Capability of $SART3_{109-118}$ peptide to induce cytotoxic T lymphocytes from prostate cancer patients with HLA class I-A11, -A31 and -A33 alleles

ELNISR RASHED MOHAMED[1], MASAYASU NAITO[2,3], YASUNOBU TERASAKI[2], YAMEI NIU[2], SHOJIRO GOHARA[2], NOBUKAZU KOMATSU[2], SHIGEKI SHICHIJO[2], KYOGO ITOH[2,3] and MASANORI NOGUCHI[1,3]

Departments of [1]Urology, [2]Immunology, and [3]Cancer Vaccine Development Division of the Research Center for Innovative Cancer Therapy, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan

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Abstract. We previously reported the *SART3* gene to be a tumor-rejection antigen gene encoding a peptide at positions 109-118 ($SART3_{109-118}$) with the ability to induce HLA-A24-restricted cytotoxic T lymphocytes. In this study, we investigated both humoral and cellular responses to this peptide in cancer patients with alleles other than HLA-A24 to explore the possibility of using this peptide as a cancer vaccine for these patients. IgG reactive to $SART3_{109-118}$ peptide was identified in sera of the vast majority of non-cancer subjects (n=50) and all cancer patients (n=50) tested without apparent HLA-A association. Levels of anti-$SART3_{109-118}$ peptide antibody in cancer patients were significantly higher than those of non-cancer subjects, but no difference was found between $HLA\text{-}A24^{+}A2^{-}$ and $HLA\text{-}A24^{-}A2^{+}$ cancer patients. This peptide induced cancer cell-reactive cytotoxic T lymphocytes from peripheral blood mononuclear cells of both healthy donors and prostate cancer patients with HLA-A11, HLA-A31 and HLA-A33 alleles, but not with HLA-A2. These results suggest that this peptide can be applicable as a cancer vaccine not only for $HLA\text{-}A24^{+}$, but also for $HLA\text{-}A11^{+}$, $HLA\text{-}A31^{+}$ and $HLA\text{-}A33^{+}$ prostate cancer patients.

Introduction

A large number of tumor antigens and their peptides that are recognized by cytotoxic T lymphocytes (CTL) have been identified from a variety of cancer types (1-9). Subsequently, many clinical trials of peptide-based immunotherapy for cancer patients have been performed in the last decade (10-15), and some of these therapies are promising as a potential new treatment modality (11,12,14,15). Peptide-based immunotherapy, however, is highly restricted by HLA-A alleles, and the currently on-going clinical trials are limited to HLA-A2 or HLA-A24-positive patients, primarily because of the higher worldwide frequency of these alleles (10,12,15-17). Subsequently, cancer patients other than HLA-A2 or HLA-A24 alleles are out of the scope of the current peptide-based immunotherapy, and development of candidate peptides for treatment of these patients is needed.

We identified the *SART3* gene from the cDNA library of a human esophageal cancer cell line (2). It encodes a protein expressed in the nuclei of the majority of the proliferating cells, including both normal and malignant cells (18-25). SART3 antigen is widely expressed in various types of epithelial cancers and hematological malignancies as well (14). It is, however, undetectable in normal tissues, except in the testes and fetal liver (2). This antigen encodes peptides to induce HLA-A24-restricted CTLs at positions 109-118 ($SART3_{109-118}$) and at positions 315-323 (2). We also reported the presence of Immunoglobulin G (IgG) specific to this peptide in sera of healthy donors (HD) and cancer patients (6,26,27). In this study, we investigated both humoral and cellular responses to this peptide in subjects with alleles other than HLA-A24 to explore the possibility of using it as a peptide vaccine for cancer patients with alleles other than HLA-A24.

Materials and methods

Patients. Peripheral blood mononuclear cells (PBMC) were obtained from prostate cancer patients (n=10) and HD (n=6) who had provided written informed consent. The patient alleles included HLA-A24, HLA-A2, HLA-A11, HLA-A31 and HLA-A33. None of the participants was infected with HIV. Twenty milliliters of peripheral blood was obtained, and PBMC were prepared by Ficoll-Conray centrifugation. All of the samples were cryopreserved until they were used for the

Correspondence to: Dr Kyogo Itoh, Department of Immunology, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan
E-mail: kyogo@med.kurume-u.ac.jp

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experiments. HLA-alleles were determined by a commercially available assay (SRL, Tokyo, Japan). The expression of HLA-A24, HLA-A2, HLA-A11, HLA-A31 and HLA-A33 molecules on PBMC was also confirmed by flow cytometry using the following antibodies: anti-HLA-A2 monoclonal antibody (mAb) (BB7.2 supernatant), anti-HLA-A24, -A11, -A31 or -A33 mAb (One Lambda, Inc., Canoga, CA). HLA-A2 subtypes of HLA-A2$^+$ patients were determined by the DNA-based HLA typing performed by Luminex Multi-Analyte Profiling system (xMAP) with WakFlow HLA typing kit (Wakunaga, Hiroshima, Japan) (28). Sera were obtained from 50 non-cancer subjects (12 HD and 38 urolithiasis patients) and 50 cancer patients (10 prostate and 40 pancreatic cancer patients) who had provided written informed consent and were used for measurement of IgG reactive to $SART3_{109\text{-}118}$ peptide.

