Human CTL Epitopes Prostatic Acid Phosphatase-3 and Six-Transmembrane Epithelial Antigen of Prostate-3 as Candidates for Prostate Cancer Immunotherapy

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Abstract

Specific immunotherapy of prostate cancer may be an alternative or be complementary to other approaches for treatment of recurrent or metastasized disease. This study aims at identifying and characterizing prostate cancer–associated peptides capable of eliciting specific CTL responses in vitro. Evaluation of peptide-induced CTL activity in vitro was done following immunization of HLA-A2 transgenic (HHD) mice. An in vitro tumor rejection test was tested by adoptive transfer of HHD immune lymphocytes to nude mice bearing human tumors. To confirm the existence of peptide-specific CTL precursors in human, lymphocytes from healthy and prostate cancer individuals were stimulated in vitro in the presence of these peptides and CTL activities were assayed. Two novel immunogenic peptides derived from overexpressed prostate antigens, prostatic acid phosphatase (PAP) and six-transmembrane epithelial antigen of prostate (STEAP), were identified; these peptides were designated PAP-3 and STEAP-3. Peptide-specific CTLs lysed HLA-A2.1+ LNCaP cells and inhibited tumor growth on adoptive immunotherapy. Furthermore, peptide-primed human lymphocytes derived from healthy and prostate cancer individuals lysed peptide-pulsed T2 cells and HLA-A2.1+ LNCaP cells. Based on the results presented herein, PAP-3 and STEAP-3 are naturally processed CTL epitopes possessing anti–prostate cancer reactivity in vivo and therefore may constitute vaccine candidates to be investigated in clinical trials. (Cancer Res 2005; 65(14): 6435–42)

Introduction

Prostate cancer is the most diagnosed noncutaneous cancer in the western male population and the second leading cause of cancer-related death in this population (1). Yet, treatment of hormone-refractory metastatic prostate cancer remains ineffective (1). Alternative approaches based on immunotherapy have used recent advances in identification of tumor-specific and tumor-associated antigens (TAA) and have shown therapeutic promise (2). Tumor-specific antigens come from mutated proteins and from viral proteins in virally associated tumors. TAs are embryonal reexpressed or overexpressed proteins and differentiation antigens (3). Clinical trials, which test peptide-based vaccination, have been conducted mainly in melanoma patients and resulted in partial clinical responses in 10% to 30% of patients (4). Moreover, metastatic melanoma regression was achieved by adoptive transfer of peptide-specific CTLs directed against differentiation antigens after a nonmyeloablative conditioning regimen (5).

Yet, relatively few prostate carcinoma–derived antigens have been identified and evaluated as potential reagents for immunotherapy. The earliest prostate-restricted antigens include prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA), and prostatic acid phosphatase (PAP), all overexpressed in prostate cancer. PSA and PSMA peptides were tested in clinical trials (6). PSA-derived peptides generate in vitro T-cell responses when cultured in peripheral blood mononuclear cells (PBMC) of HLA-A2+ or HLA-A24+ donors (7, 8). In a phase II trial, dendritic cell vaccines pulsed with HLA-A2+-restricted PSA peptides resulted in CTL induction of 2 of 33 patients not accompanied by clinical manifestations (9). In accordance, both peptides failed to induce effective CTL responses against prostate tumor cells expressing PSMA (10). There is evidence of CD8 T-cell response against PAP (11, 12). Yet, to the best of our knowledge, there are no reports demonstrating in vivo antitumor reactivity of PAP-derived peptide vaccines. Recent studies describe differentially expressed prostate-specific genes, which may be a potential source of candidates for peptide-based vaccination. These include six-transmembrane epithelial antigen of prostate (STEAP; ref. 13), prostate stem cell antigen (PSCA; ref. 14), prostate carcinoma tumor antigen-1 (PCTA-1; ref. 15), prostate tumor-inducing gene-1 (PTI-1; ref. 16), prostate-specific gene with homology to G protein–coupled receptor (17), and prostase, an androgen-regulated serine protease (18).

