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Study of HLA class I restriction and the directed antigens of cytotoxic T lymphocytes at the tumor sites of ovarian cancer

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Abstract The molecular basis of T-cell-mediated recognition of ovarian cancer cells remains to be fully addressed. In this study we investigated HLA class I restriction and directed antigens of cytotoxic T lymphocytes (CTL) at the sites of ovarian cancer. Three HLA-class-I-restricted CTL lines were established from the tumor sites of ovarian cancer by culturing tumor-infiltrating lymphocytes or tumor-associated ascitic lymphocytes with interleukin-2: (1) HLA-A2402-restricted and ovarian-adenocarcinoma-specific CTL, (2) HLA-A2-restricted CTL recognizing histologically different cancers, and (3) HLA-B52-restricted and ovarian-cancer-specific CTL. HLA-A0201, HLA-A0206 and HLA-A0207 tumor cells were lysed by the HLA-A2-restricted CTL. HLA-B52 restriction of the third CTL line was confirmed by the transfection of *HLA-B5201* cDNA into the tumor cells. The HLA-A2-restricted CTL recognized the SART-1, but not the MAGE-1 or MAGE-3 antigen. These results may facilitate a better understanding of the molecular basis of tumor-specific immunity at the tumor site of ovarian cancer.

Key words Cytotoxic T lymphocytes · Ovarian cancer · Tumor antigen · HLA restriction

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Introduction

Although the majority of ovarian cancers are relatively sensitive to initial chemotherapy, recurrence is frequent and the recurrent tumors are generally resistant to the available therapeutic approaches [12]. It is hoped, therefore, that the development of immunotherapy will be able to provide alternative treatment modalities. Although HLA-class-I-restricted cytoxic T lymphocytes (CTL) at the tumor sites of ovarian cancer have been reported in past studies [4, 5, 8, 15–17, 24], the molecular basis of T-cell-mediated recognition remains to be fully investigated, because these studies used serological determination of HLA phenotypes of the target and effector cells in most cases. The serotype contains various subtypes and each subtype presents different antigenic peptides [1, 21]. Further, the directed tumor antigens recognized by the CTL from ovarian cancer patients were not clearly determined, although Her-2/neu-derived peptides were suggested to be the directed antigen recognized by the HLA-A2-restricted CTL [5, 17, 24]. Therefore, we studied the HLA class I restriction and directed antigens of the CTL at the sites of ovarian cancer to improve our understanding of the molecular basis of host defense against autologous cancer cells.

Materials and methods

Establishment of CTL lines

Tumor specimens from patients with ovarian cancer were obtained from the Gynecology Clinic of Kurume University Hospital. Tissues of ovarian cancers were obtained from patients undergoing ovarian surgery. Tumor specimens were finely minced, washed with minimal essential medium (MEM), and suspended in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), antibiotics and 100 units/ml recombinant interleukin-2 (IL-2; a generous gift from Shionogi Pharmaceutical Co., Osaka, Japan). Tumor-associated ascitic cells were also obtained from some patients and suspended in the same medium after washing with MEM. Minced

tumor cell suspensions or ascitic cells were cultured at 37 °C in a 5% CO_2 incubator for up to 90 days. In the case of OC72, $CD8^+$ cells were enriched from the ascitic cell culture on day 50 with anti-CD8-mAb-coupled DynaBeads (Dynal, Oslo, Norway) and further cultured with IL-2.

Cell lines and genotyping of HLA

Ovarian cancer cell lines, KOC-2S, KOC-3S, KOC-5C, OC72 tumor, and OC90 tumor were established in our department. The other ovarian cancer cell lines were obtained as follows: RTSG, RMG-I, RMG-II, RKN, RMUG-L, and RMUG-S from the Institute for Fermentation, Osaka, Japan; MCAS and TYK-nu from the Health Science Research Resources Bank, Osaka, Japan; HTOA and OMC-3 from Riken Cell Bank, Tsukuba Science City, Japan. The other tumor cell lines were as previously described [14]. The genotypes of both the HLA-A and HLA-B alleles were determined by the polymerase chain reaction (PCR)/sequence-specific oligonucleotide probe hybridization method [13].