Cell lines. PC93 [wild-type (WT): HLA-A68$^+$, HLA-A2$^-$, HLA-A24$^-$), PC93-A24 (*HLA-A*2402* gene transfected PC93 cell) and TSU-PR (HLA-A11) are prostate carcinoma cell lines. LC-1 is an HLA-A31/A33 lung cancer cell line. KWS (HLA-A*0206) and COLO201 (HLA-A*0201) are stomach and colon cancer cell lines, respectively. We used T2 cells (HLA-A2, T-B hybridoma) to pulse a peptide. C1R-A24 was kindly provided by Dr Takiguchi (Kumamoto University, Japan). C1R-A11, C1R-A31 and C1R-A33 are lymphoma sublines that have been stably transfected with the *HLA-A*1101*, *HLA-A*3101* and *HLA-A*3303* genes, respectively, by methods reported previously (2). In brief, to generate the C1R-subline expressing each of the HLA-A11, HLA-A31 or HLA-A33 molecules, an *HLA-A*1101*, *HLA-A*3101* or *HLA-A*3303* plasmid cDNA was inserted into the eukaryotic expression vector pCR3.1 (Invitrogen, Carlsbad, CA) by a method reported previously (2). Electroporation was carried out using a Gene Pulser (Bio-Rad, Richmond, CA). The expression of HLA-A24, HLA-A11, HLA-A31 and HLA-A33 molecules on these sublines was confirmed before use by a previously reported method (7). RMA-S cells were from a mouse mutant cell line found to be deficient in antigen processing that showed decreased cell surface expression of major histocompatibility complex (MHC) class I molecules (29). The *HLA-A*0201* and *HLA-A*0206* genes were also individually transfected into RMA-S cells using the FuGENE transfection reagent (Roche, Mannheim, Germany), and stable cloned cells were established from a separate well in the presence of genetecin (0.5 mg/ml). All the cell lines and clones were maintained in RPMI-1640 (Invitrogen) with 10% FCS.

Peptides. The following peptides with >90% purity were purchased from Hokkaido System Science (Sapporo, Japan) or Genenet (Fukuoka Japan) and dissolved in dimethyl sulphoxide (DMSO) at a concentration of 10 mg/ml: $SART3_{109\text{-}118}$, Epstein-Barr virus (EBV)-derived (30-32), Tyrosinase-related protein 2 (TRP-2)-derived (33) and HIV-derived (30) peptides (Table I).

Measurement of IgG reactive to $SART3_{109\text{-}118}$ peptide. The levels of IgG reactive to $SART3_{109\text{-}118}$ peptide in sera of non-cancer subjects and cancer patients were measured by the Luminex method as reported previously (34). In brief, 100 μl of diluted serum was incubated with 5 μl suspension of color-coded beads (Luminex Corp., Austin, TX, USA) coated with $SART3_{109\text{-}118}$ peptide on 96-well filter plates (MABVN1250; Millipore Corp., Bedford, MA, USA) for 2 h at room temperature on a plate shaker. The plates were then washed with phosphate-buffered saline (PBS) with 5% Tween-20 (Tween-PBS) and incubated with 100 μl of biotin-conjugated goat anti-human IgG (BA-3080: Vector Laboratories, Burlingame, CA, USA) for 1 h at room temperature on a plate shaker. After the plates were washed, 100 μl of streptavidin-phycoerythrin (PE) was added to the wells, and the samples were incubated for 30 min at room temperature on a plate shaker. The bound beads were washed four times, and 100 μl of Tween-PBS was added to each well. Fifty microliters of sample was examined using the Luminex system.

Table I. The peptide sequences used in this study.