Direct and reverse strategies are used for identification of TAA epitopes. The direct approach depends on patient-derived established CTL lines and genetic or biochemical screening. However, it is difficult to establish human carcinoma lines as well as carcinoma-associated CTL lines. Moreover, conceptually CTL lines from cancer patients may partially represent the repertoire of the anergized immune system. Furthermore, the in vitro propagation of CTL lines may enhance emergence of sporadic clones rather than tumor-specific clones. Alternatively, the modified reverse approach involves prediction of putative TAA-derived epitopes with amino acid anchor motifs specific for the defined human HLA allele (often A2.1, a prevalent allele in the population). Next, the predicted peptides are screened in HLA transgenic mice to identify immunodominant epitopes. In the last few years, several studies have determined concordance between the CTL repertoires of HLA-A2.1-restricted peptides in human PBMC and the CTLs (19).
The dominant murine H-2-restricted responses that take place upon immunization with multi-epitope proteins have been overcome in second-generation HLA transgenic mice [i.e., HHD mice (D\(^{b/-}\) × I\(^{b/-}\)-null mice transgenic for a recombinant HLA-A2.1/D\(^{b/-}\)/2m single chain (20)]. Recently, novel breast tumor-associated MUC1 and BA46-derived peptides were characterized (21, 22). Noticeably, consistent with their immunogenity in the HHD mice, BA46-derived peptides stimulated cytotoxic activity in PBMC from HLA-A2.1-positive breast carcinoma patients (22). Thus, the HHD model is an effective means for screening of candidate TAA peptides.

In the current study, we used the HHD mouse system to screen candidate prostate-associated TAA peptides for immunogenic HLA-A2.1-restricted CTL epitopes. The results show that CTLs against PAP-3 (ILLWQPIPV) and STEAP-3 (LLLGTIHAL) lyse HLA-A2.1 and antigen-positive tumor cells in vitro and inhibit tumor growth in vivo. Furthermore, PAP-3 and STEAP-3 prime antitumor CTLs in human PBMC from healthy and prostate cancer donors.

**Materials and Methods**

**Mice.** The derivation of HLA-A2.1/D\(^{b/-}\)/2m monochain, transgenic, H-2\(^{D/-}\)/I\(^{b/-}\)/2m/- double-knockout mice (HHD mice) was described previously (20). Male CD\(^{m/-}\)/2m mice (6-12 weeks old) were bred in Weizmann Institute of Science (Rehovot, Israel). All experiments were conducted in accordance with Weizmann Institute of Science Animal Facility and NIH guidelines.

**Cell lines.** LNCaP-1740 (HLA-A2\(^{b/-}\)), LNCaP-10995 (HLA-A2\(^{m/-}\)), and PC-3 and DU 145 (both HLA-A2\(^{-}\)) are human prostate carcinoma cell lines obtained from the American Type Culture Collection (Manassas, VA). The PC-3/HHD clone is a HLA-A2.1/2m single-chain (HHD) transfectant of PC-3 cells. EL4-HHD and RMA-S-HHD clones are HHD transfectants of the murine T lymphoma EL4 and TAP-2-deficient RMA-S cells, respectively. The RMA-S-HHD-B7.1 clone is a HHD transfectant expressing the murine B7.1 immunostimulatory molecule. T2 is a TAP-2-deficient lymphoblastoid line of HLA-A2 genotype. PC-3, DU 145, and T2 cells were maintained in RPMI 1640 (Sigma, St. Louis, MO) supplemented with 10% FCS (Life Technologies, Rockville, MD) and combined antibiotics. Viable cells (effector cells) were separated by FACScan and CellQuest software (Becton Dickinson, San Jose, CA).

**Reverse transcription-PCR analysis.** Total cytoplasmic RNA was isolated from logarithmically growing cell cultures. Tissue samples were obtained from patients who underwent radical prostatectomy at Barzilai Medical Center (Ashkelon, Israel). All tissues were histologically confirmed obtained from patients who underwent radical prostatectomy at Barzilai Medical Center (Ashkelon, Israel). All tissues were histologically confirmed.

**In vitro cytotoxicity assay.** HHD mice were immunized i.p. thrice at 7-day intervals with 2 × 10\(^7\) irradiated (5,000 rad) peptide-pulsed RMA-S-HHD-B7.1 or 10\(^6\) peptide-pulsed (100 μmol/L for 2 hours at 37°C) dendritic cells. In mixed synthetic vaccines, RMA-S-HHD-B7.1 cells were loaded separately with each peptide and admixed before vaccination.

