Generation of Human Cytolytic T Lymphocyte Lines Directed Against Prostate-Specific Antigen (PSA) Employing a PSA Oligopeptide Peptide

Pierpaolo Correale, Konstantin Walmsley, Sam Zaremba, MingZhu Zhu, Jeffrey Schlom and Kwong Y. Tsang

*J Immunol* 1998; 161:3186-3194; ; http://www.jimmunol.org/content/161/6/3186

References

This article cites 54 articles, 33 of which you can access for free at: http://www.jimmunol.org/content/161/6/3186.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Generation of Human Cytolytic T Lymphocyte Lines Directed Against Prostate-Specific Antigen (PSA) Employing a PSA Oligopeptidotope Peptide

Pierpaolo Correale, Konstantin Walmsley, Sam Zaremba, MingZhu Zhu, Jeffrey Schlom, 1 and Kwong Y. Tsang

Prostate-specific Ag (PSA), which is expressed in a majority of prostate cancers, is a potential target for specific immunotherapy. Previous studies have shown that two 10-mer PSA peptides (designated PSA-1 and PSA-3) selected to conform to human HLA class I-A2 motifs can elicit CTL responses in vitro. A longer PSA peptide (30-mer) designated PSA-OP (oligoepitope peptide), which contains both the PSA-1 and PSA-3 HLA-A2 epitopes and an additional potential CTL epitope (designated PSA-9) for the HLA-class I-A3 allele, was investigated for the ability to induce cytotoxic T cell activity. T cell lines from different HLA-A2 and HLA-A3 donors were established by in vitro stimulation with PSA-OP; the CTL lines lysed PSA-OP as well as PSA-1- or PSA-3-pulsed C1R-A2 cells, and PSA-OP and PSA-9-pulsed C1R-A3 cells, respectively. The CTL lines derived from the PSA-OP peptide also lysed PSA-positive prostate cancer cells. PSA-OP-derived T cell lines also lysed recombinant vaccinia-PSA-infected targets but not targets infected with wild-type vaccinia. PSA-OP did not bind HLA-A2 and HLA-A3 molecules. The decrease in cytotoxicity in the presence of protease inhibitors suggests that the PSA-OP is cleaved into shorter peptides, which in turn can interact with HLA-class I molecules and, as a consequence, induce CTL-mediated lysis. We have also demonstrated that it is possible to induce CTL responses in HLA-A2.1/Kb transgenic mice by immunization with PSA-OP with adjuvant. These studies thus provide evidence that oligopeptides such as PSA-OP may be useful candidates for peptide-based cancer vaccines. The Journal of Immunology, 1998, 161: 3186–3194.

Protein Ags are presented to CTLs as small peptides (~9–10 amino acids long) associated with class I molecules of the MHC (1, 2). Much progress has recently been made in the identification of tumor Ags that can be recognized by human T cells. A number of tumor Ags recognized by CTLs have been characterized, including members of the MAGE gene family (3), BAGE (4), GAGE (5); MART-1/melan-A (6, 7), tyrosinase (8–11), gp-100 (12, 13), β-catenin (14), HER-2/neu (15–17), p21 Burton (18–21), p53 (22, 23), HPV6 (24), carinoembryonic Ag (CEA) 2 (25), and prostate-specific Ag (PSA) (26, 27).

Prostate cancer is one of the most common cancers found in man and is associated with increased serum levels of PSA (28). The expression of PSA is cell type specific and is thought to be produced exclusively in males by the epithelial cells of the prostate (29). PSA is also a kallikrein-like serine protease, and human PSA has a high degree of homology with human pancreatic kallikrein (30). The majority of prostate cancers and epithelial cells lining the acini and ducts of the prostate gland express PSA (31). Immuno- peroxidase staining indicates that PSA is found in the cytoplasmic portion of these cells (31). For this reason, PSA is a potential target for the induction of T cell-directed immunity against prostate cancer. One human PSA peptide, which consists of amino acids 146–154 of PSA molecules, is recognized by CTLs and has been described; however, CTLs generated in response to this peptide have not been shown to lyse prostate cancer cells (26). The identification of two HLA-A2-binding PSA peptides capable of eliciting cytotoxic T cell responses has recently been reported (27). These peptides were designated PSA-1 (amino acids 141–150) and PSA-3 (amino acids 154–163). The T cell lines were capable of lysing PSA-positive, HLA-A2-positive LNCaP human prostate carcinoma cells (27).

