A Potent Vaccination Strategy That Circumvents Lymphodepletion for Effective Antitumor Adoptive T-cell Therapy

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Abstract

Adoptive cell therapy using tumor-reactive T lymphocytes is a promising approach for treating advanced cancer. Successful tumor eradication depends primarily on the expansion and survival of the adoptively transferred T cells. Lymphodepletion using total body irradiation (TBI) and administering high-dose interleukin (IL-2) have been used with adoptive cell therapy to promote T-cell expansion and survival to achieve maximal therapeutic effects. However, TBI and high-dose IL-2 increase the risk for major complications that impact overall survival. Here we describe an alternative approach to TBI and high-dose IL-2 for optimizing adoptive cell therapy, resulting in dramatic therapeutic effects against established melanomas in mice. Administration of a potent, noninfectious peptide vaccine after adoptive cell therapy dramatically increased antigen-specific T-cell numbers leading to enhancement in the survival of melanoma-bearing mice. Furthermore, combinations of peptide vaccination with PD1 blockade or IL-2/anti-IL-2 antibody complexes led to complete disease eradication and long-term survival in mice with large tumors receiving adoptive cell therapy. Our results indicate that PD1 blockade and IL-2/anti-IL-2 complexes enhance both the quantitative and qualitative aspects of the T-cell responses induced by peptide vaccination after adoptive cell therapy. These findings could be useful for the optimization of adoptive cell therapy in cancer patients without the need of toxic adjunct procedures. Cancer Res. 72(8): 1986–95. ©2012 AACR.

Introduction

CD8 T lymphocytes recognize and destroy tumor cells through perforin/granzyme B-mediated lysis or via the production of cytostatic lymphokines (1–4). Tumor-reactive CD8 T cells recognize peptide antigens that associate with MHC class I molecules on the surface of tumor cells (5). In the case of malignant melanoma, peptides can be derived from melanosomal differentiation antigens such as gp100 and tyrosinase-related proteins (6–8). One factor limiting the effectiveness of T cells to recognize tumors is related to the T-cell receptor (TCR) antigen affinity, which requires being sufficiently high to enable T-cell activation when tumor cells express low density of peptide/MHC-I complexes (9, 10). Because in many instances normal tissues also express the tumor-associated proteins, immunologic tolerance precludes the induction of T cells expressing high-affinity TCRs, limiting the effectiveness of many therapeutic vaccines (11, 12). In view of this, adoptive immunotherapy using high avidity CD8 T cells has been explored to treat established and aggressive malignant diseases such as melanoma (13, 14). In addition to TCR affinity, other factors may determine the effectiveness of adoptive cell therapy, such as the ability of the T cells to expand and survive in vivo after adoptive transfer into the tumor-bearing hosts. Lymphokines such as interleukin (IL)-2, IL-7, and IL-15 are critical for expansion and survival of T cells and generating long-lasting memory CD8 T cells (15–17). Some procedures have been used to increase the access of the transferred T cells to these lymphokines such as the coadministration of high-dose IL-2 (18, 19) and lymphodepletion using total body irradiation (TBI) or chemotherapy (14, 20–23). Unfortunately these procedures generate severe toxic effects that can be life threatening.

The B16 mouse melanoma model has been widely used and proven to be valuable for developing effective adoptive cell therapy strategies for melanoma patients (24). In this model, the use of high avidity CD8 T cells obtained from Pmel-1 TCR transgenic mice was effective against large-established tumors but required lymphodepletion, high-dose IL-2, and active immunization using a recombinant vaccinia virus vaccine after the T-cell transfers (25). Our goal was to determine whether effective adoptive cell therapy against established B16 melanoma could be attained in the absence of the concomitant harmful procedures (high-dose IL-2, live vaccines, and lymphodepletion). We assessed the ability of TriVax (26), a potent, noninfectious peptide-based vaccine to elicit antitumor effects of adoptively transferred Pmel-1 T cells. TriVax induced significant tumor regressions in the absence of lymphodepletion and without the need of high doses of IL-2. Furthermore, the addition of low-dose IL-2 in the form of IL-2/anti-IL-2 antibody complexes (IL2x) or...
PD1 blockade to TriVax resulted in total tumor eradication. These findings may facilitate the implementation of adoptive cell therapy in humans in circumstances that may reduce the overall toxicity of this therapeutic approach.