CTL assays

A standard 6-h ^{51}Cr-release assay was used to measure the cytotoxicity of T cell lines as previously reported [14]. If the mean value of the percentage specific lysis in triplicate assays was more than 10%, the level of cytotoxicity was considered to be significant. Inhibition experiments with anti-CD3 (OKT3, ATCC, Rockville, Md.), anti-CD4 (Nu-Th/i), anti-CD8 (Nu-Ts/c), anti-CD11a (YH384), anti-(class I HLA) (W6/32, ATCC), and anti-HLA-DR (H-DR1) mAb have been described elsewhere [14]. All of these ascites mAb were used at 1:100 dilution for the blocking study. The mAb Nu-Th/i, Nu-Ts/c and YH384 were previously established and characterized in our laboratory [18, 23].

Quantification of interferon γ (IFNγ)

Reactivities of CTL lines to appropriate target cells, including transfectants, were measured by the IFNγ production assay as reported [20]. CTL (4×10^4 cells) were cultured with 1×10^4 target cells in the wells of a 96-well flat-bottom culture plate in the presence of IL-2 (100 units/ml) at 37 °C for 18 h. The amounts of IFNγ in the culture supernatants were measured by enzyme-linked immunosorbent assay (ELISA). The limit of sensitivity of the ELISA system was 5 pg/ml.

Flow-cytometric analysis of CTL lines

Cells were stained with fluorescein-isothiocyanate-conjugated anti-CD3 (Nu-T3, established in our laboratory), -CD4 (Nu-Th/i), -CD8 (Nu-Ts/C), -TcRα/β (WT31, Becton-Dickinson, Mountainview, Calif.) mAb and further analyzed on a FACScan flow cytometer (Becton-Dickinson).

Cloning of HLA cDNA

HLA cDNA was amplified from peripheral blood mononuclear cells of HLA-identified donors by the reverse transcription/polymerase chain reaction (RT-PCR) with a sense primer specific for common HLA-ABC (5′-GAATCTCCCCAGACGCCGA-3′) and an anti-sense primer specific for HLA-A (5′-CACAGGTCAGCGTGGGAAG-3′) or HLA-B (5′-AAACACAGGTCAGCATGGGAAC-3′). PCR was performed as follows: 35 cycles of denaturing at 94 °C for 1 min, annealing at 63 °C for 2 min and extension at 72 °C for 3 min. PCR products were subsequently cloned into a mammalian expression vector, pCR3.1 (InVitrogen, San Diego, Calif.). The HLA alleles and the direction of the cloned cDNA were confirmed by the nucleotide sequencing.

Transfection experiments

Ovarian cancer cell lines, TYK-nu (*HLA-B35*/*-B15*) and TOC-2 (*HLA-B51*/*-B54*) cells, were plated at 2×10^4 cells/well in a 96-well plate 1 day before the transfection. A 200-ng sample of the *HLA-B5101* or *HLA-B5201* cDNA was mixed with 1 μl Lipofectoamine (Gibco-BRL, Gaithersburg, Md.) in 70 μl Opti-MEM (Gibco-BRL) for 30 min at room temperature, and 30 μl mixture was added to the cells. After 6 h incubation at 37 °C, 200 μl 10% FCS/RPMI-1640 medium was added to the culture. The transfectants were used as target cells after 2 days of culture. Human fibroblast line VA13 cells were plated at 1×10^4 cells/well in a 96-well-plate 1 day before the transfection. A 200-ng sample of the HLA cDNA (*HLA-A0206*, *-A2402*, *-B5201*, *-B4601*) and 200 ng tumor antigen gene (*SART-1*, *MAGE-1*, *MAGE-3*) were mixed with 1 μl Lipofectoamine (Gibco-BRL, Gaithersburg, Md.) in 70 μl Opti-MEM (Gibco-BRL), and the mixtures were used for the transfection. Construction of the tumor antigen genes, *SART-1*, *MAGE-1* and *MAGE-3* with mammalian expression vectors (*pSVSPORT* for *SART-1*, *pGEM3SR*αneo for the *MAGE* genes) was previously described [6, 20].