The identification of peptides that conform to HLA-class I A2 motifs was pursued in the previous study (27) because the HLA-A2 allele is the most common class I allele, represented in ~50% of North American Caucasians and 34% of African-Americans (32). Other common HLA class I alleles of North American populations are HLA-A3, -A11, -A24, and -B53. Indeed, these class I molecules, along with A2, are present in 90% of the human population.

Herein we report identification of a PSA oligopeptidotope peptide (designated PSA-OP) that contains motifs for HLA class I-A2, -A3, -A11, and B53 alleles. The ability of this oligopeptidotope peptide to induce cytotoxic T cell activity to multiple epitopes in vitro and in vivo in HLA-A2.1/Kb transgenic mice was investigated. Our results suggest that PSA-OP may be a potential candidate for use in peptide-based vaccines for human prostate carcinoma.

Materials and Methods

Cell cultures

The human prostate carcinoma cell line LNCaP (HLA-A2-positive and PSA-positive) and K562 chronic myelogenous leukemia cells were purchased from American Type Culture Collection (Manassas, VA). The cultures were free of mycoplasma and were maintained in complete medium
of complete medium supplemented with 10% pooled human AB serum and after treatment of targets with 2.5% Triton X-100. Total releasable radioactivity was obtained with 111In-labeled oxyquinoline (Medi-tex, Milan, Italy) using T2 cells and single-color analysis. After cells were washed three times in DPBS as described above, they were incubated for 1 h with HLA-A2–specific mAb (One Lambda, Canoga Park, CA), using 10 μl of a 1× working dilution per 105 cells. UPC-10 (Cappel/Organon Teknika, West Chester, PA) was used as isotopic control. The cells were then washed three times, resuspended as described above, and immediately analyzed using a Becton Dickinson FACSort equipped with a blue laser with an excitation of 15 nW at 488 nm and equipped with the CellQuest program.

**Generation of T cell lines**

The protocol described by Tsang et al. (25) was used for the generation of T cell lines. PBMCs were obtained from the heparinized blood of apparently normal HLA-A2 and HLA-A3 donors by the use of lymphocyte separ- ation medium gradients (Organon Teknika, Durham, NC) as described previously (37). Washed PBMCs were resuspended in complete medium (RPMI 1640, Life Technologies) supplemented with 10% pooled human AB serum (Valley Biomedical, Winchester, VA), 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml of streptomycin (Life Technologies). Cells (2 × 105) in complete medium in a volume of 100 μl were put into each well of a 96-well flat-bottom assay plate (Corning Costar, Cambridge, MA). PSA-OP peptide was added to cultures at a final concentration of 50 μg/ml. Cultures were incubated for 5 days at 37°C in a humidified atmosphere containing 5% CO2. After removal of the peptide-containing medium, the cultures were then supplemented with human rIL-2 (provided by the National Cancer Institute Surgery Branch) (10 U/ml) for 11 days, with IL-2-containing medium being replenished every 3 days. The 5-day incubation with peptide and 11 days with IL-2 constituted one in vitro stimula- tion (IVS) cycle. Primary cultures were restimulated with PSA-OP peptide at 24 h intervals for 6 IVS. Irradiated (4000 rad) autologous PBMCs (5 × 106) were added in a volume of 50 μl in complete medium as APCs. The T cell lines derived from two HLA-A2–donors were designated T1–PSA-OP and T2–PSA-OP. The T cell lines derived from two HLA-A3 donors were designated T3–PSA-OP and T4–PSA-OP.