	Amino acid sequence	Binding HLA	Reference
$SART3_{109\text{-}118}$	VYDYNCHVDL	HLA-A24	2
EBV-A2	GLCTLVAML	HLA-A2	30
EBV-A24	TYGPVFMCL	HLA-A24	31
EBV-A3	AVFDRKSDAK	HLA-A11	32
TRP-2	LLGPGRPYR	HLA-A31/A33	33
HIV-A2	SLYNTVATL	HLA-A2	30

Reverse transcriptase polymerase chain reaction (RT-PCR). Total RNA was isolated from cancer cell lines using RNAzol™ B (Tel-Test Inc., Friendswood, TX). The cDNA was prepared using the SuperScript™ Preamplification System for first strand cDNA Synthesis (Invitrogen), and was amplified using the following primers: 5'-AAGTACGCCA ACATGTGGC-3' (sense) and 5'-CTCTGCTCATTGACAC GAGC-3' (anti-sense) for *SART3*, and 5'-CTTCGCGGGCGA TGC-3' (sense) and 5'-CGTACATGGCTGGGGTGTTG-3' (anti-sense) for *ß-actin*. PCR was performed using TaqDNA polymerase in a DNA thermal cycler (iCycler, Bio-Rad Laboratories, Hercules, CA) for 30 cycles of 95˚C for 1 min, 60˚C for 1 min and 72˚C for 1 min. The PCR products were separated by electrophoresis on 2% agarose gel.

Induction of peptide-specific CTL from PBMC. The assay for the detection of peptide-specific CTL was performed according to a previously reported method with slight modification (35). In brief, PBMC ($1x10^5$ cells per well) were incubated with 10 μM of $SART3_{109\text{-}118}$, a positive control peptide, or a negative control peptide in wells of a U-bottom-type 96-well microculture plate (Nunc, Roskilde, Denmark) in 200 μl of culture medium in quadruplicate assays. The culture medium consisted of 45% RPMI-1640, 45% AIM-V medium (Invitrogen), 10% FCS, 100 U/ml of interleukin-2 (IL-2), and 0.1 μM MEM nonessential amino acid solution (Invitrogen). Every 3 days, half the culture medium was removed and replaced by new medium containing the

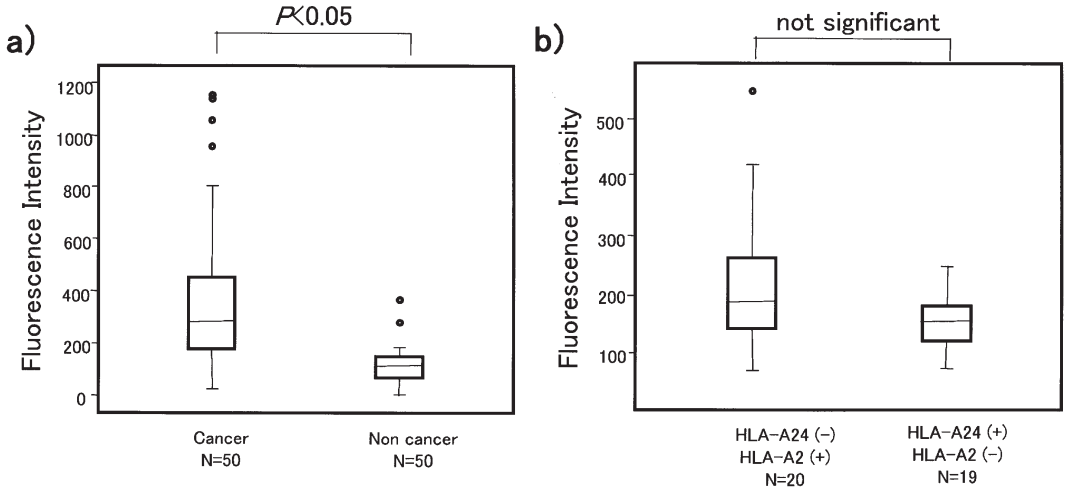

Figure 1. IgG levels of anti-SART3$_{109-118}$ peptide antibody. IgG levels specific to SART3$_{109-118}$ peptide were measured by Luminex. (a) Anti-SART3$_{109-118}$ IgG levels in sera of cancer patients (n=50) and non-cancer subjects (n=50). There is a significant difference (P<0.05) between the two groups. (b) Anti-SART3$_{109-118}$ IgG levels in sera of HLA-A24$^-$A2$^+$ (n=20) and HLA-A24$^+$A2$^-$ (n=19) cancer patients. There was a significant difference between the two groups. *A P-value <0.05 was considered as statistically significant.

corresponding peptide (20 μM) and 100 U/ml IL-2. On day 14 of the culture, the cultured cells were separated into four wells. These cells were harvested and then tested for their ability to produce interferon (IFN)-γ in response to the peptide. Two wells were used for the culture with the corresponding peptide-pulsed T2, C1R-A24, C1R-A11, C1R-A31 or C1R-A33 cells. The other two wells were used for the culture with HIV peptide-pulsed T2, C1R-A24, -A11, -A31 or -A33 cells as a negative control. After 18 h of incubation, the supernatant was collected, and the amount of IFN-γ was measured by ELISA.

