Molecular Basis of T Cell-mediated Recognition of Pancreatic Cancer Cells

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ABSTRACT

Pancreatic cancer continues to be a major unsolved health problem in the world. The prognosis of pancreatic cancer is extremely poor with a median survival of 3–4 months and the 5-year survival being 1–4%. This poor prognosis is primarily because of a lack of effective therapies, and thus development of new treatment modalities is needed. One of these treatments could involve specific immunotherapy, for which elucidation of the molecular basis of T cell-mediated recognition of cancer cells is required. We report here six different genes and 19 immunogenic epitopes from pancreatic adenocarcinoma cells and T-cell receptor β usage of HLA-A2-restricted CTL clones reacting to some of these epitopes. Sixteen of 19 epitopes were found to possess the ability to induce HLA-A2-restricted CTL activity in the peripheral blood lymphocytes of patients with pancreatic and also colon adenocarcinomas. These results should provide a scientific basis for the development of specific immunotherapy for pancreatic and colon cancer patients.

INTRODUCTION

Pancreatic cancer is one of the major causes of cancer death in the world, with ~27,000 deaths annually in the United States and 50,000 deaths in Europe (1). Only 1–4% of pancreatic cancer patients survive the disease; the incidence rates are virtually identical to the mortality rates. Therefore, the development of new treatment modalities is needed; one of which could involve specific immunotherapy. Molecules involved in specific immunity, including tumor rejection antigen genes, HLA class I-binding immunogenic epitopes capable of inducing CTLs, and TCRs have been identified in melanomas and other cancers in the past decade (2–10). These results have opened the door to peptide-based specific immunotherapy for advanced or metastatic cancer patients (11–13). However, little information is available regarding pancreatic cancer cells (14, 15). Here, we report on the molecular basis of T cell-mediated recognition of pancreatic cancer cells. These results should provide a scientific basis for the development of specific immunotherapy for pancreatic cancer patients.

MATERIALS AND METHODS

Generation of HLA-A2-restricted CTLs. The HLA-A2-restricted and tumor-specific CTL (OK-CTL) line was established from tumor-infiltrating lymphocytes of a patient with colon cancer (HLA-A0207/3101, HLA-B46/51, HLA-Cw1) by incubation with interleukin 2 (100 units/ml) for 6–8 days followed by incubation for 14 days of culture, the cells were restimulated with irradiated (30 Gy) tumor cells and an IFN-γ assay with ELISA (limit of sensitivity, 10 pg/ml) as reported previously (10). Genotypes of HLA class I alleles of the tumor cells were reported previously (8–10). HLA class I of PBMCs was serotyped by the conventional method, whereas HLA-A2 subtypes were determined by the sequence-specific oligonucleotide probe method and also by direct DNA sequencing. The surface phenotypes of CTLs were investigated by a direct immunofluorescence assay with FITC conjugated anti-CD3, anti-CD4, or anti-CD8 mAb (Nichirei, Tokyo, Japan) or anti-TCRβ mAb (WT31; Becton Dickinson, Mountain View, CA). To inhibit CTL activity, 20 μg/ml of anti-HLA-class I (W6/32, IgG2a), anti-HLA-A2 (BB7.2, IgG2b), anti-CD8 (Nu-Ts/C, IgG2a), anti-HLA-class II (H-DR-1, IgG2a), and anti-CD4 (Nu-Thi, IgG1) mAbs were used. Anti-CD13 (MCS-2, IgG2a) and anti-CD14 (JML-H14, IgG1) served as isotype-matched control mAbs.

Identification of the cDNA Clones. A previously reported expression cloning method (8–10) was used to identify genes coding for tumor antigens recognized by the OK-CTLp. In brief, cDNA of Panc-1 was inserted into the expression vector pCMV-SPORT-2 (Life Technologies, Inc., Rockville, MD). HLA-A0207, HLA-A2402, or HLA-A2601 was amplified by reverse transcription-PCR and cloned into the pcR3 (Invitrogen, San Diego, CA). COS-7 cells (5 × 105) were cotransfected with the Panc-1 cDNA library and HLA-A0207 plasmid DNA by Lipofectamine (Life Technology) and cultured for 2 days, followed by addition of the OK-CTLp (5 × 105 cells/well). After an 18-h incubation, 10 μl of supernatant were collected to measure IFN-γ by ELISA. DNA sequencing was performed with a dideoxynucleotide sequencing method and analyzed by an ABI PRISM 377 DNA Sequencer (Perkin-Elmer, Foster, CA). The two clones of WHSC were obtained from a Panc-1 cDNA library by the standard colony hybridization method using the 32P-labeled cDNA clone no. 3 as a probe.