Materials and Methods

**Mice and cell lines**

C57BL/6 (B6) mice were from Charles River. Congenic B6 (CD45.1) and Pmel-1 mice (CD90.1) were from The Jackson Laboratory. Animal care and experiments were conducted according to our institutional animal care and use committee guidelines. Murine melanoma B16F10 and RMA-S cells were from the American Type Culture Collection. Transfected RMA-S/CD80 cells were prepared using a cDNA plasmid encoding for mouse CD80.

**Peptides, antibodies, and tetramers**

Synthetic peptides representing the CD8 T-cell epitopes hgp10025 (KVPRNQDWL), mgp10025 (EGSRNQDWL), LCMV33 (KAVYNFATM), Trp1455 (TAPDNLGYA), the heteroclitic anagram LCMV33 (KAVYNFATM), Trp1455 (TAPDNLGYA) and Ova55 (KVVRFDKL) were from A&A Labs. Monoclonal anti-mouse CD40 (FGK45.5) and anti-4-1BB/CD137 (2A) were isolated from hybridoma culture supernatants. Anti-mouse programmed death ligand-1 (PD-L1; clone FKG45.5), and 50 μg/dose anti-CD40 monoclonal antibody (mAb; clone FKG45.5) were provided by the NIAID Tetramer Core Facility (Emory University, Atlanta, GA). Fluorescent antibodies for flow cytometry were from eBioscience, Inc.

**Adoptive cell transfers and immunizations**

B6 mice were injected intravenously with 0.3 to 3 × 10⁶ splenocytes from naive Pmel-1 mice. Mice were vaccinated one day after adoptive cell therapy. TriVax consists of 200 μg synthetic peptide, 100 μg anti-CD40 monoclonal antibody (mAb; clone FKG45.5), and 50 μg poly-IC (Poly-ICLC, Oncovir, Inc.), which was administered as a mixture via the intravenous (tail vein) route. Some mice were immunized subcutaneously with 200 μg of hgp10025 emulsified in incomplete Freund’s adjuvant (IFA/hgp10025) or intravenously with 3 × 10⁶ Den-dritic cells (DC) pulsed with 10 μg of hgp10025 for 18 hours. DCs were generated from bone marrow macrophages cultured for 6 days with 10 ng/mL granulocyte macrophage colony-stimulating factor and 5 μg/mL IL-4 and matured with 20 ng/mL TNFα (PeproTech). For PD1 blockade, anti-PD-L1 mAb was administered intraperitoneally (200 μg/dose) on days 2, 4, 6, 8, and 10 after TriVax. IL-2/anti-IL-2 mAb complexes (IL2Cx) were prepared by incubating 2 μg recombinant mouse IL-2 with 10 μg anti-mouse IL-2 (JES6-5H4) per dose for 18 hours at 4°C. IL2Cx were administered intraperitoneally on days 2, 3, 5, and 7 after TriVax. For adoptive transfer using endogenous, vaccine-generated T cells, B6 mice received 3 × 10⁶ Trp1455-specific CD8 T cells generated in B6 mice immunized with Trp1455/9A/CD80. CD8 T cells were purified from spleens (Miltenyi Biotec) 10 days after the second immunization, followed by culture with irradiated (60 Gy) B16 cells for 7 days in the presence of 50 IU/mL IL-2 and 5 ng/mL IL-7 (PeproTech).

The cells used in adoptive cell therapy were more than 50% tetramer positive for Trp1455.

**Evaluation of cellular immune responses**

For measuring antigen-specific CD8 T-cell responses, peripheral blood samples or splenocytes were stained with fluorescein isothiocyanate (FITC)-anti-MHC class II, PerCP Cy5.5-anti-CD8α, and PE-conjugated tetramers (or in some instances APC-anti-CD90.1, for Pmel-1 cells). For intracellular staining, splenocytes were incubated with 5 μg/mL peptide for 6 hours at 37°C and stained for intracellular cytokines, IL-2, IFNγ, and TNFα. FlowJo software. To evaluate the in vitro T-cell tumor recognition, 1 × 10⁶ purified antigen-specific CD8 T cells (adjusted by tetramer staining) from TriVax immunized mice were cocultured with various numbers of irradiated (60 Gy) B16 cells in 96-well plates for 2 days. Antigen-induced IFNγ was measured in culture supernatants by ELISA (eBioscience).

