TABLE 2. Response of Tumor Volume

No.	MRI Image	Pre Size	Post Size	Response Rate (%)	Response
1	Detect.	11 × 9	7 × 4	71.7	PR
2	Undetect.				NE
3	Undetect.				NE
4	Undetect.				NE
5	Undetect.				NE
6	Detect.	20 × 10	11 × 8	56.0	PR
7	Detect.	19 × 17	17 × 12	36.8	NC
8	Detect.	15 × 13	7 × 7	74.9	PR
9	Detect.	11 × 8	7 × 6	52.3	PR
10	Undetect.				NE
11	Detect.	7 × 5	6 × 5	14.3	NC
12	Undetect.				NE
13	Detect.	13 × 9	10 × 6	48.7	NC
14	Detect.	12 × 10	8 × 7	53.3	PR
15	Detect.	13 × 12	8 × 5	74.4	PR
16	Undetect.				NE

MRI, magnetic resonance imaging; NC, no change; NE, not evaluated; PR, partial response.

rate of patients who received radiation therapy was approximately 60%.[11–13] In contrast, the 10-year progression-free rate observed in pathologic T1-T2 patients who underwent radical prostatectomy was 90%.[14] However, direct comparison of these results would be misleading because the methodology varied greatly. The definition of "progression-free" is different depending on whether the study used PSA. Furthermore, clinical stage is not accurately indicative of pathologic stage in prostate cancer, and pathologic staging is not available in radiation studies. A randomized study using numerous patients is needed to directly compare these two treatment modalities.

The majority of urologists assume that radical prostatectomy gives a higher rate of curability of disease in ex-

TABLE 3. Response of PSA

No.	Pre PSA	Post PSA	Nadir PSA	Latest PSA	Follow-up (Months)	Response	Total Response
1	12.0	3.5	1.5	1.5	14.5	CR	CR
2	0.7	1.0	0.5	0.6	12.9	NE	NE
3	22.0	5.3	2.2	2.2	10.0	CR	CR
4	12.0	3.1	0.5	0.5	12.3	CR	CR
5	0.8	3.1	0.8	1.6	12.1	NE	NE
6	8.7	2.1	1.0	1.0	11.3	CR	CR
7	23.0	5.7	2.1	2.1	7.9	CR	CR
8	13.0	4.1	0.9	0.9	12.1	CR	CR
9	6.6	4.1	2.2	2.2	12.1	CR	CR
10	0.2	0.6	0.2	0.5	12.1	NE	NE
11	8.4	4.7	2.0	2.0	11.8	CR	CR
12	10.0	3.4	1.1	1.1	11.7	CR	CR
13	17.0	5.6	1.1	1.7	11.8	CR	CR
14	10.0	9.2	2.9	2.9	10.1	CR	CR
15	8.2	1.7	0.7	0.7	12.0	CR	CR
16	0.2	0.6	0.6	0.7	11.2	NE	NE

CR, complete response; NE, not evaluated; PSA, prostate-specific antigen.

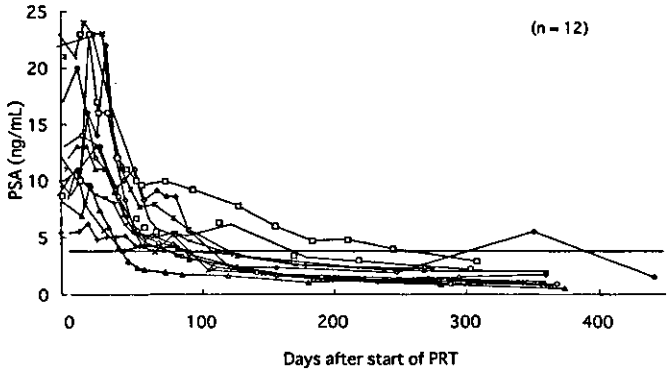

FIGURE 1. Changes in PSA value of the patients who underwent proton therapy as initial therapy (n = 12).

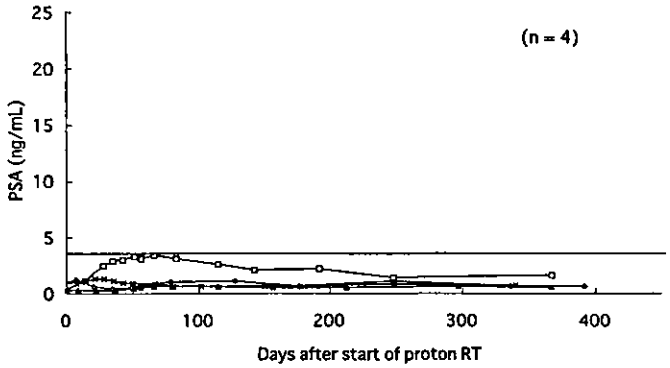

FIGURE 2. Changes in PSA value of the patients who had undergone hormonal therapy before proton therapy.

change for surgical complications. In fact, urinary incontinence and sexual dysfunction are two major complications following radical prostatectomy. The rate of these two complications in radiation therapy is clearly lower than that in radical prostatectomy.[15,16] However, the side effects of radiation therapy cannot be considered negligible. The severity of side effects from radiation therapy is closely related to total irradiated dose, and serious side effects occur when the dose is increased. The maximum dose of external beam radiation that can be tolerated is 70 Gy.[8,17] Complications of radiation therapy are divided in acute and late complications. Acute complications are usually temporary, but late complications sometimes present a serious problem. Urinary tract symptoms and bowel symptoms are two major complications of radiation therapy. Bowel complications in particular lead to limited total irradiation doses. Therefore, numerous techniques have been attempted to deliver sufficient radiation doses to the prostate without increasing the risks of bowel complications.

One technique that has been developed is 3D-conformal radiotherapy (3D-CRT). Multiple studies have reported encouraging initial results following treatment with 3D-

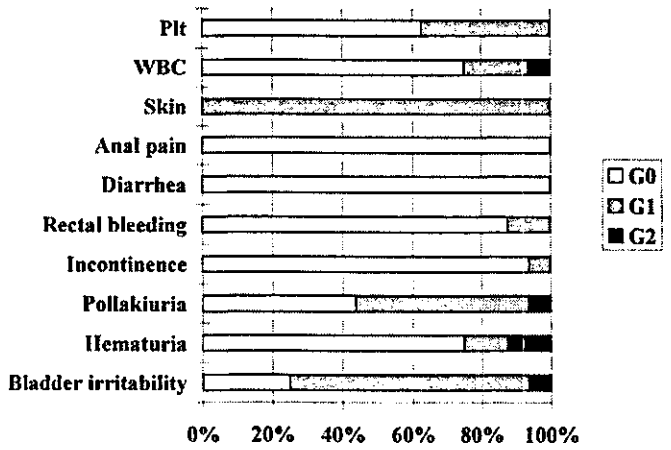

FIGURE 3. The rate of side effects. Acute treatment-related toxicity was graded using the NCI Common Toxicity Criteria Grading System (version 2.0, April 1999), and late treatment-related toxicity was graded using the Radiation Therapy Oncology Group (RTOG) late morbidity criteria. No grade III or grade IV toxicity was observed.

CRT.[18,19] These studies reported successfully increasing radiation doses without increasing the risks of serious acute complications. Because long-term clinical outcomes are not yet available, the antitumor effects have been evaluated based on PSA values in the short term. To date, the clinical outcomes in these studies seem to be good, although long-term observation is necessary before conclusions can be drawn. Another concern is the late complications of 3D-CRT, although longer follow-up periods are also needed to evaluate this concern.

Brachytherapy is also an optional treatment for early prostate cancer. Several improvements such as ultrasound guidance for more precise needle location, novel isotopes, and minute treatment planning for dose distribution have made brachytherapy a powerful treatment modality. Zelefsky et al. conducted a comparison of clinical outcomes and morbidity in early-stage prostatic cancer patients undergoing 3D-CRT and transperineal permanent iodine-125 implantation.[20] The 5-year non-PSA failure rates of brachytherapy and 3D-CRT were 88% and 82%, respectively. Urinary symptoms were more frequently observed in the brachytherapy group. No late bowel complications greater than grade III were observed in either group. This result demonstrates the feasibility of brachytherapy in the treatment of prostatic cancer.

Proton therapy is another promising treatment modality. The unique dose distribution of protons offers a potential advantage over other forms of conformal therapy: each individual proton beam can conform to the target volume in three dimensions. However, very little information has been reported relating to clinical outcomes of proton therapy because

the availability of equipment is limited due to the high cost. Loma Linda University reported clinical outcomes in a large number of patients.[21–23] According to these reports, the 5-year biochemical disease-free survival rate of 319 patients with T1-T2b prostate cancer was 88%.[22] When 106 T2b-T3 cases were analyzed, 13 patients (12.2%) showed PSA failure in the 20.2 month median follow-up period.[23] In both papers, no severe treatment-related morbidity was seen. Both studies demonstrate excellent clinical outcomes.

We received a proton beam radiation system in our prefecture in 2001.[24] We wanted to determine whether excellent curability with minimum toxicity could be obtained using this system. Although the follow-up period was relatively short, we successfully showed a reduced number of complications, particularly relating to rectal damage. We also demonstrated an excellent response rate in the short term. Of course, further studies employing longer follow-up periods and using larger numbers of patients will be necessary to form reliable conclusions. However, we believe the present results indicate that proton beam therapy is less damaging than conventional forms of external beam radiotherapy.