Cytotoxicity assay. Peptide-stimulated PBMC were further incubated for 5-7 days followed by the testing of their cytotoxicity against PC93-A24, TSU-PR (HLA-A11), LC-1 (HLA-A31/A33), COLO201 (HLA-A*0201) and KWS (HLA-A*0206) by a standard 6-h ^{51}Cr-release assay as reported previously (1). Phytohaemagglutinin (PHA)-activated T cells were also used as a target cell. Two thousand ^{51}Cr-labelled cells per well were cultured with effector cells in 96-well round bottom plates at the indicated effector/target (E/T) ratio in the triplicate assays.

Stabilization assay. Binding of peptide to HLA molecules was examined using the stabilization assay according to a previously reported method with several modifications (3,36). Briefly, RMA-S-A*0201 and -A*0206 (5x10^5 cells per well in a 24-well plate) were cultured at 26˚C for 20 h in 500 μl RPMI-1640 supplemented with 20% fetal bovine serum followed by incubation with 500 μl Opti-MEM (Invitrogen) containing 0.1-100 μM peptides and human ß$_2$ microglobulin (2 μg/ml) at 26˚C for 2 h and then at 37˚C for 3 h. After washing with PBS, the cells were incubated for 30 min on ice with an appropriate dilution of BB7.2 supernatant (anti-HLA-A2). After washing twice with PBS, the cells were incubated for 30 min on ice with Alexa Fluor 488 goat anti-mouse IgG (Invitrogen).

Statistics. The statistical significance of the data was determined using a two tailed Student's t-test. A P-value <0.05 was considered statistically significant.

Results

Measurement of IgG reactive to SART3$_{109-118}$ peptide. We measured IgG levels reactive to SART3$_{109-118}$ peptide in sera of cancer patients (50 patients, 10 prostate cancer and 40 pancreatic cancer patients) and non-cancer subjects (12 HD, 38 urolithiasis patients). Significant levels of anti-SART3$_{109-118}$ IgG were detectable in the vast majority of samples tested with significantly higher levels (P<0.05) in the cancer patient group (Fig. 1a). Namely, the levels of anti-SART3$_{109-118}$ IgG in cancer patients ranged from 24 to 1155 with a median fluorescence intensity unit (FIU) of 282, while those in the non-cancer subjects ranged from 0 to 366 with a median FIU of 112. These results showed no apparent association between HLA and humoral responses to this peptide. To confirm no HLA association, we investigated anti-SART3$_{109-118}$ IgG levels in both HLA-A24$^+$A2$^-$ cancer patients (20 patients, 11 prostate and 9 pancreatic cancer patients) and HLA-A24$^-$A2$^+$ patients (19 patients, 12 prostate cancer and 7 pancreatic cancer patients). As expected, there was no difference in the IgG levels between the two groups (Fig. 1b).

Induction of peptide-specific CTL. We initially attempted to determine whether or not SART3$_{109-118}$ peptide has the potential to generate peptide-specific CTL from the PBMC of HD (Table II) and prostate cancer patients (Table III) by means of IFN-γ release assay. PBMC with different HLA alleles were stimulated *in vitro* with SART3$_{109-118}$ peptide or relevant control peptides followed by measurement of IFN-γ production in response to the appropriate peptide-pulsed cells. The pulsed target cells were C1R-A24, T2, C1R-A11, C1R-A31 and C1R-A33 for HLA-A24$^+$, HLA-A2$^+$, HLA-A11$^+$, HLA-A31$^+$ and HLA-A33$^+$ PBMC, respectively.

Table II. Induction of peptide-specific CTL from HD.

	HLA allele	Target cells	IFN-γ production[a]
HLA-A24			
HD1	A24/A24	C1R-A24	81
HD2	A24/A2	C1R-A24	94
HLA-A2			
HD3	A2/A11	T2	0
HD4	A2/A2	T2	0
HD2	A24/A2	T2	0
HLA-A11			
HD5	A11/A11	C1R-A11	265
HD3	A2/A11	C1R-A11	130
HLA-A31			
HD6	A31/A33	C1R-A31	148
HLA-A33			
HD6	A31/A33	C1R-A33	29

[a]IFN-γ production (pg/ml) in response to $SART3_{109-118}$. The PBMC from HLA-A24$^+$, HLA-A2$^+$, HLA-A11$^+$, HLA-A31$^+$ and HLA-A33$^+$ healthy donors (HD) were induced *in vitro* with $SART3_{109-118}$ peptide. On day 14, the cultured PBMC were tested for their reactivity to C1R-A24, T2, C1R-A11, C1R-A31 and C1R-A33 cells, respectively, which were pre-pulsed with a corresponding peptide or the HIV peptide. The $SART3_{109-118}$ peptide-specific CTL activity was measured by means of an IFN-γ release assay with ELISA. The representative results are shown. Background IFN-γ production in response to the HIV peptide was subtracted from that in response to $SART3_{109-118}$ peptide. Significant values ($P<0.05$, the two-tailed Student's t-test) are shown in the table.