**Cytotoxicity assays**

Target cells were labeled with 50 μCi of 111In-labeled oxyquinoline (Medi-Physics, Arlington, IL) for 15 min at room temperature. Target cells (0.5 × 103) in 100 μl of complete medium were added to each of 96 wells in U-bottom assay plates (Corning Costar). The labeled target cells were incu- bated with peptides at various concentrations for 60 min at 37°C in 5% CO2, before adding effector cells. Effector cells were suspended in 100 μl of complete medium supplemented with 10% pooled human AB serum and added to target cells. The plates were then incubated at 37°C in 5% CO2 for 4 or 16 h. Supernatant was harvested for gamma counting with the use of harvesters frames (Skatron, Sterling, VA). Determinations were conducted in triplicate, and SDs were calculated. All experiments were conducted three times. Specific lysis was calculated with the following formula: % specific lysis = [(observed release (cpm) – spontaneous release (cpm))/ [total release (cpm) – spontaneous release (cpm)]) × 100. Spontaneous release was determined from wells to which 100 μl of complete medium was added. Total releasable radioactivity was obtained after treatment of targets with 2.5% Triton X-100.

**Exopeptidases and inhibitors**

Captopril (Sigma Chemical, St. Louis, MO) and potato carboxypeptidase inhibitor (Calbiochem, La Jolla, CA) were dissolved in PBS and used at a concentration of 10–6 M and 10–5 M, respectively. E64 and Plummer’s inhibitor (Calbiochem) were dissolved in acidified deionized water and 33% DMSO (final DMSO concentration, 1.7%). Both E64 and Plummer’s inhibitor were used at a concentration of 10–8 M.

**Flow cytometry**

**Single-color flow cytometric analysis.** The method for single-color flow cytometric analysis has been described previously (38). Briefly, cells were washed three times with cold Ca2+ and Mg2+-free Dulbecco’s phosphate-buffered saline (DBPBS) and then stained for 1 h with mAb against CD3, CD4, CD8, CD56, CD19, HLA-DR (Becton Dickinson, San Jose, CA), and HLA class I (W6/32) (Serotec, Sussex, U.K.). MOPC-21 (Cappel/Organon Teknika, West Chester, PA) was used as isotopic control. The cells were then washed three times and incubated with 1:100 dilution of FITC-labeled goat anti-mouse IgG (Kirkegaard & Perry, Gaithersburg, MD). The cells were washed a third time in DBPBS and resuspended in a concentration of 1 × 106 cells/ml. The cells were immediately analyzed using a Becton Dickinson FACScan equipped with a blue laser with an excitation of 15 nW at 488 nm. Data were gathered from 10,000 live cells, stored, and used to generate results.

**Dual-color flow cytometric analysis.** The procedure for dual-color flow cytometric analysis was similar to that for single-color analysis, with the following exceptions. The Abs used were anti-CD54 FITC/anti-CD28 phycoerythrin conjugate and anti-IgG1 FITC/anti-IgG2a phycoerythrin (iso- type controls). All of the Abs mentioned above were purchased from Becton Dickinson. Staining was done simultaneously for 1 h, after which cells were washed three times, resuspended as described above, and immediately analyzed using a Becton Dickinson FACSort equipped with a blue laser with an excitation of 15 nW at 488 nm and equipped with the CellQuest program.

**HLA typing**

HLA phenotyping was performed on PBMCs by the Blood Bank of the National Institutes of Health with a standard Ab-dependent microcytotoxic- ity assay and a defined panel of anti-HLA antisera or with a DNA assay. The following HLA phenotypes were found for donors in this study: donor 1: HLA-A2, 24; B50, 62; Cw, 111; DR, DR*B1*0701,0901; DR*B4*0101; donor 2: HLA-A2;–; B7, 60; Cw3; DR*B1*0405, 0801; DQ B1*0302,04; DQ*B4*0101; donor 3: HLA-A3, 34; B14, 58; Cw6;–; DR*B1*0102, 1102; DQ*B1*0501, 0301; DR*B3*0202; and donor 4: HLA- A3, 11; B7, 51; Cw7;–; DR*B1*0409; DQ*B1*03,03; DR*B4*01.

