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CTLA-4 blockade in combination with xenogeneic DNA vaccines enhances T-cell responses, tumor immunity and autoimmunity to self antigens in animal and cellular model systems

Polly D. Gregor[a,*], Jedd D. Wolchok[a], Cristina R. Ferrone[a], Heidi Buchinshky[a], Jose A. Guevara-Patiño[a], Miguel-Angel Perales[a], Fariborz Mortazavi[a], Dean Bacich[b], Warren Heston[b], Jean-Baptiste Latouche[a], Michel Sadelain[a], James P. Allison[c], Howard I. Scher[a], Alan N. Houghton[a,d]

[a] *Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, USA*
[b] *Department of Cancer Biology, The Cleveland Clinic, Cleveland, OH, USA*
[c] *Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA*
[d] *Weill Medical and Graduate Schools of Cornell University, New York, NY, USA*

Abstract

Xenogeneic DNA vaccination can elicit tumor immunity through T cell and antibody-dependent effector mechanisms. Blockade of CTLA-4 engagement with B7 expressed on APCs has been shown to enhance T cell-dependent immunity. We investigated whether CTLA-4 blockade could increase T-cell responses and tumor immunity elicited by DNA vaccines. CTLA-4 blockade enhanced B16 tumor rejection in mice immunized against the melanoma differentiation antigens tyrosinase-related protein 2 and gp100, and this effect was stronger when anti-CTLA-4 was administered with booster vaccinations. CTLA-4 blockade also increased the T-cell responses to prostate-specific membrane antigen (PSMA) when given with the second or third vaccination. Based on these pre-clinical studies, we suggest that anti-CTLA-4 should be tested with xenogeneic DNA vaccines against cancer and that special attention should be given to sequence and schedule of administration.

Keywords: CTLA-4; DNA vaccine; Tumor immunity

1. Introduction

Recent studies have shown that self antigens, particularly differentiation antigens, are frequently recognized on human cancers [1–4]. Melanoma and prostate cancer provide useful models to explore immunization against differentiation antigens [5–13]. We have previously shown that tumor immunity targeting differentiation antigens is feasible in mouse models. These studies have demonstrated that: (1) there is immune tolerance to differentiation antigens; (2) tolerance can be broken by immunizing with altered forms of antigen; and (3) immunity against differentiation antigens can elicit tumor immunity and not surprisingly autoimmunity (although the threshold for autoimmunity can be much higher than for tumor immunity). We hypothesize that the immune system is either ignorant of these differentiation antigens or is tolerant because they are self antigens. Therefore, a major prerequisite in the quest to develop immunotherapies for cancer is to induce a potent immune response that overcomes tolerance to self.

We have used immunization with xenogeneic DNA as a means for overcoming immunologic tolerance to otherwise poorly immunogenic differentiation antigens, such as tyrosinase-related proteins-1 and-2 (gp75 and tyrosinase-related protein 2 [TRP-2]) and gp100. Vaccination of mice with DNA encoding human gp75, TRP-2 or gp100 results in the production of auto-reactive antibodies (gp75) and T cells (TRP-2 and gp100), that mediate autoimmune depigmentation and rejection of syngeneic tumor challenge [5–7,9].

Despite the ability of xenogeneic DNA vaccines to induce tumor immunity in mouse models, additional immune modulation may be needed to achieve a meaningful clinical response to spontaneous tumors in human patients. Therefore, while assessing DNA vaccines as single agents in phase I clinical trials, we are also exploring adjuvants to enhance the response to DNA vaccines. We and others

* Corresponding author. Tel.: +1-212-639-6075.
E-mail address: gregorp@mskcc.org (P.D. Gregor).

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have previously shown that the effect of DNA vaccines is enhanced with the use of cytokine and chemokine genes as molecular adjuvants [14–24]. The focus of this report is on the preclinical development of CTLA-4 blockade as an adjuvant for xenogeneic DNA vaccines.

CTLA-4 is a ligand for B71/B72 that is expressed on the surface of activated T cells. Interaction between CTLA-4 and B7 on antigen presenting cells (APCs) results in down-regulation of activated T cells, attenuating the immune response [25]. Prior studies have shown that blockade of CTLA-4 results in disruption of the binding of CTLA-4 to the costimulatory molecules B71/B72, and enhances the response to antigenic tumors and foreign antigens [25–31]. 9H10 is a hamster mAb that blocks CTLA-4-B7 interactions, and thus enhances the potency of immune responses through activated T cells [25,26]. This antibody has been shown to enhance anti-tumor effects in combination with active immunization in animal models, including the TRAMP mouse, a transgenic model of prostate cancer [30–32]. A humanized mAb specific for human CTLA-4 (MDX-CTLA-4) is being evaluated in phase I trials as a single agent for patients with cancer and in combination with antigen-specific vaccines or chemotherapy [33].