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Identification of parathyroid hormone-related protein-derived peptides immunogenic in human histocompatibility leukocyte antigen-A24$^+$ prostate cancer patients

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Parathyroid hormone-related protein (PTHrP) is a key factor in the development of bone metastases, which are a major barrier in treating prostate cancer patients. In this study, we attempted to identify PTHrP-derived peptides immunogenic in human histocompatibility leukocyte antigen (HLA)-A24$^+$ prostate cancer patients. Among four different PTHrP peptides carrying the HLA-A24 binding motif, both the $PTHrP_{36-44}$ and $PTHrP_{102-111}$ peptides efficiently induced peptide-specific cytotoxic T lymphocytes from peripheral blood mononuclear cells (PBMCs) of HLA-A24$^+$ prostate cancer patients. Peptide-stimulated PBMCs showed cytotoxicity against prostate cancer cells in an HLA-A24-restricted manner. Experiments using antibodies and cold inhibition targets confirmed that their cytotoxicity was dependent on PTHrP peptide-specific and CD8$^+$ T cells. Immunoglobulin G reactive to the $PTHrP_{102-111}$ or $PTHrP_{110-119}$ peptide was frequently detected in the plasma of prostate cancer patients, suggesting that the $PTHrP_{102-111}$ peptide is able to elicit cellular and humoral immune responses in cancer patients. These results indicate that the PTHrP could be a promising target molecule for specific immunotherapy of HLA-A24$^+$ prostate cancer patients with metastases.
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Prostate cancer is one of the most common cancers among elderly men (Greenlee *et al*, 2000). Prostate cancer frequently metastasises to bone. Androgen withdrawal therapy has been applied for patients with bone metastases. Although hormone therapy can temporarily inhibit the progress of the disease in these patients, a progression to hormone-refractory prostate cancer inevitably occurs in most cases. Therefore, the development of new therapeutic modalities is needed.

Recent advances in tumour immunology have allowed us to identify the genes encoding human cancer-related antigens, and the epitopes, which are recognized by cytotoxic T lymphocytes (CTLs), in patients with various types of cancers (Boon *et al*, 1997; Rosenberg, 1999; Renkvist *et al*, 2001). The identified tumour antigens and their peptides have been applied for specific immunotherapy (Nestle *et al*, 1998; Rosenberg *et al*, 1998; Marchand *et al*, 1999). In the case of prostate cancer, tissue-specific antigens, which are expressed in the normal prostate, can also be target molecules for specific immunotherapy for patients with this disease. Immunotherapy targeting prostate-specific antigens or prostate-specific membrane antigens has been carried out, and antitumour effects have been observed in limited cases (Murphy *et al*, 1996, 1999; Tjoa *et al*, 1998; Small *et al*, 2000; Gulley *et al*, 2002).

Parathyroid hormone-related protein (PTHrP) is an autocrine or paracrine factor that binds to receptors on osteoblasts, and stimulates bone formation and reabsorption. Parathyroid hormone-related protein has limited homology with PTH at its NH2 terminus, and can bind to the same receptor as PTH, resulting in similar biological activity (Suva *et al*, 1987; Juppner *et al*, 1991). Parathyroid hormone-related protein plays a variety of physiological roles, including calcium transport, keratinocyte differentiation, smooth muscle relaxation, and cartilage development (Philbrick *et al*, 1996). In parathyroid cells, a high extracellular calcium concentration inhibits parathyroid hormone (PTH) secretion and the proliferation of parathyroid cells as a result of negative feedback regulation, whereas it evokes further PTHrP secretion and promotes worsening bone resorption (Sanders *et al*, 2001). Therefore, PTHrP has been considered to be responsible for the hypercalcemia associated with malignancy (Guise, 1997). In addition, prostate cancers have been reported to produce PTHrP (Francini *et al*, 2002). These lines of evidence suggest that PTHrP could be a promising target molecule for the immunotherapy of prostate cancer patients with bone metastases. In this study, we

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attempted to identify new, PTHrP-derived peptides that are immunogenic in HLA-A24$^+$ prostate cancer patients.

MATERIALS AND METHODS

Patients

Informed consent was obtained from all of the HLA-A24$^+$ prostate cancer patients and HLA-A24$^+$ healthy volunteers who were enrolled in this study. None of the participants were infected with HIV. In total, 20 ml of peripheral blood was obtained, and the PBMCs were prepared by Ficoll-Conray density gradient centrifugation. The expression of HLA-A24 molecules on the PBMCs of the cancer patients and healthy donors was determined by flow cytometry.

Cell lines

C1R-A24 is an HLA-A*2402-expressing subline of C1R lymphoma (Dr M Takiguchi, Kumamoto University, Japan). LNCaP is an HLA-A24 negative prostate cancer cell line. To establish LNCaP cells that stably express HLA-A24 molecules (designated as LNCaP-A24), an *HLA-A*2402* gene was inserted into a pcDNA3.1/Hygro vector (Invitrogen, CA, USA), and electroporated into the LNCaP cell line (ATCC, Manassas, VA, USA), and selection was carried out with hygromycin B (Invitrogen) at a dose of 170 $\mu g\,ml^{-1}$. All cell lines were maintained in RPMI-1640 medium (Gibco BRL, Grand Island, NY, USA) supplemented with 10% FCS.

Peptides

Four PTHrP-derived peptides (listed in Table 1) were prepared based on the HLA-A24 binding motif (Parker *et al*, 1994; Rammensee *et al*, 1995). All peptides were of >90% purity and were purchased from Biologica Co., Nagoya, Japan. Influenza (Flu) virus-derived (RFYIQMCYEL), EBV-derived (TYGPVFMCL), and HIV-derived peptides (RYLRQQLLGI) with the HLA-A24 binding motif were used as controls. All peptides were dissolved with DMSO at a dose of 10 $mg\,ml^{-1}$.

Assay for peptide-specific CTLs in PBMCs

The assay for the detection of peptide-specific CTLs in PBMCs was performed according to a previously reported method (Hida *et al*, 2002). In brief, PBMCs (1×10^5 cells per well) were incubated with 10 $\mu g\,ml^{-1}$ of each peptide in a U-bottom-type 96-well microculture plate (Nunc, Roskilde, Denmark) at a volume of 200 μl of culture medium. The culture medium consisted of 45% RPMI-1640, 45% AIM-V medium (Gibco BRL), 10% FCS, 100 $U\,ml^{-1}$ of IL-2, and 0.1 mM MEM nonessential amino-acid solution (Gibco, BRL). Half of the culture medium was removed and replaced with new medium containing a corresponding peptide (20 $\mu g\,ml^{-1}$) every 3 days. On the 15th day of culture, the cultured cells were separated into four wells; two wells were used for the PTHrP peptide-pulsed C1R-A24 cells, and the other two wells were used for the HIV peptide-pulsed C1R-A24 cells. After an 18-h incubation period, the supernatants were collected, and the level of IFN-γ was determined by ELISA (limit of sensitivity: 10 $pg\,ml^{-1}$).

Table 1 Reactivity of PTHrP peptide-stimulated PBMCs from HLA-A24$^+$ healthy donors and prostate cancer patients

	Peptides					
	Name	$PTHrP_{36-44}$	$PTHrP_{102-111}$	$PTHrP_{25-34}$	$PTHrP_{110-119}$	Flu	EBV
PBMCs	Amino-acid sequence	RAVSEHQLL	RYLTQETNKV	RSVEGLSRRL	KVETYKEQPL	RFYIQMCTEL	TYGPVFMCL
Derived from	Score[a]	14.4	19.8	17.3	14.4		
		IFN-γ production (pg/ml)[b]					
Healthy donors							
#1		154	352	10	394	306	0
#2		156	132	8	17	0	207
#3		497	0	7	20	17	0
#4		0	0	37	2	59	14
#5		184	0	166	38	0	27
#6		1354	0	0	357	124	168
#7		166	38	0	0	1017	0
#8		0	194	0	1017	0	0
#9		0	5624	5	61	123	228
#10		0	168	1354	0	0	3
Total		6/10	5/10	2/10	3/10	4/10	3/10
Cancer patients							
#1		180	154	145	0	0	15
#2		122	138	15	9	5	0
#3		699	8	17	38	0	21
#4		31	105	24	19	0	159
#5		799	28	16	10	130	20
#6		500	4	1	14	198	15
#7		317	0	0	0	ND	ND
#8		4	1060	411	23	115	189
#9		17	101	1	0	709	3
#10		180	198	196	118	40	27
Total		7/10	6/10	3/10	1/10	4/9	2/9

[a]The score represents the estimated half-time of dissociation of the PTHrP peptides binding to HLA-A24 molecules. [b]The PBMCs of HLA-A24$^+$ healthy donors and prostate cancer patients were stimulated *in vitro* with the indicated PTHrP peptide, as described in Material and Methods. On the 15th day, the cultured PBMCs were tested for their reactivity to C1R-A24 cells, which were prepulsed with the corresponding peptide or the HIV peptide. The values represent the mean of two wells, and the background IFN-γ production in response to the HIV peptide was subtracted. Significant values ($P<0.05$ by two-tailed Student's *t*-test) are underlined. ND = not done.

Cytotoxicity assay

After *in vitro* stimulation with the PTHrP peptides, the peptide-stimulated PBMCs were additionally cultured with 100 U ml^{-1} IL-2 for approximately 10 days, in order to obtain a sufficient number of cells to carry out a cytotoxicity assay. These cells were then tested for cytotoxicity against both LNCaP and LNCaP-A24 by a 6-h ^{51}Cr-release assay. A total of 2000 ^{51}Cr-labelled cells per well were cultured with effector cells in 96-round-well plates at the indicated effector/target ratios. In some experiments, either anti-HLA class I (W6/32: mouse IgG2a), anti-HLA-DR (L243: mouse IgG2a), anti-CD4 (NU-TH/I: mouse IgG1), anti-CD8 (NU-TS/C: mouse IgG2a), or anti-CD14 (H14: mouse IgG2a) mAb was added to the wells at a dose of 20 μg ml^{-1} at the initiation of the assay.

Cold inhibition assay

The specificity of the PTHrP peptide-stimulated CTLs was confirmed by a cold inhibition assay. In brief, ^{51}Cr-labelled target cells (2×10^3 cells per well) were cultured with the CTLs (4×10^4 cells per well) in 96-round-well plates with 2×10^4 cold target cells. C1R-A24 cells, which were prepulsed with either the HIV peptide or a corresponding PTHrP peptide, were used as cold targets.