Results

Establishment of CTL lines

T cells from 7 out of 20 ovarian tumors adapted to the culture and proliferated well under the conditions employed. The cytotoxicity of the established T cell lines was measured against a panel of HLA-class-I-A- and -B-genotyped tumor cell lines. Out of the 7 T cell lines, 3 expressed no cytotoxicity against any of these tumor cells, whereas 1 T cell line expressed cytotoxicity against all of the target cell lines examined (data not shown). The remaining 3 T cell lines expressed selected cytotoxicity. Representative results of these 3 cases are shown in Table 1. The unseparated OC72-CTL line (*HLA-A2402*/*A3101*, *B5901*/*B3501*) from endometrioid adenocarcinoma had very low cytotoxicity, since the $CD4^+$ T cells had proliferated predominantly from the ascitic cells (data not shown). Therefore, the $CD8^+$ cells were enriched at day 50 of the culture and further expanded with IL-2. This $CD8^+$-enriched OC72 CTL line (approximately 50% of the cells were $CD3^+CD4^-CD8^+$ at the time of the experiment) showed significant levels of cytotoxicity (more than 10% specific lysis) against the autologous tumor cells and the KOC-3S ovarian cancer (serous adenocarcinoma) cells (*HLA-A2402*/*A3101*, *B40*/*B50*), but did not lyse any other tumor cells tested including 11 *HLA-A2402*$^+$ cancer cells (2 ovarian, 4 esophageal, 2 lung, 1 stomach, 1 cervical, and 1 bladder cancer). The results suggest that this CTL line recognized an antigen(s) of the autologous tumor cells in an HLA-A2402-restricted and ovarian-adenocarcinoma-specific manner. The directed antigen was not widely shared among either the ovarian cancer cells or the other epithelial cancer cells.

The unseparated OC88-CTL line (*HLA-A0206*/*A2402*, *B35*/*B61*) from serous adenocarcinoma showed significant levels of lysis against of OC90 tumor (clear-cell adenocarcinoma) and TYK-nu (undifferentiated

Table 1 Establishment of cytotoxic T lymphocyte (*CTL*) lines from tumor-infiltrated or tumor-associated lymphocytes of patients with ovarian cancers. Genotypes of HLA-A and HLA-B are indicated as 2-, 4- or 5-digit numbers. Cytotoxicity is shown at an E/T ratio of 10. Values represent the mean percentage specific lysis of triplicate assays. *a.c.* adenocarcinoma, *c.* carcinoma

Target			Lysis (%)		
Cell name	HLA-A	HLA-B	OC72-CTL endometrioid a.c. (*A2402*/*A3101*) (*B5901*/*B3501*)	OC88-CTL serous a.c. (*A0206*/*A2402*) (*B35*/*B61*)	OC90-CTL clear-cell c. (*A0201*/*A3101*) (*B5101*/*B5201*)
Ovarian cancers					
OC72-tumor (endometrioid a.c.)	*2402*/*3101*	*5901*/*3501*	15	5	3
OC90-tumor (clear cell c.)	*0201*/*3101*	*5101*/*5201*	8	12	40
KOC-2S (serous a.c.)	*2402*/*2402*	*51*/*52*		7	37
KOC-3S (serous a.c.)	*2402*/*2601*	*40*/*52*	17	8	13
KOC-5C (clear cell c.)	*2402*/*2603*	*52*/*15*		0	4
MCAS (mucinous a.c.)	*0206*/*2402*	*46*/*40*	1	0	3
RMUG-L (mucinous a.c.)	*2402*/*2601*	*40*/*52*		4	15
RMUG-S (mucinous a.c.)	*0207*/*3302*	*46*/*15*	3	7	1
TOC-2 (undifferentiated c.)	*0207*/*2402*	*51*/*54*	2	0	5
TYK-nu (undifferentiated c.)	*0201*/*2603*	*35*/*15*	5	13	5
OMC-3 (mucinous a.c.)	*2402*/*2402*	*4002*/*5201*		4	19
RTSG (poorly differentiated a.c.)	*2402*/*2601*	*40*/*52*		6	13
Other adenocarcinomas					
SW620 (colon)	*0201*/*2402*	*0702*/*1518*		26	0
HCT116 (colon)	*0101*/*0201*	*4501*/*4501*	0	10	5
KM12LM (stomach)	*02*/*2402*	*0702*/*0702*		0	1
MKN28 (stomach)	*3101*/*3101*	*5101*/*5101*	0	2	0
MKN45 (stomach)	*2402*/*2402*		0	0	
1-87 (lung)	*0207*/*1101*	*4601*/*5401*	4	21	3
11-18 (lung)	*0201*/*2402*	*5201*/*5401*		5	3
PC-9 (lung)	*0206*/*2402*	*0702*/*5502*	0	0	0
LK87 (lung)	*0207*/*1101*		5	31	
Squamous cell carcinomas					
HSC-3 (oral)	*0201*/*2402*	*4801*/*5201*			6
Sq-1 (lung)	*1101*/*2402*	*1501*/*5201*		4	0
QG56 (lung)	*2601*/*2601*	*4601*/*4601*	3	0	1
RERF-LC-A1 (lung)	*2402*/*2402*	*5201*/*5201*	7	0	4
TE-10 (esophagus)	*02*/*2402*	*55*/*51*	0		0
TE-11 (esophagus)	*2402*/*2601*	*56*/*51*	0	0	5
KE3 (esophagus)	*2402*/*0206*	*15*/*55*	0	21	0
KE4 (esophagus)	*2402*/*2601*	*5101*/*5501*	0	0	4
OMC-4 (cervix)	*0207*/*2402*	*4001*/*4601*		0	3
Others					
HT-1376 (bladder)	*2402*/*2402*	*1501*/*1501*	0	5	4
TCCSUP (bladder)	*0201*/*0301*	*0702*/*4402*	2		3

