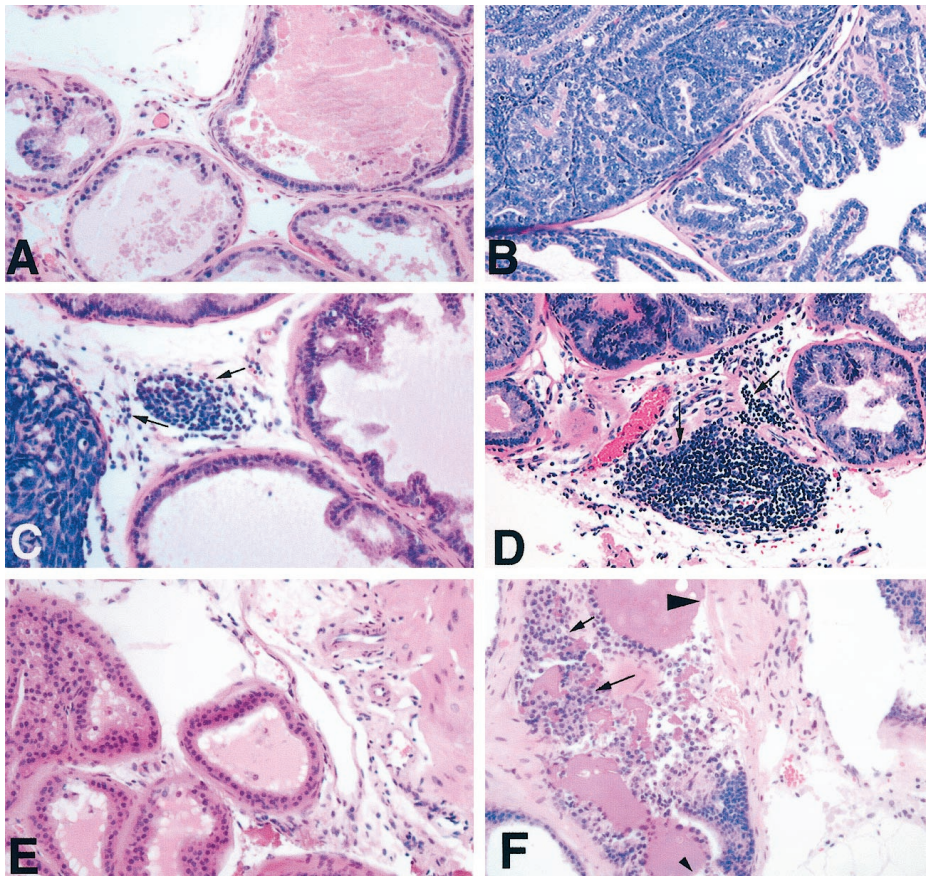

Fig. 3. Treatment of TRAMP mice with GMTRAMP-C vaccines and anti-CTLA-4 results in accumulation of inflammatory infiltrates in the prostatic acinar structures. In contrast to nontransgenic dorsolateral prostate (*A*), severe transformation of TRAMP prostatic tissues was observed (*B–D*). Varying degrees of histological severity were observed in *ctrl Ig*-treated TRAMP mice, ranging from advanced cribiform structures with occlusion of ductal structures to anaplastic adenocarcinoma (*B*). Less severe disease was observed in mice treated with GMTRAMP-C1/C2 and anti-CTLA-4 (*C* and *D*), with significant accumulation of inflammatory cells (*arrows*) in the interductal spaces, closely associated with the vasculature. *E* and *F*, generation of prostatitis in wild-type mice treated with a GMTRAMP-C vaccine and anti-CTLA-4. Nontransgenic C57BL/6 male mice, 12 weeks of age, were sensitized with GMTRAMP-C1/C2 vaccines and given anti-CTLA-4, as described in "Materials and Methods." In contrast to dorsolateral prostate of mice treated with GMTRAMP-C1/C2 alone (*E*), inflammatory infiltrates (*arrows*) were observed in vaccinated mice treated with anti-CTLA-4, 4 weeks after treatment (*F*). In some areas, tissue damage included destruction of acinar structures (*arrowheads*). The data presented are representative of three experiments.

transcription-PCR (18), nor were the tumor cells lysed by CTL reactive against H-2^b-restricted epitopes of TAg.[5] To determine whether the immune response elicited by the therapeutic regimen was limited to oncogene-encoded antigens, nontransgenic C57/BL6 mice received vaccinations and the prostates examined for evidence of inflammation 28 days later. Examples of tissue sections from mice that received vaccinations are shown in Fig. 3, *E* and *F*. There was no evidence of significant inflammation or tissue damage in the dorsolateral or ventral lobes of the prostates of mice vaccinated with the GMTRAMP-C1/C2 vaccine only. However, there was mononuclear cell infiltration and destruction of glandular epithelium of the male reproductive tract (including the dorsolateral prostate) in some mice vaccinated with GMTRAMP-C1/C2 and treated with anti-CTLA-4. These results demonstrate that the response elicited by the vaccination regimen is directed, in part, to antigens expressed by normal prostate cells.