Detection of peptide-specific IgG

The peptide-specific IgG levels in the plasma were measured by ELISA, as previously reported (Nakatsura *et al*, 2002; Ohkouchi *et al*, 2002). In brief, peptide (20 μg per well)-immobilised plates were blocked with Block Ace (Yukijirushi, Tokyo, Japan) and washed with 0.05% Tween-20-PBS, after which 100 μl per well of plasma sample diluted with 0.05% Tween-20-Block Ace was added to the plate. After a 2-h incubation at 37°C, the plates were washed and further incubated for 2-h with a 1 : 1000-diluted rabbit anti-human IgG (γ-chain-specific) (DAKO, Glostrup, Denmark). The plates were washed, and then 100 μl of 1 : 100-diluted goat anti-rabbit IgG-conjugated horseradish peroxidase (EnVision, DAKO) was added to each well, and the plates were then incubated at room temperature for 40 min. After the plates were washed once, 100 μl per well of tetramethyl benzidine substrate solution (KPL, Guildford, UK) was added, and the reaction was stopped by the addition of 1 M phosphoric acid. The values are shown as optical density (OD) units ml^{-1}. IgG reactive to a corresponding PTHrP peptide was judged to be positive when the difference of the OD in 1 : 100-diluted plasma exceeded 0.05. To confirm the specificity of IgG to the indicated PTHrP peptide, sample plasma was cultured with plates coated with either the corresponding PTHrP peptide or an irrelevant PTHrP peptide. Thereafter, the levels of PTHrP peptide-specific IgG in the resulting supernatant were determined by ELISA.

Statistics

The statistical significance of the data was determined using a two-tailed Student's *t*-test. A *P*-value of less than 0.05 was considered to be statistically significant.

RESULTS

Induction of PTHrP peptide-specific CTLs from HLA-A24$^+$ healthy donors and prostate cancer patients

First, four PTHrP-derived peptides were prepared based on their binding affinity to HLA-A24 molecules (Parker *et al*, 1994; Rammensee *et al*, 1995) (Table 1). Although the PTHrP$_{1-36}$ peptide is a propeptide (Suva *et al*, 1987; Juppner *et al*, 1991), the PTHrP$_{25-34}$ peptide was included. With regard to the difference in amino acids, three amino acids were found to differ between the PTHrP$_{36-44}$ peptide and PTH, and all of the amino acids were found to differ between the other three PTHrP peptides and PTH. Next, to investigate the immunogenicity of these four PTHrP peptides, the PBMCs of 10 HLA-A24$^+$ healthy donors and 10 HLA-A24$^+$ prostate cancer patients were stimulated with each of four PTHrP peptides, and were then examined for their IFN-γ production in response to C1R-A24 cells, which were prepulsed with either a corresponding PTHrP peptide or the HIV peptide (Table 1). Flu- and BEV-derived peptides were used as controls. The assay was carried out in quadruplicate. The cultured cells in one well were separated into four wells. Two wells were used for the PTHrP peptide-pulsed C1R-A24 cells, and the other two wells for the HIV peptide-pulsed C1R-A24 cells. The background IFN-γ production in response to the HIV peptide was subtracted, and the results that showed the best response are shown in Table 1. The successful induction of peptide-specific CTLs was judged to be positive when significant values ($P < 0.05$ by two tailed Student's *t*-test) were observed. The results showed that the PTHrP$_{36-44}$, PTHrP$_{102-111}$, PTHrP$_{25-34}$, and PTHrP$_{110-119}$ peptides induced peptide-specific CTLs in six, five, two, and three of 10 HLA-A24$^+$ healthy donors, respectively. These PTHrP peptides also induced peptide-specific CTLs in seven, six, three, and one of 10 HLA-A24$^+$ prostate cancer patients, respectively. The net IFN-γ production of the cases with 10 HLA-A24$^+$ prostate cancer patients in response to the corresponding PTHrP peptide or the HIV peptide are shown in Figure 1. In total, these findings indicate that both the PTHrP$_{36-44}$ and PTHrP$_{102-111}$ peptides are promising candidates to generate peptide-specific CTLs from HLA-A24$^+$ prostate cancer patients.

Induction of prostate cancer-reactive CTLs using PTHrP$_{36-44}$ and PTHrP$_{102-111}$ peptides

In order to investigate the HLA-A24-restricted and prostate cancer-reactive cytotoxicity of peptide-stimulated PBMCs, we prepared an HLA-A24-expressing LNCaP cell line, which we designated LNCaP-A24 (Figure 2). LNCaP has previously been reported to produce PTHrP (Francini *et al*, 2002). A parental LNCaP cell line was negative for the cell surface expression of HLA-A24 molecules, whereas the LNCaP-A24 cell line expressed HLA-A24 molecules on their cell surface. It was then determined whether PBMCs stimulated by either the PTHrP$_{36-44}$ or PTHrP$_{102-111}$ peptide could induce prostate cancer-reactive CTLs from HLA-A24$^+$ healthy donors and prostate cancer patients. PBMCs from HLA-A24$^+$ healthy donors and cancer patients were repeatedly stimulated with the indicated PTHrP peptide, based on the culture protocol described in Materials and Methods. After confirming that these peptide-stimulated cells could produce IFN-γ in response to PTHrP peptide-pulsed C1R-A24 cells, the peptide-stimulated PBMCs were examined for their cytotoxicity against three targets. It was found that the PTHrP peptide-stimulated PBMCs from HD#2, Pt#1, and Pt#2 produced higher levels of IFN-γ in response to the corresponding PTHrP peptide-pulsed C1R-A24 cells than to the HIV peptide-pulsed C1R-A24 cells (Figure 3A). These peptide-stimulated PBMCs also showed higher levels of cytotoxicity against the LNCaP-A24 cell line than against the LNCaP line and HLA-A24$^+$ PHA-induced T cell blasts (Figure 3B). In addition, their cytotoxicity against LNCaP-A24 was significantly inhibited by the addition of anti-HLA-class I and anti-CD8 mAbs, but not by the addition of other anti-HLA-class II, anti-CD4, or anti-CD14 mAbs (Figure 4A). Furthermore, their cytotoxicity against the LNCaP-A24 cell line was significantly suppressed by the addition of the corresponding PTHrP peptide-pulsed C1R-A24 cells, as a cold target, but this suppression was not observed with the addition of HIV peptide-pulsed C1R-A24 cells (Figure 4B). In

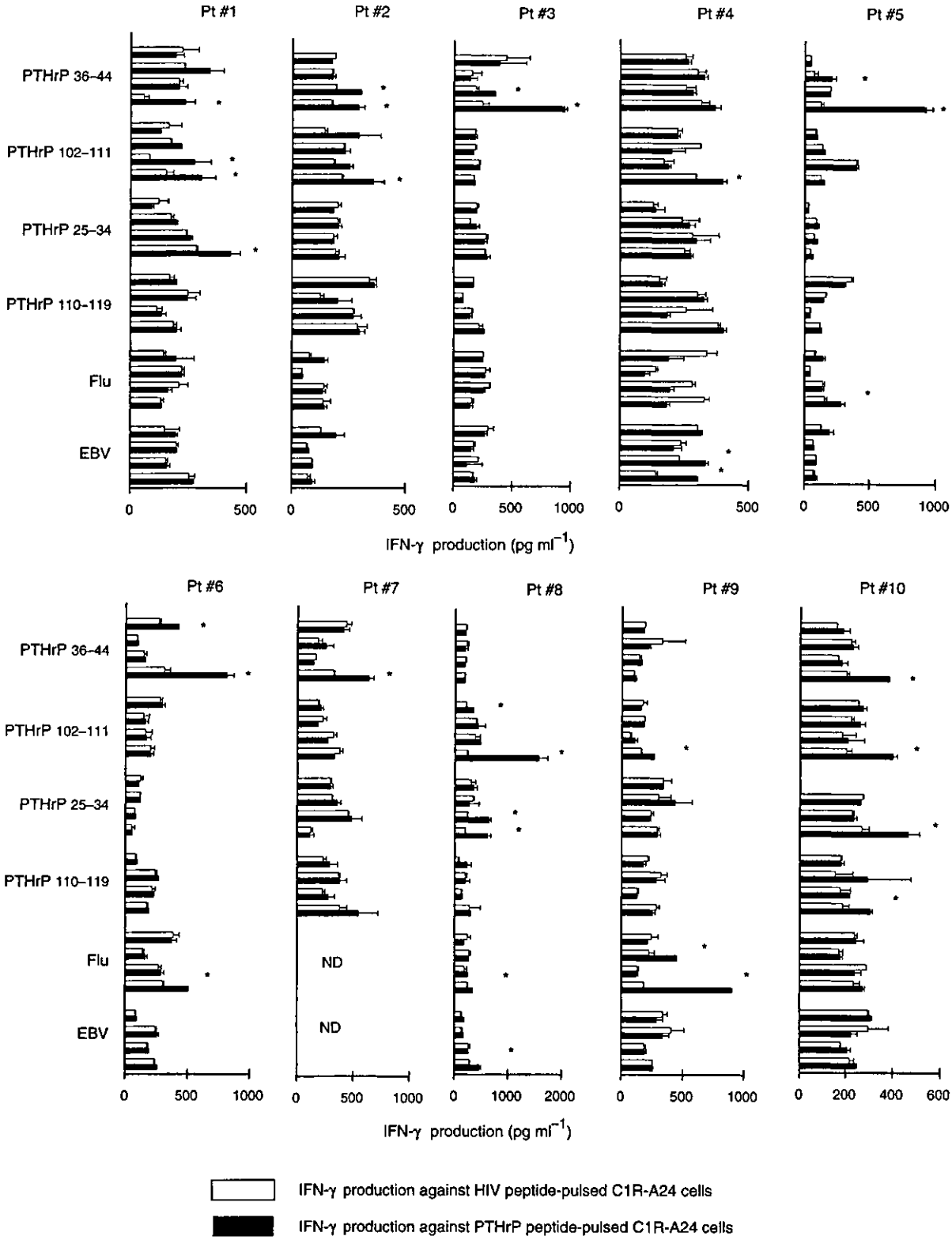

Figure 1 Induction of PTHrP peptide-specific CTLs from the PBMCs of HLA-A24$^+$ prostate cancer patients. PBMCs from 10 HLA-A24$^+$ prostate cancer patients were stimulated *in vitro* with the PTHrP peptides indicated, as described in Materials and Methods. On the 15th day, the peptide-stimulated cells were cultured with C1R-A24 cells, which were prepulsed with an HIV peptide (open bar) and the indicated PTHrP peptide (closed bar) for 18-h. The levels of IFN-γ in the supernatants were then determined by ELISA. *$P<0.05$ was considered statistically significant.