carcinoma) ovarian cancer cells, 2 colon and 2 lung adenocarcinoma cell lines, and 1 esophageal squamous cell carcinoma cell line (Table 1). The dominant population (83%) of the OC88 CTL line was $CD3^+CD4^-CD8^+$ and the remaining cells possessed the $CD3^+CD4^+CD8^-$ phenotype. The shared HLA class I allele of these 7 carcinomas is HLA-A2 with different subtypes (*A0201*, *A0206*, *A0207*). Kinetic study showed that the OC88 CTL produced significant levels of IFNγ by recognition of the *HLA-A0201*$^+$ SW620 colon cancer with the maximal level on day 14 (Fig. 1A). However, it failed to produce IFNγ by recognition of the *HLA-A2402*$^+$ HT1376 bladder cancer cells throughout the culture period. An assay for IFNγ production to measure the activity of established CTL lines is essentially correlated with the cytotoxicity assay in most cases. In this study, similar results were also obtained by the ^{51}Cr-release assay (data not shown). These results suggest that the OC88 CTL line possessed HLA-A2-restricted cytotoxicity against histologically different cancer cells and the directed antigen(s) were shared.

The unseparated OC90 CTL line (*HLA-A0201*/-*A3101*, -*B5101*/-*B5201*) from clear-cell adenocarcinoma lysed the 6 different ovarian cancer cell types (2 serous, 2 mucinous, 1 clear-cell, and 1 poorly differentiated adenocarcinoma), but not any of the other tumor cells tested. The shared HLA class I allele of these 6 types of tumor cell was *HLA-B52*. The kinetics study showed that a maximum level (40% lysis) of lysis was observed at day 54 of the culture with gradual decrease of the cytotoxicity (Fig. 1B). The OC90 CTL did not lyse the TOC-2 serous adenocarcinoma cells (*HLA-A0207*/-*A2402*, -*B51*/-*B54*) during the culture period. The levels of the OC90-CTL-mediated cytotoxicity were dependent on the increase in number of the effector cells (Fig. 2A). The surface phenotype of the OC90-CTL line was

