DETECTION AND INDUCTION OF CYTOTOXIC T LYMPHOCYTES SPECIFIC FOR SYT-SSX- DERIVED PEPTIDES IN HLA-A24+ PATIENTS WITH SYNOVIAL SARCOMA

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INTRODUCTION.
Current immunotherapeutic strategies depend largely on identification of tumor antigenic peptides. Although a number of antigenic peptides have been identified by using autologous tumor cell-CTL pairs, the difficulty in establishing such autologous pairs has hampered identification of antigenic peptides from bone and soft tissue sarcomas. Recently, chimeric fusion genes resulting from tumor-specific chromosomal translocations have been identified in several types of soft tissue sarcomas including SYT-SSX in synovial sarcoma. These chimeric genes have been considered to play a key role in the genesis of soft tissue sarcomas. At the same time, identification of the chimeric genes has provided a unique opportunity to examine the immunological response against the tumor-specific chimeric sequences, which serve as putative antigenic peptides. In the present study, we chose synovial sarcoma as a representative tumor of soft tissue sarcomas with chromosomal translocation and investigated the immunogenic property of synthetic peptide derived from the SYT-SSX fusion gene sequence.

MATERIALS AND METHODS.
The entire sequence of SYT-SSX1 and SYT-SSX2 fusion genes was searched for identifying regions that contain anchor motif residues required for binding to HLA-A24 class I molecules (the most common allele in the Japanese population and also frequently present in Caucasians). Consequently, 4 synthetic peptides were designed. Two peptides, SS391 (PYGYDQIMPK) and SS393 (GYDQIMPKK), were derived from the breakpoint and the remaining 2 peptides, SS449a (AWTHRLERK) and SS449b (AWTHRLERK), from an SSX region. The immunogenic property of these SYT-SSX peptides were analyzed with respect to (i) binding activity to HLA-A24 molecules, (ii) frequency of cytotoxic T lymphocyte precursors (CTLp) that specifically interact with the SYT-SSX peptides, and (iii) induction of CTLs by in vitro peptide stimulation. (i) The binding activity of peptides was evaluated by flowcytometric analysis. In this assay, mean fluorescence intensity (MFI) of HLA-A*2402 molecules on RMA-S-A*2402/K cells after peptide pulsation (100ng/mL), which reflected the property of peptides to stabilize HLA-A*2402 molecules on the cell surface, was regarded as the binding activity. (ii) Peripheral blood samples were obtained from 10 normal donors, 16 synovial sarcoma patients, and 10 patients with other sarcomas. Frequency of CTLp to the synthetic peptides was determined using peptide/HLA-A24 tetramers (SS391/HLA-A24, SS393/HLA-A24, SS449a+HLA-A24). Peripheral blood mononuclear cells (PBMCs) were stained with tetramers and FITC-conjugated anti-CD8 monoclonal antibody and analyzed by two color FACS. The frequency of CTLp was calculated as the number of tetramer positive cells / the number of CD8+ cells. Also, association between increased frequency of CTLp and clinical parameters of 16 synovial sarcoma patients was analyzed with respect to age, gender, and the state of primary tumor, pulmonary metastasis and chemotherapy using the Student t-test and the Fisher’s probability test. (iii) CD8+ T cells isolated from PBMCs of 4 HLA-A24+ synovial sarcoma patients were induced for CTL activity by in vitro stimulation with peptides. The cytotoxic activity of stimulated CD8+ T cells was measured using a conventional 51Cr-release assay. Cell lines used as target were synovial sarcoma cell lines (Fuji, HS-SY-II, SW982-A24, K562) and lymphoblastoid cell lines (CIR-A*2402 and CIR-A*31012). After a 4-h incubation of the labeled target cells and stimulated CD8+ T cells, the release of the 51Cr label was measured by collecting the supernatant, followed by quantification in an automated gamma counter.

RESULTS
(i) Among the SYT-SSX-derived peptides, pulsation of SS393 led to the highest MFI (0.8), whereas other 3 peptides showed relatively low MFI (SS391: 0.15, SS449a: 0.2, and SS449b: 0.1). (ii) According to the average number and standard deviation of the frequency of CTLps for the synthetic peptides in 10 healthy individuals and 10 other sarcoma patients (0.11±0.09), the frequency of 0.25% or higher was determined as significantly high. Such high CTLp frequency was observed in none of 10 healthy donors, a patient with Ewing’s sarcoma and 6 out of the 16 synovial sarcoma patients. Notably, all of the 6 synovial sarcoma patients who showed high CTLp frequency to the SYT-SSX peptides had either a present or past history of pulmonary metastasis. Statistical analysis revealed the significant association of the increased CTLp frequency to the SYT-SSX peptides with development of pulmonary metastasis (p<0.03). (iii) CTLs were induced from PBMCs of 2 synovial sarcoma patients who showed high CTLp frequency to the peptides. 51Cr-release assay using CIR-A*2402 cells and CIR-A*31012 cells, which had been pulsed with peptides, revealed peptide-specific, HLA-restricted, cytotoxic activity. CTLs induced with the peptide cocktail of SS391+SS393 lysed synovial sarcoma cell lines, Fuji and HS-SY-II (Figure). On the contrary, no such cytotoxic activity was observed against K562 cells or SW982-A24 synovial sarcoma cells that lacked SYT-SSX.

DISCUSSION
In the present study, we found that 1 or more of 4 SYT-SSX-derived peptides reacted in the context of HLA-A24/peptide tetramer with 0.25% or more of circulating CTLp in 6 (66.7%) out of 9 synovial sarcoma patients who had either a present or past history of pulmonary metastasis. In addition, CTLs were induced from 2 synovial sarcoma patients who showed high CTLp frequency, which lysed HLA-A24+ synovial sarcoma cells expressing SYT-SSX as well as the peptide-pulsed target cells in an HLA class I restricted manner. These findings suggest that aberrantly expressed SYT-SSX gene products have primed SYT-SSX-specific CTLp in vivo and increased their frequency in synovial sarcoma patients, especially during the process of systemic blood borne metastasis. The identification of the SYT-SSX peptides offers the opportunity to design peptide-based immunotherapeutic approaches that might prove to be effective in treating HLA-A24 positive patients with SYT-SSX-positive synovial sarcoma.