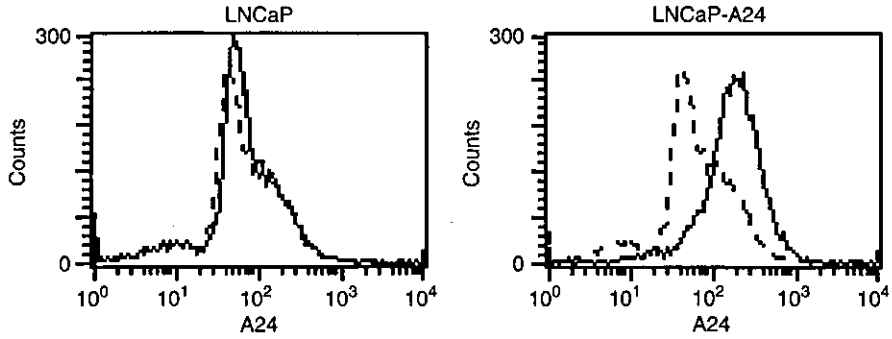

Figure 2 An HLA-A24-expressing LNCaP cell line. Flow cytometric analysis was performed on the LNCaP and LNCaP-A24 cells. These cells were stained with anti-HLA-A24 mAb, followed by FITC-conjugated anti-mouse IgG mAb. The dotted lines represent staining without the first mAb.

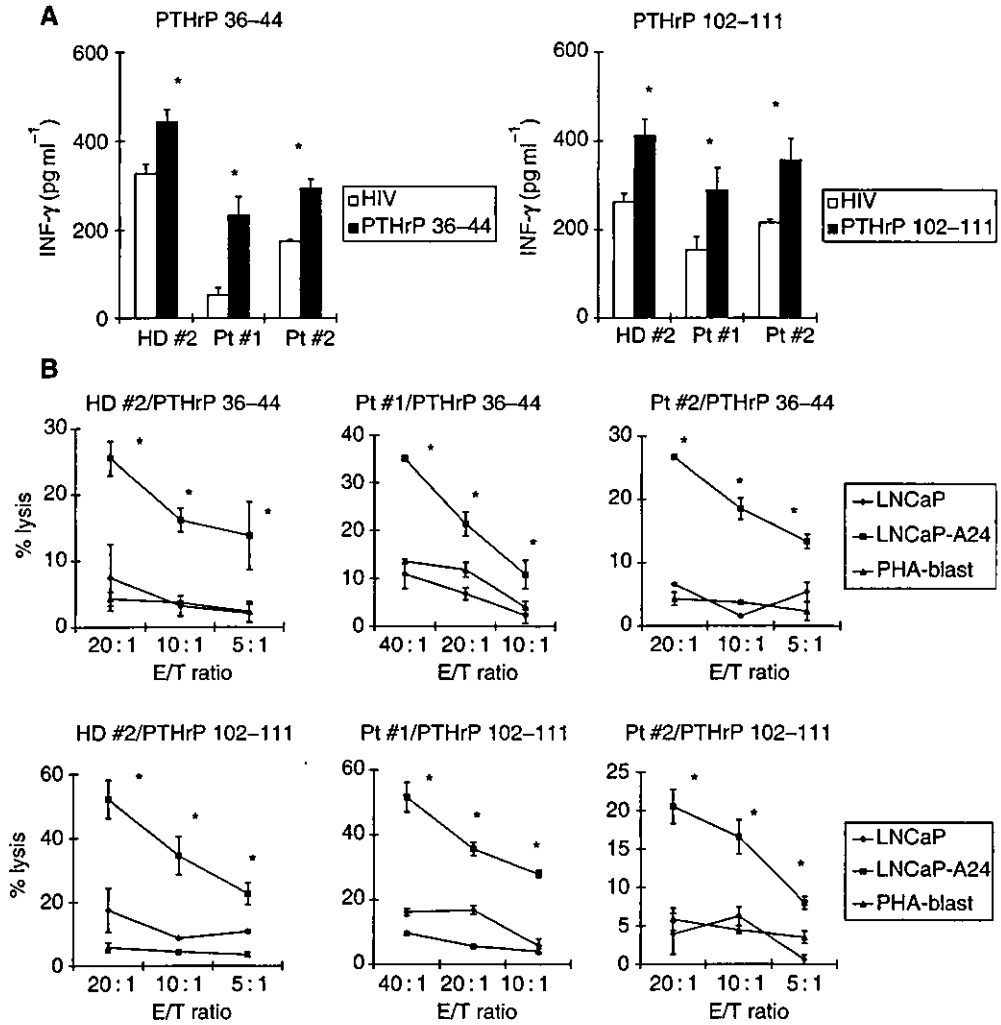

Figure 3 Induction of HLA-A24-restricted and prostate cancer-reactive CTLs from the PBMCs of healthy donors and cancer patients. (**A**) PBMCs from one HLA-A24$^+$ healthy donor (HD #2) and from two HLA-A24$^+$ prostate cancer patients (Pt #1 and Pt #2) were stimulated *in vitro* with the indicated PTHrP peptides, as described in Materials and Methods. On the 15th day, half of the cultured cells were harvested, pooled from four wells, and cultured with C1R-A24 cells, which were prepulsed with an HIV peptide (open symbol) and the indicated PTHrP peptide (closed symbol) for 18-h. The levels of IFN-γ in the supernatants were then determined by ELISA. (**B**) Thereafter, these cells were examined for their cytotoxicity against the LNCaP cells (HLA-A24$^-$), LNCaP-A24 cells (HLA-A24$^+$), and PHA-blastoid T cells (HLA-A24$^+$). A 6-h ^{51}Cr-release assay was performed. Values represent the mean of triplicate assays. *$P<0.05$ was considered statistically significant.

addition, we observed that these PTHrP peptide-stimulated PBMCs from cancer patients showed cytotoxicity against another prostate cancer cells PC-93-A24, stably expressed the HLA-A24 molecules and produced PTHrP (data not shown). These results indicate that both the $PTHrP_{36-44}$ and $PTHrP_{102-111}$ peptides have the potential to induce prostate cancer-reactive CTLs from HLA-A24$^+$ prostate

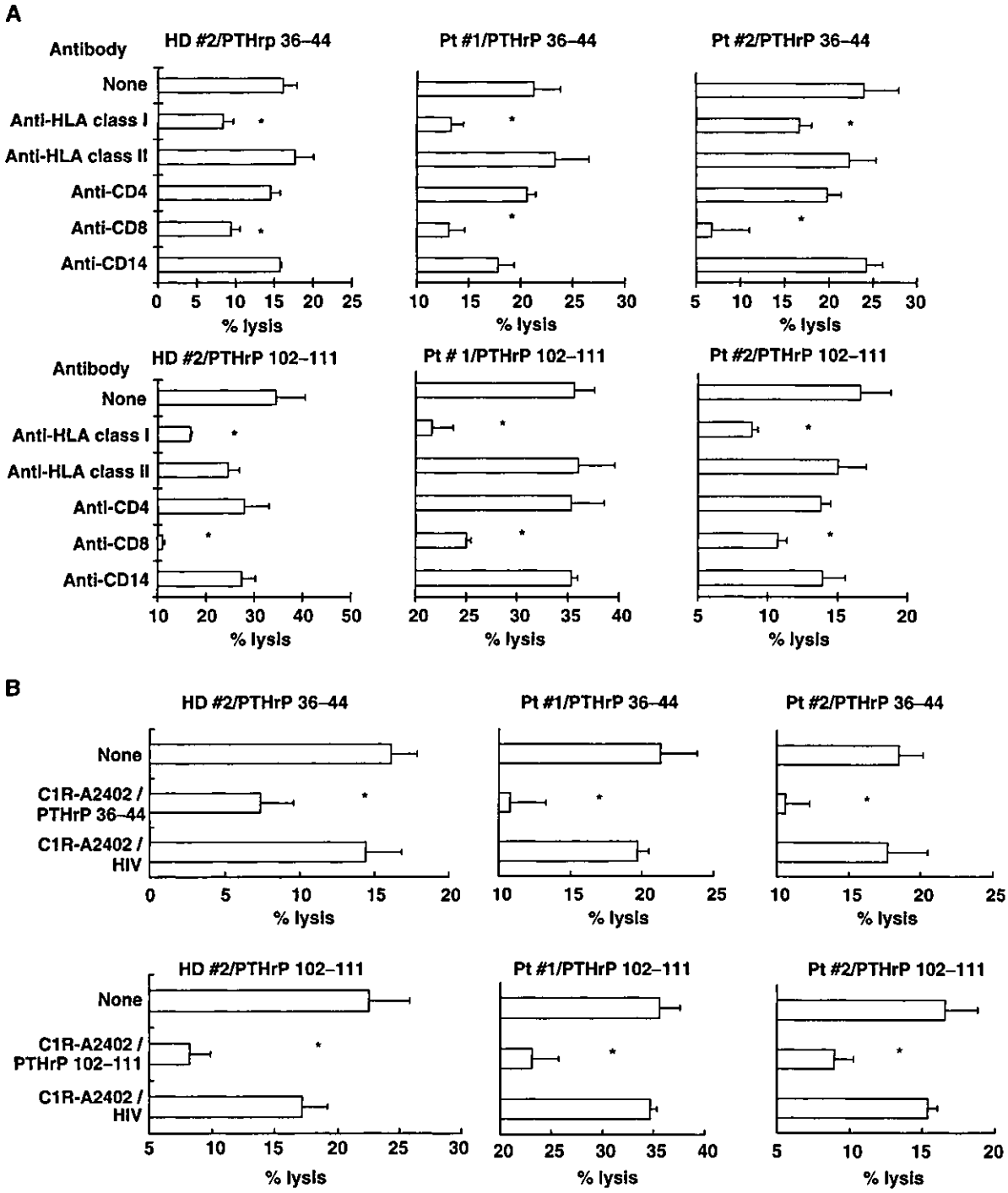

Figure 4 CD8$^+$ T-cell-dependent and PTHrP peptide-specific cytotoxicity against LNCaP-A24 cells. (**A**) The PTHrP peptide-stimulated PBMCs, described in Figure 2, were examined for their cytotoxicity against the LNCaP-A24 cell line, with or without anti-HLA class I, anti-HLA class II, anti-CD4, anti-CD8, or anti-CD14 mAb at a dose of 20 μg ml^{-1}. The values represent the mean of triplicate assays. *$P < 0.05$ was considered statistically significant. (**B**) The cytotoxicity against the LNCaP-A24 cell line (2×10^3 cells per well) was also examined in the presence of unlabelled C1R-A24 cells (2×10^4 cells per well), which were prepulsed with the HIV peptide or a corresponding PTHrP peptide. The values represent the mean of triplicate assays. *$P < 0.05$ was considered statistically significant.

cancer patients, and that their cytotoxicity against prostate cancer was dependent on PTHrP peptide-specific CD8$^+$ T cells.