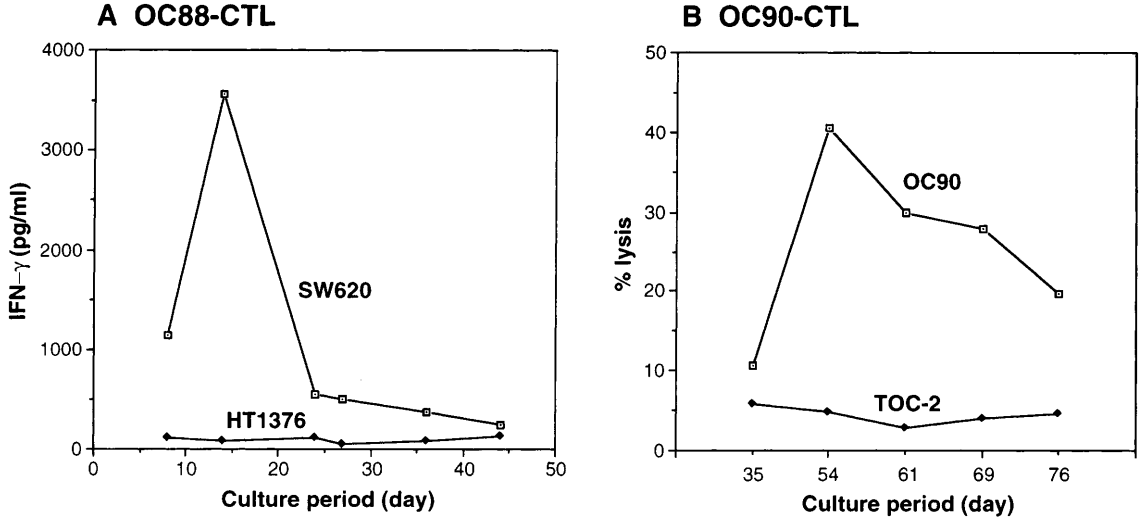


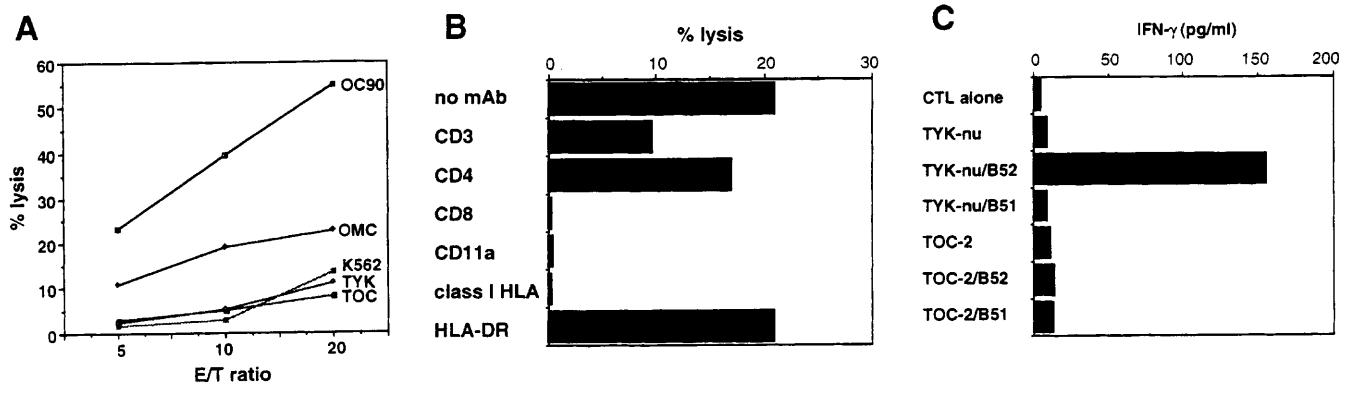
Fig. 1A,B Kinetic study of the cytotoxic T lymphocyte (CTL) lines. **A** Reactivities of the OC88 CTL (*HLA-A0206/-A2402*) against the SW620 (*HLA-A0201/-A2402*) colon adenocarcinoma and HT1376 (*HLA-A2402/-A2402*) bladder cancer cells (E/T ratio = 4) after various culture periods. An assay of IFNγ production was employed in this study. **B** Cytotoxicities of the OC90 CTL (*HLA-B5101/-B5201*) to the autologous OC90 tumor and TOC-2 (*HLA-B51/-B54*) ovarian cancer cells (E/T ratio = 10) after various culture periods

$CD3^+TcR\text{-}\alpha/\beta^+CD4^-CD8^+$ (data not shown). Inhibition experiments showed that the cytotoxicity against autologous tumor cells was completely inhibited by anti-CD8, anti-CD11a (LFA-1), or anti-class I HLA (W6/32) mAb, and partially blocked by anti-CD3 mAb (Fig. 2B). In contrast, it was not affected by anti-HLA-DR or anti-CD4 mAb. Furthermore, TYK-nu cells that were transfected with *HLA-B5201*, but not the control *HLA-B5101* cDNA stimulated the OC90-CTL to produce significant levels of IFNγ (Fig. 2C). Because of a low efficiency of expression of the transfected gene in the transient transfectants, the levels of IFNγ production in the transfectants were lower than that of the autologous tumor cells. The CTL failed to produce IFNγ by recognition of the undifferentiated TOC-2 adenocarcinoma cells, even when transfected with *HLA-B5201* cDNA. All these results suggest that the OC90-CTL line possessed HLA-B52-restricted and ovarian-cancer-specific cytotoxicity. The antigen would be widely shared among ovarian cancer cells, although it might not be expressed on the TOC-2 cells.

