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Histocompatibility Leukocyte Antigen-A2-restricted and Tumor-specific Cytotoxic T Lymphocytes from Tumor-infiltrating Lymphocytes of a Patient with Testicular Embryonal Cancer

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T lymphocytes play an important role in tumor rejection. To understand T cell-mediated specific immunity at the tumor site of testicular embryonal cancer, we investigated whether interleukin-2 (IL-2)-activated tumor-infiltrating lymphocytes (TIL) of a patient with testicular embryonal cancer show histocompatibility leukocyte antigen (HLA)-class I-restricted and tumor-specific cytotoxicity. We established a $CD3^+CD4^-CD8^+$ cytotoxic T lymphocyte (CTL) line from the IL-2-activated TIL of a 37-year-old patient with testicular embryonal cancer. A 6 h ^{51}Cr-release assay was performed to measure the cytotoxicity of the CTL. The $CD3^+CD4^-CD8^+$ CTL line showed cytotoxicity against $HLA\text{-}A2^+$ tumor cells, including freshly isolated autologous tumor cells, adenocarcinoma cell lines from various organs (lung, breast, pancreas, colon and kidney) and squamous cell carcinomas (esophagus and oral cavity). No other cell lines examined, including an autologous tumor cell line and $HLA\text{-}A2^-$ tumor cell lines, were lysed by this CTL line. These results suggest the existence of HLA-A2-restricted and tumor-specific CTL at the tumor site of testicular embryonal cancer.

Key words: Cytotoxic T lymphocytes — HLA-A2 — Tumor-infiltrating lymphocytes — Testicular embryonal cancer

Histocompatibility leukocyte antigen (HLA)-class I-restricted tumor-specific cytotoxic T lymphocytes (CTL) were initially reported in tumor-infiltrating lymphocytes (TIL) of patients with metastatic melanomas.[1,2] These CTL recognize tumor-rejection antigens in an HLA-class I-restricted manner,[3–8] and some of them are currently under clinical trial as cancer vaccines.[9] HLA-class I-restricted and tumor-specific CTL have also been observed in other cancers, including ovarian, breast, esophageal, gastric, colon and non-small cell lung cancers.[10–15] However, a search of the literature showed that the presence of these CTL in TIL of testicular cancer has not previously been reported. T cell-mediated specific immunity plays a central role in tumor rejection. Therefore, it is important to know whether HLA-class I-restricted and tumor-specific CTL exist at the site of testicular cancer. We have established a $CD3^+CD4^-CD8^+$ CTL line from TIL of a 37-year-old man with testicular embryonal cancer. This CTL line showed HLA-A2-restricted and tumor-specific cytotoxicity, suggesting the existence of T cell-mediated specific immunity at the tumor site of testicular embryonal cancer.

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MATERIALS AND METHODS

Subject and tumor cell lines A 37-year-old male patient presented with a testicular tumor. A sample of the tumor was obtained at the time of surgery in Kurume University Hospital and a pathological diagnosis was made of embryonal carcinoma at the Department of Pathology of Kurume University. For establishment of the tumor cell line, single cell suspensions of tumor samples were cultured *in vitro* with RPMI-1640 medium (Gibco-BRL, Grand Island, NY) plus 10% fetal calf serum (FCS) (Whittaker, Walkersville, MA). Most of the HLA-class I-identified allogenic tumor cell lines used in this study were previously reported.[12]

Genotyping of HLA-class I alleles Genotypes of HLA-A alleles of the tumor cell lines were determined by polymerase chain reaction (PCR) and the sequence-specific oligonucleotide probe (SSOP) hybridization method as reported.[16] HLA-B and -C alleles of some tumor cell lines were also determined by the PCR-SSOP and PCR-restriction fragment length polymorphism (RELF) methods.[17]

Transfection HLA-A0206 cDNA was isolated from the autologous tumor cell line (OT-T) as reported.[12] HLA-A2402 cDNA was isolated from KE-4. COS7 cells were transfected with cloned HLA-A0206 and HLA-A2402 cDNA using Lipofectin (Gibco-BRL) as reported.[12]

