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Induction of Tc2 cells with specificity for prostate-specific antigen from patients with hormone-refractory prostate cancer

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Abstract Prostate-specific antigen (PSA) is a potentially useful antigen for targeted T-cell immunotherapy of prostate cancer (CaP). Our laboratory has identified a synthetic nonamer peptide (PSA 146–154) homologue of PSA, which binds to the prevalent human leukocyte antigen, HLA-A2, and elicits specific cytotoxic T-lymphocyte (CTL) responses from normal individuals of the HLA-A2 phenotype. In the present study, we report on the induction of CTL from peripheral blood mononuclear cells (PBMC) of patients with hormone-refractory CaP, which exhibit the same specificity. T-cell lines were established from two patients by stimulation of PBMC with PSA 146–154 peptide in vitro. The T-cell lines exhibited specific cytolytic activity against T2 cells pulsed with PSA 146–154 peptide, but not a control HLA-A2 binding peptide (HIV-RT 476–484) via chromium release assay (CRA). The T-cell lines also showed PSA 146–154 peptide-specific IL-4 responses, but no detectable interferon-gamma (IFN-γ) responses via enzyme-linked immuno-spot assays. Magnetic immuno-selection studies of one of the T-cell lines demonstrated that both cytolytic and interleukin-4 (IL-4) responses were mediated by $CD8^+$, but not by $CD4^+$ T cells. This Tc2 line was further characterized for the ability to recognize endogenously processed PSA epitopes. The line specifically secreted IL-4 in response to $HLA\text{-}A2^+$ target cells transfected to express PSA and specifically lysed the PSA^+ target cells, but not control transfected cells. The results indicate that the PSA 146–154 peptide emulates a naturally processed and presented peptide epitope of PSA that is within the T-cell repertoire of $HLA\text{-}A2^+$ patients with CaP.

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Abbreviations *CaP* prostate cancer · *CRA* chromium release assay · *CTL* cytotoxic T lymphocytes · *DC* dendritic cells · *DTH* delayed-type hypersensitivity · *ELISA* enzyme-linked immunosorbent assay · *PBMC* peripheral blood mononuclear cells · *PSA* prostate-specific antigen · *IL-4* interleukin-4 · *IFN-γ* interferon-gamma

Introduction

Prostate cancer (CaP) is the most common malignancy and the second leading cause of cancer-related mortality among the male population in the United States [1]. Although mortality rates from CaP have declined recently, there remains a compelling need for the development of novel therapeutic strategies, particularly for advanced stages of the disease, where current treatment approaches are limited to a palliative role. New avenues for targeted immunotherapy of CaP have been opened by the identification of prostate-specific antigen (PSA).

PSA is a 34-kDa kallikrein-like serine protease, which is produced by ductal and acinar epithelial cells of the prostate gland in males [2, 3]. PSA is detected in most adenocarcinomas of the prostate, and hence may be a useful target antigen for T-cell immunotherapy [4]. Our laboratory has identified a nonameric segment of PSA (amino-acid position 146–154; sequence KLQCVDLHV) which binds to the prevalent human leukocyte antigen, HLA-A2, and elicits specific cytotoxic T lymphocyte (CTL) responses in normal individuals of the HLA-A2 phenotype [5]. It has been shown that CTL induced by immunization with dendritic cells (DC) pulsed with killed tumor cells that express PSA protein recognize target cells pulsed with the PSA 146–154 peptide [6]. These findings imply that the PSA 146–154 peptide is naturally processed and presented by PSA^+

tumors of the HLA-A2 phenotype. Importantly, homologous segments of other known members of the human kallikrein family do not share key HLA-A2 binding anchor residues of the PSA 146–154 sequence. These observations predict the usefulness of the PSA 146–154 peptide as a target antigen for T-cell immunotherapy of CaP.

Although normal individuals have the capacity to mount specific CTL responses against PSA 146–154 peptide, it has not been determined whether patients with CaP also are able to respond specifically to the same epitope. A number of investigators have reported that patients with advanced malignancies, including CaP, have impaired T-cell immune responses [7, 8]. Successful attempts to elicit specific CTL from patients with CaP have been few, and no reports of the induction of specific CTL from patients with hormone refractory CaP have been published [9, 10, 11]. In this paper, PSA 146–154 peptide-specific CTL were induced from two different patients with hormone-refractory CaP. The T-cell lines recovered from both patients evidenced a Tc2 cytokine profile, and one of the lines also demonstrated the ability to recognize naturally processed PSA protein. The results provide a basis for the evaluation of the immunogenic and therapeutic potential of the PSA 146–154 epitope in HLA-A2$^+$ patients with CaP.

Materials and methods

Patient characteristics

Two patients with stage D3a CaP, UPN6 and UPN44, aged 77 and 75 years, respectively, were selected for study. Both patients had hormone-refractory disease with increased serum PSA levels but normal bone scans. Their tumors were pathologically classified as well to moderately differentiated adenocarcinomas. Peripheral blood (50 ml) was collected in heparinized syringes from each patient after informed consent. Serum PSA levels at the time of study were 29 and 40 ng/ml in patients UPN6 and UPN44, respectively. The total PSA levels were quantified using an AxSYM PSA kit (Abbot Laboratories, Abbott Park, Ill.) at the clinical pathology laboratory of the University of Illinois at Chicago. The phenotype of both patients was HLA-A0201 via HLA-A2 SSP-PCR subtyping (Genovision, Exton, Pa.). Peripheral blood was obtained from both patients following primary therapy with recombinant Flt3-ligand (Immunex Corporation, Seattle, Wash.) at a concentration of 25 μg/kg/day for 14 days.