**Antitumor effects**

B6 mice were injected subcutaneously with 3 or 5 × 10⁶ B16 cells and 7 (or 12 days) later, they received Pmel-1 splenocytes or activated Trp1455 reactive T cells. The following day, the mice were vaccinated and subsequently received IL2Cx or PD1 blockade as described above. Tumor growth was monitored every 3 to 4 days in individual tagged mice by measuring 2 opposing diameters with a set of calipers. Results are presented as the mean tumor size (area in mm²) ± SD for every treatment group at various time points until the termination of the experiment (usually when tumors in the control group reached 2 cm diameter).

**Peptide/MHC-I elution and quantification**

A mild acid peptide elution procedure (27) using viable fractions derived from the B16 cell acid eluate were estimated for mgp10025 and Trp1455. For measuring antigen-specific CD8 T-cell responses, peripheral blood samples or splenocytes were stained with fluorescein isothiocyanate (FITC)-anti-MHC class II, PerCP Cy5.5-anti-CD8α, and PE-conjugated tetramers (or in some instances APC-anti-CD90.1, for Pmel-1 cells). For intracellular staining, splenocytes were incubated with 5 μg/mL peptide for 6 hours at 37°C and stained for intracellular cytokines, IL-2, IFNγ, and TNFα. FlowJo software. To evaluate the in vitro T-cell tumor recognition, 1 × 10⁶ purified antigen-specific CD8 T cells (adjusted by tetramer staining) from TriVax immunized mice were cocultured with various numbers of irradiated (60 Gy) B16 cells in 96-well plates for 2 days. Antigen-induced IFNγ was measured in culture supernatants by ELISA (eBioscience).
by measuring the amount of IFNγ produced and comparing these measurements to the values obtained in the linear portion of the synthetic peptide standard curves, taking into account dilution factors and adding the values obtained for all fractions for each peptide that stimulated the T cells.

MHC binding and stabilization assays

Peptide/MHC binding and dissociation assays were carried out following similar procedures as described by van Stipdonk (28). TAP-deficient RMA-S cells were incubated with serial dilutions of various peptides for 18 hours. For peptide-dependent stabilization assays, RMA-S cells were loaded with 20 μg/mL peptides for 1 hour, washed, and incubated in AIM-V medium at 37°C for various time points. After these incubations, sake dilution of H-2Db was measured by flow cytometry using FITC-conjugated mAb (28-14-8: eBioscience).

Statistical analyses

The results are representative of data obtained from at least 2 independent experiments. Statistical significance to assess numbers of antigen-specific CDB8 T cells was determined by unpaired Student t tests. Tumor sizes between 2 populations throughout time were analyzed for significance using 2-way ANOVA. All analyses and graphics were done using Prism 5.01 software (GraphPad).

Results

Effects of vaccination in antitumor efficacy of adoptive cell therapy

Three peptide vaccination modes were compared for their ability to enhance the efficacy of adoptive cell therapy with Pmel-1 T cells against 7-day (subcutaneous) B16 tumors. Pmel-1 T cells recognize the mgp10025 (EGSRNQDWL), H-2Db-restricted epitope expressed by B16 and normal melanocytes (25). The hgp10025 peptide (KVRPRNQDWL) function as a heteroclitic T-cell epitope because it binds with higher affinity to H-2Db (29). Peptide hgp10025 administered in IFA did not decrease the tumor growth rate as compared with the unvaccinated mice (Fig. 1A). DCs pulsed with hgp10025 had a modest therapeutic effect, whereas TriVax (hgp10025 peptide mixed with poly-IC, or peptide plus anti-CD40 antibody and administered intravenously; ref. 26) had a substantially higher antitumor effect. TriVax play in the generation of the antitumor effect with Pmel-1 had a negligible therapeutic effect.