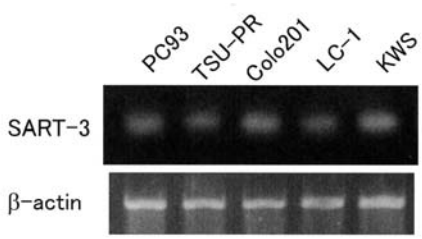

Figure 2. Expression of SART3 mRNA. Messenger RNA level of *SART3* in the tumor cell lines was examined by a RT-PCR-based method. PC93, TSU-PR, COLO201, LC1 and KWS tumor cells expressed *SART3* mRNA. The level of *β-actin* was used as a control for the concentration of mRNA in total RNA.

$SART3_{109-118}$ peptide induced significant levels of IFN-γ in PBMC from all HD with HLA-A24, HLA-A11, HLA-A31 and HLA-A33 alleles, but not from any of the 3 HD with the HLA-A2 allele. Similar results were obtained with the PBMC from prostate cancer patients. PBMC from all patients tested except for Pt. 4 showed CTL activity in response to the relevant control peptides (Table II).

We next addressed whether the PBMC stimulated with $SART3_{109-118}$ peptide were cytotoxic to cancer cells by means of a 6-h ^{51}Cr release assay. Target tumor cells were prostate cancer cell lines PC93 (WT), PC93-A24 (HLA-A24/A68) and TSU-PR (HLA-A11), lung cancer cell line LC-1 (HLA-A31/A33), colon cancer cell line COLO201 (HLA-A*0201), and stomach cancer cell line KWS (HLA-A*0206). Expression of *SART3* mRNA on PC93, TSU-PR, LC-1, COLO201 and

Table III. Induction of peptide-specific CTL from prostate cancer patients.

	HLA allele	Target cells	IFN-γ production (pg/ml) in response to				
			$SART3_{109}$	EBV-A24	EBV-A2	TRP-2	EBV-A3
HLA-A24							
Pt. 1	A24/A24	C1R-A24	375	424			
Pt. 2	A24/A2	C1R-A24	100	182			
HLA-A2							
Pt. 3	A*0201/A*3303	T2	0		46		
Pt. 4	A*0206/A*3101	T2	0		0		
HLA-Al1							
Pt. 5	A11/A11	C1R-A11	265				702
Pt. 6	A2/A11	C1R-A11	376				92
HLA-A31							
Pt. 7	A2/A31	C1R-A31	26			41	
HLA-A33							
Pt. 8	A33/A33	C1R-A33	367			310	

The PBMC from HLA-A24$^+$, HLA-A2$^+$, HLA-Al1$^+$, HLA-A31$^+$ and HLA-A33$^+$ prostate cancer patients were induced *in vitro* with $SART3_{109-118}$ peptide. On day 14, the cultured PBMC were tested for their reactivity to C1R-A24, T2, C1R-A11, C1R-A31 and C1R-A33 cells, respectively, which were pre-pulsed with a corresponding peptide or the HIV peptide. The peptide-specific CTL was measured by means of an IFN-γ release assay with ELISA. The representative results are shown. Background IFN-γ production in response to the HIV peptide was subtracted from that in response to $SART3_{109-118}$ peptide. Significant values ($P<0.05$, the two-tailed Student's t-test) are shown in the table.