**Peptide binding to HLA-A2**

Binding of the PSA-OP peptide to HLA-A2 molecules was evaluated by up-regulation of these molecules’ expression on the surface of T2 cells as demonstrated by flow cytometry. The method described by Nijman et al. (39) was used for T2 cell peptide binding assays. Briefly, 1 × 105 cells in serum-free Iscove’s modified Dulbecco’s complete medium were incu- bated with peptides at a concentration of 50 μg/ml in 24-well culture plates at 37°C in 5% CO2. Flow cytometry for peptide binding was performed using T2 cells and single-color analysis. After cells were washed three times in DPBS as described above, they were incubated for 1 h with HLA-A2–specific mAb (One Lambda, Canoga Park, CA), using 10 μl of a 1× working dilution per 105 cells. UPC-10 (Cappel/Organon Teknika) was used as isotopic control. The cells were then washed three times and incubated with a 1:100 dilution of FITC-labeled anti-mouse IgG (Becton Dickinson). A similar method was used for the analysis of PSA-OP, PSA-3, and PSA-9 binding to HLA-A3 molecules using T2A3 cells and anti-HLA-A3–specific mAb (One Lambda). Analysis was performed with the FACScan, as described above. Cells were maintained on ice during all cell preparation and staining, unless otherwise stated.

**Transgenic mice**

HLA-A2.1/Kb transgenic mice were kindly provided by Dr. L. Sherman (Scripps Laboratories, San Diego, CA). Transgenic mice express the product of the HLA-A2.1/Kb chimeric gene in which the α3 domain of the heavy chain was replaced by the mouse HLA-Bα11 domain, but the HLA-A2.1 α1 and α2 domains are unaltered (40, 41).

**In vivo immunization and marine T cell cultures**

Groups of HLA-A2.1/Kb transgenic mice (three mice per group) were immunized in the base of the tail with 250 μg of PSA-OP emulsified in Detox adjuvant. Detox adjuvant was formulated as a stable emulsion with either Sequalene (C30H62) or Sequalene (C30H50) plus cell wall skeleton and monophosphoryl lipid A in a 10:1 ratio, with an emulsifier (provided by RBIImmunoChem Research, Hamilton, MT). Mice injected with ad- juvant were used as controls. A total of three injections of PSA-OP were
The standard deviations. Similar significant lysis was observed at E:T ratios of 25:1.  

Studies were conducted employing all four T cell lines to determine cytotoxic potential at different E:T ratios. As seen in Figure 1, A and B, increased lysis of the C1R-A2 cells, pulsed with either PSA-OP or PSA-3 peptide, was seen with E:T ratios for both the T1-PSA-OP and the T2-PSA-OP T cell lines. The control peptide CAP-1 elicited no lysis at any of the E:T ratios. Similar results were seen when C1R-A3 cells were pulsed with either PSA-OP or PSA-3 but not with the PSA-2 peptide or the control CEA CAP-1 peptide.  

Vaccinia virus infection of C1R cells  
A recombinant vaccinia virus expressing PSA (designated rV-PSA) was generated as described previously (43). C1R-A2 and C1R-A3 target cells were pulsed with the control PSA-3 peptide, which was previously shown to bind HLA-A3, but not HLA-A2 (see Table I). These studies were conducted at E:T ratios of 25:1 (Table III). No lysis was detected with these two cell lines when C1R-A2 cells were pulsed with PSA-1, PSA-3, or PSA-OP peptides (Table II). PSA-OP were established from HLA-A2 donors 1 and 2, respectively. T1-PSA-OP and T2-PSA-OP were cytotoxic to C1R-A2 cells that had been pulsed with PSA-1, PSA-3, or PSA-OP peptides (Table II). These studies were conducted at E:T ratios of 25:1. No lysis was observed when C1R-A2 cells were pulsed with the PSA-2 peptide or the control CEA CAP-1 peptide (25).  

T cell lines were established from the PBMCs, two of two different HLA-A2 and two of two different HLA-A3 donors by in vitro stimulations of PBMCs with the PSA-OP peptide and IL-2, as described in Materials and Methods. Six to seven IVS cycles were required for the generation of these PSA peptide-specific T cell lines. Consequently, T cell lines designated T1-PSA-OP and T2-PSA-OP were established from HLA-A2 donors 1 and 2, respectively. T cell lines designated T3-PSA-OP and T4-PSA-OP were established from HLA-A3 donors 3 and 4, respectively. T1-PSA-OP and T2-PSA-OP were cytotoxic to C1R-A2 cells that had been pulsed with PSA-1, PSA-3, or PSA-OP peptides (Table II). These studies were conducted at E:T ratios of 25:1. No lysis was observed when C1R-A2 cells were pulsed with the PSA-2 peptide or the control CEA CAP-1 peptide (25).  