**Adaptive immunotherapy of CD1\(^{m/-}\)/2m mice bearing human prostate carcinoma.** HHD male mice were immunized i.p. six times at 3-day intervals with 2 × 10\(^7\) peptide-pulsed syngeneic dendritic cells. Spleens were removed 10 days after the last immunization and stimulated as above. Lympholyte-M gradient purified cells were seeded in 24-well plates.
from BD PharMingen, San Diego, CA). Male CD1, CD8, and CD45R was detected by FACScan (all antibodies were purchased in lymphocyte medium with hIL-2 for an additional 4 days. Expression of CD4, total body irradiation from a 137Cs source followed by s.c. inoculation in the upper back of 5 x 10^6 PC-3-HHD cells admixed 1:1 with Matrigel (Becton Dickinson). Seven days later, the mice were injected around the tumors with the indicated number of HHD-derived activated T cells followed by local s.c. administration of 1,000 units hIL-2 twice daily for 5 days. Tumor size was measured in two perpendicular dimensions thrice weekly. Mice were sacrificed 44 days from tumor inoculation.

In vitro cytotoxicity assays of human peripheral blood mononuclear cells. HLA-A2.1-positive healthy volunteers and patients (after informed consent) with localized prostate carcinoma, after surgery, were recruited for in vitro generation of human CTLs. Leukapheresed PBMCs (Barzilai Medical Center) were isolated by centrifugation on Ficoll-Plaque Plus gradients (Amersham, Uppsala, Sweden). Mature autologous dendritic cells were prepared from adherent PBMC by incubation in the presence of IL-4 (1,000 units/ml, R&D, Minneapolis, MN) and GM-CSF (1,000 units/ml; R&D) followed by maturation with a cocktail of IL-1β, IL-6, tumor necrosis factor-α (each at 10 ng/ml, R&D), and prostaglandin E2 (1 μg/ml, Alexis, Carlsbad, CA). Dendritic cells were pulsed with synthetic peptides and then overlaid with PBMC in the presence of IL-7 (20 ng/ml; R&D). Two days later, hIL-2 (12 units/ml, Prospek) was added and renewed every 2 days. Two additional restimulations were done every 7 days over peptide-pulsed monocytes. Seven days later, lymphocytes were harvested and cytolytic assays were done using peptide-pulsed T2 cells or human prostate carcinoma cells as targets.

Results

Expression patterns of candidate tumor-associated antigens in prostate cancer. Candidate targets for immunotherapy include prostate selectin antigens overexpressed in prostate cancer. To evaluate the expression profile of such genes, we used reverse transcription-PCR (RT-PCR) analysis. We examined the expression patterns of PSA, PSMA, PAP, STEAP, PCTA, PTI-1, and prostate in human prostate cancer cell lines LNCaP, DU 145, and PC-3, in five prostate cancer specimens and in six BPH samples. PAP, STEAP, PSA, and PSMA were overexpressed in most prostate cancer samples compared with BPH (Fig. 1; data not shown). In our hands, PTI-1, PCTA-1, and prostate were not overexpressed in most prostate cancer specimens (data not shown). Thus, PAP, STEAP, PSA, and PSMA are a possible source for immunogenic peptides.

Screening for HLA-A2.1-restricted peptides. The four selected antigens were screened for potential TAA peptides predicted to bind HLA-A2.1 molecules and scored according to their ability to stabilize MHC using "HLA-binding predictions" software (24). This software scores every possible peptide along the protein sequence in a dose-dependent manner as determined by FACS analysis. MHC binding at 100 μmol/L gave the highest level of stabilization that was comparable with that of reference tyrosinase-derived, HLA-A2.1-restricted peptide (Fig. 3). A high correlation between predicted and actual binding has been confirmed (22).

Confirmation of predicted peptide binding by stabilization of cell surface MHC. To examine the actual peptide binding to HLA-A2.1 molecules, we used TAP-2-deficient, HLA-A2.1-transfected RMA-S cells (RMA-S-HHD), which express low levels of peptide-free and therefore unstable class I MHC molecules. External loading of RMA-S-HHD cells with selected synthetic peptides at 0.1 to 100 μmol/L stabilized HLA-A2.1 molecules at a dose-dependent manner as determined by FACS analysis. MHC binding at 100 μmol/L gave the highest level of stabilization that was comparable with that of reference tyrosinase-derived, HLA-A2.1-restricted peptide (Fig. 3). A high correlation between predicted and actual binding has been confirmed (22).

