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Different immunodominance of HIV-1-specific CTL epitopes among three subtypes of HLA-A*26 associated with slow progression to AIDS

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Abstract

It is speculated that HLA-A*26-restricted HIV-1-specific CTLs can control HIV-1, since HLA-A*26 is associated with a slow progression to AIDS. In three major HLA-A*26 subtypes, HLA-A*2601-restricted, and HLA-A*2603-restricted HIV-1 epitopes have been identified, but HLA-A*2602-restricted ones have not. We here identified HLA-A*2602-restricted HIV-1 epitopes by using reverse immunogenetics and compared the immunodominance of the epitopes among the three subtypes. Out of 110 HIV-1 peptides carrying HLA-A*26 anchor residues, only the Gag169-177 peptide, which had been previously identified as an HLA-A*2601- and HLA-A*2603-restricted immunodominant epitope, induced Gag169-177-specific $CD8^+$ T cells from only two of six HLA-A*2602^+ HIV-1-infected individuals. No difference in affinity of this epitope peptide was found among these three HLA-A*26 subtypes, indicating that Gag169-177 was effectively presented by HLA-A*2602 but recognized as a subdominant epitope in HIV-1-infected HLA-A*2602^+ individuals. These findings indicate different immunodominance of Gag169-177 epitope among 3 HLA-A*26 subtypes.

Keywords: HLA-A*26; HLA-A*2602; HIV-1; Epitope; CTL

Human immunodeficiency virus type-1 (HIV-1)-specific cytotoxic T lymphocyte (CTL) responses play an important role in the control of HIV-1 infections [1–5]. However, it is thought that HIV-1 can escape from the host immune system, since it fails to completely eradiate HIV-1 from infected individuals. There are several proposed mechanisms that would allow HIV-1-infected cells to escape from being killed by HIV-1-specific $CD8^+$ T cells [6–11]. A mutation within the viral epitopes recognized by CTLs is one of these mechanisms [6,7]. Identification and characterization of HIV-1 CTL epitopes are therefore necessary for studies on the immunopathogenesis of AIDS. In addition, since HIV-1-specific CTLs are expected to suppress HIV-1 replication *in vivo*, characterization of these epitopes is also necessary for studies aimed at developing HIV-1 vaccines and immunotherapy to induce HIV-1-specific CTLs, either of which might be expected to prevent HIV-1 infection and the progression to AIDS.

HLA-A*26 is one of the alleles associated with a slow progression to AIDS [12]. Therefore, identification and characterization of HIV-1-specific epitopes presented by this allele are necessary for studies on the immunopathogenesis of AIDS and vaccine development. Three HLA-A*26 subtypes, i.e., HLA-A*2601, HLA-A*2602, and HLA-A*2603, are found at a gene frequency of 7.7%, 2.3%, and 1.5%, respectively, in the Japanese population [13]. We previously identified four HLA-A*2601- and two HLA-A*2603-restricted HIV-1 epitopes by using reverse immunogenetics [14,15]. Both HLA-A*2601 and -A*2603 presented one immunodominant epitope, Gag169-177

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(EVIPMFSAL), which overlaps with the HLA-B*57-restricted immunodominant epitope KF11 (KAFSPEVIPMF) [16]. Gag169-177-specific CTLs have been speculated to control HIV-1 replication.

In the present study, we sought to identify HLA-A*2602-restricted HIV-1 epitopes by using reverse immunogenetics and to compare them with HLA-A*2601- and HLA-A*2603-restricted ones [14,15].

Materials and methods

Cells. C1R and TAP-defective cells of mouse cell line RMA-S were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS). C1R cells expressing HLA-A*2601, -A*2602 or -A*2603 (C1R-A*2601, C1R-A*2602 or C1R-A*2603, respectively) were generated by transfecting the C1R cells with the HLA-A*2601, -A*2602 or -A*2603 gene, respectively [17]. RMA-S transfectants expressing HLA-A*2602 (RMA-S-A*2602) were previously generated [18]. C1R-A*2601, -A*2602 and -A*2603 were maintained in RPMI 1640 medium supplemented with 10% FCS and 0.2 mg/ml neomycin; and RMA-S-A* 2602, in RPMI 1640 medium supplemented with 10% FCS and 0.15 mg/ml hygromycin B.