Cell lines

The human colon carcinoma cell line, SW480, the prostate cancer cell line, LNCaP, and the peptide transport-deficient lymphoblastoid cell line, T2, were obtained from the American Type Culture Collection (ATCC; Manassas, Va.). Epstein–Barr virus (EBV)-transformed lymphoblastoid lines (LCL) were established in our laboratory from peripheral blood mononuclear cells (PBMC) as described previously [5]. The SW480 cell line was maintained in Leibovitz medium (Sigma, St. Louis, Mo.) supplemented with 10% fetal bovine serum (FBS; BioWhittaker, Walkersville, Md.), while the LNCaP, T2 and LCL cell lines were grown in RPMI-1640 containing 10% FBS.

Induction of PSA 146–154 peptide-specific cytotoxic T lymphocytes

PBMC were repetitively pulsed in vitro with PSA 146–154 peptide at a concentration of 50 μg/ml (Research Genetics, Huntsville, Ala.) as previously described [5]. Briefly, PBMC were separated over Ficoll (Sigma, St. Louis, Mo.) by gradient centrifugation, washed twice with phosphate-buffered saline (PBS; BioWhittaker, Walkersville, Md.), and re-suspended in RPMI-1640 supplemented with 10% heat-inactivated autologous plasma. PBMC (1×10^6 cells/ml) were plated in 24-well flat-bottomed plates (Nunc, Naperville, Ill.) with PSA 146–154 peptide (50 μg/ml) and IL-2 (10 IU/ml) in a final volume of 2 ml per well. Every 7 days (1 cycle), effector cells were restimulated with autologous, irradiated (2,500 rad) PBMC (1.0×10^5 cells/well) and additional IL-2 for ten to 12 cycles. Peptide-specific CTL activity is generally observed by 10 cycles, and hence in subsequent cycles, CTL were maintained in 10% FBS containing low dose IL-2 and restimulated weekly with irradiated autologous LCL (3,000 rad) pulsed with PSA 146–154 peptide. Reactivity to bovine serum proteins was not observed under these culture conditions. The T-cell lines obtained were analyzed for specific cytolytic and cytokine responses, and phenotyped for surface markers.

Chromium release assay

Peptide-specific cytolytic activity was analyzed by standard 4-h chromium release assay (CRA) as previously described [5]. Briefly, target cells were labeled with 100 μCi of $Na^{51}CrO_3$ (Amersham Pharmacia Biotech, Piscataway, N.J.) and then pulsed with PSA 146–154 peptide or HIV-RT 476–484 control peptide (Research Genetics, Huntsville, Ala.) or no peptide. Effector cells were plated in triplicate at indicated effector concentrations and incubated with target cells (1×10^4 cells/well) for 4 h in 96-well round-bottomed plates (Nunc, Naperville, Ill.). Supernatants recovered from CRA were assayed for gamma emission using a Top-count NXT scintillation counter (Packard, Meriden, Conn.) and percentage specific lysis was calculated as previously described [5].

Cytokine analysis

T cells secrete distinct cytokine patterns in vitro and in vivo [12]. In order to study the cytokine profile exhibited by the T-cell lines, enzyme linked immuno-spot assays were performed as described previously [13]. Varying concentrations of PBMC (100,000, 50,000 and 10,000/well) from normal individuals were stimulated with 5 μg/ml of PHA (Sigma, St. Louis, Mo.) and standardized for IFN-γ and IL-4 assays. In subsequent experiments, PHA-stimulated PBMC (cryopreserved from the same individual at 50,000/well) served as positive controls and as a quality control for assay reproducibility. T2 cells (target cells) at a concentration of 10,000 cells/well were pulsed with 50 μg/ml of PSA 146–154 peptide, HIV-RT 476–484 control peptide or no peptide, and co-cultured with indicated numbers of effector cells in RPMI-1640 containing 10% FBS with 30 IU/ml of IL-2 for 24 h. In experiments where SW480 cells were used as targets, the parent cell line, a vector-only transfected control line or a PSA-positive-transfected line (10,000/well) was co-cultured with indicated numbers of effector cells and incubated as above. T-cell lines obtained from UPN6 and UPN44 served as effector cells. All assays were performed in quadruplicate wells in 96-well, polystyrene, enzyme-linked immunosorbent assay (ELISA) plates (Greiner, Labortechnik, Germany). The number of spot-forming cells was visualized by a two-step, purified mouse anti-human IL-4/IFN-γ capture (catalog nos. 18651D and 18891D, respectively) and biotinylated mouse anti-human IL-4/IFN-γ (catalog nos. 18502D and 18902D, respectively) detection monoclonal antibody system (Pharmingen, San Diego, Calif.). Assays were developed with an avidin–biotin complex (ABC) substrate conjugate system (Santa Cruz Biotechnology, Santa Cruz, Calif.) followed by tetramethylbenzidine (TMB) liquid color developer

(Sigma, St. Louis, Mo.). The blue spots were counted microscopically, and the results were represented as the number of spot-forming cells.

