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# Histocompatibility Leukocyte Antigen-A2402-restricted Cytotoxic T Lymphocytes Recognizing Adenocarcinoma in Tumor-infiltrating Lymphocytes of Patients with Colon Cancer

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**To cast light on T cell-mediated specific immunity at the tumor site of colon cancer, we investigated whether interleukin-2 (IL-2)-activated tumor-infiltrating lymphocytes (TIL) from colon cancer show histocompatibility leukocyte antigen (HLA)-class I-restricted cytotoxicity against adenocarcinoma. IL 2-activated TIL from all four HLA-A24 patients examined lysed HLA-A2402$^+$ adenocarcinomas, but not HLA-A2402$^-$ tumors. Those of two of the four cases also lysed HLA-A2402$^+$ squamous cell carcinomas. CD8$^+$ cytotoxic T lymphocyte (CTL) clones recognizing HLA-A2402$^+$ adenocarcinomas were established from one CTL line. This CTL line produced IFN-$\gamma$ upon recognition of an HLA-A2402$^-$ adenocarcinoma transfected with HLA-A2402 cDNA. These results suggest the presence of HLA-A2402-restricted CTL recognizing adenocarcinoma at the tumor site of colon cancer. Furthermore, HLA-A31-restricted CTL activity was found in IL-2-activated TIL from one of two HLA-A31$^+$ patients, suggesting the existence of HLA-class I-restricted CTL involving an allele other than A24.**

Key words: HLA-class I — HLA-A2402 allele — Cytotoxic T lymphocyte — Tumor-infiltrating lymphocyte — Colon cancer

T cells play an important role in tumor rejection. Histocompatibility leukocyte antigen (HLA)-class I restricted and tumor-specific cytotoxic T lymphocytes (CTL) have been observed in interleukin (IL)-2-activated tumor-infiltrating lymphocytes (TIL) of patients with melanoma, esophageal cancer, and ovarian cancer.[1-4] Genes encoding tumor antigens recognized by CTL have been cloned from melanoma.[5-12] However, little is known about the existence of HLA-class I-restricted and tumor-specific CTL in TIL of colon cancer. Several authors have suggested the existence of such CTL,[13,14] but others reported conflicting results.[15,16] In this investigation, we have studied HLA-A locus-restriction and tumor-specificity of IL-2-activated TIL from patients with colon cancer. The results suggest the presence of HLA-A2402 restricted CTL recognizing adenocarcinoma at the tumor site of primary colon cancer.

## MATERIALS AND METHODS

**Subjects and tumor cell lines** Six patients with colon cancer were enrolled in the study at the Kurume University Hospital. A part of the primary colon cancer samples (cases 1, 2, 3, 5 and 6) or metastatic lymph nodes (case 4) were obtained at the time of major surgery in our University Hospital. The profile of the patients is shown in Table I. All research followed the tenets of the Declaration of Helsinki. Most of the tumor cell lines, transformed cell lines and normal cell lines used in this study and their HLA-class I alleles have been previously reported.[3] The LC-1 tumor cell line was newly established from pleural effusion of a patient with lung cancer in our laboratory. The other cell lines used were: HST-2 and SSTW-9 gastric cancers provided by Dr. Sato (Sapporo Medical College, Sapporo); a 1-87 lung cancer provided by Dr. Kobayashi (Tohoku University, The Research Institute for Tuberculosis and Cancer, Sendai); and a HAK-2 hepatocellular carcinoma and a KMCH-1 hepatocholangiocellular carcinoma from Dr. Kojiro of our University. The other cell lines listed in Table II were either established in our laboratory, provided by JCRB (Tokyo) or purchased from American Type Culture Collection (ATCC, Rockville, MD). Autologous tumor cell lines were not available from any of the six cases due to the lack of tumor cell proliferation *in vitro* in culture.

Genotypes of HLA-A alleles of the tumor cell lines were determined by the PCR-sequence specific oligonucleotide probe (SSOP) method, as reported.[17] HLA-B and -C alleles of some tumor cell lines were also determined by the PCR-SSOP and PCR-restriction fragment length polymorphism (RELP) methods.[18] The results of genetic analyses of HLA-A, -B and -C alleles are shown in Table I. The results of serological HLA-class I typing of PBMC with HLA Monoclonal Reagent (One Lambda, Canoga Park, CA) are shown when genotyping data were not available.