CTL Activity and TCR β Usage of OK-CTL Clones. The OK-CTL clones were established from the parental OK-CTLp line by methods reported previously (10). Cloned CTLs were tested for CTL activity to tumor cells, peptides were loaded on T2 cells and also to the COS-7 cells cotransfected with each of the six genes and HLA-A0207 plasmid DNA. Total RNA was isolated from CTL clones (5 × 106 cells), and cDNA was prepared. Single-stranded cDNA was amplified using one of the 22 different Vβ primers (Vβ1–20) and 3′ Cβ primer (16). PCR was performed for 35 cycles (at 94°C for 1 min, 58°C for 2 min, and 72°C for 3 min). The PCR product was inserted into the pcR2 (Invitrogen, San Diego, CA), followed by transformation into competent Escherichia coli cells, selection of colonies, and plasmid preparation for sequencing.

Peptides, CTL Assay, and CTL Induction. Peptides capable of binding to the HLA-A2 molecules were searched at the literature level with regard to peptides for HLA-A2 binding motifs (17), and 128 different peptides (>70% purity) were synthesized for screening. For further studies, 26 peptides with >95% purity were synthesized. The binding activity of peptides to HLA-A2, and ELISA. DNA sequencing was performed with a dideoxynucleotide sequencing method and analyzed by an ABI PRISM 377 DNA Sequencer (Perkin-Elmer, Foster, CA). The two clones of WHSC were obtained from a Panc-1 cDNA library by the standard colony hybridization method using the 32P-labeled cDNA clone no. 3 as a probe.

Received 8/4/00; accepted 1/9/01.

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The abbreviations used are: TCR, T-cell receptor; PBMC, peripheral blood mononuclear cell; mAb, monoclonal antibody; SCC, squamous cell carcinoma; EBV-B, EBV-transformed B cell; PHA, phytohemagglutinin; CDR, complementarity-determining region; MFI, mean fluorescence intensity.
same peptide at the same dose for 2 h. Effector cells were harvested at day 21 of culture and tested for both the surface phenotypes and the activity to produce IFN-γ in response to various target cells by ELISA. These PBMCs were further expanded in the presence of autologous antigen-presenting cells, interleukin 2, and a corresponding peptide, and the cells were retested for their surface phenotypes and CTL activity at around days 21–28 of reculture.

RESULTS

HLA-A2-restricted CTL Subline Used for Study. The HLA-A2-restricted CTL line (OK-CTL) was established from tumor-infiltrating lymphocytes of a patient HLA-A0207/3101 with colon adenocarcinoma. One of the sublines (OK-CTLp) with an 80% CD3⁺CD4⁻CD8⁺ phenotype (the remaining cells were CD3⁺CD4⁺CD8⁻) showed HLA-A2-restricted and tumor-specific CTL activity at day 58 of culture, as measured by both ⁵¹Cr release (Fig. 1A) and IFN-γ production assays (Fig. 1B). The OK-CTLp showed significant levels of cytotoxicity to HLA-A0201⁺ Panc-1 pancreatic adenocarcinoma cells and SW620 colon adenocarcinoma cells, HLA-A0206⁺ KE3 esophageal SCC cells, and HLA-A0207⁺ CA9–22 oral SCC cells but failed to lyse either HLA-A2⁺ tumor cells or autologous EBV-B cell line and PHA-activated T cells (Fig. 1A). The OK-CTLp also lysed all of the HLA-A2⁺ tumor cells tested (HLA-A0201⁺ R27 breast adenocarcinoma, HAK-2 hepatocellular carcinoma, SK-MEL-5 melanoma, SF126 astrocytoma, HLA-A0206⁺ PC9 lung adenocarcinoma, HLA-A0207⁺ 1–87 lung adenocarcinoma, and OMC-4 cervical SCC cells; data not shown). The CTL activity was inhibited by anti-HLA-class I, anti-CD8, or anti-HLA-A2 mAb but not by any other mAbs tested (Fig. 1B). This OK-CTLp, which showed the highest response to Panc-1 (Fig. 1B), was used as an indicator in the following experiments.