Flow cytometry

SW480 cells (5×10^5) were incubated with 3 μg/ml of fluorescein isothiocyanate (FITC) conjugated anti-HLA-A2 monoclonal antibody (BB7.2 hybridoma; kindly donated by P. Weiss, flow cytometer facility, University of Illinois at Chicago). FITC-conjugated mouse immunoglobulin (MIgG2b) served as the isotype control. The T-cell lines were phenotyped for surface markers using CD3–FITC, CD8–FITC, and CD4–PE (phycoerythrin), with MIgG1–FITC and MIgG2a-PE as isotype controls. All antibodies and isotype controls were purchased from Pharmingen (San Diego, Calif.). All incubations were carried out at 4°C for 15 min in PBS containing 2% FBS and 0.01% sodium azide. After staining, propidium iodide (Sigma, St. Louis, Mo.) was added at 1 μg/ml final concentration to each sample to distinguish viable cells from dead cells. The relative log fluorescence of viable cells was measured at 495 nm by FACSCalibur (Becton Dickinson, Mountain View, Calif.).

Transfection of SW480 cells

An HLA-A2-positive cell line, SW480, was transfected with the full length PSA-cDNA (a kind gift from C. Young, Mayo Clinic) or empty vector, pCR3 (Invitrogen, Carlsbad, Calif.) by electroporation at 250 V and 650 μF (Bio-Rad Laboratories, Hercules, Calif.). The SW480 cells so obtained were called SW480-PSA$^+$ and SW480-PSA$^-$, respectively. The stable transfectants were obtained by selection with G418 (Gibco BRL, Grand Island, N.Y.) at 500 μg/ml for three weeks. Supernatants were collected from confluent cultures of SW480-PSA$^+$ and SW480-PSA$^-$ and analyzed for PSA protein expression as described above. The analysis was performed twice, and the results presented as the average of the two readings.

Sorting of the T-cell line

The T-cell line obtained from UPN6 was separated into CD4$^+$ and CD8$^+$ T cells by magnetic selection using CD4 micro-beads (Miltenyi Biotech, Auburn, Calif.) according to the manufacturer's instructions. The purity of the negative fraction (CD8$^+$) was checked by flow cytometry. The total number of cells in the positive fraction (CD4$^+$) was low and reserved for functional assays. Both fractions were analyzed for cytokine and cytolytic activity.

Statistical analysis

All analyses were performed with StatView software (Adept Scientific, Acton, Mass.). Statistical comparisons of enzyme-linked immuno-spot assays and CRA results for T2 cells incubated with PSA 146–154 peptide versus HIV-RT 476–484 control peptide or no peptide and, similarly, SW480 PSA-positive transfectants versus PSA-negative vector-control cells were performed using the Student's *t*-test for paired samples.

Results

Establishment of PSA 146–154 peptide-specific T-cell lines from patients with CaP

T-cell lines were induced from the PBMC of two HLA-A2$^+$ patients, UPN6 and UPN44, by multiple cycles of stimulation with PSA 146–154 peptide in vitro. Peptide-specific responses were observed after ten to 12 cycles of stimulation. The T-cell lines from both patients evidenced specific release of IL-4, but not IFN-γ, via enzyme-linked immuno-spot assays (Fig. 1). The T-cell