Detection of IgG reactive to the PTHrP peptides

We previously reported that IgGs reactive to CTL epitope peptides were detected in healthy donors and cancer patients (Nakatsura *et al*, 2002; Ohkouchi *et al*, 2002). IgGs reactive to prostate-related antigens were also detected in healthy donors and prostate cancer patients (Harada *et al*, 2003a; Kobayashi *et al*, 2003; Matsueda *et al*, 2004) Therefore, we attempted to determine whether IgG reactive to four PTHrP-derived peptides could be detected in the plasma of cancer patients and healthy donors. The result was that IgG reactive to either the $PTHrP_{102-111}$ or the $PTHrP_{109-119}$ peptide

Table 2 IgG reactive to the PTHrP peptides in plasma of HLA-A24$^+$ healthy donors and prostate cancer patients

	Healthy donors											Prostate cancer patients										
Peptides	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**#9**	**#10**	**Total**	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**#9**	**#10**	**Total**
$PTHrP_{36-44}$	−	−	−	−	−	−	+	+	+	−	3/10	−	−	−	−	+	−	−	−	−	−	1/10
$PTHrP_{102-111}$	+	−	+	+	+	−	+	+	+	+	8/10	+	+	−	−	+	+	+	+	+	−	7/10
$PTHrP_{25-34}$	−	−	−	−	−	−	−	−	−	−	0/10	−	−	−	−	−	−	−	−	−	−	0/10
$PTHrP_{110-119}$	+	+	+	−	+	−	+	+	+	+	8/10	+	+	−	−	−	+	+	+	+	+	7/10

IgG reactive to the corresponding peptide was judged to be positive when the difference in the OD in 1 : 100-diluted plasma exceeded 0.05. The cutoff level (OD: 0.05) was determined based on the levels of anti-HIV peptide IgG in HIV-negative healthy donors.

was detected in eight of 10 healthy donors and in seven of 10 prostate cancers (Table 2). Representative results are in Figure 5A. However, IgG reactive to the $PTHrP_{36-44}$ peptide was detected in three of 10 healthy donors and one of 10 prostate cancer patients, respectively. No IgG reactive to $PTHrP_{25-34}$ was detected in any of the healthy donors or cancer patients. The levels of PTHrP peptide-specific IgG were significantly diminished by culturing the plasma in the corresponding PTHrP peptide-coated wells (Figure 5B). This peptide-specific absorption demonstrated the validity of the present assay system.

DISCUSSION

Prostate cancer appears to be a good target for the development of specific immunotherapies (Harada *et al*, 2003b). In recent years, our group has attempted to identify epitope peptides derived from prostate-related antigens that would be able to generate prostate cancer-reactive CTLs from prostate cancer patients (Inoue *et al*, 2001; Kobayashi *et al*, 2003; Harada *et al*, 2003a; Matsueda *et al*, 2004). However, one major obstacle encountered when treating prostate cancer patients is the treatment of bone metastases, as prostate cancer frequently metastasises to the bone tissue. Therefore, we undertook the present study to identify epitope peptides that could potentially be suitable for specific the immunotherapy of HLA-A24$^+$ prostate cancer patients with metastases.

PTHrP is known to be a key agent in the development of bone metastasis in cases of prostate cancer, and prostate cancer cells has been reported to produce PTHrP (Francini *et al*, 2002). These lines of evidence indicate that PTHrP could be a good target for the development of specific immunotherapies against metastatic prostate cancer. Indeed, $PTHrP_{59-68}$ and $PTHrP_{165-173}$ peptides have been reported to be candidates for such specific immunotherapy of HLA-A2$^+$ prostate cancer patients (Guise, 1997; Francini *et al*, 2002). In this study, we identified new PTHrP peptides that have the potential to generate prostate cancer-specific CTLs in HLA-A24$^+$ prostate cancer patients, in order to extend the possibility of PTHrP peptide-based anticancer vaccine. We revealed that both the $PTHrP_{36-44}$ and the $PTHrP_{102-111}$ peptides have the potential to induce prostate cancer-reactive CTLs in HLA-A24$^+$ prostate cancer patients. PBMCs from HLA-A24$^+$ prostate cancer patients showed peptide-specific IFN-γ production in six or seven of 10 patients when stimulated with the $PTHrP_{102-110}$ and $PTHrP_{36-44}$ peptide, respectively. More importantly, PBMCs that were stimulated with these PTHrP peptides showed cytotoxicity against prostate cancer cells in an HLA-A24-restricted manner. These results indicate that these two PTHrP peptides are immunogenic, and therefore potentially useful for the specific immunotherapy of HLA-A24$^+$ prostate cancer patients with metastases.

The $PTHrP_{36-44}$ and the $PTHrP_{102-110}$ peptides also induced peptide-specific and tumour-reactive CTLs from the PBMCs of HLA-A24$^+$ healthy donors. This result is consistent with that of a previous report demonstrating the induction of PTHrP peptide-specific CTLs from the PBMCs of HLA-A2$^+$ healthy donors (Francini *et al*, 2002). As the $PTHrP_{36-44}$ peptide shares three amino acids with PTH, and because there is no homology between the $PTHrP_{102-111}$ peptide and PTH, crossreactivity between the PTHrP peptides and PTH could be excluded. Low levels of PTHrP have been sporadically detected in keratinocytes, uterus, and mammary glands during lactation (Tian *et al*, 1993). Recent advances in tumour immunology have revealed that self-antigens on human cancer cells are the most prevalent antigens recognized by the immune system (Rosenberg, 1999; Renkvist *et al*, 2001). CTL precursors reactive to nonmutated self-antigens may circulate in the peripheral blood of both certain healthy donors and cancer patients.

Here, we investigated whether or not IgG against PTHrP peptides would be detectable in plasma from HLA-A24$^+$ healthy donors and prostate cancer patients, because the antibodies against CTL epitope peptides had already been observed in certain cancer patients and healthy donors (Nakatsura *et al*, 2002; Ohkouchi *et al*, 2002). We also previously reported that IgG reactive to peptides derived from prostate-related antigens was frequently detectable in healthy donors and prostate cancer patients (Harada *et al*, 2003a; Kobayashi *et al*, 2003; Matsueda *et al*, 2004). In this study, IgG reactive to either the $PTHrP_{102-111}$ peptide or $PTHrP_{110-119}$ peptide was frequently detected in healthy donors as well as in prostate cancer patients. This means that the $PTHrP_{102-111}$ peptide was recognized by both the cellular and humoral immune systems. Although we do not yet have a clear understanding of the roles played by peptide-specific IgG in antitumour immune responses, our clinical trials revealed that a peptide vaccination frequently resulted in the induction of IgG reactive to the CTL epitope peptides which were administered (Noguchi *et al*, 2003; Tanaka *et al*, 2003). In addition, the induction of IgG reactive to the vaccinated peptides was positively correlated with longer survival of advanced lung cancer patients (Mine *et al*, 2003). As regards the use of a peptide vaccination in cases of gastric cancer, prolonged survival has been observed in patients showing not only cellular, but also humoral immune responses to vaccinated peptides (Sato *et al*, 2003). In addition, the induction of IgG reactive to the administered peptides was correlated with a clinical response among patients with recurrent gynecologic cancer (Tsuda *et al*, 2004). Furthermore, we recently analysed 113 vaccinated patients with various types of cancers, and revealed that the augmentation of peptide-specific IgG after peptide vaccination could be a laboratory marker for the prediction of prolonged survival in vaccinated cancer patients compared to the induction of peptide-specific CTLs or the delayed-type hypersensitivity test (Mine *et al*, 2004). Moreover, we recently observed that peptide vaccination with a 9-mer CTL epitope peptide could induce peptide-specific and HLA-DR-restricted CD4$^+$ T cells *in vivo* (Harada *et al*, 2004). As these findings provide circumstantial evidence, further clinical study is needed to