Next, we assessed the roles that each of the components of TriVax play in the generation of the antitumor effect with Pmel-1 adoptive cell therapy. The vaccine containing all 3 components (TriVax) was significantly superior to the administration of peptide alone, peptide plus poly-IC, or peptide plus anti-CD40 antibody (Fig. 1B). Substitution of the hgp10025 peptide for an irrelevant peptide (Ova55) in TriVax slightly delayed tumor growth (compared with the no treatment control) but was clearly not as effective as hgp10025TriVax. Moreover, the small therapeutic effect observed with TriVax Ova55 was independent of adoptive cell therapy and did not enhance the expansion of the Pmel-1 cells when adoptively transferred into the tumor-bearing hosts (Fig. 1C). Measurements of the Pmel-1 cell numbers in blood at various time points showed that TriVax was the most effective vaccine in expanding the Pmel-1 cells (Fig. 1C). However, these numbers decreased substantially by days 25 to 35 (50%–75%, respectively), which correlated with the observed tumor growth (Fig. 1B). Similar experiments conducted in tumor-free mice also indicated that TriVax was substantially more effective than peptide alone, peptide plus poly-IC, or peptide plus anti-CD40 antibody in expanding Pmel-1 cells (Supplementary Fig. S1A and B). Moreover, the large decrease in Pmel-1 cell numbers observed in TriVax immunized tumor-bearing mice in which the cells disappeared by day 45 (Fig. 1C) was not apparent in tumor-free mice, in which more than 50% of the cells remained at a similar time point (Supplementary Fig. S1C).

Effects of IL-2, PD1 blockade, and T-cell dose in antitumor efficacy of adoptive cell therapy

We have reported that TriVax induces massive T-cell responses that are substantially higher than those generated by other modes of vaccination (26, 30). We hypothesized that the number of T cells that expand after vaccination determine the antitumor effects of adoptive cell therapy. To further increase T-cell numbers, we evaluated the use of IL-2 in the form of IL2Cx, which have been shown to potentiate in vivo CDB8 T-cell expansion (31, 32). In addition, because it has been reported that B16 cells express PD-L1, a ligand for the inhibitory receptor PD1 found on activated T cells, we evaluated the use of PD1 blockade using anti–PD-L1 antibodies (33–35). The results shown in Fig. 2A indicate that in the presence of adoptive cell therapy, the addition of either IL2Cx or PD1 blockade increase dramatically the efficacy of TriVax, in which complete tumor regressions were now attained. The improved antitumor effects observed in adoptive cell therapy with the TriVax IL2Cx (or PD1 blockade) combination were accompanied by a substantial improvement in the sustained numbers of expanded Pmel-1 cells (Fig. 2B). Interestingly, the administration of TriVax with IL2Cx or PD1 blockade in the absence of adoptive cell therapy had a small but significant therapeutic effect that did not correlate with the presence of endogenously generated T cells by the hgp10025 peptide (Fig. 2A and B, open symbols). Next, we compared 3 doses of Pmel-1 cells and the use of TriVax with IL2Cx or PD1 blockade for their therapeutic effects against 7-day established B16 tumors. Indeed, the number of Pmel-1 cells used for adoptive cell therapy correlated with the antitumor efficacy (Fig. 2C). However, regardless of the Pmel-1 cell dose, TriVax alone was not able to achieve complete tumor rejection. Nevertheless, the implementation of IL2Cx or PD1 blockade together with TriVax resulted in numerous tumor rejections.

The tumor size may impact the effectiveness of T-cell immunotherapy. Adoptive cell therapy using Pmel-1 cells followed by TriVax was used to treat 12-day established B16 tumors (~1 cm diameter). Under these circumstances, adoptive cell therapy followed by TriVax alone reduced the median tumor growth rate only by approximately 5 days (Fig. 3A). The addition of PD1 blockade to the therapy reduced significantly the median tumor growth rate (~22 days), but no tumor rejections were observed. In contrast, IL2Cx with TriVax
resulted in tumor rejection in 80% of the mice. When the frequency of Pmel-1 cells in blood was measured, it became evident that the combination using IL2Cx helped to maintain higher numbers of the adoptively transferred T cells as compared with TriVax alone or TriVax/PD1 blockade (Fig. 3B).