CTLA-4 blockade has not been used with DNA vaccines previously. We have assessed the use of CTLA-4 blockade in combination with xenogeneic DNA vaccination and have further determined an optimal immunization schedule. Our overall goal was to design clinical trials in which anti-CTLA-4 is combined with DNA vaccines that have been first tested as single agents in phase I trials. Here, we report the adjuvant effect of anti-CTLA-4 in three xenogeneic DNA vaccine models of tumor immunity, and we show that immune enhancement is dependent on timing of CTLA-4 blockade.

2. Materials and methods

2.1. Mice

C57Bl/6J and BALB/c mice (4–8-week-old females) were obtained from The Jackson Laboratory (Bar Harbor, ME) and were used at 6–12 weeks of age. B16F10 is a pigmented mouse melanoma cell line of C57BL/6 origin (kind gift of Dr. Isaiah Fidler, M.D. Anderson Cancer Center, Houston, TX) [34]. Animals were treated in accordance with the Animal Welfare Act and NIH Guidelines for the Care and Use of Laboratory Animals.

2.2. Cell lines and tissue culture

CT-26 is a colon carcinoma of BALB/c origin [35]. CT-26 cells were obtained from the ATCC and were transduced with recombinant retroviruses SFG [36] encoding human or mouse PSMA. Transduced cells were selected in 10 μg/ml puromycin. Expression of PSMA in transduced cells was confirmed by immunostaining and flow cytometry, using polyclonal and monoclonal antibodies specific for mouse and human PSMA [37]. All cells were grown in RPMI medium.

2.3. Plasmid DNA

The hTRP-2 and hgp100 plasmids have been described [7,9]. Full-length hPSMA [37,38] and mPSMA [39] cDNAs were cloned into the clinical grade vector pING [40]. Plasmids were purified from bacterial lysates using Qiagen columns (Qiagen, Santa Clara, CA), and DNA was precipitated on 1.0 μm gold particles.

2.4. DNA immunizations

Plasmid DNA was coated on plastic tubing and injected in the skin of mice as described previously [6,41,42]. Briefly, abdominal hair of mice was removed and plasmid DNA was delivered using a helium-driven gun (Accell, PowderJect, Madison, WI) into each abdominal quadrant (1 μg plasmid DNA per quadrant).

2.5. 9H10 antibody

The hybridoma has been previously described [25]. Hybridoma cells have been produced in the Monoclonal Antibody Core Facility at Memorial Sloan-Kettering Cancer Center. 9H10 was purified by Protein-G Sepharose (Pharmacia, Piscataway, NJ). The concentration was adjusted to 1 mg/ml with sterile PBS.

Mice were injected with 100 μl sterile 9H10 in PBS intraperitoneally.

2.6. Tumor challenge

Mice were injected intradermally with 1×10^5 B16 melanoma cells on the right flank 5 days after the final DNA immunization. The mice were then followed for tumor onset by palpation every other day. Tumors were scored as present once they reached a 2 mm diameter and continued to grow. Mice were sacrificed once it was assured the tumors were progressing (usually at a size of ∼1 cm). Kaplan–Meier tumor free survival curves were constructed and analyzed.

2.7. ELIspot assays

Five or 12 days after the last immunization, mice were sacrificed and lymph nodes and spleen were harvested. $CD8^+$ T cells were isolated (Miltenyi, Auburn, CA). IP-Multiscreen plates (Millipore, Burlington, MA) were coated with 100 μl anti-mouse IFN-γ antibody (10 μg/ml; MabTech, Sweden) in PBS, incubated overnight at 4 °C, washed with PBS to remove unbound antibody and blocked with RPMI/FCS for 2 h at 37 °C. Purified $CD8^+$ T cells were plated at a concentration of 1×10^5 per well. For antigen presentation,

5×10^4 syngeneic naïve spleen cells per well pulsed with 0.1–10 μg/ml peptide were added to a final volume of 100 μl per well. In other experiments, irradiated CT-26 cells were used. After incubation for 20 h at 37 °C, plates were extensively washed with PBS/0.05% Tween and 100 μl per well biotinylated detection-antibody against mouse IFN-γ (2 μg/ml) were added. Plates were incubated for an additional 2 h at 37 °C and spot development was performed as described [43]. Spots were evaluated with an Automated ELIspot Reader System with KS 4.3 software (Carl Zeiss, Thornwood, New York).