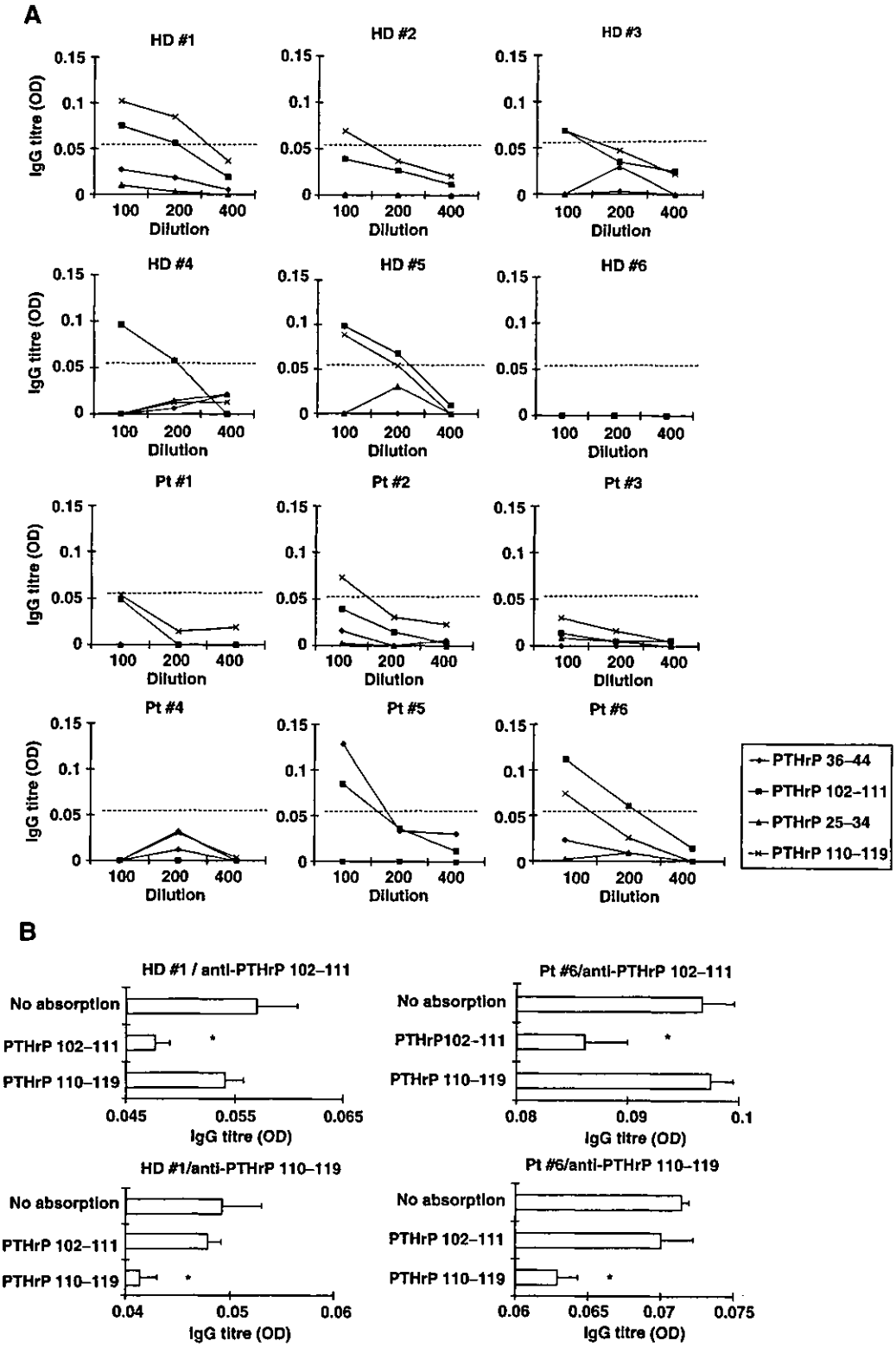

Figure 5 IgG reactive to the PTHrP peptides in plasma from healthy donors and prostate cancer patients. (**A**) Representative results from six healthy donors and six prostate cancer patients are shown. These values are shown as optical density (OD), and the responses to the HIV peptide were subtracted. IgG reactive to a corresponding PTHrP peptide was judged to be positive when the difference of the OD in 1 : 100-diluted plasma exceeded 0.05. The cutoff level (OD: 0.05) was determined based on the levels of anti-HIV peptide IgG in HIV-negative healthy donors. (**B**) To confirm the specificity of IgG to the indicated PTHrP peptides, 100 μl of sample plasma from either HD #1 and Pt #6 was cultured in a plate precoated with either a corresponding PTHrP peptide or an irrelevant PTHrP peptide. Thereafter, the levels of IgG reactive to the $PTHrP_{102-111}$ peptide or the $PTHrP_{110-119}$ peptide in the resultant samples were determined by ELISA.

elucidate the role and meaning of peptide-specific IgG in anti-cancer immunotherapy.

In conclusion, we identified new two PTHrP-derived peptides that are immunogenic in HLA-A24$^+$ prostate cancer patients. The frequencies of the HLA-A24 allele are relatively high throughout the world (Imanishi *et al*, 1992). The information provided here might increase the possibility of treating HLA-A24$^+$ prostate cancer patients with metastases using peptide-based immunotherapy.

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COMPARISON OF QUALITY OF LIFE FOLLOWING LAPAROSCOPIC AND OPEN PROSTATECTOMY FOR PROSTATE CANCER

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ABSTRACT

Purpose: We compare the quality of life after laparoscopic prostatectomy to that after standard radical prostatectomy.

Material and Methods: The quality of life of 52 and 54 patients who underwent laparoscopic and open radical prostatectomy, respectively, was analyzed using the European Organization for the Research and Treatment of Cancer Prostate Cancer quality of life questionnaire for general health related quality of life, International Index of Erectile Function 5 for screening erectile dysfunction and International Continence Society MaleSF questionnaire to evaluate urinary status. These questionnaires were given to patients before and 6 months after surgery.

Results: The general health related quality of life survey revealed no significant differences in health before and after laparoscopic and open prostatectomy. However, sexual quality of life was markedly lower after surgery ($p < 0.01$). In addition, the International Index of Erectile Function score was markedly abrogated by surgery ($p < 0.05$) and quality of life due to urinary incontinence was significantly disturbed by surgery ($p < 0.05$). In contrast, quality of life due to voiding dysfunction was impaired before surgery and significantly improved by surgery ($p < 0.05$). Patients were also asked if they would choose the same treatment if suffering from the same disease, with more patients treated laparoscopically choosing the same treatment than those treated with open surgery ($p < 0.05$).

Conclusions: While general health related quality of life was not impaired, sexual quality of life was diminished by surgery. Patients were generally satisfied with postoperative urinary status. Although patients who underwent laparoscopic prostatectomy expressed a more favorable attitude toward surgery, there was no significant difference in quality of life at 6 months after surgery between the 2 groups.

KEY WORDS: quality of life, prostatic neoplasms, prostatectomy, laparoscopy

Laparoscopic radical prostatectomy has now become a realistic option for localized prostate cancer.[1–3] To date, the clinical outcomes of cancer control, complications, postoperative urinary status and sexual function appear to be almost identical to those of open radical prostatectomy. Generally speaking, laparoscopic surgery allows earlier oral intake and shorter hospital stay after surgery, which results in better recovery. In particular, with laparoscopic prostatectomy a more secure vesicourethral anastomosis allows removal of the urethral catheter 2 to 4 days after surgery. However, the duration of surgery is longer and the learning curve is steep with laparoscopic prostatectomy.

Quality of life is an important factor in cancer treatment. Although quality of life after radical prostatectomy has been well studied,[4–6] to our knowledge it has not been evaluated after laparoscopic prostatectomy. Thus, we compared the quality of life after laparoscopic and open prostatectomy.

PATIENTS AND METHODS

Patient characteristics. Between 1999 and 2002, 111 consecutive patients who underwent prostatectomy (open 57 and laparoscopic 54) due to prostate cancer were enrolled in this study. To assess the quality of life of these patients 3 questionnaires examining general health related, sexual and urinary quality of life were given to patients before and at least 6 months after surgery. The patients were asked to complete the questionnaires at home and to mail them to the Department of Urology, Kobe University Hospital. Of the patients 106 (95.5%) completed the questionnaires (open 54 and laparoscopic 52). Patient characteristics are shown in table 1. No significant differences between the open and laparoscopic prostatectomy groups were found with regard to patient age, followup, distribution of pathological stage and rate of prostate specific antigen failure.

Questionnaire for general health related quality of life. We used the European Organization for the Research and Treatment of Cancer (EORTC) prostate cancer quality of life questionnaire to evaluate general health related quality of life.[7] This questionnaire consists of 33 questions categorized into 6 groups representing functional status, urological symptoms, physical comfortableness, sexual life, psychological distress and social activity. The reliability and validity of this questionnaire for Japanese patients with prostate cancer have been previously confirmed by Isaka et al.[8] The degree of disturbance in each group was scored.

Evaluation of sexual quality of life. Sexual quality of life was evaluated using the International Index of Erectile Function 5 (IIEF-5),[9] which was simplified from the original IIEF. IIEF-5 is suitable for screening erectile dysfunction and is widely accepted by urologists. The full score of IIEF-5 is 25 points, with less than 21 points considered indicative of erectile dysfunction.

Evaluation of urinary quality of life. Although urinary quality of life is addressed in the EORTC questionnaire, a

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TABLE 1. *Patient characteristics*

	Open	Laparoscopic
No. pts.	54	52
Mean age	66.5	68.2
Mean followup (mos.)	8.1	7.9
No. pT:		
pT0	1	1
pT2	31	42
pT3	22	9
No. prostate specific antigen failure (%)	4 (7.4)	3 (5.8)

more detailed questionnaire with regard to urinary quality of life was also used. The International Continence Society questionnaire (ICSmaleSF) is a short form of the ICSmale questionnaire, which was originally developed to evaluate the lower urinary tract symptoms caused by benign prostatic disease.[10] The ICSmaleSF questionnaire has 11 items evaluating the 2 distinct factors of voiding and incontinence symptoms. Although this questionnaire was originally developed to evaluate patients with benign prostatic hyperplasia (BPH), it was deemed appropriate since there are few questionnaires that evaluate not only voiding dysfunction but also male urinary incontinence, which is an important factor in postoperative status.

Statistical methods. The comparison of quality of life between open and laparoscopic surgery was assessed by Mann Whitney U test. The comparison of quality of life before and after surgery was assessed by Wilcoxon signed rank test. The comparison of acceptance for open and laparoscopic prostatectomy was also analyzed by Wilcoxon signed rank test.

RESULTS

Comparison of general health related quality of life between laparoscopic and open surgery. Mean score was calculated by totaling scores of each question in the 6 categories of functional status, urological symptoms, physical comfortableness, sexual life, psychological distress and social activity (fig. 1). Zero indicates no disturbance and a higher score indicates disturbed quality of life. Percent disturbance quality of life was defined as the mean score divided by the full score (most disturbed situation) in each category. When quality of life was compared between laparoscopic and open prostatectomy, no significant differences were observed in any of the items of general health before or after surgery (Mann Whitney U test). There were no significant differences in functional status, urological symptoms, physical comfortableness, psychological distress and social activity before and after surgery. Only sexual quality of life was markedly diminished after surgery independent of surgical method (Wilcoxon signed rank test $p < 0.01$).

Urinary quality of life. Percent disturbance of urinary quality of life was calculated by same manner as health related quality of life. Although no difference was found between laparoscopic and open surgery, quality of life due to difficulty of urination was impaired before surgery and significantly improved by surgery (Wilcoxon signed rank test $p < 0.05$, fig. 2). In contrast, quality of life due to urinary incontinence was significantly disturbed by surgery independent of surgical method (Wilcoxon signed rank test $p < 0.05$, fig. 2). There were no significant differences in urinary frequency before and after surgery for either group. The overall degree of satisfaction with urination was also surveyed (full score 10). Although a slight increase in satisfaction was noted after surgery, it did not reach statistical significance (table 2).