Synthetic peptides. Sequences derived from four proteins of the human immunodeficiency virus type-1 SF2 strain (HIV-1: Env, Gag, Pol, and Nef) were screened for HLA-A*2602-binding motifs. Peptides were prepared by utilizing an automated multiple peptide synthesizer, with the Fmoc strategy followed by cleavage. The purity of the synthesized peptides was examined by mass spectrometry. Peptides with more than 90% of purity were used in the present study.

HLA-stabilization assay. Binding of HIV-1-derived peptides to HLA-A*2602 was measured as previously described by using RMA-S-A*2602 cells [18]. RMA-S-A*2602 cells were cultured for about 16 h. Then they were incubated with peptides at 26 °C for 1 h and subsequently at 37 °C for 3 h. Peptide-pulsed cells were stained with the HLA class I α_3 domain-specific mAb TP25.99 [19] and the FITC-conjugated IgG fraction of sheep anti-mouse Ig (Silenius Laboratories, Hawthorn Victoria, Australia). The mean fluorescence intensity (MFI) was measured by using a FACS Calibur (BD Bioscience, San Jose, CA, USA). HLA-A*2602-binding peptides were defined as those which at a concentration of 10^{-3} M caused a >25% increase in MFI compared with the MFI of control RMA-S-A*2602 cells cultured at 26 °C. The peptide concentration that yielded the half-maximal levels of the MFI was calculated and was reported as the BL50 value.

Patients. Blood samples were collected with informed consent from six HIV-1 clade B-infected patients with HLA-A*2602 (KO-003, KI-021, KI-030, KI-082, KI-382, and KI-478), 11 those with HLA-A*2601, and eight those with HLA-A*2603 at the AIDS Medical Center, National Hospital Organization, Osaka National Hospital or the AIDS Clinical Center, International Medical Center of Japan. Clinical stage of all patients tested was chronic one. Significant difference of CD4 count was not found among three subtype groups (HLA-A*2601:458±257, HLA-A*2602:564±299, HLA-A*2603:314±109). This study was approved by the ethical committees of Kumamoto University, International Medical Center of Japan, and Osaka National Hospital. Informed consent was obtained from all subjects, according to the Declaration of Helsinki.

Intracellular cytokine staining (ICC assay). After C1R-A*2601, C1R-A*2602 or C1R-A*2603 cells had been incubated for 60 min with each peptide (1 μM) or each peptide cocktail (1 μM concentration of each peptide), they were washed twice with RPMI-1640 containing 10% FCS. These C1R-A*26 cells and cultured PBMCs were incubated at 37 °C for 6 h at an effector-to-stimulator ratio of 1:4 after the addition of Brefeldin A (10 μg/ml). Next, the cells were stained with anti-CD8 mAb (DAKO Corporation, Flostrup, Denmark), fixed with 4% paraformaldehyde at 4 °C for 20 min, and then permeabilized at 4 °C for 10 min with PBS supplemented with 0.1% saponin containing 20% NCS (permeabilizing buffer). The cells were resuspended in the permeabilizing buffer and then stained with anti-IFN-γ mAb (BD Bioscience Pharmingen, San Diego, CA). The cells were finally resuspended in PBS containing 2% paraformaldehyde, and then the percentage of $CD8^+$ cells positive for intracellular IFN-γ was determined by using the FACSCalibur.

*ICC assay using C1R-A*2602 cells infected with recombinant HIV-1 vaccinia.* C1R-A*2602 cells were infected for 1 h at 37 °C with 10 plaque-forming units (per target cell) of recombinant vaccinia virus expressing HIV-1 SF2 Gag protein or of WT vaccinia virus and cultured for 16 h. These infected cells were washed twice with RPMI 1640 containing 10% FCS and then incubated with cultured effector cells at 37 °C for 6 h after the addition of Brefeldin A (10 μg/ml). The ability of the effector cells to produce IFN-γ was tested at an E:S ratio of 1:4. The cells were then stained with anti-CD8 mAb and anti-IFN-γ mAb.