2.8. Intracellular cytokine flow-cytometry

Anti-CD8-peridin chlorophyll protein (PerCP) (clone 53-6.7) was used for cell-surface staining. Intracellular staining for cytokines was performed using phycoerythrin (PE) anti-IFN-γ (clone XMG1.2), PE-anti-TNF-α (clone MP6-XT220). Isotype controls for the intracellular cytokines used the irrelevant rat IgG_1 (clone R3-34) and rat IgG_{2b} (clone A95-1) conjugated to PE. All antibodies were purchased from PharMingen (San Diego, CA).

In order to assess antigen-specific cytokine production by $CD8^+$ T cells, 5×10^6 lymph node cells were stimulated for 16 h in the presence of 10^6 irradiated CD8-depleted splenocytes, 10 μg/ml of peptide. Brefeldin A (10 μg/ml, Sigma, St. Louis, MO) was added 1 h after the peptide. Following stimulation, the cells were stained for cell-surface markers and intracellular cytokines using the Cytofix/Cytoperm Kit (PharMingen) according to the manufacturer's instructions and analyzed on a FACScalibur (Becton Dickinson Immunocytometry Systems, San Jose, CA). Fluorescence voltages and compensation values were determined using cells single-stained with anti-CD8-FITC, anti-CD8-PE or anti-CD8-PerCP. Acquisition and analysis were performed using CellQuest software (Becton Dickinson Immunocytometry Systems). For each tube, 30,000 events were acquired in a live lymphocyte and CD8 double-gate.

2.9. Statistical analysis

Two-tailed Student's *t*-test was performed on cytokine and ELIspot data sets and the ANOVA test, analysis of variants, was used for the ICCA. Log Rank analysis was performed to determine differences in tumor-free survival based on Kaplan–Meier plots.

3. Results

3.1. CTLA-4 blockade increases anti-tumor immunity when combined with a human TRP-2 DNA vaccine

We have shown previously that three weekly injections of xenogeneic human TRP-2 DNA (hTRP-2) conferred 90–100% protection from challenge with syngeneic B16 melanoma in both lung metastasis [7] and intradermal (this report) models. Autoimmune depigmentation was observed in the majority of these animals. Mice that received two injections of hTRP-2 showed partial (10–30%) protection. Therefore, we began our study by combining anti-CTLA-4 with this sub-optimal TRP-2 DNA regimen to assess its effect when combined with a well-characterized xenogeneic DNA vaccine.

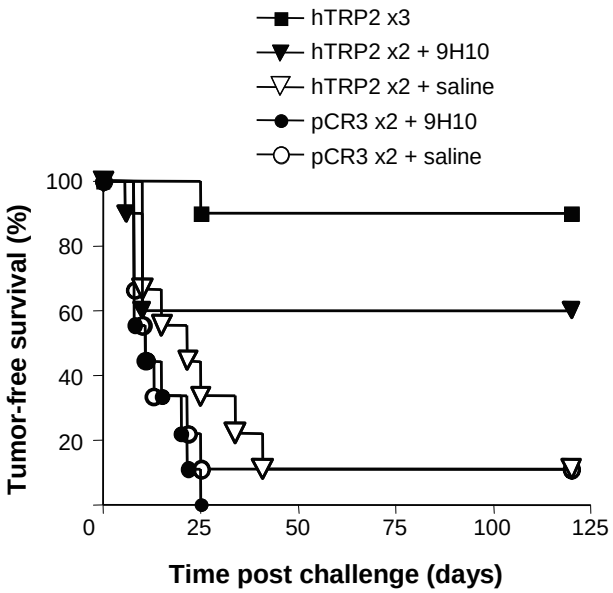

Fig. 1. Anti-CTLA-4 (9H10) increases tumor immunity after DNA immunization. C57BL/6 mice (10/group) were immunized 1–3 times with hTRP-2 or pCR3 DNA via particle bombardment. 9H10 was given with the first vaccine, on days −1, 0 and +1. Five days after the last immunization, all mice were challenged with B16 tumor cells cutaneously. Tumors were measured every 2–3 days with calipers and the proportion of tumor-free mice was plotted against time.

The anti-CTLA-4 mAb 9H10 blocks CTLA-4-B7 interactions in vivo and in vitro [25–31]. Because 9H10 is of hamster origin, it is immunogenic in mice, and cannot be used over multiple DNA injections. Therefore, we administered 9H10 with only one vaccine injection in any given cohort of mice. In our initial studies, C57BL/6 mice were immunized one to three times with either hTRP-2 or control vector PCR3 (Fig. 1). Some mice also received 100 μg 9H10 intraperitoneally on days −1, 0 and +1 relative to the first hTRP-2 DNA injection, while control groups received saline. Injections were staggered such that all mice received their final DNA vaccine on the same day, and all were challenged with B16 melanoma cutaneously 5 days later.