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Table I. Patients' Profile and Surface Phenotypes of IL-2-activated TIL

| Patient | Age | Sex | HLA class I | | | Stage[a] | Surface phenotypes of IL-2-activated TIL[b] | |
|---|---|---|---|---|---|---|---|---|
| | | | A | B | C | | $CD3^+CD4^+CD8^-$ (%) | $CD3^+CD4^-CD8^+$ (%) |
| Case 1 | 81 | Male | 24/33 | 52/44 | | II | 2 | 95 |
| Case 2 | 50 | Female | 24/26 | 36/52 | 3 | IV | 20 | 75 |
| Case 3 | 60 | Male | 24/33 | 52/44 | | IV | 52 | 47 |
| Case 4 | 85 | Female | 24/ | 7/52 | 7 | III | 85 | 15 |
| Case 5 | 63 | Female | 26/31 | 51/ | | IV | 67 | 20 |
| Case 6 | 61 | Male | 31/33 | 46/44 | | IV | ND[c] | ND |

*a*) These six cases with colon cancer were classified according to the TNM classification of UICC (1987).
*b*) IL-2-activated TIL around 40 days of culture are shown in the table.
*c*) Not done.

**Preparation, expansion and functional assay** Tumor tissues were finely minced using surgical blades. These paste mixtures of tumor cells, tissue cells and TIL were then cultured in RPMI-1640 medium (GIBCO BRL, Grand Island, NY) supplemented with 25% AIM-V medium (GIBCO BRL), 10% fetal calf serum (FCS) (Whittaker, Walkersville, MA), 0.1 m*M* MEM non-essential amino acids solution (GIBCO BRL) and 100 units/ml of recombinant IL-2 (a generous gift from Shionogi Pharmaceutical Co., Osaka) at 37°C in a 5% $CO_2$ atmosphere for up to 11 weeks. TIL proliferated under these conditions in five of six cases (cases 1 to 5), whereas those of the remaining case (case 6) did not.

The surface phenotypes of the proliferating TIL (cases 1 to 5) were studied by direct immunofluorescence assay with FITC-conjugated anti-CD3, -CD4, and -CD8 (Nichirei, Tokyo) monoclonal antibodies (mAbs) and FACScan (Becton Dickinson, Mountain View, CA), as reported.[3] The results at around 50 days of culture are shown in Table I. A standard 6-h $^{51}Cr$-release assay was used to measure CTL activity, as reported.[3] When the mean of triplicate measurements exceeded the negative control (no effector cells) by 5% in these studies, the difference was considered statistically significant (at least $P<0.05$) in most cases. If the mean of triplicate measurements of percent specific lysis exceeded 10%, cytotoxicity was evaluated as significant in these studies. Inhibition experiments with anti-CD3 (OKT3) (ATCC), -CD4 (Nichirei), -CD8 (Nichirei), anti-class I (W6/32) (ATCC) and anti-DR mAb (Becton Dickinson) were performed as described previously.[3] Interferon gamma (IFN-$\gamma$) production by IL-2-activated TIL in response to various tumor cells was also measured. Twenty thousand target cells were added to the wells of a 96-well flat-bottomed microplate in 100 $\mu$l of RPMI-1640 medium containing 10% heat-inactivated FCS, and were incubated at 37°C for 12 h, followed by addition of effector CTL at an effector-to-target cell (E/T) ratio of two to one. After an 18 h incubation, the supernatants were collected and assayed for cytokine production using IFN-$\gamma$ ELISA (Genzyme Corp., Cambridge, MA). The sensitivity limit of this ELISA was 5 pg/ml.

Two $CD8^+$ T cell clones were established from the IL-2-activated TIL line of case 1 (bulk CTL line) as reported previously.[1] Culture conditions for establishment of $CD8^+$ CTL clones were as follows; different numbers (0.3, 1, 4, 16, or 64 cells/well of U-bottomed 96-well microplate) of IL-2-activated TIL were incubated in 0.2 ml of the medium (50% RPMI 1640 medium and 50% AIM-V medium) supplemented with 10% FCS, 100 U/ml rIL-2, 0.4 $\mu$g/ml PHA-P (Difco Laboratories, Grand Island, NY), 1% "T-STIM" with PHA (Becton Dickinson Labware, Bedford, MA) in the presence of $2\times10^5$ cells/well of irradiated allogenic PBMC from 3 different healthy donors. The culture medium was replaced with the medium mentioned above in the absence of allogenic PBMC every 7 days and the proliferating cells were transferred into a well of a 48-well culture plate at around 21 days of culture. The cells were further transferred into a well of a 24-well culture plate at around 28 days of culture followed by testing of IFN-$\gamma$ production in response to target cells. Surface phenotypes of expanded cells were analyzed, and only $CD3^+CD4^-CD8^+$ T cells were used as potential T cell clones in this study.