Results and discussion

HLA-A*2602-binding peptides have two anchor residues, Val, Phe, Ile, Leu or Thr at position 2 and Tyr, Phe, Met or Leu at the C-terminus [17]. A previous study demonstrated that acidic amino acids (Asp and Glu) and a broad range of amino acids with the exception of positively charge amino acids function as an anchor at position 1 and the C-terminus, respectively [18]. Therefore, to identify HLA-A*2602-binding HIV-1 peptides, 8-mer to 11-mer sequences containing the anchor residues Asp or Glu at position 1, Val, Thr, Ile, Leu or Phe at position 2, and any amino acids except positively charged ones at the C-terminus were selected from the sequence of Gag, Pol, Nef, and Env proteins in the HIV-1 SF2 strain; and then 110 peptides matching these sequences were synthesized. The binding affinity of these synthesized peptides for the HLA-A*2602 molecule was tested by using the HLA-stabilization assay [18,20]. Representative results are shown in Fig. 1. Thirty-two out of these 110 peptides bound to HLA-A*2602 (Table 1). The frequency of HLA-A*2602-binding HIV-1 peptides was similar to that of HLA-A*2601- or HLA-A*2603-binding HIV-1 peptides previously identified by using the same 110 peptides [14,15].

PBMCs from three HLA-A^*2602^+ HIV-1-infected individuals (KO-003, KI-030 and KI-082) were stimulated

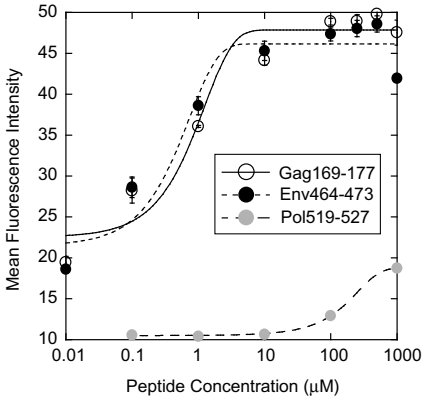

Fig. 1. Binding to HLA-A*2602 of HIV-1 peptides carrying HLA-A*2602 motif. Binding of the peptides carrying A*2602 anchors was measured by a stabilization assay using RMA-S-A*2602 cells. Representative results showing high-affinity peptides (Gag169-177 and Env464-473) and a very low-affinity peptide (Pol519-527) are given in this figure.

Table 1
HLA-A*2602-binding peptides

Sequence	Position	BL50[a]
EVFRPGGGDM	Env464-473	3.98×10^{-7}
EVIPMFSAL	Gag169-177	5.84×10^{-7}
ELKKIIGQV	Pol872-880	9.88×10^{-6}
EVNIVTDSQY	Pol647-656	1.21×10^{-5}
EVVIRSDNF	Env272-280	4.1×10^{-5}
ELNKRTQDF	Pol234-242	1.02×10^{-4}
EIKGEIKNCSF	Env147-157	1.62×10^{-4}
DIVIYQYMDDL	Pol332-342	1.94×10^{-4}
DTTNQKTEL	Pol626-634	2.78×10^{-4}
EIVASCDKCQL	Pol750-760	3.50×10^{-4}
ETVPVKLKPGM	Pol161-171	4.26×10^{-4}
ETWEAWWMEYW	Pol551-561	6.28×10^{-4}
ETWEAWWMEY	Pol551-560	$>1 \times 10^{-3}$
EICGHKAIGTV	Pol121-131	$>1 \times 10^{-3}$
EVVLGNVTENF	Env82-92	$>1 \times 10^{-3}$
DLRSLCLFSY	Env758-767	$>1 \times 10^{-3}$
DLWIYHTQGYF	Nef115-125	$>1 \times 10^{-3}$
EVIPLTEEA	Pol446-454	$>1 \times 10^{-3}$
ETPGIRYQY	Pol293-301	$>1 \times 10^{-3}$
EVYYDPSKDLV	Pol471-481	$>1 \times 10^{-3}$
EVYYDPSKDL	Pol471-480	$>1 \times 10^{-3}$
ETKLGKAGYV	Pol604-613	$>1 \times 10^{-3}$
EVHNVWATHAC	Env63-73	$>1 \times 10^{-3}$
EVQLGIPHPA	Pol244-253	$>1 \times 10^{-3}$
ELYPLTSLRS	Gag484-493	$>1 \times 10^{-3}$
DLNTMLNTV	Gag185-193	$>1 \times 10^{-3}$
DVKQLTEAV	Pol519-527	$>1 \times 10^{-3}$
ELYPLTSLRSL	Gag484-494	$>1 \times 10^{-3}$
DIQKLVGKL	Pol411-419	$>1 \times 10^{-3}$
DIAGTTSTL	Gag235-245	$>1 \times 10^{-3}$
ELRQHLLRW	Pol359-367	$>1 \times 10^{-3}$
DTKEALEKI	Gag96-104	$>1 \times 10^{-3}$

