Single T Cell Receptor-Mediated Recognition of an Identical HIV-Derived Peptide Presented by Multiple HLA Class I Molecules[1]

Takamasa Ueno, Hiroko Tomiyama, and Masafumi Takiguchi[2]

A dual specific human CTL clone harboring one β and two inframe α transcripts of TCR was previously reported to recognize an HIV Pol-derived nonapeptide (IPLTEEAEL) endogenously presented by both syngeneic HLA-B*3501 and HLA-B*5101. In the current study, a retrovirus-mediated TCR transfer of individual α- and β-chains to TCR-negative hybridoma showed that Vα12.1 TCR in complex with Vβ5.6 were responsible for the peptide-specific response in the context of both HLA-B*3501 and HLA-B*5101, confirming single TCR-mediated dual specificity. The second TCR-α chain was not somehow expressed on the cell surface. Remarkably, the Vα12.1/Vβ5.6 TCR also recognized the same peptide presented by allogeneic HLA class I molecules that share the similar peptide-binding motifs, such as HLA-B*5301 and HLA-B*0702. The sensitivity of peptide recognition by the Vα12/Vβ5.6 TCR appeared to be comparable when the peptide was presented by syngeneic and allogeneic HLA class I molecules, with changes in T cell responsiveness caused largely by peptide-binding capacity. Moreover, the CTL clone bearing Vα12.1/Vβ5.6 TCR showed substantial cytolytic activity against the peptide-loaded cells expressing HLA-B*3501, HLA-B*5101, HLA-B*5301, or HLA-B*0702, providing further evidence that a single TCR complex can recognize the same peptide presented by a broad range of HLA class I molecules. A TCR with fine specificity for an HIV Ag but broad specificity to multiple HLA molecules may provide an advantage to the generation of allorestricted, peptide-specific T cells, and thus could be a potent candidate for immunotherapy against HIV infection. ***The Journal of Immunology*****, 2002, 169: 4961–4969.**

T cells recognize immunogenic peptides that are noncovalently complexed to MHC class I and II Ags expressed on the surface of other cells, such as virus-infected cells. The specificity of this recognition is determined not only by the primary sequence of the peptide but also by the particular allele of the self MHC molecule to which the peptide is bound. Allogeneic MHC molecules have also been shown to induce strong T cell responses. Many studies have focused on cross-reactivity of T cells to different peptides and MHC class I molecules. Several alloreactive T cell clones have been shown to be cross-reactive, recognizing two unrelated peptides presented by two different allogeneic MHC class I molecules (1, 2). Cross-reactive CTLs showing dual recognition for both HLA class I and class II molecules have similarly been reported (3, 4). Alloreactive CTLs recognizing not only an endogenously processed peptide bound to allogeneic MHC molecules, but also recognizing a different peptide presented by syngeneic MHC class I molecules, have also been described (5). In addition, self HLA-restricted CTL clones have been shown to be cross-reactive, recognizing the same peptide presented by two different syngeneic HLA class I molecules that share similar peptide-binding motifs (6–8). In several of these previous studies, cold-target inhibition and mAb-mediated inhibition experiments were performed to confirm that one clonal T cell population was mediating the apparent cross-reactivity. However, these studies did not determine whether cross-reactivity was mediated by one or two $\alpha\beta$ TCR complexes. Indeed, a significant portion (10–30%) of human and mouse peripheral T cells express two $\alpha\beta$ TCR complexes on their cell surface (9). In a recent study, TCR gene transfer showed that the dual specificity of an alloreactive T cell clone that recognizes undefined peptides presented by HLA class I and class II molecules was mediated by a single TCR complex (10).

It has been reported, despite their genetic diversity, that different HLA class I molecules share peptide-binding motifs (11–14) and that some antigenic peptides that bind to more than one HLA class I molecule are recognized by CD8 T cells in the context of one or more HLA class I restriction elements (6, 7, 15). However, the important question of whether a single TCR complex can recognize a single peptide presented by distinct HLA class I restriction elements remains to be examined. We previously described two CTL clones established from an HIV-infected individual that recognized a naturally processed peptide derived from the HIV-1 Pol protein (HIV $Pol_{448-456}$; H-IPLTEEAEL-OH) presented by two syngeneic HLA class I molecules, HLA-B35 and HLA-B51 (7, 16). However, analysis of TCR-encoding genes revealed that one of these clones had two different inframe TCR-α transcripts and one TCR-β transcript (7). Thus the possibility that the two TCR-α chains played a role in this dual specificity could not be excluded.

In the current study, we used a retroviral gene transfer system to transfer the TCR-β chain and the different TCR-α chains of the CTL clones into a TCR-negative T cell hybridoma. We clearly showed that a single TCR complex recognizes the same peptide (IPLTEEAEL) presented by both syngeneic HLA-B35 and HLA-B51 molecules. Remarkably, this TCR complex could also specifically respond to the same peptide presented by allogeneic HLA class I molecules, such as HLA-B7 and HLA-B53 which share a

Division of Viral Immunology, Center for AIDS Research, Kumamoto University, Kumamoto, Japan

Received for publication May 17, 2002. Accepted for publication August 27, 2002.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This research was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sport and Culture, the government of Japan, and a grant from the Japan Health Science Foundation.