Statistical analysis  
Statistical analysis of differences between means was done using a two-tailed paired t test (StatView statistical software; Abacus Concepts, Berkeley, CA).
the PSA-9 peptide. Increased lysis was observed with increasing E:T ratios employing both the T3-PSA-OP or the T4-PSA-OP T cell lines (Fig. 2, A and B). In these studies, the PSA-3 peptide was used as a control peptide.

Studies were undertaken to titrate the concentration of the PSA-OP required to mediate lysis of C1R-A2 cells by both the T cell lines from two different donors, established by pulsing PBMCs with the PSA-OP peptide and IL-2. Two different E:T ratios were employed. As seen in Figure 3A, lysis by the T1-PSA-OP line was greater at the E:T ratio of 25:1, increasing to 80% lysis with increasing peptide. Similar results were seen employing the T2-PSA-OP cell line (Fig. 3B). Titration experiments employing the C1R-A3 cell line were also conducted. As seen employing both the T3-PSA-OP T cell line (Fig. 4A) and the T4-PSA-OP T cell line (Fig. 4B), lysis of C1R-A3 cells reached a plateau at 25 μg/ml oligopeptide at E:T ratios of 25:1.

Studies were undertaken to determine the MHC class I-restricted nature of the lysis mediated by these T cell lines. Anti-HLA-A2 mAb (mAb A2, 69) inhibited the cytotoxic activity of the T1-PSA-OP and T2-PSA-OP cell lines (Table IV). No blocking was observed using the control UPC-10 mAb. Inhibition of T3-PSA-OP and T4-PSA-OP activity was observed by employing the anti-HLA-A3 antisera (Table IV); no inhibition was observed using the control UPC-10 antisera.

Flow cytometric studies were conducted to phenotype the four T cell lines using the mAbs and methods described in Materials and Methods. All four cell lines were CD3+ and CD56+. T1-PSA-OP was a mixture of CD4+CD8+ and CD4+CD8+ cells; T2-PSA-OP was a mixture of CD4+CD8+ and CD4+CD8+ cells; T3-PSA-OP was predominantly CD4+CD8+ cells; and T4-PSA-OP was predominantly CD4+CD8+ cells.

**Cytotoxicity of PSA-specific T cell lines against rV-PSA-infected target cells**

Studies were conducted with HLA-matched target cells ± rV-PSA infection to further demonstrate specificity of T cell lysis for PSA. The ability of T1-PSA-OP, T2-PSA-OP, T3-PSA-OP, and T4-PSA-OP cells to lyse target cells endogenously expressing PSA...
was investigated. These T cell lines were tested for cytotoxicity against C1R-A2 cells and C1R-A3 cells infected with either wild-type vaccinia virus (Wyeth strain) or rV-PSA. C1R-A2 cells infected with rV-PSA, and not with wild-type vaccinia, were lysed by T cell lines T1-PSA-OP and T2-PSA-OP (Table V). Similarly, C1R-A3 cells infected with rV-PSA were lysed by T cell lines T3-PSA-OP and T4-PSA-OP (Table V). No lysis was observed of wild-type vaccinia-infected cells by any of the T cell lines. These results demonstrate that CTL lines generated with the PSA-OP oligopeptide indeed recognize and mediate lysis via the product of the human PSA gene.

Cytotoxicity of PSA-specific T cell lines against prostate carcinoma cells

Studies were conducted to determine whether T1-PSA-OP and T2-PSA-OP cells could lyse the PSA-positive and HLA-A2-positive prostate carcinoma cell line LNCaP. As shown in Table VI, LNCaP cells were lysed by the T1-PSA-OP and T2-PSA-OP (Table V). Similarly, C1R-A3 cells infected with rV-PSA were lysed by T cell lines T3-PSA-OP and T4-PSA-OP (Table V). No lysis was observed of wild-type vaccinia-infected cells by any of the T cell lines. These results demonstrate that CTL lines generated with the PSA-OP oligopeptide indeed recognize and mediate lysis via the product of the human PSA gene.