[a] The half maximal binding level was calculated as the peptide concentration yielding the half-maximal MFI.

in vitro for 14 days with a cocktail of HLA-A*2602-binding peptides containing 5–7 peptides (cocktail 1: Env464-473, Gag169-177, Pol647-656, Pol872-880, and Pol551-560; cocktail 2: Env272-280, Pol121-131, Env82-92, Pol551-561, Env758-767, Pol234-242, and Nef115-125; cocktail 3: Pol446-454, Pol293-301, Pol471-481, Pol471-480, Pol604-613, Env147-157, and Env63-73; cocktail 4: Pol244-253, Gag484-493, Gag185-193, Pol626-634, Pol519-527, Gag484-494, and Pol411-419; and cocktail 5: Gag235-243, Pol359-367, Pol161-171, Gag96-104, Pol750-760, and Pol332-342). IFN-γ production by each bulk culture in response to C1R-A*2602 prepulsed with the corresponding peptide cocktail was assessed by intracellular IFN-γ staining. All five cocktails failed to induce specific $CD8^+$ T cells among the cells in bulk culture obtained from patients KI-030 and KI-082. On the other hand, only cocktail 1 induced specific $CD8^+$ T cells among the cells in bulk culture obtained from patient KO-003 (Fig. 2A). To determine which peptides in the cocktail induced the specific $CD8^+$ T cells, we re-stimulated the cells of this bulk culture with C1R-A*2602 cells prepulsed with each single peptide in this cocktail to detect the specific $CD8^+$ T cells. Only the Gag196-177 peptide induced $CD8^+$ T cells producing IFN-γ (Fig. 2B).

To clarify whether Gag169-177 was a naturally occurring peptide, we investigated the ability of these peptide-specific $CD8^+$ T cells to produce IFN-γ after having stimulated them with C1R-A*2602 cells infected with recombinant HIV-1 vaccinia virus (r-HIV vaccinia). IFN-γ-producing cells were induced in the Gag169-177-specific $CD8^+$ T cell culture after stimulation with r-HIV vaccinia-infected C1R-A*2602 cells, whereas they were not detected in that stimulated with WT vaccinia-infected C1R-A*2602 cells or r-HIV vaccinia-infected C1R cells (Fig. 2C). These results indicate that Gag169-177 is indeed a naturally occurring HIV-1 epitope peptide presented by HLA-A*2602.

Gag169-177-specific $CD8^+$ T cells were induced from only 1 of the 3 HLA-A^*2602^+ HIV-1-infected individuals; whereas two HLA-A*2601 epitopes, Pol647-656 and Env464-473, which were also HLA-A*2602-binding peptides, failed to induce specific T cells in these individuals. To address lower frequency of these peptide-specific $CD8^+$ T cells, we investigated the induction of the specific $CD8^+$ T cells by stimulating PBMC from three additional donors carrying HLA-A*2602 with these three peptides. Only Gag169-177 peptide induced the specific $CD8^+$ T cells in one donor. Thus, Gag169-177-specific $CD8^+$ T cells were induced in 2 of 6 HLA-A^*2602^+ HIV-1-infected individuals whereas they were induced in 8 of 11 HLA-A^*2601^+ and 7 of 8 HLA-A^*2603^+ HIV-1-infected ones (Fig. 2D). These results indicate that Gag169-177 is a subdominant epitope in the HLA-A^*2602^+ donors.