Sexual quality of life. Since only sexual life in general health related quality of life was markedly impaired, the IIEF score (full score 25, complete erectile function) was significantly abrogated by surgery (Wilcoxon signed rank test $p < 0.05$) dependent on surgical approach (laparoscopic versus open, fig. 3). In addition, the degree of satisfaction with sexual function was similarly affected (Wilcoxon signed rank test $p < 0.05$). To survey the acceptance of surgery, patients were asked if they would have the same treatment if they suffered from the same disease (table 3). More patients would have chosen the same treatment in the laparoscopic group than the open surgery group (Wilcoxon signed rank test $p < 0.05$).

DISCUSSION

Although retropubic radical prostatectomy is the gold standard for localized prostate cancer, laparoscopic prostatectomy has now become a realistic alternative.[1-3] Thus, comparing open and laparoscopic surgery from various aspects is important. However, to our knowledge no previous study has compared the quality of life after laparoscopic prostatectomy with that after open prostatectomy.

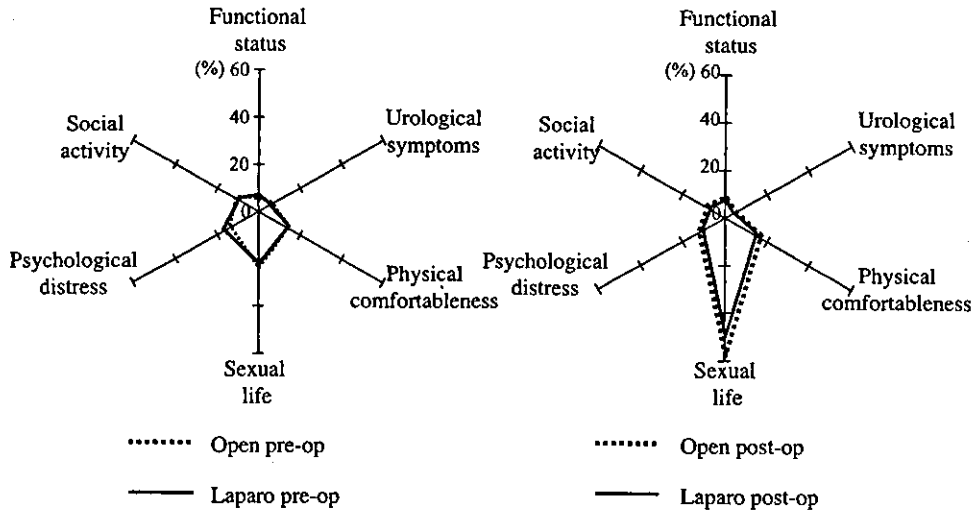

FIG. 1. Percent disturbance quality of life in each category of general health related (EORTC prostate cancer questionnaire). Mean score was calculated by totaling scores of each question in 6 categories of functional status, urological symptoms, physical comfortableness, sexual life, psychological distress and social activity. Zero means no disturbance and higher score means more disturbed quality of life. Percent disturbance quality of life was defined as mean score divided by full score (most disturbed situation) in each category. Higher numbers indicate greater disturbance to quality of life in that category. *Laparo*, laparoscopic.

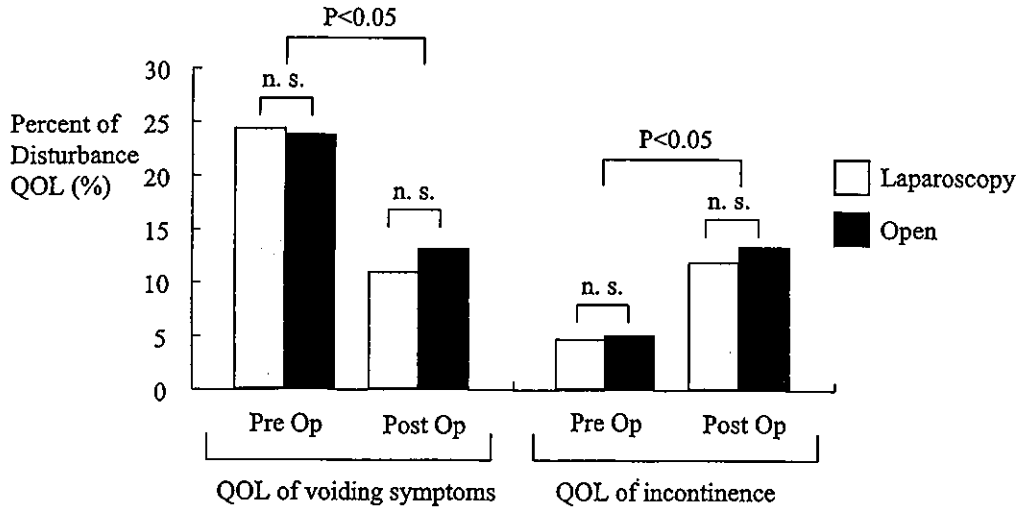

FIG. 2. Percent disturbance of urinary quality of life (*QOL*) (ICSmaleSF). ICSmaleSF is divided into distinct categories of voiding and incontinence symptoms. Higher numbers indicate greater disturbance to quality of life in that category. Calculation of percent disturbance of urinary was followed by that of health related quality of life.

TABLE 2. *Urination quality of life*

	Mean Preop.	Mean Postop.
Daytime urinary frequency: (0—greater than 4 hrs., 1—3 hrs., 2—2 hrs., 3—1 hr.)		
Laparoscopic	1.36	1.53
Open	1.19	1.50
Nighttime urinary frequency (times/night):		
Laparoscopic	1.86	1.83
Open	1.81	2.08
Satisfaction with urination (full score 10):		
Laparoscopic	6.56	6.80
Open	6.93	7.36

The importance of quality of life measurement cannot be overemphasized in current cancer treatment. Urinary diversion is typically assumed to have a great impact on quality of life and has been well studied.[11] Although a significant difference in quality of life was thought to exist between ileal conduit and orthotopic neobladder, patients with an orthotopic neobladder had marginal quality of life advantages over those with an ileal conduit.[11]

Similarly, the quality of life of patients with prostate cancer has been extensively examined. In most studies general health related and disease targeted quality of life was analyzed separately. SF-36,[12] Functional Living Index-Cancer[13] and EORTC QLQ-C30[14] are commonly used in general health related quality of life measurement. In our study we used the EORTC prostate cancer quality of life questionnaire,[7] validity and reliability of which have been proven for Japanese patients with prostate cancer.[8] General health related quality of life was not changed by either laparoscopic or open prostatectomy. This finding is consistent with data from other studies involving patients with prostate cancer treated with radical prostatectomy.[4,15–17]

In contrast, disease targeted quality of life drastically changed following prostatectomy. Erectile dysfunction and urinary incontinence are the 2 major concerns for patients after radical prostatectomy. The EORTC prostate cancer quality of life questionnaire contains items about sexual life and urological symptoms. Sexual life was significantly impaired by surgery with no difference between the laparoscopic and open prostatectomy groups. This result was confirmed by the IIEF data, with the IIEF score significantly decreasing after surgery. The nerve sparing technique is thought to improve sexual activity greatly, as Walsh reported that of the patients treated with radical prostatectomy 86% were potent and 84% considered sexual bothersomeness to be zero or small.[18] However, several reports show poor recovery of sexual life after prostatectomy.[17,19] In a large Stanford University radical prostatectomy series with results comparable to ours only 16% of patients who underwent bilateral nerve preservation reported "good erections."[18] Our data also imply less favorable outcomes with regard to the sexual function and no advantage of laparoscopic surgery in this respect.

Urinary quality of life is the other important concern of prostatectomy. We applied the ICSmaleSF questionnaire, which was originally developed to assess lower urinary tract symptoms due to BPH. Since ICSmaleSF is also suitable for evaluating BPH treatment, voiding and incontinence symptoms can be assessed separately.[10] As expected, quality of life due to incontinence symptoms deteriorated following surgery, whereas that due to voiding symptoms was improved by surgery. In terms of satisfaction with urination, a slight but not significant improvement was reported after surgery. Interestingly, these tendencies were similar in the laparoscopic and open prostatectomy groups. Schwartz and Lepor reported that open retropubic prostatectomy reduced symptom scores and improved quality of life for men with moderate and severe lower urinary tract symptoms.[20] Taken together, patients treated with laparoscopic or open prostatectomy are generally satisfied.

Quality of life is affected after prostatectomy but these changes are almost identical for laparoscopic and open prostatectomy. The only difference between these surgical approaches was that patients treated with laparoscopic prostatectomy displayed a significantly more favorable attitude toward surgery. Although it is difficult to speculate from our present data, a possible reason for this preference is the quick recovery resulting in decreased period of indwelling urethral catheter and hospital stay in laparoscopic prostatectomy.[1]

CONCLUSIONS

General health related quality of life was not changed but sexual quality of life was significantly impaired after radical prostatectomy. The quality of life due to incontinence symptoms worsened but voiding symptoms improved. These characteristics were exactly the same between the laparoscopic and open prostatectomy groups, although the patients

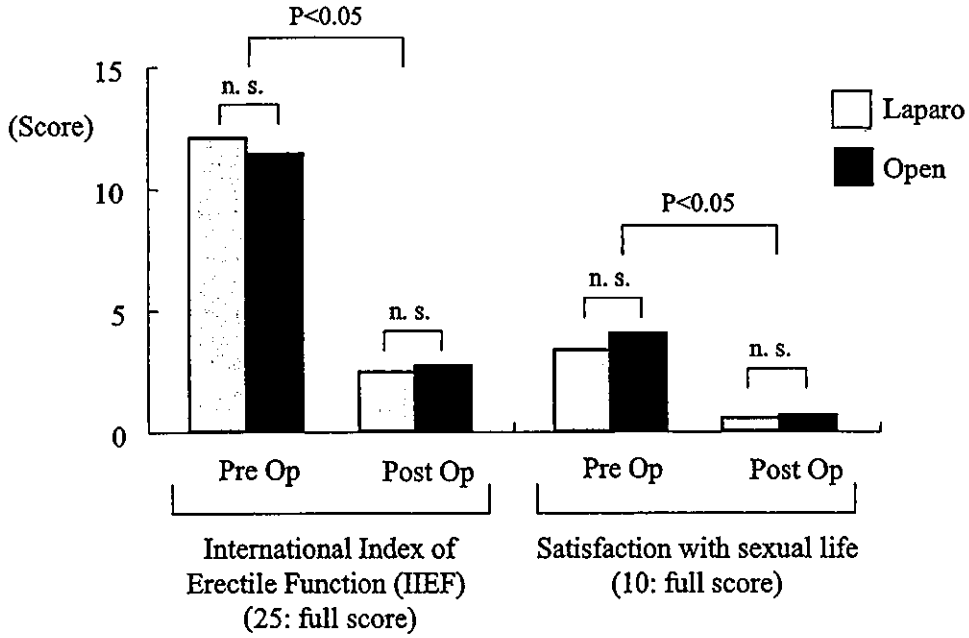

FIG. 3. IIEF and satisfaction scores before and after surgery. Higher score means more active sexual life. *Laparo*, laparoscopic

TABLE 3. *Acceptance of surgical method*

	No. Open	No. Laparoscopic
Exactly the same treatment	12	29
Probably the same treatment	34	22
Not the same treatment	7	0
Unknown	1	1

treated with laparoscopic prostatectomy displayed a more favorable attitude toward surgery.