[2] Address correspondence and reprint requests to Dr. Masafumi Takiguchi, Division of Viral Immunology, Center for AIDS Research, Kumamoto University, 2-2-1 Honjo, Kumamoto 860-0811, Japan. E-mail address: masafumi@kaiju.medic.kumamoto-u.ac.jp

0022-1767/02/$02.00

similar peptide-binding motif (HLA-B7-like supertypes). Together, these findings indicate an additional level of cross-reactivity in T cells, demonstrating that the TCR complex interacts predominantly with the peptide Ag, with the HLA class I molecule behaving solely as a scaffolding component for the peptide rather than as a restriction element for the TCR.

Materials and Methods

Cell lines and Abs

CTL clones were maintained in RPMI 1640 and 10% FCS supplemented with 100 U/ml recombinant human IL-2 and were stimulated weekly with irradiated T2 cells expressing the HLA-B*3501 molecule in the presence of the $Pol_{448-456}$ peptide. The TCR-negative mouse T cell hybridoma TG40 (17) was kindly provided by Dr. T. Saito (Chiba University, Chiba, Japan) and was transduced as described below by a retroviral vector containing a human CD8α gene. Four days later, transduced cells that were brightly stained by anti-CD8 mAb were selected by fluorescence-activated cell sorting (FACSVantage; BD Biosciences, San Jose, CA). The selected TG40 cells expressing human CD8α (TG40/CD8) were used to reconstruct human TCR complexes as described below. A series of C1R and RMA-S transfectants expressing different HLA class I molecules were previously generated (18, 19).

The following Abs were used: FITC-conjugated anti-human CD8 mAb (DAKO, Carpinteria, CA), PE-conjugated anti-mouse CD3ϵ mAb (2C11; BD PharMingen, San Diego, CA), PE-conjugated anti-human Vβ2.1 TCR mAb (Beckman Coulter, Fullerton, CA), and FITC-conjugated anti-human Vα12.1 TCR mAb (Endogen, Woburn, MA). An anti-HLA class I mAb, w6/32 was prepared from culture supernatants of a w6/32-secreting hybridoma (American Type Culture Collection, Manassas, VA).

Preparation of tetrameric peptide-MHC complex (tetramer)

A tetrameric complex of peptide (Sawady Technologies, Tokyo, Japan), β_2-microglobulin (β_2M),[3] and HLA-B*3501 was prepared as described (7). Briefly, an ectodomain of HLA-B35 (15 mg) and β_2M (100 mg) produced in *Escherichia coli* as inclusion bodies were first solubilized in denaturing buffer containing 8 M urea and then refolded in refolding buffer (100 mM Tris-HCl, pH 8.0, 400 mM arginine, 2 mM EDTA acid, 5 mM reduced glutathione, 0.5 mM oxidized glutathione, 0.1 mM PMSF) in the presence of 5 mg of a chemically synthesized peptide for 48 h at 4°C. The resultant 45-kDa ternary complex was purified by size-exclusion and anion-exchange chromatographies. Purified complexes were enzymatically biotinylated at a birA recognition sequence located at the C terminus of the H chain, and were mixed with PE-conjugated avidin (extravidin-PE; Sigma-Aldrich, St. Louis, MO) at a molar ratio of 4:1 to give the HLA-tetramer complex.

Cloning and sequencing analysis of TCR-encoding genes

Total RNA was prepared from CTL clones ($\sim 1 \times 10^5$ cells) using a total RNA isolation kit (Qiagen, Valencia, CA). cDNA clones encoding α and β TCR were obtained by reverse transcription using the SMART PCR cDNA synthesis kit (Clontech Laboratories, Palo Alto, CA) with the isolated total RNA ($\sim$50 ng) and a primer specific for Cα (5′-actggatttagagtctctcagctggtaca-3′) or Cβ (5′-ttgggtgtgggagatctctgcttctgatg-3′), respectively. cDNA was then amplified by PCR using a high fidelity *Pfu* polymerase (Promega, Madison, WI) and was cloned into a plasmid. The DNA sequence of the α and β TCR-encoding genes was determined using an automatic DNA sequencer (LI-COR, Lincoln, NE). To obtain full-length TCR genes (i.e., containing not only the V and J regions, but also the C, transmembrane, and cytoplasmic domains), we used an overlapping PCR strategy using cDNA clones encoding α (pY1.4) and β (JUR-β-2) TCR chains from Jurkat cells (provided by Dr. T. W. Mak, University of Toronto, Toronto, Ontario, Canada) as templates.

Construction of retroviral vectors and gene transfer

Retrovirus-mediated gene transfer was used to reconstruct $\alpha\beta$ TCR complexes on TG40/CD8 cells essentially as described by Kitamura et al. (20). Briefly, the genes of interest, i.e., full-length α and β TCR, were subcloned into the retroviral vector pMX, which was kindly provided by Dr. T. Kitamura (Tokyo University, Tokyo, Japan). The resulting constructs were first transfected into the ecotropic virus packaging cell line Platinum-E (also a gift from Dr. T. Kitamura) (21) using the transfection reagent Lipofectamine 2000 (Invitrogen, Groningen, The Netherlands). Two days later, culture supernatant containing recombinant virus was collected and then incubated with TG40/CD8 cells in the presence of 10 μg/ml polybrene for 6 h. The cells were cultured for an additional 2 days for analysis of TCR gene expression or selection of cells using appropriate antibiotics. Transduced TG40/CD8 cells showing bright staining by anti-mouse CD3ϵ mAb were selected by fluorescence-activated cell sorting and used for further functional assays.