Generation of CTL A sample containing tumor cells and TIL was finely minced with scissors, then digested with collagenase (2 mg/ml) (Sigma, St. Louis, MO) and deoxyribonuclease I (0.4 mg/ml) (Takara, Kyoto) as reported.[1] Single cell suspensions were extensively washed and some of the cells were then cultured with RPMI-1640 medium, supplemented with 10% FCS, and 100 U/ml of recombinant interleukin-2 (rIL-2, a generous gift from Shionogi Pharmaceutical Co., Osaka) at 37°C in an atmosphere of 5% CO_2 for up to 59 days. Most of the freshly isolated tumor cells were aliquoted and cryopreserved at −130°C in a nitrogen tank, thawed on the morning of experiments, radiolabeled with $Na_2{}^{51}CrO_4$ and used as target fresh autologous tumor cells (OT-F). The surface phenotype of the TIL was analyzed by direct immunofluorescence assay with FITC-conjugated anti-CD2 (Nu-TER), anti-CD3 (Leu4), anti-CD5 (Nu-Tpan), anti-CD11a (YH384), anti-CD16 (JH-169), anti-CD18 (MHM23), anti-CD19 (B4), anti-CD20 (Nu-B2), anti-CD25 (TA75), anti-CD28 (KOLT-2), anti-TCR-$\alpha\beta$ (WT31), anti-TCR-$\gamma\delta$ (TCR-$\gamma\delta$-1), PE-conjugated anti-CD4 (NU-Th/i), anti-CD40L (TRAP-1), anti-CD56 (Leu19), and PerCP (peridinin chlorophyll protein)-conjugated anti-CD8 (NU-Ts/c) monoclonal antibody (mAb) as previously reported.[12, 18]

Cytotoxicity assay A standard 6 h ^{51}Cr-release assay was used to measure CTL activity, as reported.[12] If the mean of triplicate measurements of percent specific lysis ex-

Table I. Kinetic Study of Surface Phenotypes of IL-2 Activated TIL[a]

Surface marker	Days of culture						
	0	14	28	35	43	50	57
$CD3^+$ (%)	84.2	77.7	93.9	100	76.4	19.3	10.8
$CD3^+CD4^+CD8^-$	42	29.5	10.2	6	2.4	2.8	0.3
$CD3^+CD4^-CD8^+$	42	48.2	83.6	94	56	14.4	1.4
$CD19^+CD20^+$		<2		<2	3		69

a) $CD3^+$, $CD3^+CD4^+CD8^-$, $CD3^+CD4^-CD8^+$, and $CD19^+$-$CD20^+$ lymphocytes (%) were analyzed by direct immunofluorescence with FITC-conjugated anti-CD3, -CD19, -CD20, PE-conjugated anti-CD4 and PerCP-conjugated anti-CD8 mAb, using a FACScan.