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Original Article

Prediction of the extent of prostate cancer by the combined use of systematic biopsy and serum level of cathepsin D

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Abstract

Background: The objective of this study was to assess the usefulness of combined systematic prostate biopsy with the serum level of cathepsin D, which has recently been shown to be a useful marker for prostate cancer, to predict the disease extension.

Methods: Seventy-two patients with clinically organ-confined disease who underwent radical prostatectomy were evaluated for serum prostate-specific antigen (PSA) and cathepsin D levels, systematic biopsy, and pathological stage.

Results: The incidence of extraprostatic disease in patients with more than half the biopsy cores positive or ≥ 15 ng/mL cathepsin D was significantly higher than that in patients with less than half the biopsy cores positive or < 15 ng/mL cathepsin D, respectively; whereas cancer in bilateral lobes or ≥ 10 ng/mL PSA could not be used as a predictor of extraprostatic disease. Furthermore, in patients with more than half the biopsy cores positive and ≥ 15 ng/mL cathepsin D or those with more than half the biopsy cores positive and ≥ 10 ng/mL PSA, extraprostatic disease was significantly more common than in those with less than half the biopsy cores positive and < 15 ng/mL cathepsin D or those with less than half the biopsy cores positive and < 10 ng/mL PSA, respectively. Furthermore, the prediction of the incidence of extraprostatic disease using these three variables was significantly more accurate than using two of the variables (percentage positive biopsy cores plus serum cathepsin D or PSA).

Conclusion: Systematic biopsy together with serum cathepsin D and/or PSA was a useful predictor of the extent of prostate cancer. Patients with more than half the biopsy cores positive, ≥ 15 ng/mL cathepsin D and/or ≥ 10 ng/mL PSA could avoid prostatectomy because there is a significantly high probability that they already have extraprostatic disease.

Key words cathepsin D, prostate cancer, PSA, radical prostatectomy, systematic biopsy.

Introduction

Radical prostatectomy is a well accepted treatment for organ-confined prostate cancer, because the survival of patients who have received prostatectomy for pathological organ-confined disease has been reported to be comparable to that of age-matched controls without prostate cancer.[1] Accordingly, the precise identification of patients with organ-confined prostate cancer as candidates for radical prostatectomy would contribute to the improvement of the survival rate of patients undergoing this surgery. Recent advances in imaging modalities, such as magnetic resonance imaging (MRI) and transrectal ultrasound (TRUS) as well as serum prostate-specific antigen (PSA) assay have markedly improved the incidence of prostate cancer detection.[2,3] However, 20–50% of patients operated based on a diagnosis of

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clinically organ-confined disease showed extraprostatic spread at the final pathological examination of prostatectomy specimens.[4] Thus, currently available methods used to preoperatively predict the extent of prostate cancer are still of limited value.

In order to accurately diagnose the extent of prostate cancer, various means have been evaluated, including MRI with endorectal coil, reverse transcriptase–polymerase chain reaction targeting the PSA gene in circulating cells, PSA-related parameters (PSA density, free-to-total PSA ratio, PSA velocity, and PSA-α 1-antichymotrypsin complex), and biopsy features (number of positive biopsy cores and percentage of cancer volume within biopsy cores),[5–7] although none of these is reliable enough to allow clinical decision-making in individual patients in clinical practice. Moreover, the usefulness of the combination of multiple variables, such as serum PSA and systematic biopsy, for predicting the extent of prostate cancer is currently being evaluated and several problems related to such methods remain to be elucidated before conclusions can be drawn.[8–10]

Cancer cells possess a high degree of proteolytic activity, resulting in the ability to degrade and consequently invade surrounding normal tissues. Several proteolytic enzymes have been demonstrated to be involved in the degradation of the extracellular matrix and basement membrane,[11] and among them, cathepsin D is one of the most important enzymes for the invasion and metastatic dissemination of cancer cells, and its production is increased in various kinds of malignant tissues, including prostate cancer.[12–15] Furthermore, we have recently reported that the serum level of cathepsin D could be a useful diagnostic marker for the differentiation of prostate cancer as well as the prediction of the extent of prostate cancer.[16] Therefore, in the present study, we retrospectively analyzed the usefulness of systematic biopsy, serum cathepsin D, or serum PSA alone and several combinations of these factors as predictors of the extent of prostate cancer.

Methods

From January 1997 to December 2001, serum samples from 72 men scheduled for radical prostatectomy and pelvic lymphadenectomy for clinically localized prostate cancer were collected prior to surgery. After the blood was allowed to clot for 60 min, serum was separated by centrifugation at 2000 × g for 15 min, and then stored at −80°C until assessed. Serum PSA values were measured using an immunofluorometric assay system (Tosoh, Tokyo, Japan). The serum concentrations of cathepsin D were determined using a quantitative sandwich EIA kit for human cathepsin D (Oncogene Research Products, Boston, MA, USA) as described previously.[16] In this series, none of the patients received hormonal therapy prior to surgery. These patients were confirmed pathologically to have prostate cancer by a TRUS-guided transrectal systematic biopsy of the prostate, as described previously,[10] and diagnosed clinically with organ-confined disease (i.e. T2N0M0) by digital rectal examination, TRUS, MRI, and computed tomography (CT).

The resected prostatectomy specimens were fixed and the whole-mount step sections cut transversely at 5 mm intervals from the apex of the prostate to the tips of the seminal vesicles. Each section was examined for cancer location, capsular penetration and seminal vesicle invasion. The clinical and pathological stages were determined according to the International Union Against Cancer (TNM) tumor stage classification.[17] The relations among the findings of systematic biopsy, including cancer location and percentage positive biopsy cores (PPBC), serum levels of PSA and cathepsin D and the pathological findings of the prostatectomy specimens were analyzed.

To determine the optimal cut-off values of serum PSA and cathepsin D, receiver operating characteristic (ROC) curve analysis was performed. Differences between two groups were compared using the chi-squared test, and probability values less than 0.05 were considered significant.

Results

In this series, the final pathological examination revealed that organ-confined and extraprostatic diseases were detected in 37 (51%) and 35 (49%) men, respectively. The patients' characteristics of these two groups are shown in Table 1. No significant differences were observed in the clinical stage or Gleason score of biopsy specimens between organ- and non-organ-confined disease.

The relationships between the extent of prostate cancer and the results of systematic biopsy are shown in Table 2. As for the cancer location determined by systematic biopsy, extraprostatic disease was found in 19 (45%) and 16 (53%) among 42 and 30 patients with cancer in one lobe and both lobes, respectively. The incidence of extraprostatic disease in patients with prostate cancer in both lobes was not significantly different from that in patients with prostate cancer in one lobe ($P = 0.50$). In contrast, the incidence of extraprostatic disease among patients with a PPBC of ≥ 50% was

Table 1 Summary of characteristics of patients according to pathological stage

Variable	Organ-confined ($n=37$)	Extraprostatic ($n=35$)
Age (years)†	68.5±4.2	67.9±4.6
Clinical stage		
T1N0M0	8	6
T2aN0M0	17	13
T2bN0M0	12	16
PSA	9.0±3.7	14.5±7.1
Cathepsin D	9.8±6.1	14.4±6.8
Grade (Gleason score)		
Well (2–4)	16	17
Moderate (5–7)	14	9
Poor (8–10)	7	9

†Values are presented as the mean±standard deviation. PSA, prostate-specific antigen.

Table 2 Relationship between systematic biopsy values, serum prostate-specific antigen and cathepsin D levels with pathological stage of prostate cancer in prostatectomy specimens

Variable	Incidence of prostate cancer		*P*-value
	Organ-confined	Extraprostatic	
Unilateral disease†	23	19	0.50
Bilateral disease†	14	16	
PPBC (%)			
< 50	29	13	< 0.005
≥ 50	8	22	
PSA			
< 10	21	13	0.10
≥ 10	16	22	
Cathepsin D			
< 15	23	11	< 0.01
≥ 15	14	24	

†Determined by systematic biopsy. PPBC, percentage of positive biopsy cores; PSA, prostate-specific antigen.

Table 3 Relationship between the combination of percentage of positive biopsy cores, serum prostate-specific antigen and/or cathepsin D levels and the pathological stage of prostate cancer in prostatectomy specimens

Variable‡	Incidence of prostate cancer		*P*-value
	Organ-confined	Extraprostatic	
PPBC < 50 + PSA < 10	17	9	< 0.05
PPBC ≥ 50 + PSA ≥ 10	7	17	
PPBC < 50 + cathepsin D < 15	19	9	< 0.005
PPBC ≥ 50 + cathepsin D ≥ 15	6	18	
PPBC < 50 + PSA < 10 + cathepsin D < 15	14	6	< 0.001
PPBC ≥ 50 + PSA ≥ 10 + cathepsin D ≥ 15	2	14	

PPBC, percentage of positive biopsy cores; PSA, prostate-specific antigen.

significantly higher than that in patients with a PPBC of < 50% ($P<0.0005$).

The cut-off values of serum PSA and cathepsin D for pathologically organ-confined disease were determined using ROC curves as 10 ng/mL and 15 ng/mL, respectively (data not shown). As shown in Table 2, the incidence of extraprostatic disease was significantly greater in patients with serum cathepsin D of ≥ 15 ng/mL than in those with < 15 ng/mL ($P<0.01$); however, no significant difference in the incidence of extraprostatic