DISCUSSION

Male TRAMP mice spontaneously develop prostatic adenocarcinoma as a consequence of expression of the SV40 TAg oncogene. A recent study demonstrated that adoptive transfer of TAg-specific T cells could reduce progression of prostatic disease in TRAMP mice (30). Here, we have examined the effects of treating TRAMP mice at 14–16 weeks of age with tumor cell vaccines in combination with anti-CTLA-4.

The reduction of both tumor incidence and histological tumor grade indicates that the combination of a cell-based vaccine together with anti-CTLA-4 was sufficient to slow the progression of primary prostatic tumors. Because the TAg transgene is under the transcriptional control of an androgen-regulated promoter and is, therefore, constitutively active in prostatic lumenal epithelial cells after sexual maturation, over time, a transformed phenotype will be observed in nearly all prostatic epithelium. It is not surprising that the immune system is unable to completely eliminate tumors in this aggressive model, but rather is remarkable that an antitumor immune response can have a significant impact on disease progression in a situation where an entire organ is undergoing transformation.

Our data in this primary tumor model indicate a synergy between CTLA-4 blockade and a tumor cell-based vaccine. TRAMP mice treated with either the vaccine or antibody alone had no reduction in tumor incidence or tumor grade, whereas the combination of both resulted in a significant reduction in both criteria. This suggests that an additional source of antigen from the cell-based vaccine contributes to T-cell priming, which is enhanced by blockade of CTLA-4/B7 interactions. The fact that tumor incidence and tumor grade were lower in mice that received the GMTRAMP-C1/C2 vaccine than those receiving the TRAMP-C1/C2 vaccine suggests that the effect is enhanced by the recruitment and activation of APCs by GM-CSF expression.

The basis for the age dependence on the effectiveness of treatment is not clear. The accessibility of the tumor to the immune system may change with the progression of neoplasia due to alterations in vasculature or intratumoral pressure. Tumor growth during this time period may begin to exceed the ability of the immune system to have a significant impact on controlling tumorigenesis. However, at 14 and 16 weeks of age, there do not appear to be any histopathological differences that might suggest that the antigenic profile might differ between these two ages.

Vaccination of nontransgenic mice with the same therapeutic strategy demonstrated to be effective for treatment of TRAMP mice led to autoimmune prostatitis and destruction of some prostatic epithelium. This finding suggests that the vaccination approach is capable of

[5] S. Tevethian and L. Mylin, personal communication.

inducing an autoimmune response against normal prostate antigens. We have also observed development of autoimmune depigmentation following rejection of a pigmented melanoma using a combination of CTLA-4 blockade and melanoma cells expressing GM-CSF (22) that is similar to the vitiligo that has been observed in patients showing clinical responses to immunotherapy of melanoma (23). The results reported here add support to the idea that effective tumor immunity is, in fact, closely tied to autoimmunity. Rather than being viewed as a troublesome side effect, an emerging concept is that intentional induction of autoimmunity to defined tissue-specific antigens may be a practical strategy for generation of effective antitumor responses (25, 31). The findings presented in this study support this approach for immunological treatment of tumors arising from nonvital tissues.

The work presented here clearly demonstrates that CTLA-4 blockade, in combination with a tumor cell-based vaccine, can elicit responses that can decrease the incidence of primary tumors in the TRAMP mice. We have previously shown that CTLA-4 blockade can synergize with tumor cell vaccines engineered to express GM-CSF and can be quite effective against murine melanoma (22) and mammary carcinoma (21). Recent clinical trials have shown that autologous melanoma cells transduced to express GM-CSF can elicit potent, although not curative (32), antitumor responses. Similarly, a recent report of a clinical trial using GM-CSF-secreting tumor cell vaccines has documented the induction of immune responses, including Th1, Th2, and antibody, in prostate cancer (33). Together, these findings make a compelling case for the use of a combination of GM-CSF-secreting tumor cell vaccines and anti-CTLA-4 in clinical trials. We are currently examining the effectiveness of CTLA-4 blockade in combination with more conventional therapies, such as androgen ablation or chemotherapy, that might induce sufficient tumor cell death to achieve priming of tumor-reactive T cells in the absence of a cell-based vaccine. Recent data provide compelling support for the therapeutic potential of the blockade of CTLA-4-mediated inhibitory signals of T-cell activation as a strategy for enhancing immunological responses to tumors. The demonstrated link between tumor immunity and autoimmunity underscore the power of this approach. A greater understanding of the role of CTLA-4 and other costimulatory molecules in the regulation of tolerance to self-antigens will facilitate the design of even more effective immunotherapies for cancer and other diseases with immune components.

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