Directed antigens

COS-7 cells were co-transfected with either the *SART-1*, *MAGE-1* or *MAGE-3* gene, together with an appropriate HLA gene, and were used as target cells to identify the antigen recognized by the OC88 CTL and OC90-CTL lines. Cells of the CTL lines were stimulated with

Fig. 2A–C Cytotoxicity of the OC90 CTL. **A** The OC90 CTL (*HLA-B5101/-B5201*) at day 54 of the culture were tested for their cytotoxicity against the autologous OC90 tumor, OMC-3 (*HLA-B4002/-B5201*), TOC-2 (*HLA-B51/-B54*), TYK-nu (*HLA-B15/-B35*), and natural-killer(NK)-sensitive K562 cells at different E/T ratios. Values represent the mean percentage lysis of the triplicate assays. **B** Effects of various mAb on the cytotoxicity of OC90 CTL. The indicated mAb were added to the culture of the cytotoxicity assay. Values represent the mean percentage lysis of the triplicate assays using autologous OC90 tumor cells at an E/T ratio of 10. **C** Reactivity of the OC90 CTL against *HLA-B5201* or *-B5101* transfectants. Ovarian cancer cell lines TYK-nu (*HLA-B35/-B15*) and TOC-2 (*HLA-B51/-B54*) cells were transiently transfected with *HLA-B5201* or control *HLA-B5101* cDNA. The OC90 CTL on day 57 of the culture were co-cultured with autologous OC90 tumor, KOC-2S (*HLA-B51/-B52*), the transfectants, or their parental cells at an E/T ratio of 4



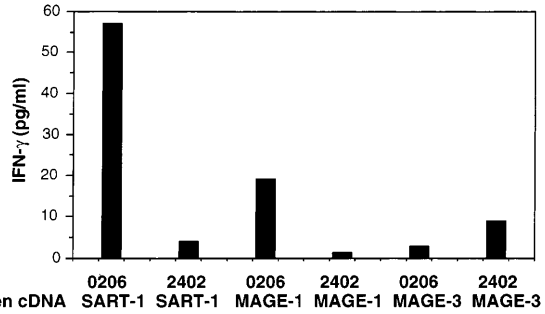


Fig. 3 Directed antigens of the OC88 CTL. COS-7 cells were co-transfected with one of the tumor antigen genes (*SART-1*, *MAGE-1*, *MAGE-3*) and *HLA-A0206* or control *HLA-A2402* cDNA. Reactivities of the OC88 CTL against the transfectants are shown as IFNγ production. Values represent the means of triplicate assays in two different experiments

these COS-7 transfectants and the IFNγ production was measured. The OC88 CTL produced significant levels of IFNγ by recognition of the *SART-1*/*HLA-A0206* transfectants but not with the control *SART-1*/*HLA-A2402* transfectants (Fig. 3). None of the *MAGE-1* or *MAGE-3* transfectants with the *HLA-A0206* gene induced a significant level of IFNγ production when compared with those with the control *HLA-A2402* gene. These results suggested that a some of the OC88 CTL recognized the SART-1 antigen in an HLA-A0206-restricted manner.

OC90 CTL were also stimulated with the transfectants of the tumor antigen cDNA and *HLA-B5201* or the control *HLA-B4601* gene, and subsequent IFNγ production was measured. However, significant levels of IFNγ production were not observed in any combinations tested (data not shown).