Nine out of 10 mice given three injections of hTRP-2 DNA were protected from tumor challenge, while relatively short delays in tumor growth were seen in mice given two injections with only one of 10 mice demonstrating long-term tumor protection (Fig. 1). Six of the 10 mice that received two injections of hTRP-2 and 9H10

were protected, an intermediate level of tumor immunity compared with that seen with three hTRP-2 injections. There was no anti-tumor effect when 9H10 was given with a control vector, pCR3, in the same system, or when mice received a single injection of hTRP-2, with or without 9H10 (Fig. 1, and results not shown). The protection afforded by three injections of hTRP-2 was significantly better than that seen in control animals ($P < 0.0001$) or in mice that received two injections of hTRP-2 + saline ($P = 0.0003$), and it was slightly better than the tumor protection seen in mice that received two injections of hTRP-2 + 9H10 ($P = 0.11$). The difference between two injections of hTRP-2 + saline and injection of pCR3 did not reach statistical significance ($P = 0.08$), but two injections of hTRP-2 + 9H10 had a significantly improved effect compared with the control, two pCR3 injections ($P = 0.01$).

3.2. Enhancement by CTLA-4 blockade is dependent on the schedule of 9H10 administration

The 9H10 antibody had not been used previously with DNA vaccines. Therefore, we varied the 9H10 schedule, comparing the adjuvant effect of 9H10 when given with the first or second of the two hTRP-2 DNA injections (Fig. 2). Tumor protection was improved when 9H10 was given with the second of the two hTRP-2 injections ($P = 0.02$). In this and other similar experiments, protection was improved in mice that received 9H10 with the second immunization, although the absolute number of protected animals varied from experiment to experiment, depending on the number of tumor cells injected and their passage number in mice.

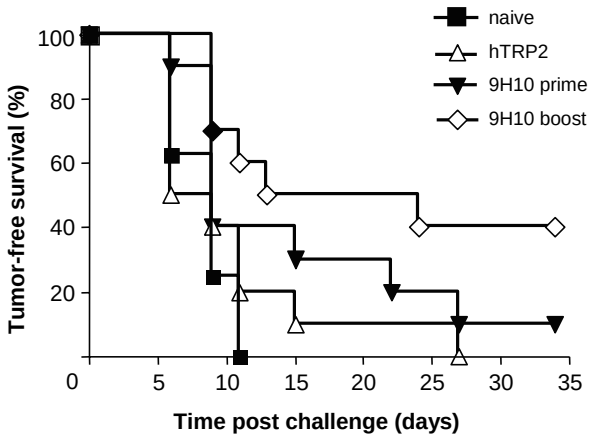

Fig. 2. Anti-tumor effects of CTLA-4 blockade are increased when antibody is given with the second vaccine. Mice (10/group) were immunized two times with hTRP-2 DNA via particle bombardment. 9H10 was given with the first (prime) or second (boost) vaccination. Five days after the last immunization, all mice were challenged with B16 tumor cells cutaneously. Tumors were measured every 2–3 days with calipers and the proportion of tumor-free mice was plotted against time.

3.3. CTLA-4 blockade increases anti-tumor immunity and T cells specific for mouse gp100 when combined with a human gp100 DNA vaccine

Using a second melanoma differentiation antigen, mice were immunized with human gp100 (hgp100) DNA. We had previously shown that three injections of hgp100 confer only partial tumor protection from B16 challenge, and that this is increased by injecting DNA encoding cytokine constructs 1 day after the hgp100 vaccine [44]. Without adjuvants, the immunity is short lived, with no survival benefit when vaccinated mice are challenged with tumor as little as 12 days following the last hgp100 vaccination.

C57BL/6 mice (15/group) were immunized three times with hgp100. Some mice were also treated with 9H10 at the first or second immunization and 10 mice/group were challenged with B16 tumor either 5 or 12 days after the last vaccine. Five mice in each group were not challenged with B16, but were sacrificed and spleen and lymph nodes were harvested in order to quantify $CD8^+$ T-cell responses to a mouse gp100 (mgp100) peptide by ELISpot and intracellular cytokine secretion assays (ICCA).