Identification of Genes. A total of 1 × 10⁷ cDNA clones from the cDNA library of Panc-1 and HLA-A0207 cDNA was cotransfected to COS-7 cells, followed by a test of their ability to stimulate IFN-γ production by the OK-CTLp. After repeated experiments, six cDNA clones, no. 1 to no. 6, were identified (Fig. 2A). This CTL line, however, did not react with COS-7 cells transfected with HLA-A0207 cDNA and cDNA clone no. 7 taken as a negative control or with COS-7 cells transfected with one of the cDNA clones no. 1 to no. 6 and HLA-A2402 or HLA-A2601 cDNA (Fig. 2A). The sequence of cDNA clone no. 1 registered in the GenBank (accession no. AB044550) was almost identical to that of the ubiquitin-conjugated enzyme variant Kua (UBE2V) gene (accession no. AF155120), and its deduced amino acids are slightly longer (3 amino acids at positions 109–111) than those of the UBE2V gene, the biological function of which has not yet been reported. The sequence of cDNA clone no. 2 (accession no. AB044547) was almost identical to that of the heterogeneous nuclear ribonucleoprotein L gene (HNRPL gene; accession no. NM 001533), but its deduced amino acids are longer at the NH₂-terminal at positions 1 to 31 than those of the HNRPL gene, which is one of the heterogeneous nuclear ribonucleoprotein complexes providing a substrate for the processing events that pre-mRNAs undergo before becoming functional and translatable mRNAs in the cytoplasm (19, 20). The sequence of cDNA clone no. 3 was 25 bp shorter at the 5′-end region than that of the Wolf-Hirschhorn syndrome candidate 2 protein (WHSC2) gene (accession no. AK001304). Subsequently, a full-length gene (accession no. AB044549) was obtained by the colony hybridization method using ³²P-labeled cDNA no. 3 as the probe. The deduced amino acid sequence of the obtained gene is 21 amino acids longer at positions 30–50 than that of the WHSC2 gene. The WHSC2 gene seems to play a role in the phenotype of WHS, a multiple malformation syndrome characterized by mental and developmental defects resulting from a partial deletion of the short arm of chromosome 4 (21). The nucleotide sequence of cDNA no. 4 (accession no. AB044548) was identical to that of eIF-4E-binding protein 1 gene (EIF4EBP1, accession no. NM 004095). This protein is known as a translation initiation factor that initiates insulin-dependent phosphorylation of 4E-BP1, making it available to form an active cap-binding complex (22). The nucleotide sequence of cDNA clone no. 5 (accession no. AB044546) was almost identical to that of the partial putative mitogen-activated protein kinase kinase gene (ppMAPkkk) gene (accession no. AJ242724), but its deduced amino acids include 230 amino acids at the NH₂-terminal and 258 amino acids at the COOH-terminal as compared with that of the ppMAPkkk gene, the function of which has not yet been reported. The sequence of cDNA no. 6 (accession no. AB044545) with 6767 bp long was almost identical to that of the 2′,5′-oligoadenylate synthetase 3 gene (2′,5′ OAS3 gene; accession no. NM006187) with a 13-amino acid difference at positions 18, 159, 249, 287, 288, 316, 393–398, and 984. The 2′-5′ OAS3 is known as an IFN-induced protein, which plays an important role in immunoprotection from microbacterial infection (23). These six genes containing full open reading frames were therefore selected for further investigation.
reading frames were all recognized by the OK-CTLp in a dose-dependent fashion (Fig. 2B). mRNA of these genes except for ppMAPkkk showed a similar expression pattern by Northern blot analysis. These genes were ubiquitously expressed in both cancer and normal cells, and their expression levels in cancer cells, including Panc-1, SW620, and CA9 –22 tumors, were significantly higher than those in the normal cells, including PHA-blastoid T cells and EBV-B cells (data not shown). However, mRNA expression of ppMAPkkk was scarcely detectable under the conditions used, which could have been attributable to rare expression, because only three clones were detected in the approximately 1 × 10⁶ cDNA library by colony hybridization using ³²P-labeled cDNA clone no. 5.