Cytotoxicity of PSA-specific T cell lines against prostate carcinoma cells

Studies were conducted to determine whether T1-PSA-OP and T2-PSA-OP cells could lyse the PSA-positive and HLA-A2-positive prostate carcinoma cell line LNCaP. As shown in Table VI, LNCaP cells were lysed by the T1-PSA-OP and T2-PSA-OP CTLs. The cytotoxic activity of these cell lines against LNCaP cells was shown to be HLA-A2 restricted, as indicated by the inhibition of lysis with the addition of anti-HLA-A2, but not with the control Ab UPC-10 (Table VI).

The addition of unlabeled T2 cells pulsed with PSA-1 or PSA-3 peptide decreased the cytotoxic activity of both T1-PSA-OP and T2-PSA-OP cells against labeled LNCaP cells. The specificity of LNCaP cell lysis was shown by the addition of K562 cells, which did not block the lysis (Table VII). This result confirmed that LNCaP cells endogenously process PSA to present PSA peptides in the context of HLA-A2 for T cell-mediated lysis. Moreover, these results demonstrate that PSA-1 and PSA-3 peptides can compete with endogenously processed PSA peptides for T cell lysis by the PSA-OP-induced CTLs.

Cytotoxic T cell responses in transgenic mice

To assess the in vivo immunogenicity of PSA-OP, HLA-A2.1/Kb transgenic mice were injected with PSA-OP. Cytotoxic T cells derived from PSA-OP-immunized mice specifically lysed PSA-OP-pulsed Jurkat A2/Kb target cells (Table VIII), whereas no cytotoxic T cell responses were observed when the control CAP-1 peptide-pulsed Jurkat A2/Kb cells were used as targets. No lysis was detected in bulk T cell cultures from control mice (Table VIII).

Effect of inhibitors on the CTL activity induced by PSA-OP

Studies were undertaken to investigate the mechanism by which a 30-mer oligopeptide can mediate MHC-restricted lysis by CTLs. We investigated the possibility that PSA-OP was cleaved into shorter peptides that can interact with HLA-A2 molecules. Four protease inhibitors—potato carboxypeptidase, captopril, E-64, and Plummer’s inhibitor—were used in CTL-blocking experiments. T1-PSA-OP cells were used as effectors. Inhibition of T1-PSA-OP cytotoxic activity against PSA-OP-pulsed target cells was detected when E64, captopril, or potato carboxypeptidase were added (Table IX). Addition of these inhibitors did not inhibit the cytotoxic activity of T1-PSA-OP CTL against PSA-3 peptide-pulsed C1R-A2 cells. This result suggested that PSA-OP can be processed.
We have investigated the ability of an oligopeptide, PSA-OP, to induce cytotoxic T cell immune responses against PSA. PSA-OP is a 30-mer peptide containing three HLA-A2-binding epitopes (27), as well as an epitope for HLA-A3 (44) and, potentially, HLA-A1 (that shares a common anchor motif of V at position 2 and K at position 9) (45). In these studies, we have generated two HLA-A2-restricted cytotoxic T cell lines (T1-PSA-OP and T2-PSA-OP) and two HLA-A3-restricted cytotoxic T cell lines (T3-PSA-OP and T4-PSA-OP) from two HLA-A2 donors and two HLA-A3 donors, respectively. The class I allelic-restricted nature of the lysis mediated by these T cell lines was confirmed by a decrease in CTL activity after adding anti-HLA-A2 mAb and anti-HLA-A3 mAb.

The specificity of T1-PSA-OP and T2-PSA-OP cell lines was similar to the specificity of the T cell line established using PSA-1 and PSA-3 peptides (27). They lysed C1R-A2 cells pulsed with PSA-OP peptide, C1R-A2 infected with rV-PSA (and not with wild-type vaccinia), and PSA-positive prostate carcinoma LNCaP cells. In addition, the T1-PSA-OP and T2-PSA-OP CTLs could lyse C1R-A2 cells pulsed with either PSA-1 or PSA-3 peptide, and the PSA-OP-specific T cell lines established from the HLA-A3 donors could lyse PSA-9 peptide-pulsed C1R-A3 cells.