All groups that were treated with gp100 showed a statistically significant increase in tumor-free survival compared to naïve mice ($P = 0.0023$, 0.0013 and <0.0001 for gp100 alone, 9H10 at prime and 9H10 at boost, respectively) (Fig. 3A). In mice that received 9H10 with the second vaccination (boost), there was a trend towards tumor growth delay at day 30 compared with mice that received 9H10 with the first vaccination ($P = 0.12$). There was no difference between mice treated with hgp100 DNA vaccine alone and those that received 9H10 with the first vaccination ($P = 0.74$). The additive effect of 9H10 was short-lived, as there were no delays in tumor growth or tumor protection in mice challenged 12 days after the third immunization (Fig. 3B). Examination of mice that survived for 77 days showed that those treated with 9H10 had increased autoimmunity, as evidenced by greater depigmentation, relative to mice that received only the hgp100 DNA vaccine (results not shown). The greatest depigmentation was seen with long-term surviving mice that received 9H10 with the second gp100 DNA injection (boost).

The data from an ELISpot assay using splenocytes from vaccinated mice are shown in Fig. 4A. We have previously shown that hgp100 DNA vaccines induce D^b-restricted $CD8^+$ T cells that recognize the syngeneic mouse $gp100_{25–33}$ peptide EGSRNQDWL (mgp100 peptide). In order to distinguish between increases in the number of low avidity and higher avidity T cells, a range of concentrations of the mgp100 peptide was used in parallel assays (10, 1 and 0.1 μg/ml). When 9H10 was given with the second, but not the first gp100 DNA injection, there was an increased number of mgp100 peptide-specific T cells in the spleen (Fig. 4A). There was also a modest increase in non-specific $CD8^+$ T cells against the control Ova peptide (SIINFEKL), but at levels well below the mgp100 response. Even at a

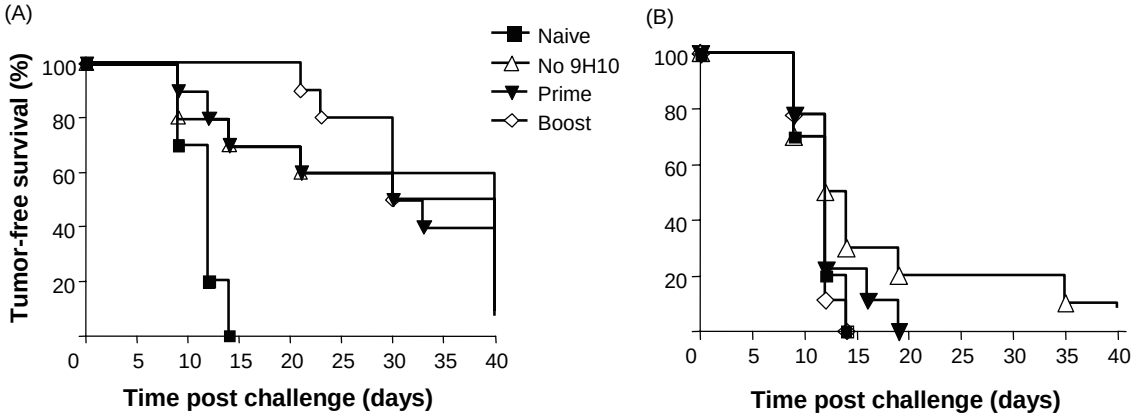

Fig. 3. Anti-tumor effects of anti-CTLA-4 are increased when antibody is given with the second vaccine (boost). Mice (10/group) were immunized three times with hgp100 DNA via particle bombardment. 9H10 was given with the first (prime) or second (boost) vaccination, or was not given (no 9H10). Five days (A) or 12 days (B) after the last immunization, all mice were challenged with B16 tumor cells cutaneously. Tumors were measured every 2–3 days with calipers and the proportion of tumor-free mice was plotted against time.

10-fold lower concentration of mgp100 peptide relative to the Ova control peptide, a significant response to mgp100 was seen, implying that 9H10 also increases the number of higher avidity T cells. The number of peptide-specific T cells decreased between day 5 and day 12, consistent with the results in tumor challenge experiments, but the pattern of 9H10 augmentation of reactive T cells against mgp100 was retained at day 12. A similar profile of T-cell reactivity