**Determination of Epitopes and TCR Usage.** Three-hundred T-cell clones were established from the parental OK-CTLp line. Eighty CTL clones among them showed HLA-A2-restricted and tumor-specific CTL activity (data not shown). All of these 80 CTL clones expressing CD3⁺CD4⁻CD8⁺ and TCR αβ⁺ phenotypes (data not shown) were tested for their reactivity to the six gene products. Among them, 2, 3, 1, 3, 2, and 4 CTL clones were reactive to UBE2V, HNRPL, WHSC2, EIF4EBP1, ppMAPkkk, and 2-5 OAS3 gene products, respectively. Representative data are shown in Table 1. Each of 27, 17, 21, 5, 19, or 39 different synthesized peptides with HLA-A2 molecule-binding motifs derived from the six gene products, respectively, was loaded onto the T2 cells, followed by testing for their ability to induce IFN-γ release by the OK-CTLp and its CTL clones. Five peptides of UBE2V at positions 43–51, 64 –73, 85–93, 201–209, and 208 –216 were recognized by the OK-CTLp, whereas the two peptides at positions 43–51 and 64 –73, but not any of the other 25
peptides, were strongly and dimly recognized by the CTL clone 2-2-H3, respectively (Fig. 3A, upper left column). Four peptides of HNRP1 at positions 140–148, 404–412, 443–451, and 501–510 were recognized by the OK-CTLp, whereas one peptide at positions 140–148, but not any of the other 16 peptides, was recognized by the CTL clone 1-2-D12 (Fig. 3A, middle left column). Similarly, the peptides recognized by the OK-CTLp were as follows: four peptides of WHSC2 at positions 103–111, 141–149, 157–165, and 267–275; two peptides of EIF4EBP1 51–59 and 52–60; three peptides of ppMAPkkk 290–298, 294–302, and 432–440; and one 2-5 OAS3 peptide lysed both the HLA-A2* tumor cells and the EBV-B cells but not the HLA-A2* tumor nor the PHA-blastoid T cells (Fig. 4A). PBMCs stimulated by each of UBE2V 43–51, 85–93, 208–216, HNRP1 140–148, 443–451, and 501–510; WHSC2 103–111, 141–149, and 267–275; EIF4EBP1 51–59 and 52–60; and ppMAPkkk 294–302 peptides lysed both the HLA-A2* tumor cells and the EBV-B cells but not the HLA-A2* tumor nor the PHA-blastoid T cells (Fig. 4A). In contrast, the PBMCs stimulated by a 2-5 OAS3 666–674 peptide were highly cytotoxic to the EBV-B cells but not any of the other target cells tested. We then investigated whether the peptide-induced CTLs were cytotoxic to the autologous PHA-blastoid T cells in the presence of excess amounts of a corresponding peptide. T2 cells served as a positive control. The PBMCs stimulated by any of the peptides tested were not cytotoxic to the PHA-blastoid T cells, even in the presence of excess amounts of a corresponding peptide, whereas they were cytotoxic in a dose-dependent manner to the peptide-pulsed T2 cells (Fig. 4B).

DISCUSSION

The OK-CTLp used for the study likely consisted of a mixture of CTL clones recognizing the shared tumor epitopes capable of binding to the HLA-A2 subtypes and were expressed on various cancers originating from different organs. Indeed, the six genes and 19 immunogenic epitopes were identified with this CTL subline. Furthermore, the results provide clear evidence that different CDR3 of the CTL clones are responsible for the recognition of different epitopes from the six gene products. There have been conflicting reports regarding the usage of TCR of CTLs reacting to melanoma cells in the early 1990s. Several studies have resulted in the observation of clonal usage (25, 26), whereas others, including our study (16), have observed polyclonal usage of CTLs (27, 28). However, recent studies using HLA class I-restricted and peptide-specific CTL clones have found evidence of multiple specificities in the repertoire of tumor-reactive CTLs (5). Therefore, CTLs at the tumor sites would consist of a mixture of CTLs with many different TCR usages reacting to many different epitopes on HLA class I-A alleles of tumor cells.