Gag169-177-specific $CD8^+$ T cells were previously detected in five of seven HLA-A^*2601^+ donors and in all four HLA-A^*2603^+ ones [14,15], suggesting that Gag169-177 is a dominant epitope in HIV-1-infected individuals carrying either of these HLA-A*26 alleles. Additional experiments in the present study confirmed the immunodominance of this epitope in HLA-A^*2601^+ and HLA-A^*2603^+ donors (Fig. 2D). In contrast, they were detected in only two of six HLA-A^*2602^+ donors, indicating Gag169-177 to be a subdominant epitope in HLA-A^*2602^+ individuals. Interestingly, they were elicited in only a long-term non-progressor and a controller having low viral load. HLA-A*1101-restricted Gag349-, Nef73-, and/or Nef84-specific $CD8^+$ T cells were induces in two of four HLA-A^*1101^+/A^*2602^+ donors who did not have Gag169-177-specific $CD8^+$ T cells (data not shown), supporting that these HLA-A^*2602^+ donors maintain HIV-1-specific cellular immunity. The affinity of Gag169-177 for HLA-A*2602 was similar to that for HLA-A*2601 and -A*2603 (Table 2), indicating that Gag169-177 was effectively presented by HLA-A*2602 but recognized as a subdominant epitope in HIV-1-infected individuals carrying HLA-A*2602. Pol604-612 and Env63-72 are HLA-A*2601 and HLA-A*2603 epitopes, respectively [14,15]. These epitope peptides failed to bind to HLA-A*2602 (data not shown). Since only one amino acid, at residue 116, differs between HLA-A*2602 and the other two subtypes (Asp