**Transfection experiments** HLA-A2402 and -2601 cDNA were isolated from a KE-4 esophageal squamous cell carcinoma cell line as reported.[3] Full-length cDNA of the HLA-A locus was amplified by PCR with HLA-A-specific oligonucleotides (sense primer, 5′-CCGAGATGGCCGTCATG and anti-sense primer, 5′-TGTCTCACACTTTACAAGCTGTGAGAG). Amplified cDNA was ligated with pCR3 (Invitrogen, San Diego, CA), cloned, and sequenced. An ovarian cancer cell line KOC-7C (HLA-A0201/A3101) was transfected with HLA-A2402 or -A2601 cDNA and Lipofectin (GIBCO BRL). Briefly, KOC-7C cells ($5\times10^5$ cells) in 2 ml of OPTI-

Table II. Cytotoxic Profile of IL-2-activated TIL of Cases 1 and 5

| Histology | Cell line | Origin | % lysis by TIL of[a] | | HLA class I | | |
|---|---|---|---|---|---|---|---|
| | | | Case 1 | Case 5 | A | B | C |
| Adenocarcinoma | SW620 | Colon | **58** | 6 | A0201/**A2402** | B0702/B1518 | |
| | HCT116 | Colon | 0 | 0 | A0101/A0201 | B4501/ | |
| | KM12LM | Colon | **25** | 0 | A0201/**A2402** | B0702/ | |
| | COLO201 | Colon | 2 | 4 | A0101/A0201 | B0702/B0801 | |
| | COLO320 | Colon | **23** | 0 | **A2402**/ | B1402/ | |
| | 1-87 | Lung | 0 | 8 | A0207/A1101 | B4601/B5401 | |
| | A549 | Lung | 4 | 0 | A2603/A3001 | B1801/B44031 | |
| | 11-18 | Lung | **10** | 9 | A0201/**A2402** | B5201/B5401 | |
| | LC1 | Lung | 30 | **30** | **A3101**/A3302 | B1511/B44031 | Cw3/ |
| | PC-9 | Lung | **30** | 3 | A0206/**A2402** | B0702/B5101 | |
| | KWS | Stomach | 0 | 7 | A0206/ | B1301/ | |
| | KATO-III | Stomach | **43** | 8 | A02/**A2402** | B1501/B4601 | |
| | MKN45 | Stomach | 2 | 3 | A2402/ | | |
| | MKN28 | Stomach | 2 | **15** | **A3101**/ | B5101/ | |
| | HST2 | Stomach | 3 | **18** | A02/**A3101** | B38/54 | Cw1/7 |
| | SSTW-9 | Stomach | 0 | 5 | A24/A26 | B1501/B4601 | Cw7/ |
| | R-27 | Breast | 2 | 0 | A0201/ | B4402/ | |
| | Panc-1 | Pancreas | 3 | 0 | A0201/A1102 | B3801/ | |
| | KMG-A | Gall bladder | 0 | 0 | A2601/ | B4002/ | |
| Squamous cell carcinoma | KE-3 | Esophagus | 8 | | A02/A24 | B7/B61 | Cw1502/2401 |
| | KE-4 | Esophagus | **12** | 2 | **A2402**/A2601 | B54/B60 | Cw0101/0302 |
| | TE-9 | Esophagus | 25 | | A3302/ | B1503/ | |
| | TE-10 | Esophagus | **10** | 0 | A02/**A2402** | | Cw0102/0302 |
| | TE-11 | Esophagus | **58** | 3 | **A2402**/A2601 | | Cw1401/ |
| | Kuma-1 | Head and neck | 7 | | A2603/A3302 | B39/B52 | Cw7/ |
| | Ca9-22 | Oral cavity | 1 | 8 | A24/ | B35/B4601 | |
| | HSC-2 | Oral cavity | **45** | 1 | **A24**/ | B44031/B5401 | |
| | HSC-4 | Oral cavity | 0 | **16** | A24/**A3101** | B0702/B5101 | |
| | LC-1sq | Lung | 0 | | A11/A24 | B44031/ | |
| | RERF-LC-AI | Lung | **13** | | **A2402**/ | B5201/ | |
| | QG56 | Lung | 8 | 3 | A2601/ | B4601/ | |
| Small cell carcinoma | LC-65A | Lung | 7 | 0 | A1101/A2402 | B4002/ | |
| | LK79 | Lung | 5 | | A2402/ | B4002/ | |
| Hepatocarcinoma | KMCH-1 | Liver | 9 | 8 | A1101/3101 | B5101/ | |
| Ovarian carcinoma | TOC-2 | Ovarium | 0 | | A26/A3302 | B7/B44 | Cw7/ |
| Leukemia | K562 | Erythrocyte | 4 | 3 | | | |
| Transformed cell | SS EBV-BCL[b] | | 6 | | A24/ | B52/B54 | Cw1/ |
| | COS7 | | 5 | | | | |
| Normal cell | VA-13 | Fibroblast | 7 | | | | |