Among the six identified gene products, UBE2V, HNRP1, and EIF4EBP1 are known as RNA- or DNA-binding proteins that are involved in cellular proliferation (19, 20, 22). A ppMAPkkk gene might also be involved in cellular proliferation if involved in the regulation of the MAPk gene (29). A mutated MAPK gene encodes tumor epitopes recognized by the CTLs in a murine model (30). We have reported other growth-related proteins (cyclophilin B, SART1, and SART3), all nonmutated forms that also include immunogenic epitopes recognized by the HLA-A24-restricted CTLs (8–10). These epitopes recognized by the HLA-A24-restricted CTLs (8–10). These epitopes might also be involved in cellular proliferation if involved in the regulation of the MAPk gene (29). A mutated MAPK gene encodes tumor epitopes recognized by the CTLs in a murine model (30). We have reported other growth-related proteins (cyclophilin B, SART1, and SART3), all nonmutated forms that also include immunogenic epitopes recognized by the HLA-A24-restricted CTLs (8–10). These growth-related proteins would be vigorously synthesized, used, and then processed in cancer cells. Subsequently, the processed peptides might be loaded onto HLA-A2 molecules from T cells over the level

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**Table 1** Recognition of CTL clones reacting to one of the six gene products*<sup>a</sup>

| Stimulator cells: COS-7 transfected with HLA-A0207 cDNA and | CTL clone | UBE2V | HNRP1 | WHSC2 | EIF4EBP1 | ppMAPkkk | 2-5 OAS3 | no cDNA | Panc-1-
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* Fifteen CTL clones were tested for their ability to produce IFN-γ by recognition of COS-7 cells that were cotransfected with one of the six genes and HLA-A0207 cDNA at an E:T ratio of 1.
of immunological ignorance, with these molecules in turn possibly being recognized by the T cells.

Among the 16 peptides with the ability to induce CTLs, 13 induced the HLA-A2-restricted CTLs reacting to all of the three tumor cell lines tested, with the remaining 3 inducing the CTLs reactive only to certain tumors. This difference might have in part been attributable to the number of epitopes on the HLA-A2 molecules of certain tumor cells. Alternatively, it could have been attributable to a lack of...
tumor-cell molecules involved in the processing and export of these particular epitopes (31). In contrast to the 16 peptides, the three peptides failed to induce CTLs. This difference might have in part been attributable to the different levels of CTL precursors reacting to peptides in circulation. Relatively large numbers of CTL precursors recognizing each of the 16 peptides with the ability to induce CTLs might be present in the circulation of cancer patients, whereas the number of precursors reacting to the latter three peptides might have been too small to be detected by the used assay for CTL induction. Alternatively, the difference might have been attributable to an immunological tolerance to certain peptides. The binding affinity of each peptide to HLA-A2 molecules might not significantly influence the ability to induce CTLs, because these binding affinities, although different from each other, did not well correlate with the ability to induce CTLs.

SW620 cells were more susceptible to lysis by the peptide-induced CTLs and also by the OK-CTLp as compared with the susceptibility of Panc-1. This might have in part been attributable to the higher expression levels of HLA-A2 molecules on SW620 tumor cells (data not shown). In addition, HLA-A2 molecules have been found to be undetectable on the cell surface of some (10–20%) Panc-1 cells (data not shown). HLA-A24+ EBV-B cells were susceptible to lysis by the CTLs induced by the peptides from EIFEBP1, ppMAPkkk, and 2-5 OAS3 but not any of the other peptides tested. We reported previously that the HLA-A24-restricted CTLs induced by a cyclophilin B peptide, but not those by a SART3 peptide, were cytotoxic to autologous EBV-B cells (9, 10). The mechanisms of the different susceptibilities of EBV-B cells to these peptide-induced CTLs are presently unknown. In contrast to EBV-B cells, PHA-activated T cells were resistant to lysis by the peptide-induced CTLs in all of the cases tested. These results are also consistent with our previous observations on the peptide-induced, HLA-A24-restricted CTLs (9, 10). Several molecules in activated T cells, including a family of serpins, might be involved in their resistance to CTL-mediated lysis (32, 33).