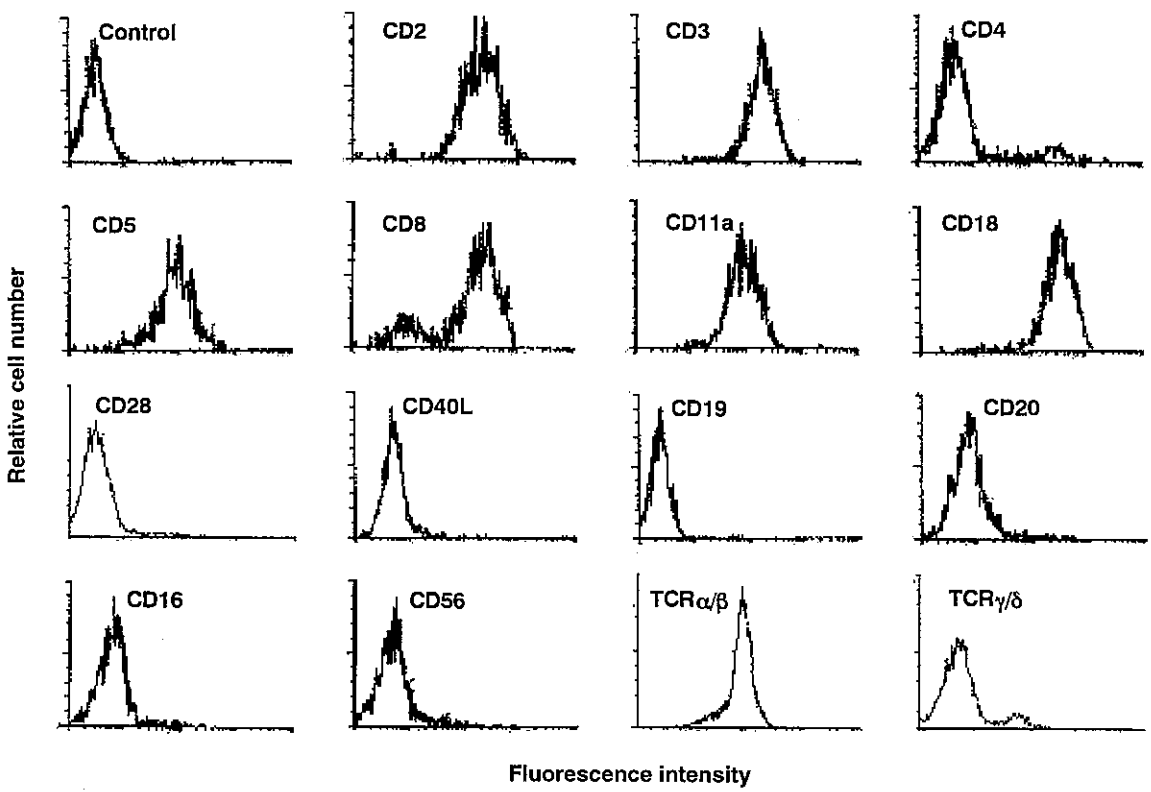

Fig. 1. Surface phenotypes of TIL. The surface phenotypes of the TIL were serially analyzed (days 0, 14, 28, 35, 43, 50, 57 of culture) by a one-, or two-, or three-color immunofluorescence technique using a FACScan. Representative results at day 43 are shown in the figure and the results are summarized in Table I.

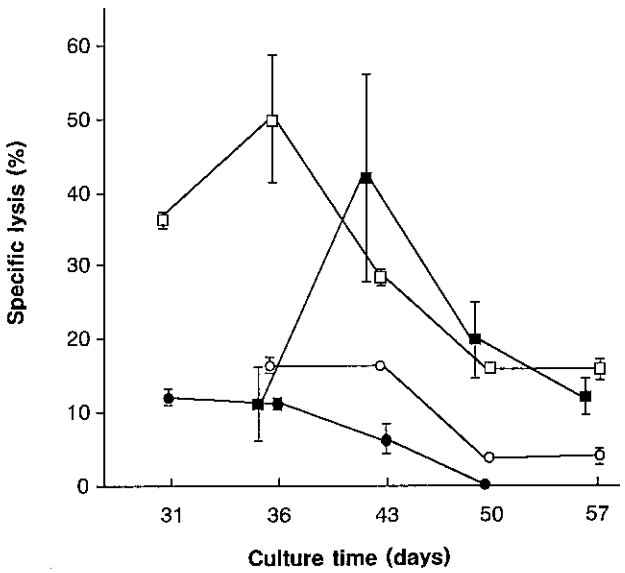

Fig. 2. Kinetic study of the cytotoxicity. IL-2-activated TIL were serially tested for cytotoxicity against freshly isolated autologous tumor cells (OT-F) (A0206/ Testicular carcinoma) (■), 1-87 tumor cells (A0207/1101 Lung adenocarcinoma) (□), YT803 tumor cells (A3101/3302 Lung adenocarcinoma) (●) and COS7 cells (○) at an E/T ratio of 20 in a 6 h ^{51}Cr-release assay.

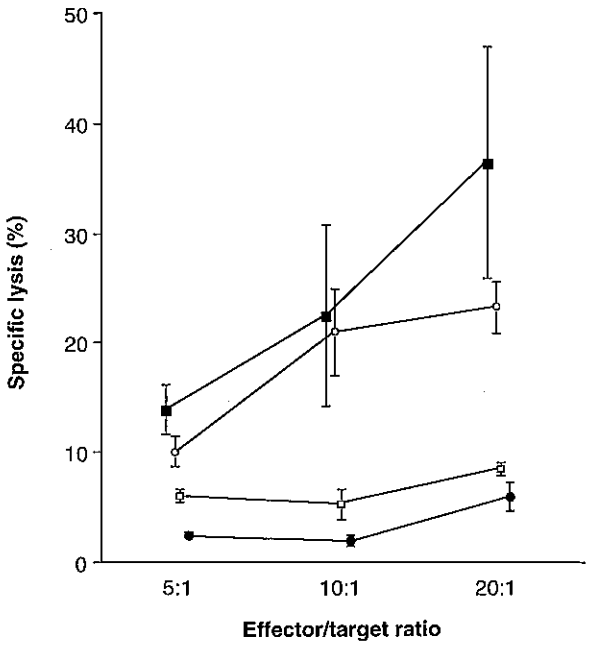

Fig. 3. Dose dependency of the cytotoxicity. Cytotoxicity of the CTL line at day 43 against OT-F tumor cells (A0206/ Testicular carcinoma) (■), OT-T tumor cells (□), SW620 tumor cells (A0201/2402 Colon adenocarcinoma) (○), and YT803 tumor cells (A3101/3302 Lung adenocarcinoma) (●) at three E/T ratios (5, 10, and 20) were analyzed in a 6 h ^{51}Cr-release assay.

ceeded 10%, the cytotoxicity was evaluated as significant in these studies. Anti-CD3 (OKT3; ATCC), -CD4 (OKT4; ATCC), -CD8 (Nu-Ts/c), anti-HLA class I (W6/32; ATCC), anti-HLA DR (H-DR1) mAb were used in blocking experiments, as reported.[12]