Figure 1. Effects of peptide-based vaccines on the therapeutic efficacy of adoptive cell therapy. A, B6 mice (4 per group) were inoculated subcutaneously on day 0 with 3 × 10^5 live B16 cells and adoptively transferred on day 7 with 3 × 10^5 naive Pmel-1 CD8 T cells followed by immunization on day 8 (arrow) with either TriVax, hgp100 (25) in IFA, or DCs pulsed with hgp100 (25). B, tumor-bearing mice that received adoptive cell therapy as described in A were immunized one day later with various combinations of peptide, poly-IC, and anti-CD40 mAb as indicated. Nonvaccinated mice (No Vax) and Ova55 TriVax-vaccinated mice with and without adoptive cell therapy were included as controls. C, presence of antigen-specific CD8 T cells in blood observed at various time points in the mice that received adoptive cell therapy from the experiment in B. For A and B, tumor sizes are presented as tumor areas in mm^2. Points, mean for each group of mice; bars, SD. P value was calculated using 2-way ANOVA test comparing with the nonvaccinated group. For C, each symbol denotes the percentage of tetramer positive cells related to total CD8 T cells in an individual mouse in each group. ACT, adoptive cell therapy.

Effects of IL2Cx and PD1 blockade in TriVax generated responses after adoptive cell therapy

The above results suggested that PD1 blockade and IL2Cx therapy potentiate the efficacy of Pmel-1 adoptive cell therapy mostly by enhancing T-cell expansion induced by TriVax. To
further study this possibility, we evaluated the overall expansion of adoptively transferred Pmel-1 cells induced by TriVax alone or in combination with PD1 blockade or IL2Cx in tumor-free hosts by enumerating the total numbers of Pmel-1 cells in their spleens. Seven days after TriVax immunization, the Pmel-1 cells expanded approximately 20-fold (Fig. 4A). With the addition of PD1 blockade or IL2Cx to TriVax, the T-cell expansion increased to 100-fold and 370-fold, respectively.

Functional assessment of the expanded Pmel-1 cells revealed that production of IFNγ, IL-2, and TNFα induced by antigen stimulation was similar in mice receiving TriVax alone or combined with PD1 blockade or IL2Cx (Fig. 4B). The expression of PD1 on the T cells could have an impact on their effectiveness against PD-L1–expressing tumor cells. Figure 4C shows that the levels of PD1 were lower on the Pmel-1 cells obtained from TriVax/IL2Cx and TriVax/PD1 blockade groups as compared with TriVax alone. The levels of PD1 in all cases were higher as compared with those observed in naive Pmel-1 T cells. The possibility that IL2Cx and PD1 blockade could also impact the qualitative response of the adoptively transferred Pmel-1 cells after TriVax was examined. Tumor recognition by the Pmel-1 cells will have an impact in the therapeutic benefit of adoptive cell therapy. Pmel-1 cells from mice that received adoptive cell therapy/TriVax combined with either IL2Cx or anti-PD-L1 antibodies were much more effective in recognizing B16 cells in vitro as compared with the Pmel-1 cells obtained from mice that received TriVax alone (Fig. 4D). Thus, the addition of IL2Cx or PD1 blockade in adoptive cell therapy/TriVax improved both quantitatively and qualitatively the Pmel-1 response. Notably, costimulatory antibodies to OX40 and 4-1BB, which are known to enhance the magnitude and quality of T-cell responses to vaccination, were not as effective as anti-PD-L1 in increasing T-cell numbers or in potentiating the therapeutic effect of adoptive cell therapy/TriVax (Supplementary Fig. S2).

Efficacy of adoptive cell therapy using TriVax generated polyclonal CD8 T cells

The source of CD8 T cells for adoptive cell therapy in humans can vary, such as using in vitro expanded tumor-infiltrating lymphocytes or genetically modified CD8 T cells.
Effective Vaccines Enhance Adoptive T-cell Therapy

Trp1455/9M peptide. Adoptive cell therapy using vaccine-T cells through TriVax immunization using the heteroclitic explored this approach by generating antigen-specific expanded in tissue culture before adoptive cell therapy. We those generated through vaccination and subsequently complete tumor regressions were observed with the Trp1455 results obtained with Pmel-1 adoptive cell therapy, no com-

tant use of PD1 blockade or IL-2Cx further increased the effective in reducing the rate of tumor growth and the con-

comitant use of PD1 blockade or IL-2Cx. Nevertheless, in contrast to the therapeutic effect (Fig. 5A). However, we observed that TBI-induced lymphodepletion, administered 1 day before adoptive cell therapy with Trp1455/463/9M-specific CDB T cells did not further augment therapeutic effects (Supplementary Fig. S3A) or antigen-specific CDB T-cell levels (Supplementary Fig. S3B) in the TriVax-treated mice regardless of whether they received IL2Cx or not.