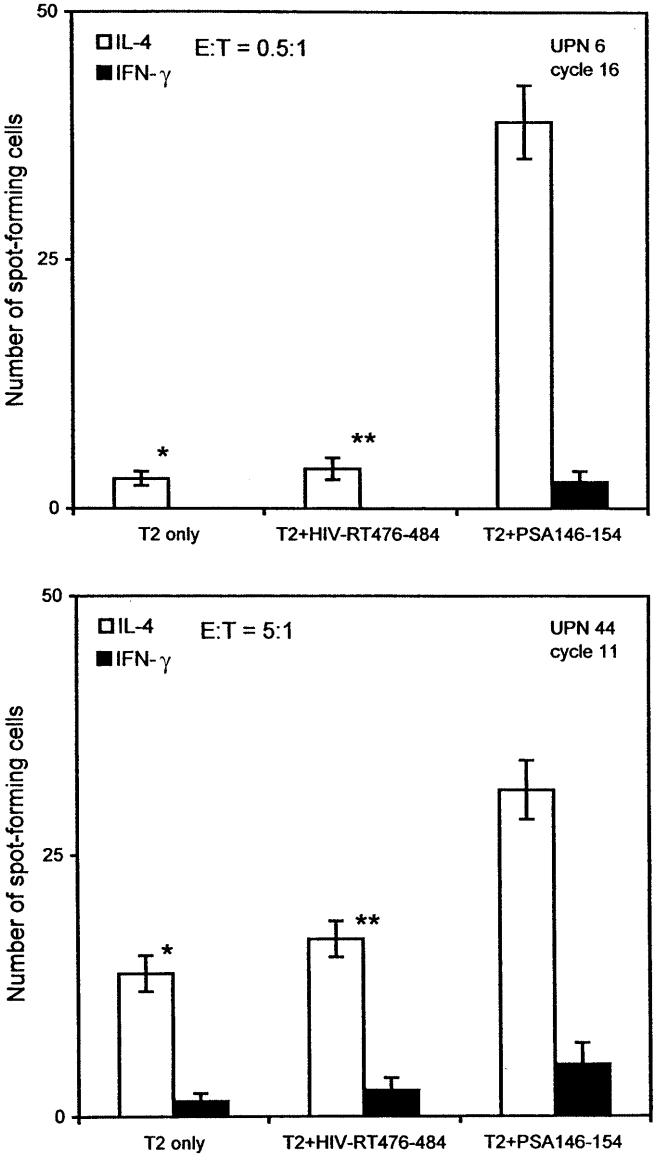

Fig. 1. Cytokine profile of T-cell lines induced with PSA 146–154 peptide per enzyme linked immuno-spot assays. Effector cells from UPN6 (*upper panel*) and UPN44 (*lower panel*) at the indicated effector to target (E:T) ratio were incubated with T2 cells (10,000/well) pulsed with PSA 146–154 peptide, HIV RT 476–484 control peptide (50 μg/ml) or in the absence of peptide. Enzyme-linked immuno-spot assays for IL-4 (*open bar*) and IFN-γ (*closed bar*) were performed as described in the Materials and methods section. T-cell lines from both patients showed polarized IL-4 responses. The number of spot-forming cells was significantly higher against T2 cells incubated with PSA 146–154 peptide compared to in the absence of peptide or HIV-RT 476–484 control peptide (*$P \le 0.005$, $t=-11.789$; **$P \le 0.025$, $t=-9.339$, respectively, *upper panel*; UPN6 and *$P \le 0.005$, $t=-7.298$; **$P \le 0.005$, $t=-6.797$, respectively, *lower panel*, UPN44). The data are representative of two experiments

line obtained from UPN6 showed significant amounts of IL-4 production at effector to target ratios as low as 0.5:1. Specific responses were observed when T2 cells were pulsed with PSA 146–154 peptide, but not in the absence of peptide or in the presence of an HLA-A2-binding control peptide, HIV-RT 476–484. In the case of UPN44, the PSA 146–154-specific IL-4 response was only seen at an effector to target ratio of 5:1 or higher. T-cell lines also were checked for cytolytic activity in CRA. The T-cell lines from both patients mediated the lysis of T2 cells pulsed with PSA 146–154 peptide, but not with HIV-RT 476–484 peptide or in the absence of peptide (Fig. 2). Similar to the enzyme-linked immunospot assays, specific lytic activity was observed only at higher effector to target ratios (20:1 or above) in the case of UPN44. The percentages of $CD3^+$ cells in the T-cell lines from UPN6 and UPN44 were 95% and 80%, respectively. The T-cell lines consisted of $CD4^+$, $CD8^-$ and $CD4^-$, $CD8^+$ T-cell populations, with a predominance of the latter (Fig. 3A and B).

Demonstration of a Tc2-pattern of cytokine expression by PSA 146–154 peptide specific $CD8^+$ T cells

The T-cell line obtained from UPN6 was sorted by selection with CD4 magnetic beads into $CD4^+$ and $CD8^+$ T-cell subpopulations to determine the source of cytokine and cytolytic activity. Over 96% of cells obtained by negative selection were $CD8^+$ T-cells, with less than 1% $CD4^+$ T-cell contamination (Fig. 3C). The total number of $CD4^+$ T-cells obtained by positive selection was limited, and therefore reserved for functional assays. T-cells obtained by both the positive and negative selection were analyzed for peptide-specific responses via IL-4-enzyme-linked immuno-spot assays and CRA. PSA 146–154 peptide-specific IL-4 response was observed only for the $CD8^+$ fraction. Similarly, $CD8^+$, but not $CD4^+$ T cells, showed specific lytic activity against T2 cells pulsed with PSA 146–154 peptide (Fig. 4). These results demonstrated the induction of PSA 146–154 peptide-specific Tc2 cells from UPN6. Sufficient numbers of sorted cells were not available from UPN44 to perform cytotoxicity assays.

Specific recognition of PSA^+ targets of the HLA-A2 phenotype by a T-cell line elicited with PSA 146–154 peptide

The ability of PSA 146–154 peptide-specific T-cells to recognize $HLA\text{-}A2^+$ target cells that endogenously produce PSA protein was examined. SW480, a colon cancer cell line of the HLA-A2 phenotype, was transfected with human PSA-cDNA or empty vector to obtain SW480-PSA^+ and SW480-PSA^- cells, respectively. Stable transfection of the gene was confirmed by PSA protein ELISA of culture supernatants. PSA protein