Characterization of the autologous tumor cell line Expression of TAP and LMP messenger RNA (mRNA) in the autologous tumor cell line was investigated by the reverse transcription-polymerase chain reaction (RT-PCR) method with the following specific primers: TACCTGCTCATAAGGAGGGTGC and ATTGGGATATGCAAAGGAGACG for TAP-2 (formerly RING11, Y1, or PSF-2), TCGCCTTCAAGTTCCAGCATGG and CCAACCATCTTCCTTCATGTGG for LMP-7 (formerly RING 10), and TTGTGATGGGTTCTGATTCCCG and CAGAGCAATAGCGTCTGTGG for LMP-2 (formerly RING 12).[19] PCR products were subjected to electrophoresis in 1.5% agarose gels, and bands of the expected sizes were isolated.

Several tumor markers of the cells of the tumor sample and of OT-T tumor cells were investigated immunohistochemically with Histofine mouse streptoavidin-biotin peroxidase kits (SAB PO[M], Nichirei, Tokyo). The antibodies used for the study were anti-cytokeratin antibody ("IMMUNON," Pittsburgh, OH), anti-placental alkaline phosphatase polyclonal antibody (Biomeda, Foster, CA), and anti-human chorionic gonadotropin (hCG) polyclonal antibody ("IMMUNON"), and the control antibody was normal mouse IgG1 (10 μg/ml, Dako, Glostrup, Denmark). Frozen sections of this testicular tumor were fixed with periodic acid sodium lysine in 2% paraformaldehyde (PLP solution) at room temperature, and washed 3 times with phosphate-buffered saline (PBS). For the examination of the autologous tumor cell line, subconfluent cells on a Lab-Tek tissue culture chamber slide (Nunc, Inc., Roskilde, Denmark) were fixed with PLP for 30 min at room temperature, washed with PBS, and frozen by spraying Freon gas from the back of the slide.

RESULTS

Cytotoxicity The minced sample from the patient was cultured with 100 U/ml IL-2 for up to 59 days. Activated lymphocytes began to be observed microscopically around the tumor cells after 7 to 10 days of culture. Lymphocytes began to proliferate vigorously after 14 to 21 days of culture, providing sufficient cells for extensive studies, including surface phenotypic analyses and serial

Table II. Cytotoxicity of Testicular Carcinoma CTL Lines

Histology	Cell line	Origin	%Cx.[a]	HLA class I		
				A	B	C
Embryonal carcinoma	OT-F[c]	Testis	**80**	**A0206**/	B4001/B3501	Cw0304/
	OT-T[d]	Testis	**0**	**A0206**/	B4001/B3501	Cw0304/
Adenocarcinoma	1-87	Lung	**50**	**A0207**/A1101	B4601/B5401	Cw0102/
	YT803	Lung	0	A3101/A3302	B44031/B5101	Cw0303/Cw1403
	11-18	Lung	**37**	**A0201**/A2402	B5201/B5401	Cw0102/Cw1201
	MKN45	Stomach	0	A2402/		
	SSTW-9	Stomach	0	A2402/A2601	B1501/B4601	Cw0102/Cw0303
	SW620	Colon	**45**	**A0201**/A2402	B0702/B1518	Cw0702/Cw0704
	KM12LM	Colon	**39**	**A0201**/A2402	B0702/	Cw0702/
	R-27	Breast	**18**	**A0201**/	B4402/	Cw0501/
	Panc-1	Pancreas	**44**	**A0201**/A1102	B3801/	Cw1203/
Squamous cell carcinoma	KE-3	Esophagus	**24**	**A0206**/A2402	B15/B53	Cw0401/Cw1502
	KE-4	Esophagus	5	A2402/A2601	B51/B53	Cw0101/Cw0302
	TE-10	Esophagus	7	A2/A2402	B55/B51	Cw0302/Cw0102
	TE-11	Esophagus	5	A2402/A2601	B56/B51	Cw1401/
	KUMA-1	Head & Neck	0	A2603/A3302	B1501/B44031	Cw0303/Cw1403
	OSC-20	Oral	**10**	**A2**/11	B54/B60	Cw1/Cw3
	LC-1Sq	Lung	0	A11/A2402	B44031/	Cw1203/Cw1601
	RERF-LC-AI	Lung	2	A2402/	B5201/	Cw1202/
	Sq-1	Lung	4	A1101/A2402	B1501/B5201	Cw0401/Cw1202
Small cell carcinoma	LC-65A	Lung	4	A1101/A2402	B4002/	Cw0304
Hepatocarcinoma	HAK-1B	Liver	**35**	**A0201**/A3301	B44031/B5401	Cw0102/Cw1403
Renal cell carcinoma	KUR-11	Kidney	9	A2402/A3303	B60/B48	Cw4
	KUR-20	Kidney	**32**	**A0201**/A0211	B60/B61	Cw3
Non-malignant cell	VA-13	Fibroblast	0	n.d.[b]		
	T2	B cell	6	A0201/		
	COS7		4			

a) Cytotoxicity of the IL-2-activated TIL around day 43 against different types of cell lines was measured by ^{51}Cr-release assay at an effector-to-target ratio of 20. Representative results are shown.
b) n.d.=not detection of HLA-A alleles.
c) OT-F=freshly isolated autologous tumor cells.
d) OT-T=autologous tumor cell line.