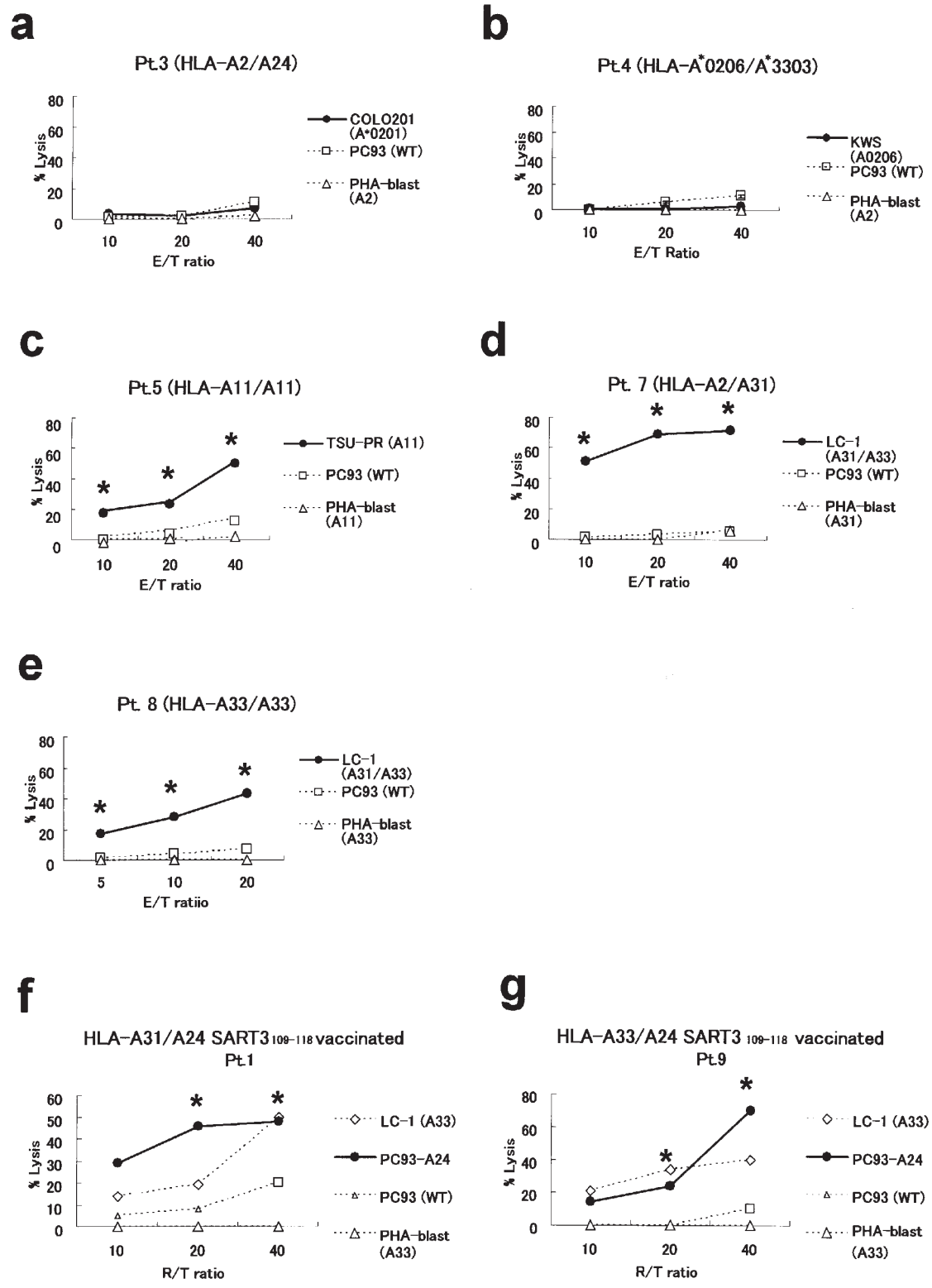

Figure 3. CTL inductions. The PBMC from HLA-A2$^+$, HLA-A11$^+$, HLA-A31$^+$ and HLA-A33$^+$ prostate cancer patients were provided for assay. The PBMC stimulated with $SART3_{109-118}$ peptide were tested for their cytotoxicity against PC93 (WT), COLO201, KWS, TSU-PR and LC-1 tumor cell lines by a 6-h ^{51}Cr-release assay. Phytohaemagglutinin (PHA)-stimulated T-cell blasts included were used as normal cells. *A P-value <0.05 was considered statistically significant compared to PC93 (WT) used as a negative control.

KWS tumor cell lines was confirmed before use as target cells (Fig. 2). As expected, all these cell lines expressed *SART3* mRNA. The PBMC from HLA-A*0201 and HLA-A*0206 prostate cancer patients which were stimulated *in vitro* with $SART3_{109-118}$ peptide did not exhibit cytotoxicity against COLO201 (HLA-A*0201) or KWS (HLA-A*0206) tumor cells, respectively, or against PC93 (WT) cells or PHA-stimulated T-cell blasts (Fig. 3a and b). On the contrary, PBMC from HLA-A11/A11, HLA-A2/A31 and HLA-A33/A33 prostate cancer patients exhibited significant levels of