Figure 1. Profile of PAP and STEAP expression in tissue samples of BPH and prostate cancer and in cell lines. mRNA expression of PAP and STEAP was examined at level of mRNA transcripts by RT-PCR using specific primers on cDNAs. Equal amounts of total RNA from surgical specimens of BPH (BPH-A-BPH-F) and prostate carcinoma (CaP-A-CaP-E) and from logarithmically growing prostate cancer-derived cell cultures (LNCaP, PC-3, and DU 145) were reverse transcribed into cDNAs that were amplified by PCR. The 748- and 260-bp PCR products for PAP and STEAP, respectively, were electrophoresed in 1.5% agarose gels.
by MHC class I molecules and therefore may serve as tumor rejection antigens. We assessed CTL responses against relevant prostate cancer cell lines as targets using the HLA-A2.1-positive LNCaP cells that were shown to express PAP and STEAP proteins as targets for CTLs raised against the peptides of interest (refs. 13, 25; Fig. 4 C and D). To this end, HHD mice were immunized with RMA-S-HHD-B7.1 cells loaded with either PAP-3 or STEAP-3, and lysis of LNCaP cells was monitored (Fig. 4 A). CTLs induced against PAP-3 or STEAP-3 showed similar and specific response profiles against target LNCaP cells. Furthermore, the lysis of HLA-A2.1-negative PC-3 cells by either PAP-3- or STEAP-3-specific CTLs was negligible, suggesting MHC-restricted lysis (Fig. 4 A). Expression of PAP in PC-3 and PC-3-HHD transfectant was confirmed by immunoprecipitation (Fig. 4 C). In contrast to previous reports describing PC-3 cells as negative for PAP (26, 27), we found that both lines express PAP (Fig. 4 D). HHD-derived anti-PAP-3 CTLs lysed efficiently and in a dose-dependent manner PC-3-HHD but not parental PC-3 cells (Fig. 4 B). Taken together, these data indicate that both PAP and STEAP proteins undergo intracellular processing in LNCaP cells.

Adoptive immunotherapy of CD1<sup>nu/nu</sup> mice bearing human prostate carcinoma. We next tested the in vivo antitumor reactivity of PAP-3- and STEAP-3-induced lymphocytes. Because neither LNCaP nor PC-3-HHD cells grow progressively in HHD mice, we established a model of adoptive transfer of HHD-derived lymphocytes into CD1<sup>nu/nu</sup> mice bearing prostate carcinoma. PC-3-HHD cells at 5 × 10<sup>6</sup> admixed with Matrigel but not LNCaP cells formed homogeneous s.c. tumors in 100% of the CD1<sup>nu/nu</sup> mice. To induce peptide-specific CTLs for adoptive transfer, we vaccinated HHD mice with PAP-3, STEAP-3, and HLA-A2.1-restricted, tyrosinase-derived peptide as an irrelevant control, all loaded on syngeneic dendritic cells. CD<sub>1<sup>nu/nu</sup></sub> mice were inoculated s.c. with the PC-3-HHD, and 7 days later, after formation of visible tumors, CTLs were injected around the tumors as described. We observed that 11 × 10<sup>6</sup> per mouse of anti-PAP-3 CTLs completely prevented progressive growth of PC-3-HHD tumors in CD1<sup>nu/nu</sup> mice, whereas the same amount of irrelevant peptide-induced CTLs failed to inhibit tumor development (Fig. 5 A). The CTLs induced by STEAP-3 (3 × 10<sup>6</sup> per mouse) were potent in decreasing the tumor volumes compared

Figure 2. Immunogenicity of PAP3 and STEAP3 peptides in HHD mice. Mice were immunized i.p. thrice weekly with 10<sup>6</sup> peptide-loaded syngeneic mature dendritic cells. DC were loaded separately with PAP-2, PAP-3, and PAP-5 (A) or STEAP-1, STEAP-2, STEAP-3, and STEAP-4 (B), washed, and pooled before immunization. DC were loaded with PAP-3 (C) or STEAP-3 (D) and injected individually. For all experiments, spleens were removed on day 10 after the last boost and splenocytes were stimulated in vitro by corresponding peptide as described. CTL assays were done on day 5 with individual peptides loaded on RMA-S-HHD (A and B) or EL4-HHD (C and D) as targets. A and B, irrelevant HIV-derived, peptide-loaded targets were used as negative controls. STEAP-3-loaded cells were used as nonspecific targets for PAP-3-induced lymphocytes (C) and vice versa (D). The percentages of specific lysis at E:T ratios of 100:1 to 12.5:1 are shown. Columns, mean of three replicates; bars, SD. Representative experiment of four. Specific lysis of PAP-3 and STEAP-3 is statistically significant (P < 0.001, unpaired Student’s t test) at all E:T ratios compared with background lysis.