cytotoxicity assays. A large number (15×10^4) of cells per sample was counted on a FACScan at days 0, 14, 28, 35, 43, 50 and 57. The percentages of $CD3^+CD4^-CD8^+$ T cells were 42%, 48.2%, 83.6%, 94%, 56%, 14.4% and 1.4% at days 0, 14, 28, 35, 43, 50 and 57, respectively (Table I). Representative histograms at day 43 are shown in Fig. 1. The majority of these cells at day 43 were $CD3^+CD4^-CD8^+TCR\alpha\beta^+CD16^-CD56^-$.

Cytotoxicity assays against autologous tumor cells and a panel of HLA-class I genotyped allogenic tumor cell lines were repeated at least twice. A kinetic study showed that IL-2-activated TIL produced significant levels (>10%) of lysis of HLA-$A2^+$ 1-87 adenocarcinoma cells at 31 days of culture, and the maximal level of cytotoxicity was seen at 36 days of culture (Fig. 2). These TIL at day 43 showed the highest cytotoxicity against the freshly isolated OT-F cells. In contrast, they failed to lyse HLA-$A2^-$ YT803 adenocarcinoma cells throughout the culture period. The cytotoxicity of the CTL line at day 43 against the OT-F cells and HLA-$A2^+$ SW620 tumor cells increased in a manner dependent on the number of effector cells (Fig. 3). Based upon these results, the IL-2-activated TIL around day 43 were used as the CTL line throughout the experiments. The CTL line mostly expressed CD2, CD3, CD5, CD8, CD11a, CD18, CD40L and TCRα/β antigens, but not CD4, CD20, CD16, CD56 or CD28 antigens (Fig. 1).

Representative results on the cytotoxicity of the CTL line are shown in Table II. This CTL line (HLA-A0206/, B4001/B3501, Cw0304/) showed significant levels of cytotoxicity (>10%) against freshly isolated autologous tumor cells and ten different HLA-$A2^+$ allogenic tumor cell lines, including six adenocarcinomas (1-87, 11-18, SW620, KM12LM, R-27, Panc-1), two squamous cell carcinomas (KE-3, OSC-20), one hepatocarcinoma (HAK-1B) and one renal cell carcinoma (KUR-20). These cell lines had been established from various organs (lung, colon, breast, pancreas, esophagus, oral cavity,