Peptide binding to HLA class I molecules

Peptide-binding affinity for HLA class I molecules was assessed by an HLA stabilization assay as described (7, 22). In brief, a series of RMA-S transfectants expressing HLA-B*3501, HLA-B*5101, or HLA-B*5301 was cultured for 18 h at 26°C and then pulsed with various concentrations of peptide for 3 h at 26°C. Cells were then incubated at 37°C for 3 h and subsequently stained by an anti-HLA class I mAb, TP25.99, and FITC-conjugated IgG fraction of sheep anti-mouse Ig (SILENUS Labs, Victoria, Australia). The surface expression level of the HLA class I molecule was evaluated by a mean fluorescence intensity (MFI) determined by flow cytometric analysis (FACSCalibur; BD Biosciences). The concentration of the peptide that yielded a half maximal binding level (BL_{50}) was calculated by defining the surface expression level of HLA class I molecules on cells incubated continuously at 26°C for 24 h as maximal.

IL-2 assays for cellular activation

TCR recognition of peptide-HLA class I complexes was analyzed by cellular activation of TCR-transduced TG40/CD8 cells, measured by IL-2 production as previously described by Zumla et al. (17) with modifications. C1R cells or transfectants expressing HLA molecules (10^4 cells/well) were preincubated in a 96-well microtiter plate for 30 min at 37°C in the absence or presence of various concentrations of peptide. TG40/CD8 cells (2×10^4 cells/well) expressing a defined TCR complex were added to the culture medium containing RPMI 1640 and 10% FCS in a total volume of 200 μl. A portion of the culture supernatant (100 μl) was removed from each well after a 48-h incubation at 37°C, transferred to another culture plate, and frozen at −20°C. The amount of IL-2 produced by the TG40/CD8 cells was determined by analyzing the proliferative activity of the IL-2 indicator cell line CTLL-2. CTLL-2 cells (2×10^4/well) were incubated with the prepared culture supernatants for 18 h at 37°C. [^{3}H]Thymidine (TdR; Amersham Pharmacia, Piscataway, NJ) was added and the cells were then incubated for an additional 6 h before being harvested onto glass fiber filters. After thorough washing of the filters with distilled water, the amount of [^{3}H]TdR incorporated by CTLL-2 cells that was retained on the filters was determined by a scintillation counter (MicroBeta TriLux; Wallac Oy, Turku, Finland). The EC_{50} value of the peptide was calculated as the concentration of peptide that exhibited a half-maximal activation of TCR-transduced TG40/CD8 cells with CD3ϵ-mAb-mediated activation of the cells defined as maximal.

Cytotoxic assay

The cytotoxic activity of the CTL clones was determined by a standard ^{51}Cr-release assay as previously described (7). When peptide-pulsed C1R cells were used as target cells, C1R cells or transfectants expressing HLA class I molecules were first incubated with 100 μCi $Na_2{}^{51}CrO_4$ (Amersham Pharmacia) in saline for 1 h at 37°C, and then washed three times with a culture medium. The ^{51}Cr-labeled C1R cells (2×10^3 cells/well) were plated in a 96-well round-bottom microtiter plate in the absence or presence of the various concentrations of peptide. After 1 h, CTL clones (2×10^3 cells/well) were added and the plate was incubated for an additional 4 h at 37°C. To determine maximum and spontaneous ^{51}Cr release, 5% Triton X-100 or culture medium alone, respectively, was also added to different wells. A portion of the culture supernatant (100 μl) was then removed from each well and analyzed by a gamma counter. The percent specific lysis was calculated as previously described (7).

Results

Cloning of TCR-encoding genes and reconstruction of the TCR complex

Two CTL clones, designated 562 and 589, originally established from an HIV-infected patient (HLA-A24/A26, B35/B51, Cw3/−) following stimulation of peripheral lymphocytes with an HIV Pol-derived synthetic epitope peptide (HIV $Pol_{448-456}$; H-IPLTEEAEL-OH) were previously found to exhibit dual specificity recognizing the same

[3] Abbreviations used in this paper: β_2M, β_2-microglobulin; MFI, mean fluorescence intensity; TdR, thymidine; GFP, green fluorescent protein; P, position; BL_{50}, half maximal binding level.

$Pol_{448-456}$ peptide presented by both HLA-B*3501 (B35) and HLA-B*5101 (B51) (7, 16). These CTL clones showed specific cytotoxic activity against several kinds of target cells expressing B35 or B51, such as C1R, T1, and .221 cells either pulsed with $Pol_{448-456}$ peptide or infected with viruses expressing HIV Gag-Pol polyproteins (7). Following cloning and sequencing analysis of TCR-encoding genes, we previously reported that CTL clone 589 had two inframe TCR-α transcripts (Vα10.1 and Vα12.1) and one TCR-β transcript (Vβ2.1). However, the CTL clone was not stained by anti-Vβ2.1 mAb (7).