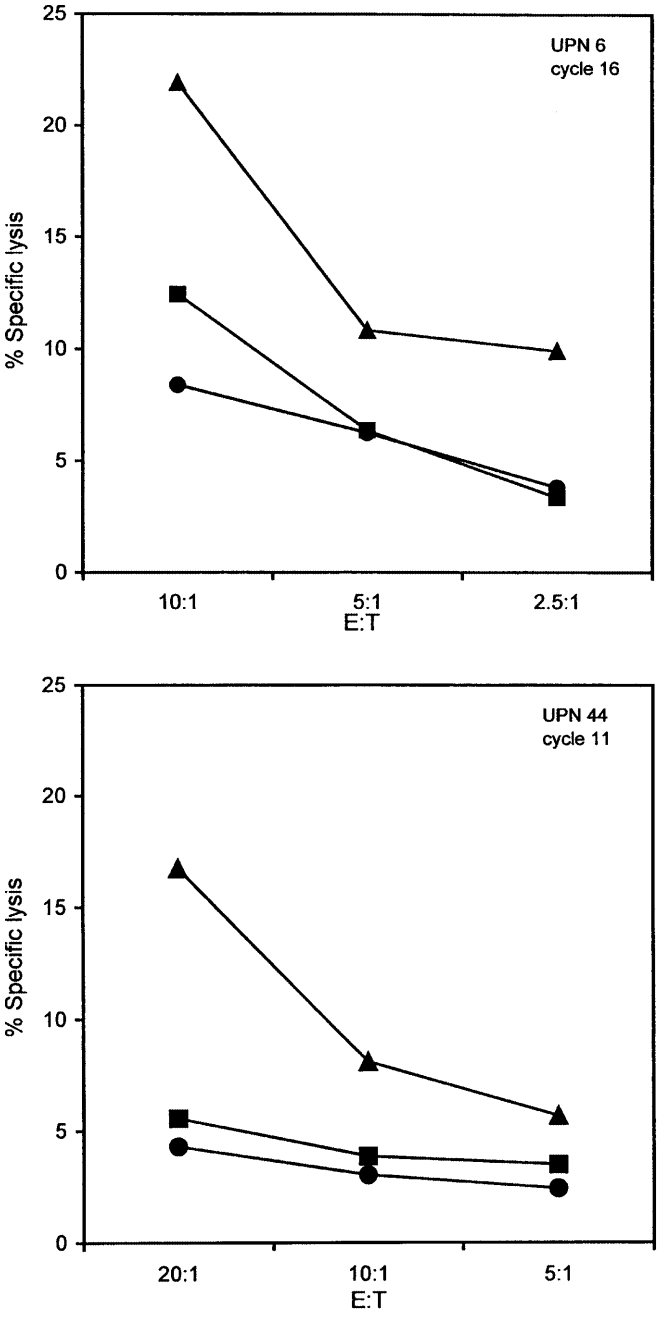

Fig. 2. Specific cytolytic activity of T-cell lines induced with PSA 146–154 peptide per CRA. T-cell lines obtained from UPN6 (*upper panel*, cycle 16) and UPN44 (*lower panel*, cycle 11) were incubated at the indicated E:T ratios with ^{51}Cr-labeled T2 cells (5,000/well), pulsed with 50 μg/ml of PSA 146–154 peptide (*closed triangle*), HIV-RT 476–484 control peptide (*closed square*) and in the absence of peptide (*closed circle*). Specific lysis was observed against T2 cells sensitized with PSA 146–154 peptide, but not with HIV RT 476–484 or in the absence of peptide. The data are representative of two experiments

levels in the supernatants from the SW480-PSA^- and SW480-PSA^+ cells were 0 and 15.5 ng/ml, respectively. Culture supernatants from LNCaP cells served as positive controls for PSA protein expression. Cell surface expression of HLA-A2 antigen on SW480-PSA^+ and