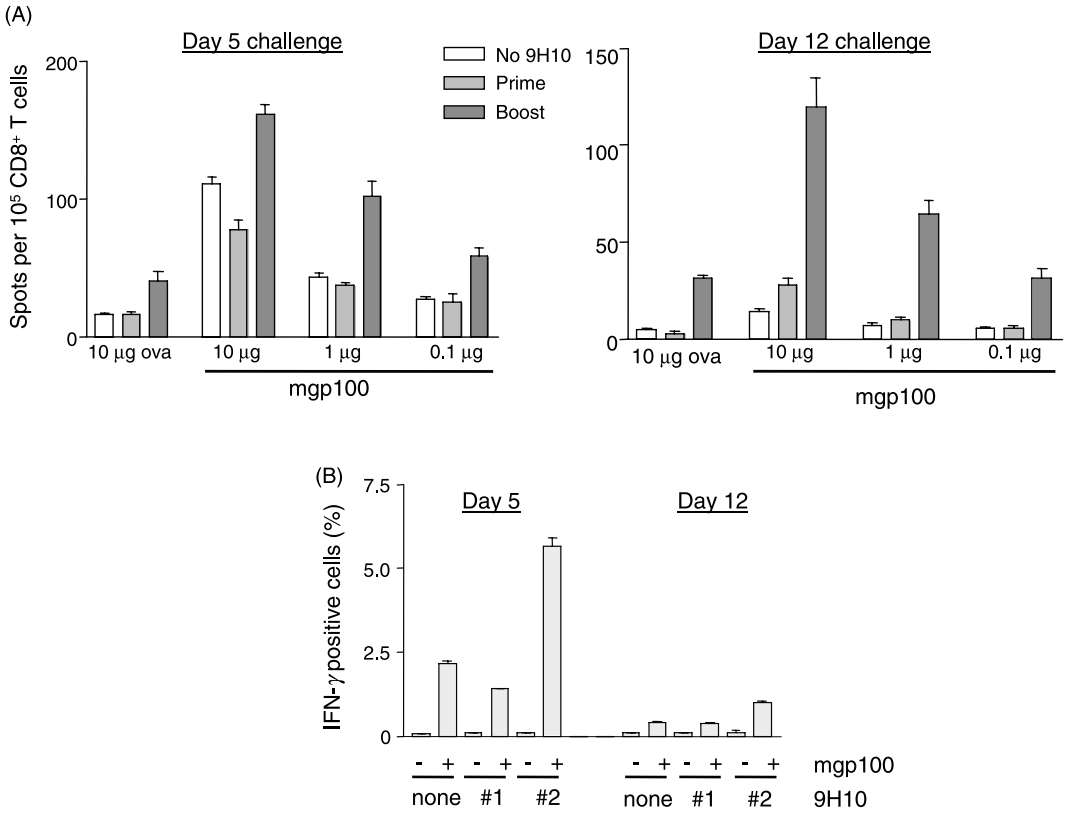

Fig. 4. The proportion of mgp100-specific T cells is increased when anti-CTLA-4 is given with the second vaccine (boost). (A) IFN-γ ELIspot assay of $CD8^+$ T cells isolated from spleen and incubated with syngeneic spleen cells pulsed with the indicated concentrations of the mgp100 peptide (EGSRNQDWL) or the control ova peptide (SIINFEKL). Splenocytes were isolated from mice sacrificed 5 days or 12 days after the third vaccine. Results represent averages ± S.E.M. of quadruple assays. (B) ICCA analysis of lymph node cells isolated from mice sacrificed 5 or 12 days after the third DNA vaccine. 9H10 was given with the first (#1) or second (#2) vaccination, or was not given (none). Cells were gated for CD8 and the percentage of IFN-γ positive cells was measured. Results represent averages ± S.E.M. of triple assays.

against mgp100 peptide was seen in the lymph nodes by ICCA (Fig. 4B). Five days after the last immunization, mice treated with 9H10 at the first vaccination showed a small decrease in the number of mgp100-specific T cells compared with mice that received vaccine only, while 9H10 given with the second vaccination increased the response approximately 2.6-fold ($P < 0.0001$).

3.4. Anti-CTLA-4 increases T-cell responses to a prostate differentiation antigen

We next applied anti-CTLA-4 to a xenogeneic DNA vaccine encoding a differentiation antigen expressed in normal prostate and in prostate cancer, prostate-specific membrane antigen (PSMA) [37,38]. We had previously shown that immunization of mice with human PSMA protein (hPSMA) or DNA results in production of autoantibodies specific for mouse PSMA (mPSMA), while there was no response to a mPSMA DNA vaccine [45].

T-cell epitopes within human or mouse PSMA have not been defined. Therefore, T cells specific for PSMA were detected by incubating effectors with class I MHC-matched tumor cells. In this case, we used CT-26, a colon adenocarcinoma cell line from BALB/c mice that was transduced with full-length human or mouse PSMA cDNAs. CT-26 cells transduced with empty vector served as a control. Using both ELIspot and ICCA, $CD8^+$ positive T cells specific for human PSMA were detected in mice immunized two or three times with hPSMA. However, in this system, no mouse PSMA-specific T cells were seen after xenogeneic DNA immunization (Fig. 5 and results not shown).