The potential advantages of using an oligopeptide rather than a combination of single-epitope peptides to induce immune responses are: 1) oligopeptide peptides are useful in generating cellular responses in a broad segment of the human population with different HLA-class I molecules; 2) the number of precursors to different single epitope peptides vary in different individuals, as indicated by our data obtained from the PSA-1 and PSA-3 peptide studies (31); thus, utilization of PSA-OP containing three HLA-A2 epitopes may activate the maximum number of PSA-specific precursors in different individuals; and (c) oligopeptide peptide may be constructed to contain both CD4+ and CD8+ T cell epitopes in an overlapping or nested configuration and, as a consequence, to form a more superior immunogen in comparison with a mixture of different peptides. For example, a single mutant 13-mer ras peptide has been shown to contain both a CD4+ and a nested CD8+ T cell epitope (46). Additional peptides recognized by CD4+ cells with MHC class II molecules and by CD8+ cells with MHC class I molecules have been described in the models of HIV (47), influenza (48, 49), and the p53 gene (50).

The use of an oligopeptide as an immunogen may thus be an appropriate compromise between the use of a 9-mer CTL peptide epitope and the entire protein. In the case of PSA, for example, there are regions of this gene product that share strong homology with normal tissue kallikrein genes. The region of the PSA-OP peptide was selected for its lack of any substantial homology for these normal sequences. Furthermore, from more practical considerations, the synthesis of a peptide is much more cost effective than the production of a recombinant protein.

The PSA-OP oligopeptide did not bind HLA-A2 and HLA-A3 molecules, as indicated by the lack of up-regulation of HLA-A2 or HLA-A3 expression on T2A2 or T2A3 cells, respectively (Tables I and II). CTLs recognize protein Ags as small peptides (~9–10 amino acids long) associated with class I molecules. There are a number of reports regarding extracellular peptides in the generation of optimal size class I-binding peptides from larger protein fragments (51–54). It has been demonstrated that T cell stimulation by peptide p18, an HIV gp160-derived peptide presented by murine H-2D MHC molecules, required extracellular processing mediated by peptidases in FCS (54). This peptide processing could be

---

**Table IV.** Blocking of cytotoxic activity with anti-HLA-A2 Ab or anti-HLA-A3 Ab

<table>
<thead>
<tr>
<th>T Cell Line</th>
<th>Anti-HLA-A2</th>
<th>Anti-HLA-A3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No peptide</td>
<td>PSA-OP</td>
</tr>
<tr>
<td>T1-PSA-OP</td>
<td>7.9 (2.7)</td>
<td>32.3 (0.9)</td>
</tr>
<tr>
<td>T2-PSA-OP</td>
<td>12.2 (1.2)</td>
<td>31.2 (0.6)</td>
</tr>
<tr>
<td>T3-PSA-OP</td>
<td>12.5 (2.7)</td>
<td>24.2 (3.9)</td>
</tr>
<tr>
<td>T4-PSA-OP</td>
<td>19.7 (0.1)</td>
<td>32.0 (2.3)</td>
</tr>
</tbody>
</table>

* An 18-h 111 In release assay was performed. Results are expressed as percentage of specific lysis at E:T ratios of 25:1. The numbers in parentheses are the standard deviation.

---

**Table V.** Cytotoxicity of human PSA-OP-specific T cell lines against target cells infected with rV-PSA

<table>
<thead>
<tr>
<th>Targets</th>
<th>Percent Lysis by T Cell Lines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1-PSA-OP</td>
</tr>
<tr>
<td>C1R-A2 (Wyeth)</td>
<td>15.0 (3.8)</td>
</tr>
<tr>
<td>C1R-A2 (rV-PSA)</td>
<td>45.2 (1.2)</td>
</tr>
<tr>
<td>C1R-A3 (Wyeth)</td>
<td>ND</td>
</tr>
<tr>
<td>C1R-A3 (rV-PSA)</td>
<td>ND</td>
</tr>
</tbody>
</table>

* An 18-h 111 In release assay was performed. The results are expressed as percentage of specific lysis at E:T of 25:1. The numbers in parentheses are the standard deviation. C1R-A2 cells were used as target for T1-PSA-OP and T2-PSA-OP, and C1R-A3 cells were used as target for T3-PSA-OP and T4-PSA-OP. Target cells were incubated for 1 h in the presence of either control Ab (UPC-10, 10 μg/mL), anti-HLA-A2 Ab (anti-HLA-A2, 69, 1:100 dilution), or anti-HLA-A3 Ab (1:100 dilution).