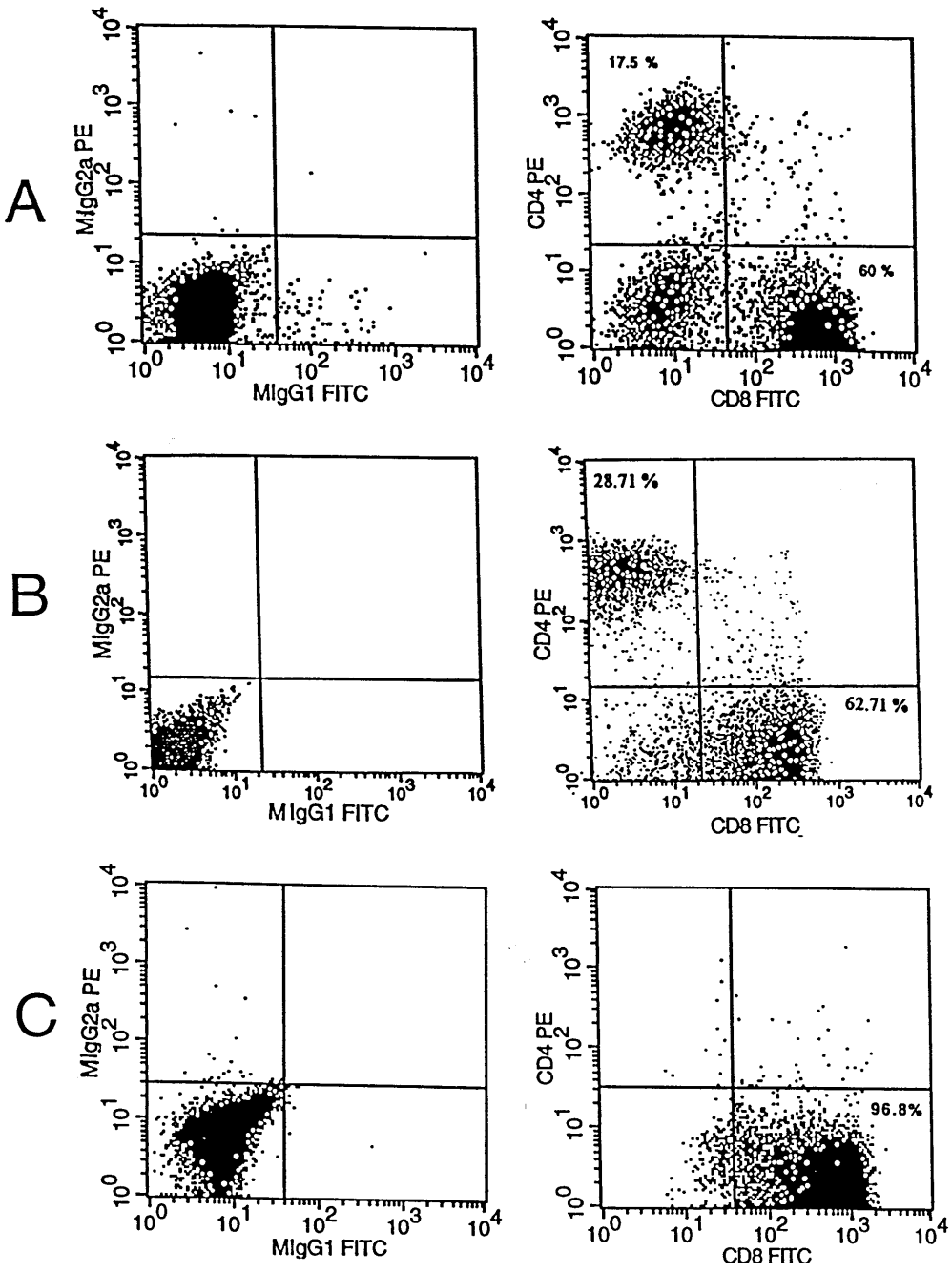

Fig. 3A–C. Cell surface phenotype of T-cell lines induced with PSA 146–154 peptide. **A, B** The phenotype of the T-cell lines (unsorted) obtained from UPN 44 (cycle 11) and UPN6 (cycle 16), respectively. The T-cell lines were stained with anti-CD4-PE and CD8-FITC antibodies. **C** Anti-CD8-FITC staining of negatively sorted T cells obtained from UPN6. Sorting was performed by selection with CD4 magnetic beads, as described in the Materials and methods section

SW480-PSA$^-$ cells were comparable to the parent SW480 cells. Thus, transfection with PSA–cDNA or empty vector did not alter the surface expression of HLA-A2 antigen. Having succeeded in obtaining HLA-A2$^+$ PSA-expressing cells, we tested the ability of the PSA 146–154 peptide-specific T-cell line obtained from UPN6 to specifically recognize target cells that had endogenous expression of PSA. The T-cell line specifically lysed SW480-PSA$^+$, but not SW480-PSA$^-$ cells via CRA (Fig. 5; upper panel). The number of IL-4-spot-forming cells also was significantly ($^*P \leq 0.025$, $t = -3.239$) higher after stimulation of the T-cell line with the SW480-PSA$^+$ cells as compared to SW480-PSA$^-$ cells, via enzyme-linked immuno-spot assays (Fig. 5; lower panel). A similar result was observed with T-cells obtained from UPN44. However, the reproducibility of these findings could not be established because the specificity of the T-cell line from UPN44 diminished over time. By contrast, the T-cell line obtained from UPN 6 was maintained for over a year without loss of specificity.

Discussion

Prostate cancers express various proteins that are potential targets for immunotherapy. However, efforts to exploit these targets may be hampered by quantitative