T-cell epitope levels on tumor cells determine the effectiveness of adoptive cell therapy

The results presented above indicated that adoptive cell therapy using Pmel-1 CDB T cells, specific for the mgp10025, was more effective than adoptive cell therapy using Trp1455-specific T cells. One possibility that might explain these differences would be if the antigen avidity of the TCR trans-
genic Pmel-1 cells was higher than the vaccine-generated Trp1455 T cells. However, peptide titration curve comparisons between these T cells revealed that the Trp1455-specific T cells exhibited a substantially higher avidity (~30-fold), as com-
pared with Pmel-1 cells (Supplementary Fig. S4A). Another possible explanation for the superior therapeutic effect of Pmel-1 cells could be if the B16 cells expressed higher levels of mgp10025/H-2Db than Trp1455/H-2Db. To study this possi-
bility, MHC-I binding peptides on B16 cells were eluted with mild acid treatment and the amounts of the 2 T-cell epitopes were measured as described in Methods. The results indicated that approximately 100-fold more of mgp10025 was obtained from the B16 acid eluates as compared with Trp1455 (Supplementary Fig. S4B).

The peptide/MHC-I density on the B16 cells for a specific epitope will be determined by numerous factors such as peptide binding affinity for its MHC-I (36) and by the amount of peptide epitope produced by the MHC-I antigen-processing pathway. Using MHC-I stabilization assays, we compared the

Figure 3. Therapeutic effects of adoptive cell therapy/TriVax against advanced B16 tumors. A, B6 mice (5 per group) were injected subcutaneously with 5 x 10⁵ B16 cells, and on day 12 received 1 x 10⁶ Pmel-1 cells followed by TriVax on day 13 (arrow) and PD1 blockade or IL2Cx. P values were calculated using 2-way ANOVA. * 4 of 5 mice rejected their tumors. B, antigen-specific CDB T cells were evaluated by tetramer analysis on day 25 using blood samples. Points, value for each mouse; horizontal lines, averages of the group. P values were calculated with unpaired Student t tests. ACT, adoptive cell therapy.
capacity of mgp10025 and Trp1455 to bind to empty H-2Db molecules expressed by RMA-S cells. Notably, Trp1455 was approximately 100 times more effective in binding to H-2Db as compared with mgp10025 and the positive control LCMV33 (Supplementary Fig. S4C). The peptide dissociation offrate from MHC-I will also affect the cell-surface density of peptide/MHC-I complexes (37). Both mgp10025 and Trp1455 had similar dissociation rates (Supplementary Fig. S4D). These results suggested that the higher amounts of mgp10025/H-2Db levels on B16, as compared with the levels of Trp1455/H-2Db are not due to stronger peptide/MHC-I–binding affinity or to slower MHC-I dissociation rates of the mgp10025 epitope.

Discussion

Although adoptive cell therapy represents one of the most promising modes of cancer immunotherapy, its implementation in the clinic remains challenging, and in some instances the results are suboptimal. The Pmel-1 TCR transgenic mouse has aided in the development and implementation of adoptive cell therapy protocols in human cancer patients. Studies by Restifo and collaborators showed that many experimental conditions play a critical role in determining the efficacy of adoptive cell therapy against established B16 melanoma (24, 25, 38–40). Early studies showed that vaccination using live recombinant vaccinia virus (rVV) and the administration of high-dose IL-2 (~6 × 10^5 IU, or 3.6 mg/Kg/day, 4 days) were necessary for obtaining tumor rejection (25). The use of live rVV vaccines generates safety issues in humans, so it is not surprising that it has not been broadly implemented in adoptive cell therapy clinical protocols. On the other hand, high-dose IL-2 has been used in the clinic, but the systemic toxic effects that sometimes are lethal are of great concern (41, 42). Other studies showed that lymphodepletion induced by TBI further increased the efficacy Pmel-1 adoptive cell therapy, and accordingly, this procedure has been translated into the clinic (14, 21, 22). It has been proposed that TBI potentiates adoptive cell therapy, by increasing the access of the transferred T cells to cytokines, removing inhibitory T regulatory cells, and through a gut-injured microbial translocation that releases immunostimulatory TLR ligands (20, 43).