*a*) Cytotoxicity of the TIL from two patients with colon cancer (cases 1 and 5) against different types of cell lines was measured in a 6-h $^{51}$Cr-release assay at an effector-to-target cell ratio of 20. Representative results are shown. HLA-A2402 and the related % lysis of case 1 are shown in bold. HLA-A3101 and the related % lysis of case 5 are also highlighted in bold. HLA-A3302 and the related % lysis are underlined.
*b*) Epstein-Barr virus-transformed B cell line from a healthy donor (S. S).

MEM (GIBCO BRL) were transfected with 2 $\mu$g of the plasmid DNA and 20 $\mu$g/ml of lipofectin. After 48-h culture, the KOC-7C cells were harvested and used for further experiments.

## RESULTS

**HLA-A2402-restricted CTL** TIL from all four HLA-A24 patients proliferated in culture with IL-2 under the conditions employed. In particular, those from case 1 proliferated vigorously and sufficient cells were available for extensive studies including serial cytotoxicity assays against a panel of HLA-class I genotyped allogenic tumor cell lines. Cytotoxicity assays were repeated three times in most tumor cell lines in separate experiments, with consistent results. Representative results at around seven weeks of culture are shown in Table II. IL-2-activated TIL of case 1 (HLA-A24/A33, B52/B54)