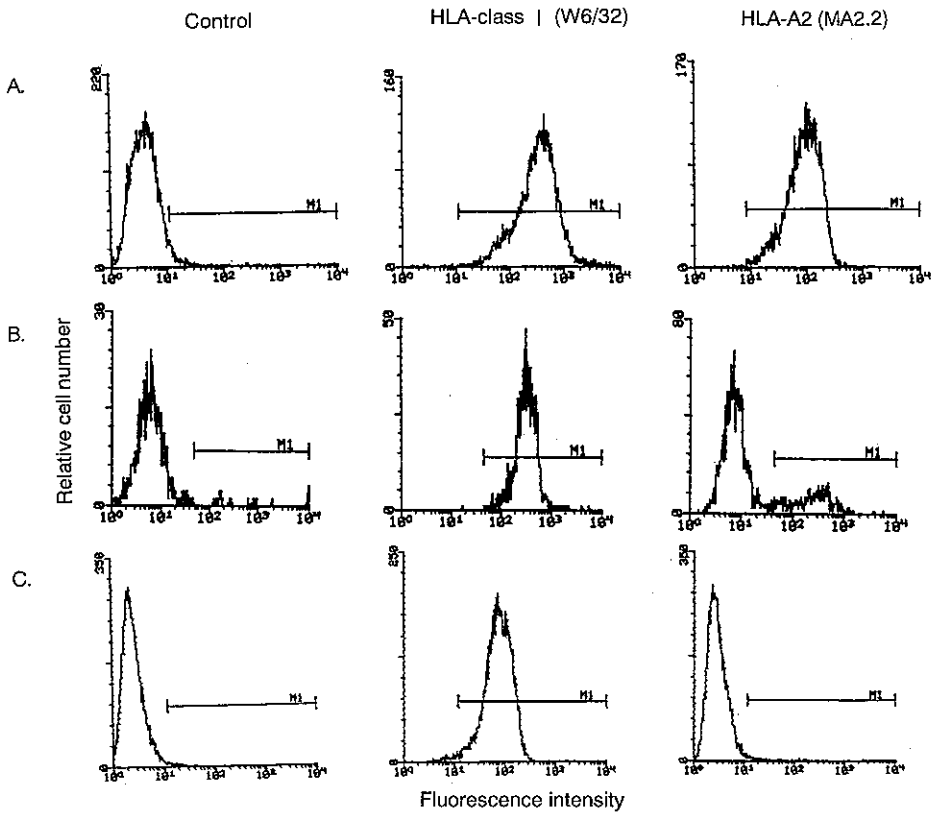

Fig. 4. Expression of HLA-A2 on the cell surface. A, Expression of HLA-A2 on the cell surface of the OT-T tumor cells was analyzed using a FACScan with anti-class I mAb (W6/32; ATCC) and anti-HLA-A2 mAb (MA2.2; ATCC). B, The COS7 cells transfected with HLA-A0206 cDNA (COS7/A0206) were analyzed. C, The COS7 cells transfected with HLA-A2402 cDNA (COS7/A2402) were analyzed.

liver, kidney). However, none of the other cell lines tested, including cells of the autologous cell line, twelve HLA-A2$^-$ tumors, and one HLA-A2$^+$ tumor (TE10), were lysed by the CTL line. Moreover, neither a VA-13 fibroblast cell line, an HLA-A2$^+$ T2 cell line nor COS7 was lysed by this CTL line.

The cytotoxicity against fresh autologous tumor cells was inhibited by anti-CD3, anti-CD8 or anti-HLA-class I (W6/32) mAb, but not by either anti-CD4 or anti-class II (DR) mAb. Namely, the percent lysis at an effector-to-target cell ratio of 40 in the absence of mAb or presence of anti-CD3, anti-CD8, anti-HLA-class I, anti-CD4, anti-class II was 18%, 2%, 0%, 5%, 22%, and 13%, respectively.

Characterization of the autologous tumor cell line This CTL line lysed freshly isolated autologous tumor cells, but failed to lyse the autologous tumor cell line (Table II, Fig. 3). To understand the mechanism of this discrepancy we examined whether OT-T tumor cells pretreated with 1×10^3 U/ml interferon-γ (IFN-γ) for 48 h before the assay became susceptible to lysis or not. However, the IFN-γ-pretreated OT-T tumor cells were not lyzed by this CTL line (data not shown).

The OT-T tumor cells were susceptible to lysis by lymphokine-activated killer (LAK) cells obtained by the culture of PBMC from a healthy donor with 100 U/ml IL-2 for 7 days. Namely, the percent lysis by LAK cells of the OT-T tumor cells at effector-to-target cell ratios of 10, 20, and 40 amounted to 14%, 25%, and 35%, respectively. These results suggested that the OT-T tumor cells were resistant to lysis by the CTL line, whereas they were susceptible to lysis by LAK cells.

We next investigated the molecular mechanism of the resistance to lysis of the OT-T tumor cells. Initially, we investigated the expression of HLA-A0206 on the OT-T tumor cells. HLA-A2 antigen was found to be highly expressed on the cell surface of the OT-T tumor cells by FACScan analyses with anti-HLA-A2 mAb (MA2.2; ATCC) and anti-class I mAb (W6/32; ATCC) (Fig. 4A). Secondly, we determined the sequence of HLA-A2

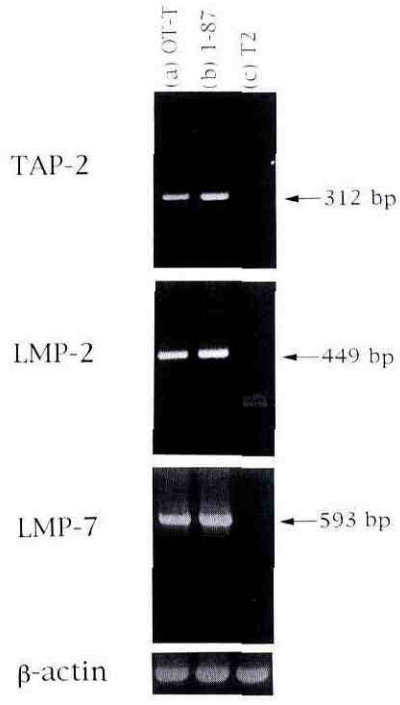

Fig. 5. Expression of TAP-2, LMP-2, and LMP-7 mRNA in the OT-T tumor cells. Levels of TAP-2, LMP-2 and LMP-7 gene expression at the mRNA level were analyzed in OT-T tumor cells (a), 1-87 lung adenocarcinoma cells (b) and T2 cells (c) by means of RT-PCR.