In experiments combining anti-CTLA-4 with a hPSMA DNA vaccine, we found that, as for hgp100, anti-CTLA-4 given with the second of three vaccinations increased T-cell responses 2.3-fold compared with hPSMA alone ($P < 0.0001$) (Fig. 5). There was no increase in antigen-specific T cells when anti-CTLA-4 was given with the first PSMA DNA vaccination ($P = 0.76$). In other experiments, we compared several schedules of PSMA DNA vaccine and 9H10 usage. All mice received three PSMA DNA vaccines at 1 or 2 week intervals. 9H10, when used, was given at days +1 and +4 relative to the first, second or third vaccine. On day 5 following the last vaccination, $CD8^+$ T cells were isolated from draining lymph nodes and analyzed by ELIspot assays. The immunizations were scheduled so that all mice received their last vaccination on the same day, and T cell assays were performed simultaneously.

These results showed that, independent of 9H10, a 2-week interval between the first and second vaccination increased the T-cell response to PSMA. Once again, 9H10

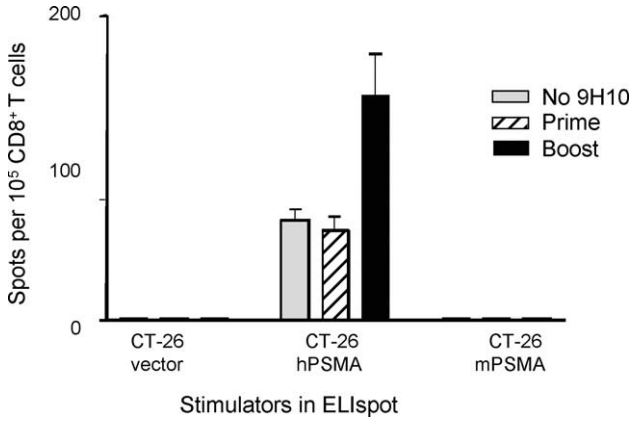

Fig. 5. The proportion of hPSMA-specific T cells is increased when anti-CTLA-4 (9H10) is given with the second vaccination (boost). BALB/c mice (four/group) were immunized three times with hPSMA DNA via particle bombardment. 9H10 was given with either the first (prime) or second (boost) vaccination. Five days after the last immunization, $CD8^+$ T cells were isolated from the draining lymph nodes and assayed by ELIspot assay in triplicate for recognition of human and mouse PSMA. Target cells were CT-26 cells transduced with empty vector, hPSMA and mPSMA. The mean number of spots/10^5 $CD8^+$ T cells is shown for each group, ±S.E.M.

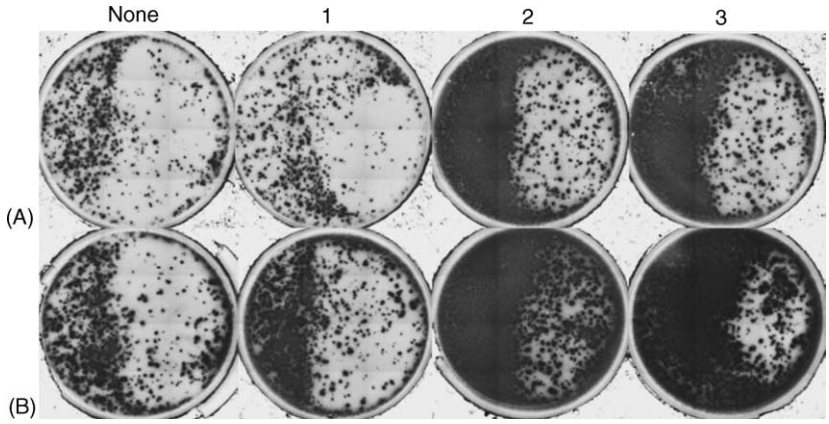

Fig. 6. The proportion of hPSMA-specific T cells is increased when DNA vaccinations are given at 2-week intervals and anti-CTLA-4 (9H10) is given with the second or third vaccination. BALB/c mice (four/group) were immunized three times with hPSMA DNA via particle bombardment at 1 (A) or 2 (B) week intervals. 9H10 was given with the first, second or third vaccine. Five days after the last immunization, $CD8^+$ T cells were isolated from the draining lymph nodes and assayed by ELIspot assay in triplicate, as described in Fig. 5. Representative wells are shown.

at the first vaccination had little or no effect, while 9H10 at the second or third vaccination increased the T-cell response two to four-fold (Fig. 6). However, even in mice hyper-immunized with hPSMA that had very large numbers of hPSMA-specific T cells, no T cells specific for mPSMA were seen.