In the current study, we first asked whether one or two TCR complexes are responsible for the dual recognition exhibited by CTL clone 589. The TCR-negative mouse T cell hybridoma cell line, TG40 (17), was transduced with human CD8α to give TG40/CD8 cells, which were then transduced with TCR genes. Cells transduced with Vα12.1 and Vβ2.1 TCR (TG40/CD8/TCRα12β2.1) were clearly stained by anti-CD3ϵ, anti-Vα12, and Vβ2.1 mAbs (data not shown), indicating that the human-derived TCR was successfully reconstructed on the surface of the TG40/CD8 cells. This result is in accordance with a previous report demonstrating that $\alpha\beta$ TCR was expressed on the surface of TG40 cells upon transduction with TCR-encoding genes isolated from Jurkat T cells (17). However, the observation that the cells were stained by anti-Vβ2.1 mAb appeared inconsistent with our previous finding that the parent CTL clone 589 was not stained by the same mAb (7). To clarify this issue, we reisolated TCR-encoding genes from CTL clone 589. This time, no TCR-β transcripts from the Vβ2.1 family were isolated, and instead a different TCR-β transcript classified in the Vβ5.6 family was isolated (Table I). CTL clone 562, isolated from the same patient as clone 589 (7), were also found to have the same TCR-β gene from the Vβ5.6 family, suggesting that the correct TCR-β chain recognizing the $Pol_{448-456}$ peptide presented by B35 is from the Vβ5.6 family and not the Vβ2.1 family (see also Figs. 1 and 2). Therefore, we used the newly isolated Vβ5.6 gene for the remainder of this study. We again isolated two inframe α transcripts of Vα10.1 and Vα12.1 families in both CTL clones (Table I), as reported previously (7).

Expression of only one TCR complex bearing Vα12.1/Vβ5.6 on the cell surface

To address the issue of whether one or two TCR complexes is responsible for the dual recognition exhibited by CTL clone 589, TG40/CD8 cells were transduced with a retroviral vector expressing the Vβ5.6 TCR and puromycin-resistance genes. Puromycin-resistant cells were isolated and then transduced with retroviral vectors expressing either the Vα10.1 or Vα12.1 TCR coupled bicistronically with green fluorescent protein (GFP). Transduced cells were stained by anti-CD3ϵ mAb and then analyzed by flow cytometry. As shown in Fig. 1*A*, TG40/CD8/Vβ5.6 cells transduced with Vα12.1 TCR were stained by anti-CD3ϵ mAb, indicating that $\alpha\beta$ TCR bearing Vα12.1/Vβ5.6 formed a complete TCR/CD3 complex on the transduced cells. In contrast, TG40/CD8/Vβ5.6 cells transduced with Vα10.1 TCR were not stained by CD3ϵ mAb (Fig. 1*A*). Because these cells showed bicistronic expression of GFP, mRNA encoding Vα10.1 TCR should have been transcribed in GFP-positive cells. Moreover, TCR-α protein was observed in an immunoprecipitate using an anti-Cα-mAb αF1 (Endogen) following detergent solubilization of GFP-positive cells (data not shown). This suggests that the Vα10.1 gene was transcribed and translated into protein in the transduced cells, but the TCR Vα10.1 protein was somehow unable to form a heterodimer complex with the Vβ5.6 TCR protein and therefore was not present on the cell surface. It has been reported that particular combinations of murine $\alpha\beta$ TCR are deficient in paring to form mature heterodimer complexes at the posttranslational stage (23), which may explain our observation. Taken together, these data suggest that only one TCR complex bearing Vα12.1/Vβ5.6 is functionally expressed on the surface of the parental CTL clones and, therefore, that this single Vα12.1/Vβ5.6 TCR complex is responsible for the dual specificity of the CTL clone for $Pol_{448-456}$ peptide presented by both B35 and B51.

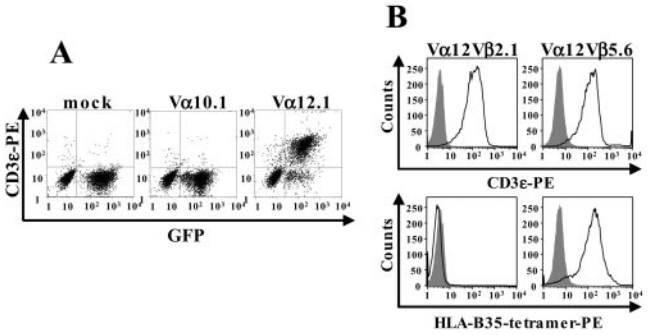

FIGURE 1. Flow cytometric analysis of surface expression of TCR/CD3 complexes in TG40/CD8 cells following transduction with TCR-encoding genes. *A*, TG40 cells transduced with human CD8α and Vβ5.6 TCR-β chain were further transduced with bicistronic retrovirus vectors containing GFP alone (mock), Vα10.1-internal ribosomal entry site-GFP (Vα10.1), or Vα12.1-internal ribosomal entry site-GFP (Vα12.1). Three days after transduction, cells were stained with anti-CD3ϵ-PE and analyzed by flow cytometry. The efficiency of retrovirus-mediated transduction as measured by GFP^+ cells was 50 $\pm$ 10% for all experiments shown. Data are shown for one of two independent experiments. *B*, Cell surface expression of TCR/CD3 complexes and their capacity to bind the $Pol_{448-456}$ peptide/HLA-B35 tetramer were examined. TG40/CD8 cells transduced with TCR genes encoding Vα12.1/Vβ2.1 or Vα12.1/Vβ5.6 were stained with anti-CD3ϵ-PE or HLA-B35 tetramer-PE as indicated, and analyzed by flow cytometry (solid lines). Mock-transduced cells stained similarly are indicated by shaded areas. Data are shown for one of two independent experiments.

A single TCR complex bearing Vα12.1/Vβ5.6 can mediate both HLA-B35 and HLA-B51-restricted peptide recognition

To directly confirm that the Vα12.1/Vβ5.6 TCR complex has dual specificity, TG40/CD8 cells were transduced with a bicistronic retroviral vector containing Vα12.1 and Vβ5.6 or Vβ2.1 genes, and then cells showing bright staining with anti-CD3ϵ mAb were sorted by flow cytometry.