---

**Table VI.** Anti-HLA-A2 Ab inhibition of LNCaP cell lysis by T cell lines

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Percent Lysis by T Cell Lines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1-PSA-OP</td>
</tr>
<tr>
<td>None</td>
<td>28.4 (4.8)</td>
</tr>
<tr>
<td>Anti-HLA-A2, 69</td>
<td>0.8 (3.3)</td>
</tr>
<tr>
<td>UPC-10 (control)</td>
<td>23.9 (1.6)</td>
</tr>
</tbody>
</table>

* LNCaP cells (5 × 10⁴) were labeled with 111 In and incubated for 1 h in the presence of medium containing either no Ab, UPC-10 (10 μg/mL), or anti-HLA-A2, 69 (1:100 dilution). Cells were then used as targets in an 18-h cytotoxic assay. The results are expressed in percentage of specific lysis at E:T ratios of 25:1. The numbers in parentheses are the standard deviation.

Statistically significant lysis (p < 0.05, two-tailed t test).
blocked by adding the angiotensin-1-converting enzyme (ACE) inhibitor, captopril. Our data suggest that proteolytic enzymes in FCS most likely degrade the 30-mer PSA-OP to smaller active forms, resulting in the induction of immune responses. To evaluate this hypothesis, four carboxypeptidase inhibitors were used in the cytotoxic activity experiments. These were E-64 (55), which blocks cathepsin B (peptidyl dipeptidase B); Plummer’s inhibitor (56), which blocks carboxypeptidase N (serum carboxypeptidase B); captorpril (57), which blocks an ACE or peptidyl dipeptidase A; and potato carboxypeptidase inhibitor (58), which blocks tissue carboxypeptidases A and B. Results reported here (Table IX) indicate a decrease in CTL activity in the presence of E-64, captopril, or potato carboxypeptidase inhibitor, suggesting that the PSA-OP must be cleaved into shorter peptides that, in turn, can interact with HLA-A2 molecules and induce CTL lysis of target cells. It is also conceivable that other extracellular proteases may be involved in the fragmentation of PSA-OP into epitopes similar to PSA-1, PSA-3, and PSA-9. The data presented here do not exclude the possible involvement of intracellular processing of the PSA-OP peptide. The mechanism for processing PSA-OP, extracellular and/or intracellular, will require further investigation.

Information obtained concerning the immunogenicity of PSA from preclinical studies of mice and rhesus monkeys have limited extrapolation to potential human immune T cell responses, since murine and monkey MHC-binding motifs are different from the human motifs. The MHC class I-binding affinity and stability of peptide-MHC complexes at the cell surface contribute to the immunogenicity of CTL epitopes. In view of this, HLA-A2.1/Kb transgenic mice provide a potential model to evaluate the immunogenicity of PSA-OP. PSA-2/Kb transgenic mice express a chimeric class I molecule composed of the α1 and α2 domains of HLA-A2.1 and the α3 transmembrane and cytoplasmic domains of H-2Kb (40, 41). Replacement of the α3 domain of the heavy chain allows interaction of the murine CD8 molecule on CD8+ T cells with the syngeneic α3 domain of the hybrid MHC class I molecule. The HLA-A2.1/Kb transgenic mouse model has been used by many investigators to examine the immunogenicity of PSA-OP in HLA-A2.1/Kb transgenic mice. The immunogenicity of PSA-OP in HLA-A2.1/Kb transgenic mice was investigated, and these results (Table VIII) illustrate that PSA-OP can also induce CTL responses in vivo.

Clinical trials employing PSA as a target are currently in progress in prostate carcinoma patients. PSA recombinant protein, encapsulated into liposomes, has resulted in the induction of PSA-specific lymphoproliferative responses and anti-PSA Ab responses (61). Phase I studies employing rV-PSA as an immunogen in prostate cancer patients are also in progress. In both studies, no unexpected toxicity has been observed thus far. The use of the PSA-OP oligopeptide as an immunogen, either in adjuvant or via pulsed dendritic cells, offers another approach to the induction of PSA-specific immunity. Moreover, the PSA-OP can be used potentially in the generation of PSA-specific CTL for adoptive transfer protocols or in the further experimental analysis of PSA-specific T cell responses.

Acknowledgments

We thank Margarita Lora for technical assistance and Nicole Ryder for editorial assistance.

References