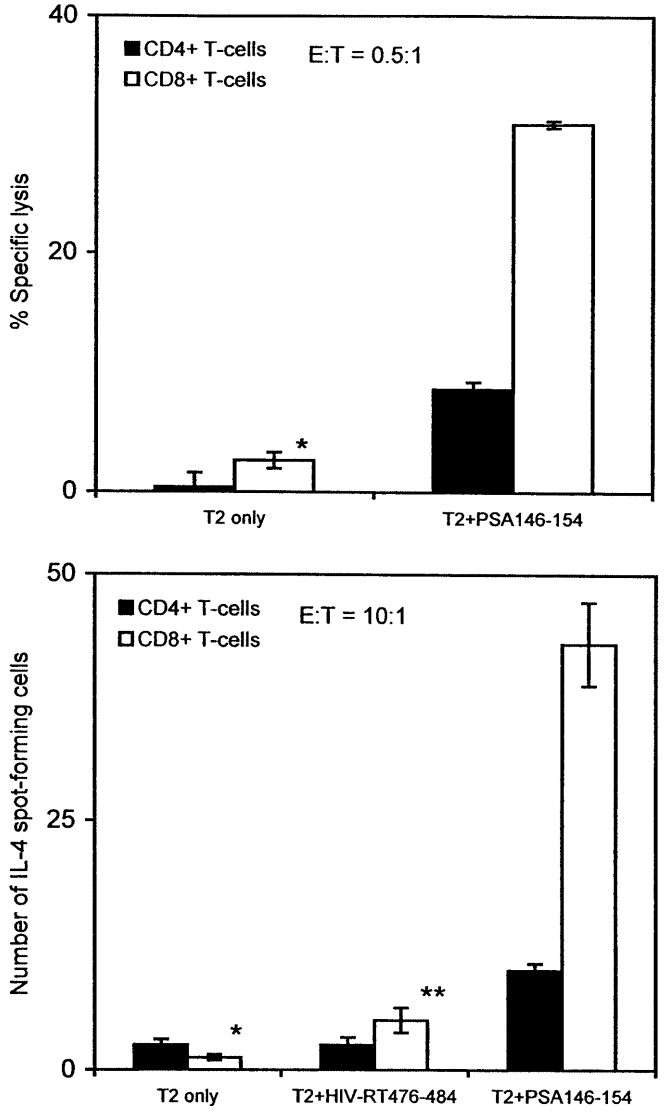

Fig. 4. Demonstration of type-2 cytokine expression by PSA 146–154 peptide-specific cytotoxic $CD8^+$ T-cells. The PSA 146–154 peptide induced T-cell line (obtained from UPN6) was sorted by magnetic selection with CD4 micro beads as described in the Materials and methods section. Both the $CD4^+$ and $CD8^+$ T-cells were assayed for cytolytic activity and IL-4 response. The $CD8^+$ but not $CD4^+$ T-cells showed specific lytic activity per CRA (*upper panel*). Statistical significance ($^*P \leq 0.005$, $t = -48.497$) of lytic activity of $CD8^+$ T cells between T2 cells incubated with PSA 146–154 peptide and in the absence of peptide. Similarly, enzyme-linked immuno-spot assay indicated an IL-4 response only in $CD8^+$ T cells (*lower panel*). The number of spot-forming cells was significantly higher against T2 cells incubated with PSA 146–154 peptide compared to in the absence of peptide or HIV-RT 476–484 control peptide ($^*P \leq 0.005$, $t = -11.626$ and $^{**}P \leq 0.005$, $t = -8.393$, respectively). The data are representative of two experiments