We have reported in this study the six genes and 16 immunogenic epitopes capable of inducing HLA-A2-restricted and tumor-specific CTLs in PBMCs from pancreatic and/or colon cancer patients. Both Panc-1 and SW620 tumors were found to be susceptible to lysis by both the OK-CTLp and the peptide-induced CTLs. These results suggest that pancreatic and colon cancers share the same tumor epitopes recognized by the host CTLs. The incidence and number of cancer deaths from colon cancer are five to six times and two to three times higher than those of pancreatic cancers (34, 35), respectively. Although resection for cure is possible in 70–75% of all colon cancer patients, 50% still die from their disease regardless of the many different treatments, and thus, the development of new treatment strategies is warranted.

Table 2 TCRβ usage of the OK-CTL clones

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<td>IYFNNVPIDDSNSERFSKMNFSFSTLKLQFSPRSASAYFCAS</td>
<td>ERVAGKFQGTRTVL</td>
<td>EDLKVVFPE</td>
<td></td>
</tr>
<tr>
<td>0.5-1-H2</td>
<td>ppMAPkkk 432–440</td>
<td>18</td>
<td>1.1</td>
<td>1.1</td>
<td>1</td>
<td>DESGMPKIFKSAFPEEPSLILIQVQGQGTRTVL</td>
<td>EDLKVVFPE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2-D1</td>
<td>2-5 OAS3 666–674</td>
<td>14</td>
<td>2.1</td>
<td>2.3</td>
<td>2</td>
<td>VSREKHERFSLIESASTQHSMYLCAS</td>
<td>GSGTDQVGTRTVL</td>
<td>EDLKVVFPE</td>
<td></td>
</tr>
<tr>
<td>2-2-B4</td>
<td>2-5 OAS3 666–674</td>
<td>14</td>
<td>2.1</td>
<td>2.3</td>
<td>2</td>
<td>VSREKHERFSLIESASTQHSMYLCAS</td>
<td>GSGTDQVGTRTVL</td>
<td>EDLKVVFPE</td>
<td></td>
</tr>
</tbody>
</table>

a Each immunogenic epitope reactive to each CTL clone is shown. The data of reactivity are presented in Fig. 3.

b The underline shows the CDR3 of the TCRβ of each CTL clone.
Table 3. Induction of HLA-A2-restricted CTL activity by the peptides in PBMCs

<table>
<thead>
<tr>
<th>Peptide</th>
<th>MFI</th>
<th>CD4(%)</th>
<th>CD8(%)</th>
<th>IFN-γ production (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>in response to</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QG56</td>
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<td></td>
<td></td>
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<tr>
<td>RERF-LC-MS</td>
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<tr>
<td>COLO320</td>
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</tr>
<tr>
<td>SW620</td>
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<tr>
<td>CA9-22</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Panc-1</td>
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<td></td>
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</tr>
<tr>
<td>Panc-1 + anti-classI</td>
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<td></td>
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<tr>
<td>Panc-1 + anti-classII</td>
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<td></td>
<td></td>
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<tr>
<td>Panc-1 + anti-CD4</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Panc-1 + anti-CD8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Panc-1 + anti-HLA-A2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**UB2EV 43-51**
- MFI: 571
- CD4: 9.9%
- CD8: 84.3%
- IFN-γ production: 0 pg/ml