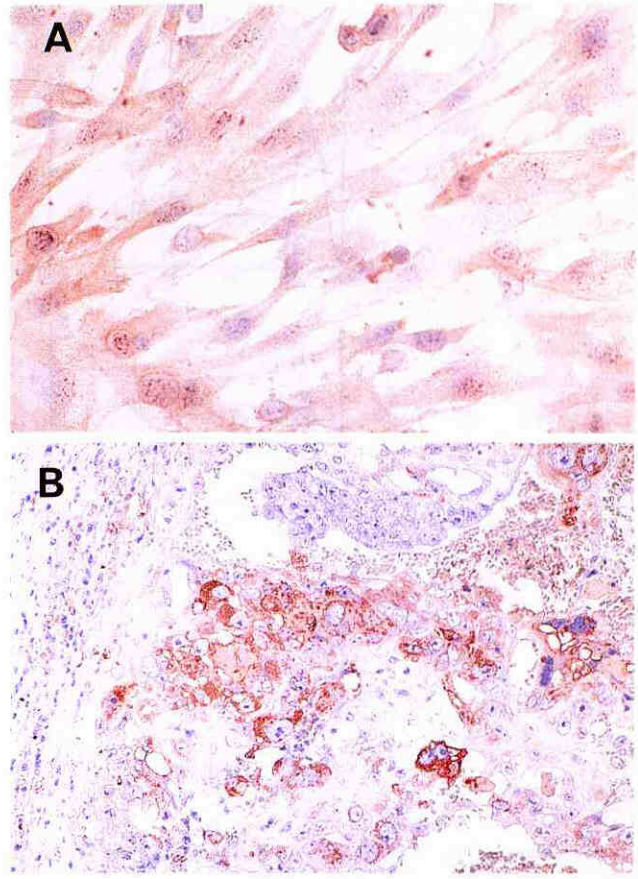

Fig. 6. Immunohistochemical study. The autologous tumor cell line (OT-T) (A) and the autologous tumor tissue (B) were stained with anti-human chorionic gonadotropin polyclonal antibody. Original magnification ×260.

cDNA of both the OT-T tumor cells and the patient's PBMC. Both had identical sequences of HLA-A0206 and there was no mutation among the sequences (data not shown). Furthermore, COS7 cells were transfected with HLA-A0206 cDNA of the OT-T tumor cells, after which the surface expression was tested. The transfectant (COS7/A0206), but not the COS7 cells transfected with HLA-A2402 cDNA as a control (COS7/A2402), expressed HLA-A2 molecules on the cell surface (Fig. 4, B and C). All these results indicate that the HLA-A0206 antigen of the OT-T tumor cells may have the ability to present antigenic peptides.

We investigated mRNA expression of the genes involved in antigen processing. Expression of the TAP-2, LMP-2 and LMP-7 genes at the mRNA level was observed in the OT-T tumor cells and 1-87 lung adenocarcinoma cells, but not in the deficient hybridoma cells (T2 cells) (Fig. 5).

Finally, we addressed the question of whether the OT-T tumor cells were different from the OT-F tumor cells with regard to tumor cell markers. All the cells of the OT-T tumor cell line were positive for cytokeratin, placental alkaline phosphatase, and hCG (Fig. 6A). In contrast, some tumor cells in the autologous tumor tissue were negative for these antigens (Fig. 6B). The results for hCG are shown in Fig. 6.

DISCUSSION

This study has provided evidence that IL-2-activated TIL of testicular cancer contained HLA-A2-restricted and tumor-specific CTL at the tumor site of testicular embryonal cancer. This is a new finding, although marked lymphocyte infiltration was previously reported in testicular cancer.[20,21] Eight HLA-A2$^+$ adenocarcinomas from six different organs (lung, colon, breast, pancreas, liver and kidney) as well as two HLA-A2$^+$ squamous cell carcinomas from the oral cavity and esophagus were lysed by this CTL line. This result suggests that the tumor antigen(s) recognized by this CTL line is expressed not only on the autologous testicular cancer

cells, but also on some adenocarcinomas or squamous cell carcinomas from other organs. Although the tumor antigen(s) recognized by this CTL line has not yet been identified, it might be one of the tumor-specific shared antigens, such as MAGE, BAGE, GAGE or RAGE that are expressed on various types of cancers.[3, 22–24] Alternatively, ubiquitous antigens such as HER-2/neu[25] are also possible candidates for the antigen(s). Testicular embryonal cancer cells retain the ability to differentiate into more mature forms of cells (seminona, choriocarcinoma, yolk sac tumor and teratoma).[20] Therefore, it is also conceivable that the tumor antigen(s) is a kind of shared tissue antigen(s) expressed on a wide spectrum of tumors with different origins. It remains necessary to identify the tumor antigen(s).