We first tested the ability of the transduced TG40/CD8 cells to bind to the PE-labeled B35 tetramer (Fig. 1*B*). Cells expressing Vα12.1/Vβ5.6 or Vα12.1/Vβ2.1 TCR were similarly stained with anti-CD3ϵ mAb (Fig. 1*B*), indicating that they expressed a comparable level of TCR/CD3 complex on their surface. In contrast, cells expressing the Vα12.1/Vβ5.6 TCR were stained by the B35 tetramer, whereas the cells expressing the Vα12.1/Vβ2.1 TCR were not stained (Fig. 1*B*). This indicates that only the Vα12.1/Vβ5.6 TCR can specifically interact with the $Pol_{448-456}$ peptide/

Table I. *TCR-encoding genes reisolated from CTL clones 562 and 589*[a]

	V Gene	J Segment	CDR3 Sequence
Vα10.1	TRAV27*01	TRAJ56*01	CAGATNSKLTFGKG
Vα12.1	TRAV19*01	TRAJ53*01	CALSHNSGGSNYKLTFGKG
Vβ5.6	TRBV5-4*01	TRBJ2–5*01	CASSFRGGKTQYFGPG

[a] Alignment of V and J regions of the TCR genes were analyzed by the International ImMunoGeneTics database (http://imgt.cnusc.fr:8104). Complementarity-determining region 3 (CDR3) sequences for α and β TCR are shown.

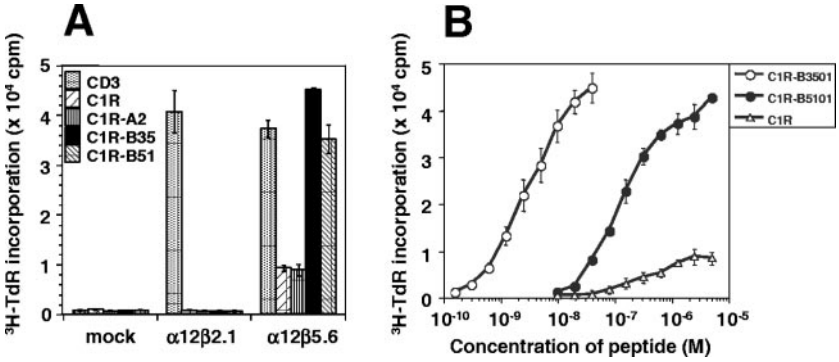

FIGURE 2. Vα12.1/Vβ5.6 TCR recognized $Pol_{448-456}$ peptide presented by both HLA-B*3501 and HLA-B*5101. *A*, TCR- (Vα12.1/Vβ2.1 or Vα12.1/Vβ5.6) or mock-transduced TG40/CD8 cells were incubated for 48 h in a 96-well microtiter plate precoated with anti-CD3ϵ mAb (10 μg/ml). Cell-free culture supernatant was collected and the amount of IL-2 released from transduced TG40/CD8 cells was determined by the proliferation capacity of CTLL-2 cells incubated in the supernatant. For Ag-specific stimulation, transduced TG40/CD8 cells were cocultured for 48 h with C1R transfectants expressing the indicated HLA class I molecule in the presence or absence of 1 μM $Pol_{448-456}$ peptide. Note that [^{3}H]TdR incorporation values were always $<$1000 cpm when TCR-transduced TG40/CD8 cells were incubated with C1R transfectants in the absence of $Pol_{448-456}$ peptide. Data are shown for one of two independent experiments as the mean $\pm$ SD of quadruplicate assays. *B*, Vα12.1/Vβ5.6-transduced TG40/CD8 cells were incubated for 48 h with C1R transfectants expressing HLA-B*3501 or HLA-B*5101 in the presence or absence of the indicated concentrations of $Pol_{448-456}$ peptide. The amount of IL-2 released from the Vα12.1/Vβ5.6-transduced TG40/CD8 cells was determined as above. Data are shown for one of two independent experiments, as the mean $\pm$ SD of quadruplicate assays.

B35 complex. We could not investigate the dual specificity of the Vα12.1/Vβ5.6 TCR for $Pol_{448-456}$ peptide/B51 complexes using tetramers as, despite many attempts to prepare a B51 tetramer in complex with the $Pol_{448-456}$ peptide, we have not yet succeeded in refolding the $Pol_{448-456}$ peptide/B51 complex under cell-free conditions. Considering that the $Pol_{448-456}$ peptide has previously been shown to be endogenously present on B51 molecules (7), distinct mechanisms might play a role in the exogenous and endogenous peptide-assembling pathway of B51 molecules (24).