Here we show that Trivax, a noninfectious subunit vaccine was capable of generating substantial antitumor effects in the Pmel-1 adoptive cell therapy model. When compared with peptide-pulsed DCs (currently the golden standard of the field), Trivax was more than twice as effective. Although increasing...
the number of Pmel-1 cells enhanced the antitumor efficacy, no complete tumor regressions were observed when TriVax alone was used. Thus, we considered 2 additional approaches to further promote the expansion of Pmel-1 cells to achieve maximal therapeutic benefits.

In vivo use of IL2Cx increases the expansion of antigen-stimulated CD8 T cells (32). Therefore a main advantage of using IL2Cx over IL-2 is that the complexes remain on for longer times in circulation, allowing the use of lower cytokine doses (0.1 mg/kg/d). Also, some anti-IL-2 antibodies, such as the one we used here, block IL-2 binding to CD25 (high-affinity IL2R), but allow binding to CD122 (intermediate affinity IL2R), promoting the expansion of activated and memory T cells without expanding T regulatory cells (31). Engagement of the PD1 receptor on T cells by its ligands (PD-L1 or PD-L2) during antigen stimulation inhibits TCR activation, limiting T-cell expansion (35). PD1 blockade using anti-PD-L1 mAbs improves T-cell activation and function (34). Our results showed that additions of IL2Cx or anti-PD-L1 to TriVax increased TriVax-driven expansion of adoptively transferred Pmel-1 cells by 10- and 4-fold, respectively, as compared with TriVax alone, and the therapeutic benefits of adoptive cell therapy were clearly evident, achieving in many cases complete tumor rejections. These results suggested that by simply increasing Pmel-1 numbers, using higher T-cell doses for adoptive cell therapy or by including IL2Cx or PD1 blockade, maximal therapeutic benefit was achieved. Nevertheless, the results also showed that IL2Cx and PD1 blockade increased the qualitative potency (antitumor recognition and decreased PD1 expression) of Pmel-1 cells. Pmel-1 cells from mice receiving adoptive cell therapy/TriVax with either IL2Cx or anti-PD-L1 antibodies recognized B16 tumor cells more than 10-fold better than Pmel-1 cells from naïve mice.
mice receiving TriVax alone. We do not know what specific changes in Pmel-1 cells are induced by IL2Cx or PD1 blockade, resulting in increased antitumor reactivity. One possible mechanism for enhancing antitumor recognition could be an improvement in T-cell avidity by increasing expression levels of costimulatory/adhesion T-cell receptors such as CD8/lck, CD2, and lymphocyte function-associated antigen-1 (LFA-1).

An important observation was that the addition of IL2Cx and to some extent PD1 blockade prevented the observed loss of the Pmel-1 cells expanded by TriVax in tumor-bearing hosts, which could be explained by the reduced levels of PD1 induced by these adjuncts. Notwithstanding, the overall results indicate that the therapeutic antitumor potentiation induced by IL2Cx and PD1 blockade in this model of adoptive cell therapy followed by TriVax is derived by an enhancement of both the quantitative and qualitative aspects of the CD8 T-cell response.

We also presented evidence that polyclonal, vaccine-generated CD8 T cells were effective in adoptive cell therapy/TriVax. However, in contrast with Pmel-1 adoptive cell therapy, no complete tumor rejections were obtained with the Trp1455/9M/TriVax-generated T cells with IL2Cx or PD1 blockade. The higher antitumor efficacy of the Pmel-1 T cells compared with the Trp1455/9M/GSK10025-generated CD8 T cells may be explained by the observations that B16 contained approximately 100-times more mgp100/H-2Db than Trp1455/H-2Db complexes. We attempted to induce endogenous, vaccine-generated CD8 T cells using hgp10025TriVax for adoptive cell therapy. However, the antigen avidity of the endogenous hgp10025TriVax generated T cells was 10-fold lower as compared with the Pmel-1 cells, and these cells failed to recognize tumor cells (Supplementary Fig. S5). It is likely that the Pmel-1 cells express a TCR in the high end of antigen affinity spectrum because of the procedure used to select the T cells from which the TCR genes were clones (6). Nonetheless, the therapeutic antitumor effects obtained in adoptive cell therapy using the vaccine-generated Trp1455/9M T cells were not meager, especially when combined with IL2Cx or PD1 blockade and offer an alternative to those instances in which high-affinity TCR genes are not available for transduction into effector T cells.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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