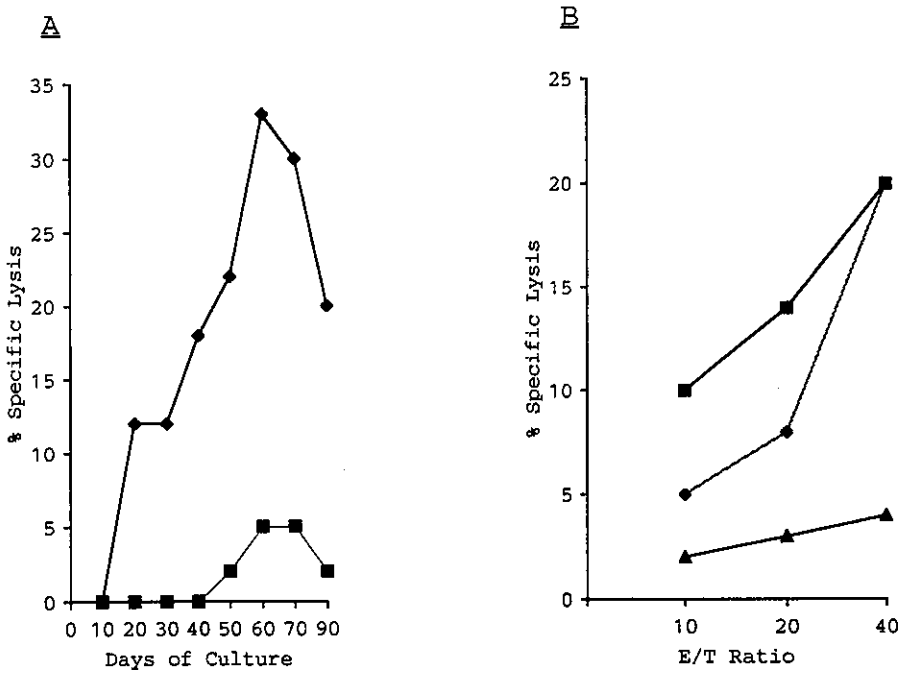


Fig. 1. HLA-A2402-restricted cytotoxicity. A, The kinetics of cytotoxicity of IL-2-activated TIL of case 1 was examined by using a 6-h $^{51}$Cr-release assay at an effector: target cell ratio of 20. IL-2-activated TIL (HLA-A24/A33) lysed HLA-A2402$^+$ PC-9 (◆), but not HLA-A2402$^-$ HCT116 (■) tumor cells. B, The CTL line of case 1 (HLA-A24/33) was tested for cytotoxicity in a 6-h $^{51}$Cr-release assay at three different E/T ratios (10, 20, 40) at 50 days of culture against HLA-A2402$^+$ tumor cell lines (SW620 (◆), PC-9 (■), and an HLA-A2402$^-$ tumor cell line (1-87) (▲)).

showed significant levels of cytotoxicity (>10%) against six different HLA-A2402$^+$ adenocarcinoma cell lines (SW620, KM12LM, COLO320, 11-18, PC-9, and KATO-III) and five different HLA-A2402$^+$ squamous cell carcinoma (SCC) cell lines (KE-4, TE-10, TE-11, HSC-2, and RERF-LC-AI). They were established from various organs (colon, lung, stomach, esophagus, and oral cavity). A kinetic study showed that IL-2-activated TIL of case 1 exhibited significant levels (>10%) of lysis against HLA-A2402$^+$ PC-9 adenocarcinoma cells at 20 days of culture, followed by increased levels of cytotoxicity up to 60 days of culture (Fig. 1A). In contrast, they failed to lyse HLA-A2402$^-$ HCT116 adenocarcinoma cells throughout the culture period. The cytotoxicity against HLA-A2402$^+$ tumors increased dependently on the number of effector cells (Fig. 1B). Furthermore, this CTL line (HLA-A24/33) also lysed two different HLA-A3302 tumor cell lines (LC-1 lung adenocarcinoma, TE-9 esophageal SCC). In contrast, it failed to lyse any of the other tumors shown in Table II, including eight HLA-A2402$^+$ tumor cell lines (two adenocarcinoma cell lines, four SCC cell lines, and two small cell carcinoma cell lines) and one HLA-A24$^+$ normal cell line (SS EBV-BCL). Neither K562 NK-sensitive tumor cells, a fibroblast cell line (VA-13), nor COS7[6] was lysed by this CTL line of case 1.

The CTL activity of case 1 was inhibited by anti-CD3, anti-CD8 or anti-HLA-class I (W6/32) mAb, but not by anti-CD4, anti-class II (DR) or an irrelevant (CD13) mAb. Namely, the mean±SD of % specific lysis of HLA-A2402$^+$ SW620 tumor cells by the CTL at day 90 of culture at an E/T ratio of 20 was 5±0.5 ($P<0.01$ vs. that with anti-CD13 mAb by Student's $t$ test), 4±1.1 ($P<0.005$), 0±0 ($P<0.001$), 13±1.6, 10±1.2 or 11±0.6% in the presence of anti-CD3, anti-CD8, anti-class I, anti-CD4, anti-class II or anti-CD13 mAb, respectively. Both the parental CTL line and one CD3$^+$CD4$^-$CD8$^+$ clone (clone 5) produced high levels of IFN-$\gamma$ by recognition of HLA-A2402$^+$ SW620 and PC-9 adenocarcinomas, as compared to those by recognition of HLA-A2603/3001$^+$ A549 adenocarcinoma HLA-A3101/3302$^+$ LC-1 adenocarcinoma and COS7 cells (Table III). The other clone 10 produced high levels of IFN-$\gamma$ in response to SW620, but not PC-9 adenocarcinoma. Furthermore, this CTL line produced higher levels of IFN-$\gamma$ by recognition of HLA-A2402$^+$ adenocarcinoma cells (SW620, positive control, 148 pg/ml) and HLA-A2402$^-$ adenocarcinoma cells (KOC-7C) transfected with HLA-

Table III. HLA-A2402-restricted IFN-$\gamma$ Production by CD8$^+$ CTL Clones[a)]

| Effector CTL | IFN-$\gamma$ production (pg/ml) in response to | | | | | |
|---|---|---|---|---|---|---|
| | None | A2402$^+$ adenocarcinomas | | A2402$^-$ adenocarcinomas | | Normal cells |
| | | SW620 | PC-9 | A549 | LC-1 | COS7 |
| Bulk CTL | 283 | 632 | 838 | 395 | 252 | 168 |
| Clone 5 | 106 | 231 | 116 | 75 | 105 | 95 |
| Clone 10 | 217 | 351 | 350 | ND[b)] | 228 | 165 |