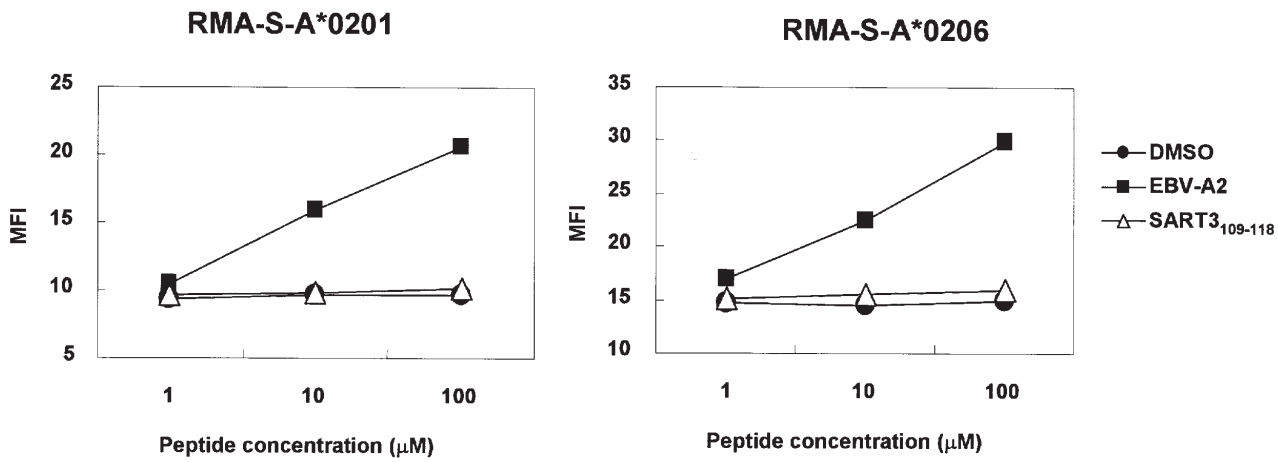

Figure 4. HLA stabilization assay with peptide binding. Expression of HLA-A*0201 and HLA-A*0206 molecules on the surface of RMA-S-A*0201 and RMA-S-A*0206 cells, respectively, was measured by HLA stabilization assay. It was induced by culturing with EBV-A2 peptide (positive control) in a dose-dependent manner, but not with $SART3_{109-118}$ (experimental peptide) or without any peptide (negative control). Representative results from three separate experiments are shown.

cytotoxicity against HLA-matched tumor cells (Fig. 3c-e). None of them, however, showed cytotoxicity against HLA-mismatched tumor cells or normal cells (PHA-blasts). Namely, the PBMC from an HLA-A11 patient which was stimulated *in vitro* with $SART3_{109-118}$ peptide exhibited significant levels of cytotoxicity against TSU-PR (HLA-A11) cells, but not against PC93 (WT) cells or HLA-A11 PHA-stimulated T-cell blasts (Fig. 3c). Similarly, $SART3_{109-118}$ peptide induced LC-1 (HLA-A31/A33)-reactive CTL from the PBMC of HLA-A31$^+$ (Fig. 3d) and HLA-A33$^+$ patients (Fig. 3e), respectively. In contrast, they failed to kill PC93 (WT) cells, HLA-A31 PHA-stimulated T-cell blasts, or HLA-A33 PHA-stimulated T-cell blasts.

We also addressed whether the PBMC of HLA-A24/A31 or HLA-A24/A33 prostate cancer patients who received the $SART3_{109-118}$ peptide vaccination *in vivo* during the clinical trials for HLA-A24$^+$ showed cytotoxicity to HLA-A31 or HLA-A33 tumor cells. As a result, the PBMC of HLA-A31/A24 and HLA-A33/A24 patients who were vaccinated *in vivo* with $SART3_{109-118}$ peptide exhibited significant levels of cytotoxicity against PC93-A24 (HLA-A24) and LC-1 (HLA-A31/A33) cells, but not against PHA-stimulated T-cell blasts or PC93 (WT) cells (Fig. 3f and g). The low level of cytotoxicity against PC93 (WT) cells in an HLA-A31/A24 patient could be due to lymphokine-activated killer cells (Fig. 3f). These results suggest that PBMC stimulated *in vivo* with $SART3_{109-118}$ peptide could exhibit cytotoxicity not only against HLA-A24$^+$ cancer cells but also against HLA-A31$^+$ or HLA-A33$^+$ cancer cells.

