IMPROVED INDUCTION OF SARCOMA-REACTIVE CTLs BY SYT-SSX JUNCTION PEPTIDE/HLA-A*2402 COMPLEX ANCHOR SUBSTITUTION

INTRODUCTION
Synovial sarcoma is an aggressive malignancy that accounts for 5-10% of soft tissue sarcomas. Since SYT-SSX1 was first identified as a resultant fusion gene of a chromosomal translocation, t(X;18)(p11;q11), accumulated evidence has consistently supported the significance of SYT-SSX genes in the pathogenesis and diagnosis of synovial sarcoma. Using synovial sarcoma as a prototype of translocation-associated sarcomas, we have analyzed the immunogenic properties of SYT-SSX gene-derived peptides. Of four peptides synthesized, a junction peptide, termed B peptide, was most immunogenic as determined by the affinity for HLA-A24 molecule and reactivity to circulating T lymphocytes in HLA-A24+ synovial sarcoma patients (1). Nevertheless, even B peptide exhibited relatively low affinity and reacted with only 38% of patients. To improve its immunogenicity, we in this study introduced single amino acid substitutions at an HLA-A24 anchor residue of B peptide and evaluated their immunogenic property compared with the parental B peptide.

MATERIALS AND METHODS
Cell lines and peptides
Synovial sarcoma cell lines (Fuji, HS-SY-II, and SW982), an erythroblast leukemia cell line (K562), and a T-B Lymphoblast hybrid transfected with HLA-A2420 (T2-A*2402) were used. A 9-mer peptide, (B : GYDQIMPKK), spanning the SYT-SSX fusion region was synthesized previously. Single amino acid substitutions were introduced at the position 9, an HLA-A24 anchor residue, changing lysine to leucine (termed K9L), isoleucine (K9I), phenylalanine (K9F), and tryptophan (K9W) according to the preferred HLA-A24 binding motif.

Peptide-binding assay
The affinity of peptides for HLA-A24 molecules was evaluated by cell surface class-I stabilization assay (2). T2-A*2402 cells were incubated with peptides at 1μM, 10μM and 100μM. Affinity of each peptide for HLA-A*2402 molecule was evaluated by percent mean fluorescence intensity (%MFI) increase of the HLA-A*2402 molecule in the following calculation. %MFI increase: [(MFI with the given peptide – MFI without peptide) / MFI without peptide] x 100.

Participants
The ethical committees of participating institutions approved this study. Peripheral blood samples were collected from 15 patients who had been diagnosed histologically with synovial sarcoma and had given informed consent. Of these, nine HLA-A24+ samples were subjected to the analysis.

CTL induction
Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples of HLA-A24+ synovial sarcoma patients using Lymphoprep (Nycomed, Oslo, Norway). On days 7, 14, 21, and 28, CD8+ T cells were repeatedly stimulated with PHA-activated CD8+ PBMCs, which had been pulsed with the peptides (50 μg/ml) and human γ2 microglobulin (25 μg/ml).

Cytotoxicity assay
The cytotoxic activity of stimulated CD8+ T cells was measured by 6-h 51Cr release assay. Target cell lines (Fuji, HS-SY-II, SW982, and K562) were labeled with 51Cr. T2-A*2402 cells were pulsed with 10 μM peptides (SS393 or K9I) and then by labeling with 51Cr. The percentage of specific cytotoxicity was calculated as the percentage of specific 51Cr release: [(experimental 51Cr release - spontaneous 51Cr release) / (maximum 51Cr release - spontaneous 51Cr release)] x 100.

Tetramer analysis
We constructed three HLA-A24/peptide tetramers (HLA-A24/B, HLA-A24/K9I, HLA-A24/HIV). Flow cytometric analysis was performed performed for PBMCs from five patients. Reactivity with HLA-A24/peptide tetramers was compared between PBMCs and those stimulated in vitro four times with B and K9I.

RESULTS
Affinity of Agretope-modified SYT-SSX peptides for HLA-A24 molecule
In a comparison between the agretope-modified peptides and the natural SS393, all four agretope-modified peptides showed higher MFI increases than did the B when pulsed to HLA-A2420 cells. Notably, K9I had the highest affinity to HLA-A2420 molecules, with MFI increases of 60% or more.

Induction of CTLs from HLA-A24+ synovial sarcoma patients
We subsequently examined the ability of peptides to induce CTLs, focusing on K9I. CTLs induced with K9I showed higher cytotoxicity against B-pulsed T2-A*2402 cells than those induced with B. Moreover, CTLs induced with K9I showed higher cytotoxicity against HLA-A2420 and SYT-SSX-bearing synovial sarcoma cell lines, Fuji and HS-SY-II, than did those induced with B (Figure). Moreover Synovial sarcoma-specific CTLs were successfully induced from 12 patients (80%) with K9I. In contrast, such CTLs were inductible from 7 patients (47%) with B.

Crisisscross-reactivity of CTLs induced with B and K9I
In vitro stimulations with K9I increased the frequency of T cells reacting with both HLA-A24/K9I and B tetramers. Conversely, in vitro stimulations with B resulted in increases in frequency of T cells reacting with both HLA-A24/K9I and B tetramers.

DISCUSSION
In the present study, we demonstrated (i) that substitutions of an HLA-A24 anchor residue (position 9) in the SYT-SSX natural junction peptide, B, enhanced the affinity for HLA-A24 molecule in all four peptide analogues examined, and (ii) that substitution of lysine to isoleucine most obviously enhanced the affinity for HLA-A24 molecule and reactivity to circulating T lymphocytes bearing synovial sarcoma cell lines, Fuji and HS-SY-II, than did those induced with B (Figure). Moreover Synovial sarcoma-specific CTLs were successfully induced from 12 patients (80%) with K9I. In contrast, such CTLs were inducible from 7 patients (47%) with B.

(Figure)

References