4. Discussion

Anti-CTLA-4 is a powerful immune adjuvant for T-cell responses that acts by suppressing the down-regulation of activated cells. Phase I clinical trials of anti-CTLA-4 as a single agent have been completed. The antibody has more recently been used in combination with peptide vaccines.

Our laboratory has a long-standing interest in xenogeneic DNA vaccines for cancer immunotherapy. More recently, we have completed a phase I clinical trial in which companion animals (outbred dogs) with spontaneously arising advanced melanoma were immunized with a human tyrosinase DNA vaccine [40]. No treatment-related toxicities were noted. The cohort of dogs showed a significant increase in Kaplan–Meier median survival time in comparison to historical controls (14 months versus 4 months), and one dog had a complete clinical response, characterized by regression of pulmonary metastases. We are currently conducting several phase I clinical trials at MSKCC in which DNA vaccines encoding xenogeneic and/or syngeneic tyrosinase, gp75, or PSMA are being used in patients with melanoma and prostate cancer, respectively.

In order to increase the effectiveness of xenogeneic vaccines, we have previously investigated cytokine adjuvants. Here, we now show that anti-CTLA-4 substantially increases the efficacy of xenogeneic DNA vaccines in preclinical models. Increased T-cell responses, anti-tumor immunity and autoimmunity were all seen, using three differentiation antigens. We further investigated the optimal schedule for administration of 9H10. Finally, in the PSMA system, we found that a 2-week gap between injections increases the overall T-cell response. These results will allow us to combine anti-CTLA-4 with xenogeneic DNA vaccines in future clinical trials.

In previous reports, 9H10 was effective in rejecting antigenic tumors when it was given with the tumor challenge and once or twice again in the first week of tumor growth [28,29]. This reflects its action upon newly activated T cells. In this report, we have observed a somewhat different requirement for 9H10 scheduling. There are three explanations for the observation that 9H10 was effective only when given with the second or third DNA vaccine. One is simply that because the number of antigen-specific T cells increases with each immunization, more cells are available for action by anti-CTLA-4 at the second or third vaccine. DNA vaccination may stimulate fewer T cells than challenge with an antigenic tumor. A second possibility is that 9H10 has a smaller effect on recently activated naïve precursor T cells. While 1–2 weeks is not sufficient to generate memory T cells, changes in surface expression of the T-cell receptor and co-stimulatory molecules (including CTLA-4) may change the dynamics of CTLA-4-B7 interactions. A third possibility is that as T cells are stimulated, higher avidity T cells are elicited, and because these cells are more reliant on CTLA-4 for their regulation, they are more sensitive to CTLA-4 blockade [46]. Finally, regulatory T cells that are induced after the priming immunization may be partly depleted or inhibited by anti-CTLA-4 treatment, although a previous study found no evidence for regulatory T-cell depletion by 9H10 [47].

Despite the very large number of T cells specific for hPSMA seen after three bi-weekly injections of hPSMA and 9H10, no responses were seen to mPSMA (Fig. 6, and results not shown). This could reflect a complete lack of auto-reactive T cells in mice due to central tolerance and T-cell deletion. We favor the hypothesis that 9H10 preferentially expands high avidity T cells that recognize hPSMA, and that the epitopes recognized by these T cells are not shared by mPSMA. It has previously been shown that 9H10 preferentially activates high avidity T cells [46]. The lack of autoreactive T cells specific for PSMA after 9H10 blockade is in contrast with our data showing that 9H10 increases and prolongs a $CD8^+$ T-cell response to the mgp100 when given with a xenogeneic (human) vaccine (Fig. 5). However, in this case, the immunodominant peptide from hgp100 is very similar to the corresponding mouse peptide, with changes at two potential anchor residues [9,48]. The T-cell receptor contact residues are conserved; therefore, T cells stimulated by hgp100 are cross-reactive with the mgp100 peptide. In the case of hTRP-2, the known epitope in hTRP-2 is completely conserved between the human and mouse sequences, so that high avidity T cells stimulated by hTRP-2 are also cross-reactive with mTRP-2 [7].

CTLA-4 blockade is emerging as an important immunological adjuvant in cancer imunotherapy, and will be tested in combination with a variety of vaccines in clinical trials over the next few years. Here, we have demonstrated that anti-CTLA-4 is a very useful adjuvant for DNA vaccines. This evidence builds upon the existing data supporting anti-CTLA-4 as an immune adjuvant. More significantly, in this report, we have highlighted the importance of optimizing the schedule of anti-CTLA-4 usage in combination with other vaccines, including DNA vaccines. It is clearly critical in pre-clinical work to compare vaccine regimens carefully, paying particular attention to detailed schedules of vaccine and adjuvant administration.

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