We next tested the ability of the TCR-expressing TG40/CD8 cells to recognize the $Pol_{448-456}$ peptide presented by B35 and B51 as assessed by a cellular activation assay (Fig. 2*A*). Both TG40/CD8/TCRα12β2.1 and TG40/CD8/TCRα12β5.6 cells were comparably activated by anti-CD3ϵ mAb (Fig. 2*A*), confirming a functional TCR/CD3-mediated signaling system in these cells. The transduced TG40/CD8 cells were then incubated in the presence or absence of 1 μM $Pol_{448-456}$ peptide either with C1R cells or with a series of transfectants expressing HLA-A*0201 (A2), B35, and B51. TG40/CD8/TCRα12β5.6 cells exhibited substantial activation when cocultured with C1R-B3501 and C1R-B5101 in the presence of the peptide, but not when cocultured with C1R-A0201 in the presence of the peptide or with any of the cells in the absence of the peptide (Fig. 2*A*). In contrast, neither TG40/CD8 nor TG40/CD8/TCRα12β2.1 cells appeared to be activated in response to any of the C1R cells tested, even in the presence of 1 μM $Pol_{448-456}$ peptide (Fig. 2*A*). These data clearly indicate that a single TCR complex bearing Vα12.1/Vβ5.6 has dual specificity recognizing $Pol_{448-456}$ peptide presented by both B35 and B51. However, it should be noted that TG40/CD8/TCRα12β5.6 cells did show a small response to peptide-pulsed C1R and C1R-A0201 cells, although these responses were significantly lower compared with their response to peptide-pulsed C1R-B3501 and C1R-B5101 cells (Fig. 2*A*). This response could be due to the low level of endogenous HLA-B*3503 expressed on C1R cells (25)(see also Fig. 4*B*), which could bind $Pol_{448-456}$ peptide and present it to the T cells.

To determine whether TG40/CD8/TCRα12β5.6 cells have a different sensitivity to $Pol_{448-456}$ peptide presented by B35 or B51, TG40/CD8/TCRα12β5.6 cells were stimulated by the C1R transfectants in the presence of various concentrations of $Pol_{448-456}$ peptide. As shown in Fig. 2*B*, the response of TG40/CD8/TCRα12β5.6 cells to both C1R-B3501 and C1R-B5101 cells was proportional to peptide concentration, with EC_{50} values of 2.6 and 140 nM for B35 and B51, respectively.

The Vα12.1/Vβ5.6 TCR recognizes the same peptide presented by multiple HLA class I molecules

A group of HLA class I alleles has been shown to share peptide-binding motifs and have the ability to bind a series of identical peptides (14). Among them, HLA-B7-like supertypes are defined

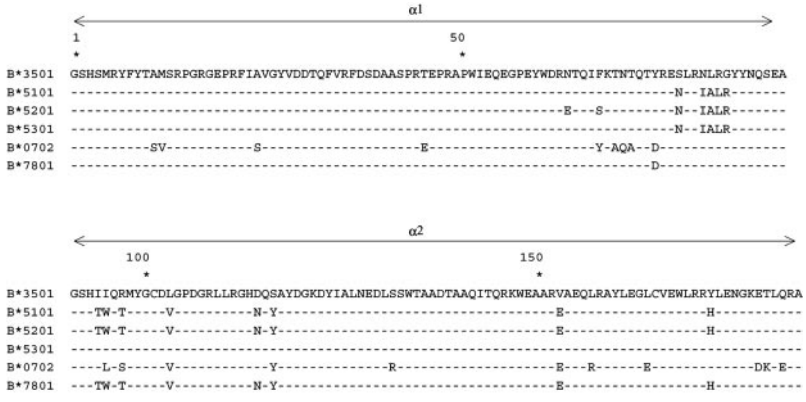

FIGURE 3. Alignment of the amino acid sequence of the α1 and α2 regions of different HLA class I molecules. The amino acid sequences of the α1 and α2 regions of HLA-B*3501, HLA-B*5101, HLA-B*5201, HLA-B*5301, HLA-B*7801, and HLA-B*0702 are shown. Dashes denote identical amino acids.

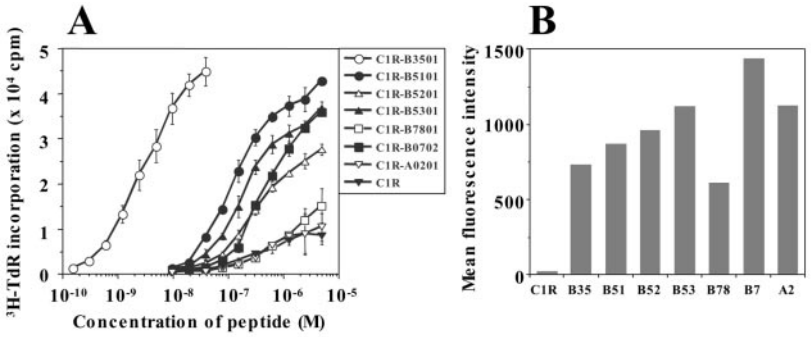

FIGURE 4. Vα12.1/Vβ5.6 TCR-mediated recognition of the $Pol_{448-456}$ peptide presented by multiple HLA molecules. *A*, Vα12.1/Vβ5.6-transduced TG40/CD8 cells were incubated for 48 h with C1R transfectants expressing different HLA class I molecules in the presence or absence of the indicated concentrations of $Pol_{448-456}$ peptide ranging from 0.15 nM to 5.0 μM. The amount of IL-2 produced by the Vα12.1/Vβ5.6-transduced TG40/CD8 cells was determined as described in the legend to Fig. 2. Data are shown for one of two independent experiments, as the mean ± SD of quadruplicate assays. *B*, The surface expression level of HLA class I molecules on C1R cells used in *A* was analyzed by staining with w6/32 mAb. Note that the parental C1R cell appeared to express a low level of HLA-Cw4 and HLA-B*3503 (MFI = 35.2), whereas the MFI value of cells treated in the absence of w6/32 mAb was 3.63. Data are shown for one of two independent experiments.