Discussion

There are several reports of cancer-specific CTL from tumor-infiltrating lymphocytes or tumor-associated ascitic lymphocytes of patients with ovarian cancer [4, 9, 15]. HLA-A2-restricted cancer-specific CTL recognizing the HER2/neu antigen were reported on the basis of results of both serological typing of HLA and antibody blocking tests [5, 17, 24]. Recent studies indicated that the serologically identified HLA-A2 allele consisted of more than 15 alleles (genotypes) at the cDNA level and that the binding peptide motifs of each allele are varied [1, 21]. We applied genotyping in this study to determine the HLA-A and -B alleles of both effector CTL and target cell lines. Cytotoxicity tests using relatively large panels of target tumor cell lines suggested that the OC72 and OC88 CTL were respectively HLA-A2402 and -A2 restricted, whereas the OC90 CTL was HLA-B52-restricted. *HLA-A0201*$^+$ and -*A0207*$^+$ adenocarcinomas, and *HLA-A0206*$^+$ squamous cell carcinoma were recognized by the OC88 CTL, which had an *HLA-A0206* allele. Although the autologous tumor cells were not available for testing as target cells, COS-7 cells transfected with the *HLA-A0206* and *SART-1* genes were recognized by the OC88 CTL. These results suggest that peptide antigen(s) recognized by the OC88 CTL had a sequence capable of binding to all three of the HLA-A2 subtypes. This sequence might have leucine or valine at position 2 and leucine at position 9 [1, 21].

The HLA-B52 restriction of the OC90 CTL was confirmed by the transfection experiments. Such HLA-B-locus-restricted tumor-specific CTL have rarely been described [3, 11] and, according to a literature search, have not been previously reported in ovarian cancer. These results suggest that investigation of HLA-B locus-restriction, in addition to the study of HLA-A locus restriction, is needed for the study of HLA class I restriction of cancer-specific CTL at the site of ovarian cancer.

Except for HER2/neu, the directed antigens of the HLA-class-I-restricted CTL from ovarian cancer have not yet been reported. Results of this study suggested that the OC90 CTL recognized an antigen(s) on an HLA-B52 groove that was shared by ovarian cancers, whereas the OC72 CTL recognized an antigen(s) on an HLA-A2402 groove that was relatively unique to the autologous tumor cells. On the other hand, the directed antigen(s) of the OC88 CTL might be widely expressed on adenocarcinoma cells originating in various tissues. HER2/neu-derived peptides were possible candidates for the directed antigen of the OC88 and OC90 CTL. However, it is unlikely since the OC90 tumor cells, target cells susceptible to both OC88 and OC90 CTL, did not express the HER2/neu, as determined by a flow-cytometric analysis (data not shown).

We previously demonstrated that MAGE-1 and -3 antigens were expressed on 20.6% and 19.0% of ovarian cancers respectively [22] and widely expressed on the other types of cancers, as well [2, 10, 19]. Therefore, MAGE-1 and -3 antigens are possible candidates for the antigen recognized by the OC88 CTL. Further the *SART-1* gene was recently identified as a gene encoding antigenic peptides of human squamous cell carcinoma recognized by CTL [20]. The 43-kDa SART-1 protein was detected in most squamous cell carcinomas and in half of the tested adenocarcinomas but not in normal tissues. Thus the SART-1 antigen is also a possible candidate for the antigen recognized by the OC88 CTL. The transfection experiments demonstrated that the *SART-1* gene product, but not the others, was one of the directed antigens recognized by the OC88 CTL. SART-1 peptides were recognized by the CTL in an HLA-A2601-restricted manner [20]. Antigenic peptide(s) of SART-1 protein involving HLA-A0206 restriction were suggested in this study. As mentioned above, this SART-1 peptide(s) may also be recognized by the CTL in an HLA-A0201- or HLA-A0207-restricted manner. The antigen frequencies of HLA-A2 in Japanese, Caucasians and South Africans (blacks in Cape Town) are 42.8%, 44.5% and 30.3% respectively [7]. Therefore, substantial numbers of patients with ovarian cancer may be appropriate

candidates for specific immunotherapy by SART-1-derived peptide(s) recognized by the ovarian-cancer-specific CTL. Identification of the HLA-A2-binding SART-1 peptide(s) recognized by the OC88 CTL is necessary.

None of the three antigens tested (SART-1, MAGE-1, MAGE-3) was recognized by the OC90 CTL. This failure was expected since these three antigens were shared antigens expressed on histologically distinct cancers, whereas directed antigen(s) of the OC72 and OC90 CTL seemed to be preferentially expressed on ovarian cancers. None of the ovarian-cancer-specific antigens has been identified in any search of the literature. Identification of these antigens would be important not only for increasing our understanding of the molecular basis of host defense against ovarian cancer cells but also for developing a specific immunotherapy to ovarian cancers.

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