HLA stabilization assay. To confirm the inability of $SART3_{109-118}$ peptide to induce CTL activity in HLA-A2$^+$ cancer patients, we tested the binding activity of $SART3_{109-118}$ peptide to HLA-A2 molecules. Namely, we carried out an HLA stabilization assay using RMA-S cells expressing HLA-A*0201 or HLA-A*0206 molecules, the two subtypes most frequently expressed in Japanese (4). Surface expression of HLA-A*0201 or HLA-A*0206 molecules in RMA-S-A*0201 or RMA-S-A*0206 cells, respectively, was induced by culturing with EBV-A2 peptide (positive control) in a dose-dependent manner (Fig. 4). In contrast, there was no surface expression of HLA-A*0201 or HLA-A*0206 molecules when RMA-S cells were cultured with DMSO (negative control) and $SART3_{109-118}$ (experimental peptide) (Fig. 4). This result indicates that $SART3_{109-118}$ peptide has no affinity to bind either HLA-A*0201 or HLA-A*0206 molecules.

Discussion

IgG reactive to $SART3_{109-118}$ peptide was detectable in sera from all the cancer patients and the majority of non-cancer subjects, regardless of different HLA-A alleles, in agreement with our previous reports (6,26,27). There was no significant difference in the IgG levels between HLA-A2$^-$24$^+$ and HLA-A2$^+$A24$^-$ prostate cancer patients. This observation led us to an assumption that humoral immunity to $SART3_{109-118}$ was not associated with HLA-A alleles. Subsequently, we addressed its ability to induce CTL from different HLA-A alleles other than the HLA-A24 allele. We found that $SART3_{109-118}$ has the potential to induce cancer-specific CTL from PBMC of HLA-A11$^+$, HLA-A31$^+$, HLA-A33$^+$ HD and also of prostate cancer patients, whereas it does not have the potential to induce cancer-specific CTL from HLA-A2$^+$ subjects. The results by means of cytotoxicity assay also revealed no CTL induction by this peptide for HLA-A2 patients. In addition, $SART3_{109-118}$ has no affinity to bind either HLA-A*0201 or -A*0206, the two most frequently expressed alleles in Japanese (4). Frequencies of HLA-A*0201 and HLA-A*0206 alleles among HLA-A2 subtypes are 47.1 and 36.5% (by the Standardization Committee of the JSHI), 52.4 and 15.9% in Chinese (37), 96.1% and <1% in Oxford Caucasians (38), 62.2% and <1% in African Blacks (38). Therefore, this $SART3_{109-118}$ peptide could not be available for the majority of HLA-A2$^+$ cancer patients worldwide. Our results indicate that this peptide has no activity to induce CTL from at least HLA-A*0201$^+$ or HLA-A*0206$^+$ PBMC.

The reasons for the discrepant results with regard to the HLA-A2 allele (positive IgG responses vs negative CTL induction to $SART3_{109-118}$ peptide) are unclear, and mechanisms of T cell recognition on this peptide remain to be solved. One assumption could be the involvement of T helper cell-mediated recognition of this peptide, but the molecules on T helper cells responsible for recognition are also unclear.

The optimal COOH-terminal amino acid of HLA-A11-binding peptides is lysine (39), whereas that of HLA-A31 and HLA-A33-binding peptides is arginine (39,40). $SART3_{109-118}$ peptide (VYDYNCHVDL) carries leucine at the COOH-terminus. Our present results indicate that $SART3_{109-118}$ peptide carrying a leucine at the COOH-terminus also has a potential to generate HLA-A11, HLA-A31 and HLA-A33 CTL. We previously reported that peptides carrying arginine at the COOH-terminus have the potential to generate HLA-A11-restricted CTLs (7,8), and these findings suggest that peptides carrying arginine at the COOH-terminus might fit the binding motifs for HLA-A11 (9). Our study suggests that a leucine at the COOH-terminus might also fit the binding motifs for HLA-A11, HLA-A31 and HLA-A33, a family of HLA-A3 supertype (HLA-A3, HLA-A11, HLA-A31, HLA-A33 and HLA-A68). Further investigation of the binding motif of CTL epitopes for the HLA-A3 supertype is needed.

In conclusion, we found that $SART3_{109-118}$ peptide induces not only HLA-A24-restricted CTL, but also HLA-A11, HLA-A31 and HLA-A33-restricted CTL. SART3 antigen is widely expressed in various types of epithelial cancers and hematological malignancies as well (7). Thus, $SART3_{109-118}$ peptide could be applicable as a cancer vaccine not only to $HLA\text{-}A24^+$ but also to $HLA\text{-}A11^+$, $HLA\text{-}A31^+$ and $HLA\text{-}A33^+$ cancer patients with various histological types, including prostate cancer. The total sum of HLA-A24, HLA-A11, HLA-A31 and HLA-A33 populations are 85% in Japanese, 34% in Caucasians, 62% in Indians and 28% in Blacks (17). Therefore, the SART3 peptide could be applicable for a large number of cancer patients worldwide.

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