Figure 3. Stabilization of cell surface MHC by PAP-3 and STEAP-3. PAP-3, STEAP-3, or a tyrosinase peptide, known to bind HLA-A2 molecules (positive control), was loaded at various concentrations (0.1-100 μmol/L) on TAP-2-deficient RMA-S-HHD cells as described. Indirect FACS analysis was done by incubating 5 × 10<sup>6</sup> loaded cells with anti-HLA-A2.1 mAb BB7.2 for 30 minutes at 4°C. Following washing with PBS containing 0.5% BSA and 0.1% sodium azide, the secondary antibody, goat anti-mouse FITC, was applied for 30 minutes at 4°C. Following another wash, the amounts of bound antibodies were detected by a FACScan. Mean fluorescence at 0.1 to 100 μmol/L peptide concentrations. Representative experiment of three.
with the control groups (Fig. 5B). Thus, using adoptive immunotherapy of human prostate carcinoma as an experimental model, we confirmed a strong in vivo antitumor reactivity of PAP-3- and STEAP-3-specific CTLs.

Activation of prostatic acid phosphatase-3–specific and six-transmembrane epithelial antigen of prostate-3–specific CTL precursors in peripheral blood mononuclear cells derived from healthy volunteers and prostate cancer patients. We then asked whether PAP-3- and STEAP-3-specific CTL precursors are found in PBMC of healthy volunteers and more importantly of prostate cancer patients. To this end, bulk lymphocytes from three healthy and two prostate cancer donors were in vitro primed over autologous dendritic cells pulsed with the synthetic peptides followed by two additional cycles of restimulation as described in Materials and Methods. Activated CTLs lysed the TAP-2-deficient HLA-A2.1 T2 target cells loaded with PAP-3 or STEAP-3 and not unloaded cells (Fig. 6A). Moreover, peptide-specific CTLs lysed the HLA-A2.1-positive LNCaP variant (ATCC CRL-1740) but not the HLA-A2.1-negative LNCaP variant (ATCC CRL-10995) and PC-3 cells (Figs. 4 and 6B and C). Based on these data, one may conclude that there are PAP-3- and STEAP-3-specific and potent CTL precursors in peripheral blood of healthy and prostate cancer donors.

Discussion

Two approaches govern active anticancer vaccination. The first uses vaccines directed against unidentified antigens as irradiated tumor cells, either intact or genetically modified, total tumor RNA, or cell lysates (28). An alternative strategy, allowing both monitoring of immune response and rational systematic improvement of vaccine design, is based on use of antigen-specific cancer vaccines that induce immune responses against defined antigens. Indeed, active vaccination with a single TAA epitope has been shown to protect from tumor challenge and to palliate established tumors in vivo (29). Relatively few prostate cancer–derived epitopes have been identified and examined in clinical trials thus far. Contrary to melanomas, prostate cancer–associated epitopes,
such as PSMA derived epitopes, were largely ineffective in specific CTL and clinical responses (9, 10).

In the current study, we identified two HLA-A2.1-restricted CTL epitopes, PAP-3 and STEAP-3, which induce antitumor immunity in vitro and in vivo. Identification of TAA peptides by the reverse approach is based on recognition of candidate TAAs, in silico prediction of HLA-binding epitopes, and testing the peptides for their ability to induce an immune response in human PBMC. This strategy is hard to reproduce and it is highly dependent on the individual heterogeneity of the population. To overcome these problems, we used the HHD mouse model for initial screening of immunogenic peptides derived from candidate TAAs. The HHD mouse combines classic HLA transgenesis (HLA-A2.1/D\textsuperscript{b}-m and Db genes) that restricts murine homologues displays 81% identity in protein sequences with those of the murine homologues and therefore induce xenogenic potential of each peptide with that of others. Two peptides, PAP-3 and STEAP-3, were highly immunogenic in HHD mice (Fig. 2). One possible explanation for the immunogenicity of PAP-3 and STEAP-3 could be that they represent sequences with minimal similarity to the murine homologues and therefore induce xenogenic potential of each peptide with that of others.