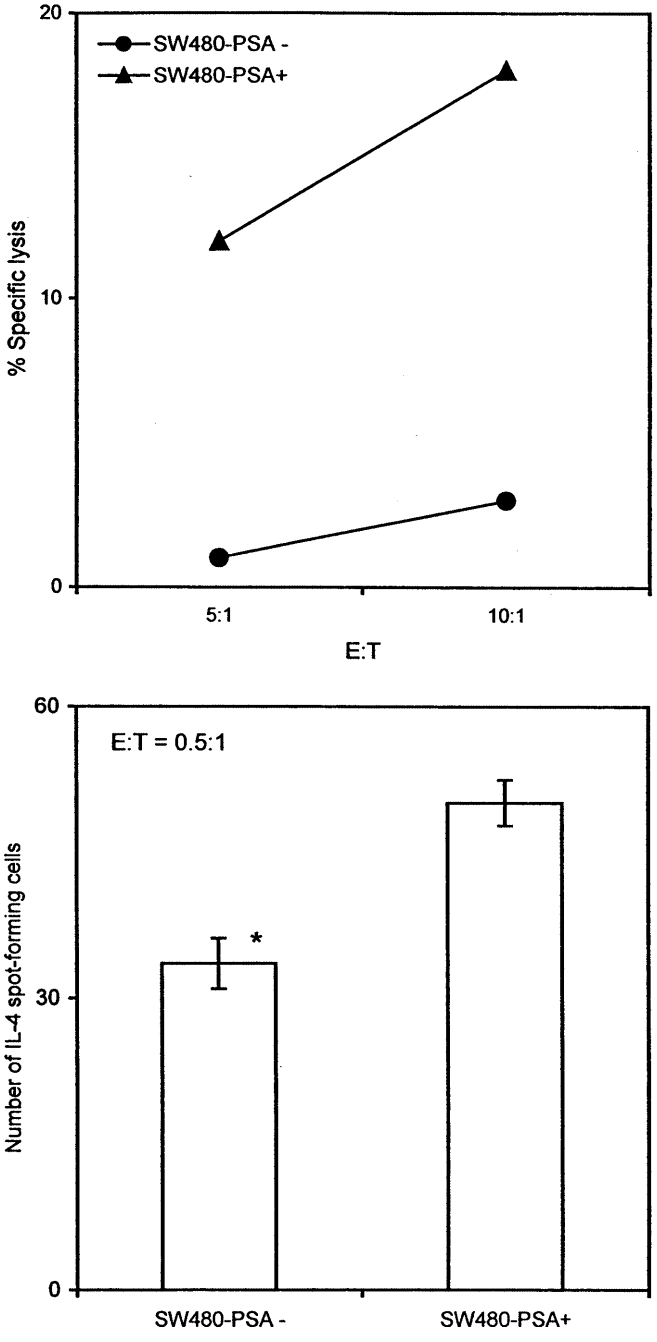

Fig. 5. Specific recognition of PSA^+ targets of the HLA-A2 phenotype by a T-cell line induced with PSA 146–154 peptide. SW480-PSA^+ and SW480-PSA^- target cells were obtained as described in the Materials and methods section. The T-cell line obtained from UPN6 (cycle 16) was incubated at the indicated E:T ratio with ^{51}Cr-labeled SW480-PSA^+ and SW480-PSA^- target cells. The *upper panel* depicts percentage specific lysis per CRA. Specific lysis was observed against SW480-PSA^+ (*closed triangle*) but not against SW480-PSA^- targets (*closed circle*). The *lower panel* shows IL-4-enzyme-linked immuno-spot assay at the indicated E:T ratio. The number of spot-forming cells was significantly higher against SW480-PSA^+ as compared to SW480-PSA^- target cells (*Statistical significance: $P \leq 0.025$, $t = -3.954$). The data are representative of two experiments

and qualitative defects of immune responsiveness in patients with CaP, especially in those with advanced stages of the disease. The purpose of the current study was to determine whether CTL with specificity for a defined epitope for PSA could be induced from patients with hormone-refractory disease. We observed that CTL

with specificity for PSA 146–154 peptide could be elicited from two patients with hormone-refractory tumors by multiple cycles of stimulation with the peptide in vitro. The findings indicate that the repertoire of HLA-$A2^+$ patients with CaP contains precursor T-cells with specificity for the PSA 146–154 peptide, and predict that the peptide might be able to elicit specific CTL by in vivo immunization.

The presence of specific T-cell precursors within the repertoire of patients with CaP is a necessary but not sufficient condition for the development of effective T-cell mediated anti-tumor immunity. It also is essential that putative target epitopes be appropriately expressed and displayed by tumor cells for recognition and killing by mature effector T cells. In order to address this question, we examined whether PSA peptide-induced CTL established from patients with CaP were able to recognize and lyse tumor cells that endogenously express PSA protein. Peptide-induced CTL from the one patient examined reproducibly showed specific recognition and lysis of tumor cells that endogenously expressed PSA and HLA-A2. This finding indicates that the PSA 146–154 peptide emulated a naturally processed and presented peptide epitope of the PSA protein, and elicited CTL which specifically targeted HLA-$A2^+$ cells that express PSA. This conclusion has been corroborated by a study showing that CTL elicited with DC pulsed with PSA^+ tumor cells include effectors that have specificity for PSA 146–154 peptide [6]. This demonstrates that the PSA146–154 peptide satisfies key criteria of a tumor rejection antigen, and thus represents an attractive target for the immunotherapy of CaP.

The PSA-peptide-specific CTL that were generated from patients with CaP displayed a Tc2 phenotype. The recovery of Tc2, as opposed to Tc1, type cells may have been the fortuitous result of protracted in vitro selection in our study. However, we have observed induction of Tc1 cells from normal individuals under similar conditions. This raises the possibility that there is an intrinsic bias towards Tc2 versus Tc1 type responses in CaP patients. Fillela et al. have shown enhanced expression of type-2 cytokines by the PBMC of CaP patients before and after activation with PMA and ionomycin [14]. Other investigators have observed reduced secretion of type-1 cytokines in patients with various types of malignancies, including CaP [15, 16, 17].

While many investigations have emphasized the utility of Tc1 effectors for the treatment of malignancies, recent studies also have established the efficacy of Tc2 effectors. Dobrzanski et al. [18] showed that adoptive transfer of either Tc1 or Tc2 subpopulations with specificity for ovalbumin cured mice with low tumor burdens and delayed mortality in mice with high tumor burdens in a B16-ovalbumin lung metastasis model. In another study, vaccination with tumor cells engineered to produce IL-4 induced type-2 $CD8^+$ T lymphocytes that cured lung metastases in mice by adoptive transfer [19]. Tc2 effector cells also have been shown to mediate graft-versus-leukemia effects after allogeneic stem cell transplantation [20, 21]. These findings suggest that the therapeutic potential of PSA-specific Tc2 for the management of CaP warrants further study.

Many cycles of stimulation were required to induce significant PSA 146–154 peptide-specific CTL responses from patients with CaP. This may indicate that the frequency of specific precursors to the PSA 146–154 epitope is low in the peripheral circulation of these patients. Alternatively, the responsiveness of T cells from patients may be attenuated. This could pose a significant obstacle to peptide vaccination for the treatment of CaP. Emerging adjuvant strategies, such as the use of DC, cytokines or specific blockade of inhibitory factors such as VEG-F may be essential to obtain optimal immunization. Alternatively, the adoptive transfer of PSA 146–154 peptide-specific CTL that have been expanded in vitro may provide sufficiently robust T-cell responses to achieve anti-tumor efficacy.

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