**UB2EV 64-73**
- MFI: 607
- CD4: 12.0%
- CD8: 83.9%
- IFN-γ production: 0 pg/ml

**UB2EV 85-93**
- MFI: 910
- CD4: 21.6%
- CD8: 75.8%
- IFN-γ production: 0 pg/ml

**UB2EV 201-209**
- MFI: 1008
- CD4: 15.6%
- CD8: 81.2%
- IFN-γ production: 0 pg/ml

**UB2EV 208-216**
- MFI: 637
- CD4: 16.6%
- CD8: 81.0%
- IFN-γ production: 0 pg/ml

**HNRPL 140-148**
- MFI: 819
- CD4: 7.7%
- CD8: 85.8%
- IFN-γ production: 0 pg/ml

**HNRPL 404-412**
- MFI: 783
- CD4: 15.2%
- CD8: 80.7%
- IFN-γ production: 0 pg/ml

**HNRPL 443-451**
- MFI: 499
- CD4: 14.2%
- CD8: 79.0%
- IFN-γ production: 0 pg/ml

**HNRPL 501-510**
- MFI: 852
- CD4: 18.1%
- CD8: 78.1%
- IFN-γ production: 0 pg/ml

**WHSC2 103-111**
- MFI: 504
- CD4: 10.8%
- CD8: 75.4%
- IFN-γ production: 0 pg/ml

**WHSC2 141-149**
- MFI: 1089
- CD4: 10.7%
- CD8: 83.1%
- IFN-γ production: 0 pg/ml

**WHSC2 157-165**
- MFI: 780
- CD4: 9.1%
- CD8: 87.9%
- IFN-γ production: 0 pg/ml

**WHSC2 267-275**
- MFI: 656
- CD4: 19.7%
- CD8: 77.1%
- IFN-γ production: 0 pg/ml

**EIF-4EBP1 51-59**
- MFI: 591
- CD4: 13.2%
- CD8: 86.8%
- IFN-γ production: 0 pg/ml

**EIF-4EBP1 52-60**
- MFI: 789
- CD4: 13.0%
- CD8: 85.6%
- IFN-γ production: 0 pg/ml

**ppMAPkk 290-298**
- MFI: 887
- CD4: 12.7%
- CD8: 79.5%
- IFN-γ production: 0 pg/ml

**ppMAPkk 294-302**
- MFI: 660
- CD4: 25.4%
- CD8: 64.2%
- IFN-γ production: 0 pg/ml

**ppMAPkk 432-440**
- MFI: 657
- CD4: 44.5%
- CD8: 53.0%
- IFN-γ production: 0 pg/ml

**2-5 OAS3 666-674**
- MFI: 775
- CD4: 92.3%
- CD8: 3.0%
- IFN-γ production: 0 pg/ml

**No peptide**
- MFI: 491
- CD4: 18.0%
- CD8: 72.3%
- IFN-γ production: 0 pg/ml

---

**a** Percentage of CD3<sup>+</sup>CD4<sup>+</sup>CD8<sup>+</sup> or CD3<sup>+</sup>CD4<sup>+</sup>CD8<sup>+</sup> T cells of the peptide-stimulated PBMCs at the time of assay.

**b** The PBMCs of a patient with colon adenocarcinoma, from which the OK-CTL clones were established, were stimulated in vitro with a peptide (10 μM) three times every 7 days, followed by a test for their ability to produce IFN-γ at day 21 of culture in response to various target cells at an E:T ratio of 5.

**c** For inhibition assay, the OK-CTL-mediated IFN-γ production by recognition of Panc-1 tumor cells at an E:T ratio of 5 was tested in the presence of 20 μg/ml of mAbs shown in the Table.
Fig. 4. Induction of CTLs by peptides. A, PBMCs from the cancer patients were stimulated without peptides or with one of the 11 different peptides (10 μM) shown in the figure, and then there was a test of their cytotoxicity against HLA-A2+ Panc-1 and SW620, HLA-A2+ RERF-LC-MS, the autologous EBV-B cells, and PHA-blastoid T cells by a 6-h 51Cr-release assay at three E:T ratios. Values are the means of triplicate assays. B, some of the peptide-induced CTLs used in A were tested by a 6-h 51Cr-release assay for their cytotoxicity to T2 cells or the autologous PHA-blastoid T cells that were pulsed with various doses of a corresponding peptide used for stimulation.
modalities is needed. The HLA-A2 allele is found in 23% of African Blacks, 53% of Chinese, 40% of Japanese, (36) and 50% of Caucasians (37). The information presented in this study should provide a better understanding of the molecular basis of T-cell-mediated recognition of pancreatic cancer cells and also of colon cancer cells. Furthermore, the molecules identified in this study could be an appropriate target for use in specific immunotherapy for a large number of cancer patients.

REFERENCES


Molecular Basis of T Cell-mediated Recognition of Pancreatic Cancer Cells

Masaaki Ito, Shigeki Shichijo, Naotake Tsuda, et al.


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