as HLA-B alleles with a preference for peptides with proline at position 2 (P2) and hydrophobic/aromatic residues at the C terminus (11, 13). These alleles include HLA-B*0702 (B7), B35, B51, HLA-B*5301 (B53), and HLA-B*7801 (B78). Although there are many polymorphic amino acid residues among different HLA class I alleles (Fig. 3), it may be possible that the $Pol_{448-456}$ peptide, which has Pro at P2 and a hydrophobic residue at the C terminus, binds to all these HLA molecules, and is recognized by the same TCR. To test this possibility, TG40/CD8/TCRα12β5.6 cells were incubated separately with C1R cells expressing different HLA class I molecules in the presence or absence of various concentrations of $Pol_{448-456}$ peptide, and then their activation was analyzed (Fig. 4*A*). The HLA expression levels were all shown to be comparable among the C1R transfectants, as assessed by staining with w6/32 mAb (Fig. 4*B*), which has a broad range of specificity to various HLA class I alleles and recognizes a conformational epitope of the HLA class I complex including β_2M and the polymorphic H chain (26). Remarkably, as shown in Fig. 4*A*, TG40/CD8/TCRα12β5.6 cells showed substantial IL-2 release in response to the $Pol_{448-456}$ peptide presented by B7 and B53 (EC_{50} values of 690 and 270 nM, respectively), and moderate release in response to peptide presented by HLA-B*5201 (B52) (EC_{50} of 1.4 μM). In contrast, the cells showed only a low level of IL-2 release when the peptide was presented by A2 and B78 ($EC_{50} > 10$ μM). Given that the Vα12.1/Vβ5.6 TCR was isolated from peripheral T cells of an individual who carries B35 and B51 alleles but not B7 or B53, it is of significant interest that this TCR showed peptide-specific recognition restricted by allogeneic B7 and B53.

Apparent differences in T cell sensitivity for peptide presented by allogeneic and syngeneic HLA class I molecules is caused by different peptide-binding activities of these molecules

We next examined whether the apparent decreased sensitivity of TG40/CD8/TCRα12β5.6 cells to $Pol_{448-456}$ peptide presented by

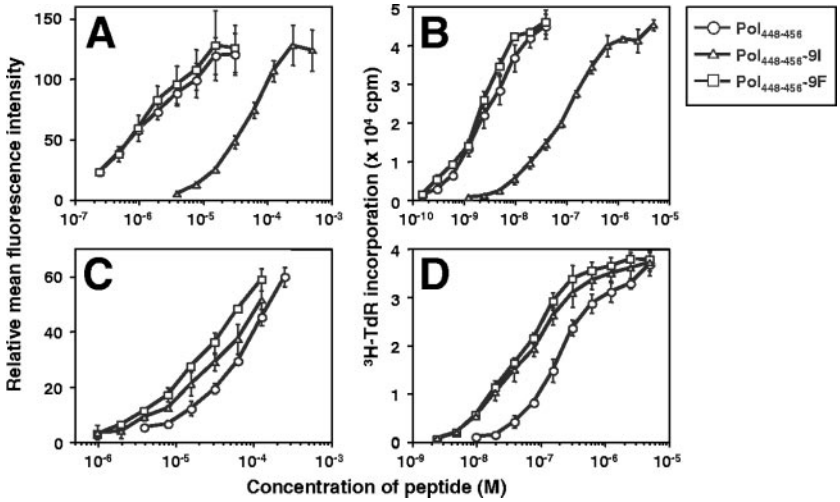

FIGURE 5. Effect of peptide-binding activity on the reactivity of T cells bearing Vα12.1/Vβ5.6 TCR in the context of HLA-B*3501 and HLA-B*5301. The binding activity of $Pol_{448-456}$ and mutant ($Pol_{448-456}$-9I and $Pol_{448-456}$-9F) peptides to HLA-B*3501 (*A*) and HLA-B*5301 (*C*) was examined using an HLA stabilization assay. The background MFI values for RMA-S-B3501 and RMA-S-B5301 cells were 50.3 ± 2.9 and 50.5 ± 0.15, respectively. The maximal MFI values for RMA-S-B3501 and RMA-S-B5301 cells were 173 ± 8.7 and 113 ± 2.5, respectively. BL_{50} values were calculated and are summarized in Table II. Data are shown for one of two independent experiments, as the mean ± SD of triplicate assays. The reactivity of T cells bearing Vα12.1/Vβ5.6 TCR against the same set of peptides was examined in the context of HLA-B*3501 (*B*) and HLA-B*5301 (*D*) similar to the experiments shown in Figs. 2*B* and 4*A*. The EC_{50} values of the peptides were calculated and are summarized in Table II. Data are shown for one of two independent experiments as the mean ± SD of quadruplicate assays.

Table II. *Summary of peptide-binding activity and T cell responsiveness in the context of self and nonself HLA class I molecules*[a]

Peptide	Sequence	HLA-B*3501			HLA-B*5301		
		BL_{50} (M)	EC_{50} (M)	EC_{50}/BL_{50} ($\times 10^{-3}$)	BL_{50} (M)	EC_{50} (M)	EC_{50}/BL_{50} ($\times 10^{-3}$)
$Pol_{448-456}$	IPLTEEAEL	1.2×10^{-6}	2.6×10^{-9}	2.2	6.8×10^{-5}	2.7×10^{-7}	4.0
$Pol_{448-456}$-9F	--------F	1.0×10^{-6}	2.1×10^{-9}	2.1	2.1×10^{-5}	7.9×10^{-8}	3.8
$Pol_{448-456}$-9I	--------I	4.3×10^{-5}	1.0×10^{-7}	2.3	3.6×10^{-5}	1.1×10^{-7}	3.1

[a] The BL_{50} and EC_{50} values were determined from the data shown in Fig. 5, *A* and *C*, and *B* and *D*, respectively. Dashes denote identical amino acids.

allogeneic HLA molecules is caused by decreased binding affinity of these molecules to the peptide. Using mutant peptides with different C-terminal anchor residues and hence with different binding activities to HLA class I molecules, we compared T cell responsiveness (EC_{50} value as determined by a cellular activation assay) and peptide-binding activity (BL_{50} value as determined by a HLA stabilization assay using RMA-S cells), and sought to analyze changes in TCR-peptide-HLA interactions (EC_{50}/BL_{50} ratio) when the peptide was presented by self (B35) and nonself (B53) HLA class I molecules.