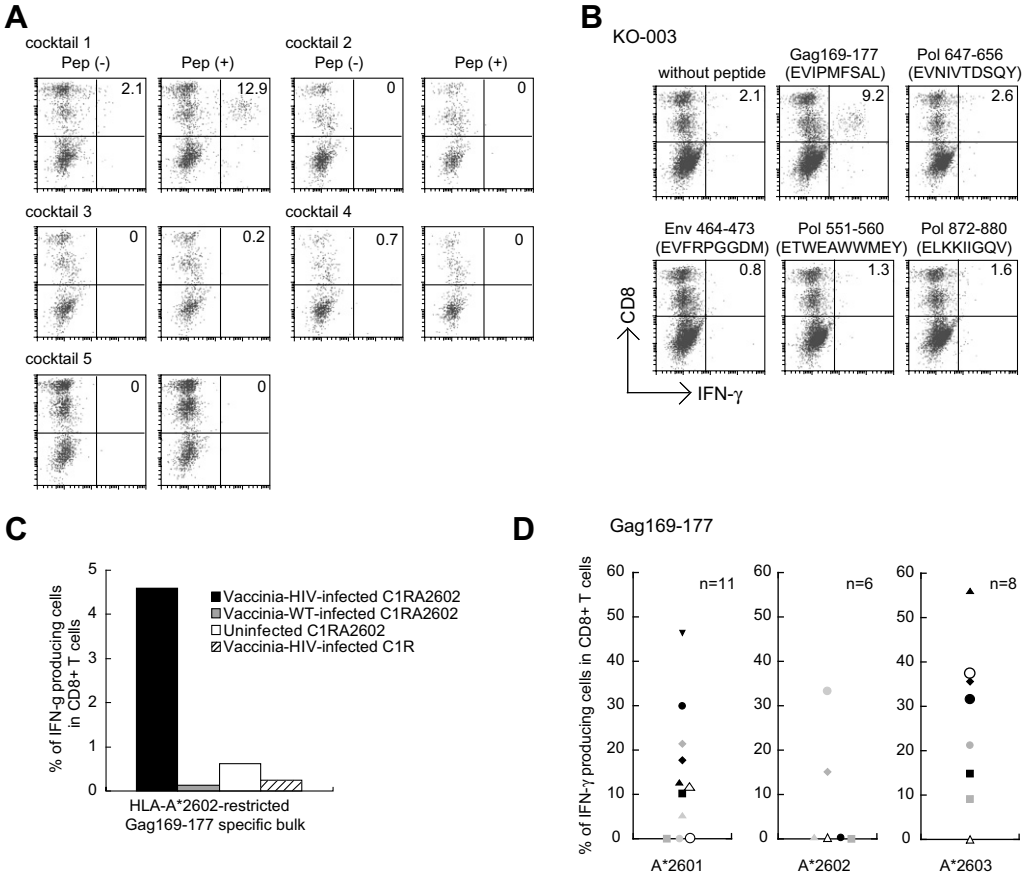

Fig. 2. Identification and recognition of Gag169-177-specific $CD8^+$ T cells. (A) PBMCs from HIV-1-infected individuals with HLA-A*2602 (KO-003) were cultured for 10–14 days after they had been stimulated with the indicated cocktails of HLA-A*2602-binding peptides. The cultured cells were then tested for IFN-γ production by $CD8^+$ T cells after stimulation with C1R-A*2602 cells prepulsed with the peptide cocktails. (B) Induction of Gag169-177-specific $CD8^+$ T cells. PBMCs from KO-003 were stimulated with peptide cocktail 1 and cultured for 10–14 days. The cultured cells were stimulated with C1R-A*2602 cells prepulsed with each single peptide included in cocktail 1. The population of IFN-γ-producing $CD8^+$ T cells was determined by using flow cytometry. The percentage of IFN-γ-producing $CD8^+$ T cells is presented at the right of the upper right-hand quadrant. (C) Presentation of Gag169-177 by HLA-A*2602 on r-HIV-1 vaccinia-infected cells. Bulk cultures containing Gag169-177-specific $CD8^+$ T cells were examined for IFN-γ production after they had been stimulated with C1R-A*2602 cells infected with wild-type vaccinia (Vaccinia-WT) or with C1R-A*2602 cells or with C1R cells infected with r-HIV-1Gag-vaccinia (Vaccinia-HIV-1), or uninfected C1R-A*2602 cells (Uninfected). The percentage of IFN-γ-producing $CD8^+$ T cells was measured by using flow cytometry. (D) Percentage of Gag169-177-specific $CD8^+$ T cells in HIV-1-infected individuals having three HLA-A*26 subtypes. The percentage of IFN-γ-producing cells among $CD8^+$ T cells from each individual was plotted in the graph. The percentage of IFN-γ-producing cells in the cultures was measured by using flow cytometry after they had been stimulated with the corresponding C1R-A*26 cells prepulsed with Gag169-177 peptide.

Table 2
Comparison of binding affinity of HLA-A*26 epitope peptides among three HLA-A*26 subtypes and induction of the peptide-specific $CD8^+$ T cells

	Binding affinity (BL50)			Comparison of binding affinity		Frequency[a]		
	A*2601	A*2602	A*2603	A*2602/A*2601	A*2602/A*2603	A*2601	A*2602	A*2603
Gag169-177	7.5×10^{-7}	5.8×10^{-7}	2.1×10^{-6}	0.77	0.28	8/11	2/6	7/8
Env63-72	1.1×10^{-4}	No binding	7.6×10^{-7}	—	—	0/11	NT	3/8
Pol604-612	6.5×10^{-5}	No binding	No binding	—	—	10/11	NT	NT
Pol647-656	6.3×10^{-5}	1.2×10^{-5}	6.6×10^{-4}	0.19	0.02	1/11	0/6	0/8
Env464-473	1.5×10^{-6}	4.0×10^{-7}	3.7×10^{-5}	0.27	0.01	1/11	0/6	0/8

NT, not tested.
[a] The number of individuals in whom peptide specific $CD8^+$ T cells were induced/the number of tested individuals.

for HLA-A*2601 and -A*2603, but Asn for HLA-A*2602), this substitution in the floor of the peptide binding groove is thought to affect the binding of these peptides.

In summary, Gag169-177 was not an HIV-1 immunodominant epitope in HIV-1-infected individuals carrying HLA-A*2602, whereas it was one in those carrying HLA-

A*2601 or -A*2603. These findings imply the possibility that HLA-A*2602 is not an allele associated with a slow progression to AIDS. However, it still remains unknown that Gag169-177-specific CTLs can control HIV-1. A further study using a cohort of a large number of subjects will clarify the association of these HLA-A*26 subtypes or Gag169-177-specific CTLs with the progression of AIDS.

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