Sequence alignment between PAP or STEAP and their murine homologues displays 81% identity in protein sequences between calculated binding affinities and immunogenicity of CTL epitopes has been shown (24, 33). Using a two-step screening in HHD mice, we compared in the same experiment the immunogenic potential of each peptide with that of others. Two peptides, PAP-3 and STEAP-3, were highly immunogenic in HHD mice (Fig. 2). Finally, analysis of CTL responses against murine PAP-3 and STEAP-3 homologues showed comparable immunogenicity with the human peptides. Thus, the immune response to PAP-3 and STEAP-3 was not the result of differences between human and mouse sequences. Lymphocytes induced against PAP-3 or STEAP-3 preferentially lysed the prostate cancer cell line LNCaP and not HLA-A\textsuperscript{2+} PC-3 cells, indicating that PAP-3 and STEAP-3 are processed and presented by LNCaP cells (Fig. 4).

To test the immunotherapeutic potential of PAP-3 and STEAP-3, we established an adoptive transfer model in which PC-3-HHD tumor-bearing nude mice were treated by peptide-specific lymphocytes transferred to the tumor vicinity from immunized HHD mice. In immunoprotection experiments on TRAMP mice, memory lymphocytes specific for T antigen have been transferred from mice immunized with SV40-transformed fibroblasts (35). In our system, we achieved a strong therapeutic effect in palpable tumor-bearing mice using peptide-induced lymphocytes (Fig. 5). PC-3-HHD cells are lysed in vitro by anti-PAP-3 (Fig. 4B) but not by anti-STEAP-3 CTLs (data not shown). Two possible mechanisms could account for the lack of lysis of PC-3-HHD by anti-STEAP-3 CTLs: low expression of STEAP by PC-3-HHD cells or low affinity of STEAP to HLA-A2.1 that decreases the effective lytic activity of STEAP-induced CTLs: low expression of STEAP by PC-3-HHD cells or low affinity of STEAP to HLA-A2.1 that decreases the effective lytic activity of STEAP-induced CTLs. We have shown, we believe for the first time, that a CD8-mediated CTL response to STEAP can be induced in vivo.

Several studies have reported that higher frequencies of survivin (36), HER-2/neu, and carcinoembryonic antigen (37) specific CTL precursors exist in patients suffering from melanoma and colorectal cancer, respectively, than in healthy subjects. On the other hand, cancer patient–derived CTLs that were successfully activated against defined TAA epitope presented by antigen-presenting cells have shown inability to lyse cancer cells presenting the same epitope (38). In our study, PAP-3 and STEAP-3 induced human CTLs in blood lymphocytes from healthy and prostate cancer donors. Importantly, peptide-specific CTLs lysed not only peptide-loaded T2 cells but also MHC-positive LNCaP cells that naturally express the TAAs of interest (Fig. 6). Yet, HLA-A2\textsuperscript{low} cells are not lysed. Thus, low MHC–expressing tumors might be resistant to peptide-based immunotherapy.
Figure 6. Specific antitumor reactivity of peptide-stimulated CTLs derived from healthy and prostate cancer donors. Leukapheresed nonadherent PBMCs from HLA-A2+ donors were primed in vitro with peptide-pulsed autologous dendritic cells. PBMC were supplemented with IL-7, and 2 days later, IL-2 was added and renewed every 3 days. Additional two cycles of restimulation were done over peptide-pulsed monocytes. CTL assays were done using peptide-pulsed T2 cells (A) or prostate cancer–derived cells (B and C) as targets. A, lymphocytes derived from healthy donor were primed and restimulated with either PAP-3 (top) or STEAP3 (bottom) pulsed dendritic cells/monocytes. Relevant (●) for PAP-3 and (●) for STEAP-3 or irrelevant (● for tyrosinase) peptide-pulsed T2 cells were used as targets. Representative of three independent experiments with leukapheresis samples derived from three healthy and two prostate cancer donors. Lymphocytes derived from a healthy volunteer (B) or a prostate cancer donor (C) were primed and restimulated with PAP-3 (top) or STEAP3 (bottom) pulsed dendritic cells/monocytes. Human prostate carcinoma LNCaP (HLA-A220P and HLA-A220B variants) and HLA-A2+ PC-3 cells were used as targets. Data obtained at E:T ratio of 10:1, 5:1, and 2.5:1 (A and 20:1, 10:1, 5:1, and 2.5:1 (B and C). The range of triplicates was <5% of the mean of the triplicates in these experiments.

In conclusion, this study presents a novel approach for in vivo two-step screening of candidate HLA-A2.1-restricted CTL epitopes for cancer immunotherapy. Using the HHD mouse as a model for in vivo evaluation of immunogenic and antigenic potential of putative TAA epitopes, PAP-3 and STEAP-3 have been identified as good vaccine candidates for immunotherapy of prostate cancer.

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References

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