*a*) The parental CTL line of case 1 (Bulk CTL) and two CD8$^+$ T cell clones were tested for IFN-$\gamma$ production in response to two HLA-A2402$^+$ adenocarcinomas (SW620 and PC-9), A2402$^-$ adenocarcinomas (A549 and LC-1) and normal cells (COS7). LC-1 cells are HLA-A3101/A3302.
*b*) Not done.

Table IV. Cytotoxicity Profile of IL-2-activated TIL of Cases 2, 3 and 4

| Histology | Cell line | Origin | % Cx.[a)] | | | HLA-A allele |
|---|---|---|---|---|---|---|
| | | | 2 | 3 | 4 | |
| Adenocarcinoma | SW620 | Colon | **46** | **13** | **17** | A0201/**A2402** |
| | HCT116 | Colon | **16** | 0 | 0 | A0101/A0201 |
| | KM12LM | Colon | 4 | **11** | 6 | A0201/**A2402** |
| | 11-18 | Lung | 7 | 5 | **15** | A0201/**A2402** |
| | PC-9 | Lung | | 0 | **38** | A0206/**A2402** |
| | MKN45 | Stomach | 2 | | 9 | A2402/ |
| | MKN28 | Stomach | 7 | 1 | 9 | A3101/ |
| | SSTW-9 | Stomach | 0 | | 2 | A24/A26 |
| | R-27 | Breast | 9 | 3 | | A0201/ |
| Squamous cell carcinoma | KE-3 | Esophagus | 6 | 0 | 0 | A2/A24 |
| | KE-4 | Esophagus | 4 | 0 | 0 | A2402/A2601 |
| | TE-10 | Esophagus | **11** | 0 | 0 | A2/**A2402** |
| | TE-11 | Esophagus | **12** | 7 | | **A2402**/A2601 |
| | Kuma-1 | Head and neck | | 0 | 0 | A2603/A3302 |
| | HSC-4 | Oral cavity | | 0 | 0 | A24/A3101 |
| | QG56 | Lung | 8 | 1 | | A2601/ |
| Hepatocarcinoma | KMCH-1 | Liver | | 0 | 0 | A1101/3101 |
| Ovarian carcinoma | TOC-2 | Ovarium | | 0 | 1 | A26/A3302 |
| Transformed cells | COS7 | | 4 | 1 | | |

*a*) Cytotoxicity of the IL-2-activated TIL from three HLA-A24$^+$ patients (cases 2, 3 and 4) with colon cancer against different types of cell lines was measured in a 6-h $^{51}$Cr-release assay at an effector-to-target cell ratio of 20. Representative results are shown.

A2402 cDNA (122 pg/ml) as compared to those by recognition of the parental KOC-7C tumor cells (82 pg/ml) and KOC-7C transfected with HLA-A2601 (71 pg/ml). All these results suggest that IL-2-activated TIL of case 1 contained HLA-A2402-restricted CTL recognizing antigens expressed on both adenocarcinoma and SCC. These TIL might also contain CTL reacting to HLA-A3302$^+$ adenocarcinoma and SCC.

IL-2-activated TIL from the other three HLA-A24$^+$ patients (cases 2 to 4) were tested for cytotoxicity against 18 different tumors and COS7 cells at around 50 days of culture (Table IV). IL-2-activated TIL from all three cases (75, 47, 15% CD3$^+$CD4$^-$CD8$^+$ T cells, respectively) primarily lysed HLA-A2402$^+$ adenocarcinomas. The TIL of case 2 also showed modest levels of lysis against two HLA-A2402$^+$ SCC (TE-10 and TE-11). In contrast, neither HLA-A2402$^-$ tumors nor COS7 cells were lysed by any of these TIL. These results suggest that the IL-2-activated TIL of these three cases possessed HLA-A2402-restricted CTL primarily recognizing adenocarcinomas.