In each cytotoxicity assay, the different lot of CTL was used. The lot of frozen OT-F target cells was also different. Therefore, the level of OT-F lysis is expected to be slightly different in each assay. Thus, we repeated the cytotoxicity assay against OT-F cells 7 times, and representative results are presented in this paper.

Antigen peptide(s) recognized by this CTL line might be able to bind to HLA-A0201, -A0206 and -A0207 since tumor cells expressing these HLA-A2 subtypes were susceptible to lysis. There is a common binding motif among these HLA-A2 subtypes. Namely, nonapeptides with leucine at position 2 and leucine at position 9 can bind to the cleft of these HLA-A2 subtypes.[26]

The percentage of $CD3^+CD4^-CD8^+$ T cells decreased after 35 days of culture (Table I). In their place, $CD19^+$ $CD20^+$ B cells that weakly expressed CD25 (IL-2R-α) and IgG on their surface increased with prolonged incubation. The percentages of $CD20^+$ B cells were 22%, 44%, 55% and 98% at days 14, 35, 43 and 57, respectively. Those of $CD19^+$ cells were $<2\%$, $<2\%$, 3% and 69%, respectively. Moreover, the IL-2-activated TIL at longer incubation times spontaneously produced higher levels of IL-2 in the supernatants. The levels of IL-6 in the supernatants were 105 pg/ml, 170 pg/ml and 1145 pg/ml, as determined by enzyme-linked immunosorbent assay, at days 35, 46 and 57, respectively. In contrast, significant levels of IFN-γ or TNF-α were not detected in the culture supernatants throughout the culture period (data not shown). None of these cytokines was produced by IL-2-activated TIL in response to various $HLA\text{-}A2^+$ tumor cells tested (OT-F, OT-T, R-27, 1-87, Panc-1, SW 620 or KE-3). These results suggest that the prolonged incubation of the TIL with IL-2 caused the proliferation of $CD3^-CD20^+$ B cells. $CD3^-CD20^+$ B cells usually do not vigorously proliferate in *in vitro* culture with IL-2. In a literature search, we found no previous report showing that IL-2-activated TIL contained a large number of $CD3^-CD20^+$ B cells. Although the mechanisms involved in this phenomenon are unclear, one possibility is that the testicular embryonal cancer cells contained immature types of cells.

HLA-A2 antigen was highly expressed on the cell surface of the OT-T tumor cells. There was no mutation in the HLA-A0206 cDNA of the OT-T tumor cells. *TAP-2*, *LMP-2* and *LMP-7* genes were highly expressed in the OT-T tumor cells at the mRNA level. These results suggest that molecules required for antigen presentation in the OT-T tumor cells function well and therefore are not involved in the mechanisms of resistance to the lysis by the CTL lines. However, expression of these molecules does not directly demonstrate that the antigen presentation is normal in these cells.

All the cells of the OT-T tumor cell line were positive for cytokeratin, placental alkaline phosphatase and hCG, whereas some tumor cells in both the autologous tumor tissue and the OT-F tumor cells were negative for these antigens. These results suggest the heterogeneity of cells in the autologous tumor tissue, which in turn might be involved in the resistance of the OT-T tumor cells. Tumor cells deficient in tumor antigen(s) recognized by the host CTL proliferated well *in vitro*, whereas the OT-F tumor cells contained both tumor cells expressing tumor antigen(s) and those which did not express it. The OT-T tumor cells were susceptible to LAK cells. These results indicate that there are at least two types of tumor cells in this testicular embryonal cancer. One type would be the tumor cells susceptible to lysis by both CTL and LAK cells, and the other would be those susceptible only to LAK cells.

Antigen frequency of HLA-A2 in Yugoslavs (Caucasians), North Americans (Amerinds), South Africans (Blacks in Cape Town) and Japanese was reported to be 45.2%, 44.5%, 30.3% and 42.8%, respectively.[27] HLA-A0201 along with A0206 and A0207 is shared by more than 95% of serologically defined HLA-A2-positive individuals. In conclusion, we have presented evidence for the existence of HLA-A2-restricted and tumor-specific CTL in IL-2-activated TIL of $HLA\text{-}A2^+$ testicular embryonal cancer from one patient. Testicular cancer is usually sensitive to chemotherapy, but recurrence or advanced stage tumors are associated with a poor prognosis. This information may be helpful for understanding host immunity against testicular cancer at the tumor site. Furthermore, the CTL could be a useful tool for identifying tumor antigens expressed on $HLA\text{-}A2^+$ testicular cancer cells.

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