**HLA-A31-restricted CTL** TIL of one (case 5) of the two HLA-31$^+$ patients proliferated well, whereas those of the other did not (case 6). IL-2-activated TIL of case 5 (A26/A31, B51/$-$) showed significant levels ($>10\%$) of cytotoxicity against three HLA-A3101$^+$ adenocarcinomas (LC-1, MKN28, and HST-2) and one HLA-A3101$^+$ SCC (HSC-4) (Table II). A kinetic study

showed that this CTL line did not lyse any of the tumors tested until 40 days of culture, and began to show significant lysis against HLA-A3101$^+$ LC-1 adenocarcinoma at 50 and 60 days of culture (data not shown). This CTL line, however, failed to lyse HLA-A3101$^-$ HCT116 tumor cells throughout the culture period (data not shown). None of the others, including HLA-A3101$^+$ KMCH-1 hepatocarcinoma cells and K562 cells, was lysed by this CTL. These results suggest that the IL-2-activated TIL of case 5 showed HLA-A31-restricted cytotoxicity against both adenocarcinoma and SCC.

## DISCUSSION

This study has provided evidence that IL-2-activated TIL of colon cancer contained HLA-A2402-restricted CTL recognizing antigens expressed on adenocarcinomas at the tumor site of colon cancer. The existence of HLA-class I-restricted CTL recognizing adenocarcinomas in colon cancer was suggested in several previous reports.[13, 14] However, other studies have yielded conflicting results.[15, 16] We have now obtained the first clear evidence for HLA-class I-restricted CTL recognizing adenocarcinomas.

Thirty-nine HLA-class I-identified allogenic tumor cell lines, including 5 colon cancers, 2 virus-transformed cell lines and 1 normal cell line were used as target cells for functional studies of TIL of solid tumors, from which autologous tumor cell lines were genetically difficult to establish. Because no autologous tumor cells were available, the method employed in this study cannot provide direct evidence that these IL-2-activated TIL are cytotoxic against autologous tumor cells, even though they showed HLA-class I-restricted cytotoxicity.

TIL of case 1 (stage II) proliferated well *in vitro* under the conditions employed and those of the other four cases (stage III–IV) proliferated modestly. All these tumors were histologically adenocarcinomas, and there were no obvious histological differences among them. Therefore, TIL of relatively early-stage colon cancer might have greater potential to proliferate *in vitro* than those of late stage.

Tumor antigens of colon cancers recognized by host CTL have not yet been identified, although a number of melanoma antigens have been determined.[5–12] Therefore, the nature of the tumor antigens recognized by either HLA-A2402 or -A31-restricted CTL is not known at the present time. However, considering the cytotoxic profile of these CTL, there might be two different types of antigens on colon cancers. One might be an adenocarcinoma-specific antigen like melanoma antigen (MART1/Melan A, gp100, or tyrosinase) that is expressed only on melanoma and melanocytes.[6–9] This type of antigen might be recognized by the HLA-A2402-restricted CTL lines of cases 3 and 4. CTL clone 10 recognized both HLA-A2402$^+$ SW620 and PC-9 adenocarcinomas, whereas CTL clone 5 recognized only SW620 tumor cells, suggesting that the latter clone reacted to an antigen expressed only on SW620 tumor cells.

The other antigen might be a tumor-specific shared antigen like MAGE, GAGE, BAGE, or NA 17-A that is widely expressed on cancers with different types of histology.[5, 10–12] This type of antigen might be recognized by the HLA-A2402-restricted CTL lines of cases 1 and 2. Ubiquitous antigens such as *HER2/neu*[19] are an alternative candidate.

A kinetic study showed that the IL-2-activated TIL of case 1 or 5 began to show HLA-A2402- or -A31-restricted cytotoxicity at day 20 or day 50 of culture, respectively. This difference could be explained by the different numbers of CTL in the samples. Namely, CTL frequency in the TIL of case 1 may have been higher than that of case 5.

Antigen frequency of HLA-A24 (or 31) in Yugoslavians (Caucasians), North Americans (Amerinds), South Africans (blacks in Cape Town) and Japanese is 20.3% (3%), 35.4% (47.4%), 12% (2.8%) and 57.9% (15.4%), respectively. HLA-A2402 is the dominant subtype of HLA-24 in Japanese.[20] In conclusion, we have presented evidence for the existence of HLA-A2402-restricted and tumor-specific CTL in IL-2-activated TIL of all four HLA-A24$^+$ colon cancer patients tested. This information is helpful for understanding host immunity against colon cancer at the tumor site. Furthermore, these CTL could be a useful tool to identify tumor antigens expressed on HLA-A2402$^+$ colon cancer.

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