As revealed by HLA stabilization assays using an RMA-S transfectant expressing B35, $Pol_{448-456}$ peptide bound to self B35 molecules, with a BL_{50} value of 1.2 μM (Fig. 5*A*, Table II). Substitution of Leu to Ile at the C terminus ($Pol_{448-456}$-9I) resulted in a ~35-fold reduced binding activity to B35, whereas substitution of Leu to Phe ($Pol_{448-456}$-9F) showed a comparable binding activity to B35 (Fig. 5*A*, Table II). In cellular activation assays, TG40/CD8/TCRα12β5.6 cells responded comparably to $Pol_{448-456}$ and $Pol_{448-456}$-9F peptides, but showed ~38-fold reduced sensitivity to the $Pol_{448-456}$-9I peptide (Fig. 5*B*). Consequently, virtually the same EC_{50}/BL_{50} values were obtained for the peptides tested (Table II), indicating that the Vα12/Vβ5.6 TCR can tolerate changes of amino acid side chains at the C terminus of peptides presented by B35.

B35 and B53 share an identical α2 region and have five amino acid differences in the α1 region (Fig. 3). X-ray structural analyses have shown that three (residues 77, 80, and 81) of these five amino acids form part of the F pocket and therefore contribute in part to the preference of these HLA molecules for particular peptide C-terminal anchor residues (27, 28). Indeed, an HLA stabilization assay showed that $Pol_{448-456}$ peptide bound to nonself B53 with a BL_{50} value of 68 μM (Fig. 5*C*, Table II), a ~50-fold decrease in binding activity compared with that for B35. Both $Pol_{448-456}$-9I and $Pol_{448-456}$-9F peptides showed 1.9- and 3.2-fold increased binding activity to B53, respectively (Fig. 5*C*), highlighting the differences between B35 and B53 in their binding peptide preference. In cellular activation assays, TG40/CD8/TCRα12β5.6 cells responded with 2.4- and 3.4-fold increased sensitivity to the $Pol_{448-456}$-9I and $Pol_{448-456}$-9F peptides presented by B53, respectively, compared with $Pol_{448-456}$ peptide (Fig. 5*D*, Table II). Thus, similarly to peptides presented by B35, virtually the same EC_{50}/BL_{50} values were obtained among the peptides tested (Table II), indicating that the Vα12/Vβ5.6 TCR can tolerate changes in amino acid side chains at the C terminus of peptides presented by B53.

The EC_{50}/BL_{50} values obtained when the same peptide (either $Pol_{448-456}$, $Pol_{448-456}$-9I, or $Pol_{448-456}$-9F) was presented by B35 or B53 were also all comparable (<2-fold differences; Table II). This indicates that the apparent decreased sensitivity of TG40/CD8/TCRα12β5.6 cells to $Pol_{448-456}$ peptide presented by allogeneic HLA molecules is largely caused by the decreased binding activity of these molecules for the peptide and that the Vα12/Vβ5.6 TCR binds to the peptide-HLA complex with a comparable affinity whether the peptide is presented by B35 or B53.

It should be noted that, although TG40/CD8/TCRα12β5.6 cells recognized the $Pol_{448-456}$ peptide presented by B51 but not by B78 (Fig. 4*A*), in the course of HLA stabilization assays we observed poor binding of $Pol_{448-456}$ peptide to both B51 and B78 (BL_{50} >1 mM). Because the ability of $Pol_{448-456}$ peptide to be endogenously presented by B51 has previously been shown in a peptide-elution study using C1R-B5101 cells infected with recombinant vaccinia virus expressing the HIV Pol protein (7), the reason for not being able to detect binding of $Pol_{448-456}$ peptide to B51 in the HLA stabilization assay is unclear. However, it has previously been documented that peptide binding to B51 is generally low (29) and that endogenous assembling of B51 for Ag presentation is slow (24) compared with B35. This could explain why $Pol_{448-456}$ peptide binding to HLA-B51 could not be detected in our HLA stabilization assay.

A parental CTL clone killed peptide-pulsed target cells in the context of multiple HLA molecules

To further confirm that the Vα12.1/Vβ5.6 TCR has multiple specificity and can recognize a single peptide presented by a broad range of B7-like HLA supertype molecules, we tested the ability of parental CTL clone 589 to kill peptide-loaded target cells expressing different HLA molecules. Although the CTL clone has two inframe TCR-α transcripts, our data showed that only one (Vα12.1) is functionally expressed on their cell surfaces and is thus the determinant for Ag specificity in the CTL clone. In support of this, staining of the clone with anti-Vα12.1 mAb as well as $Pol_{448-456}$/B35 tetramer showed virtually no negative subsets in the cell populations (data not shown).

As shown in Fig. 6, the CTL clone showed specific lysis activity against peptide-loaded C1R cells expressing B35, B51, B53, or

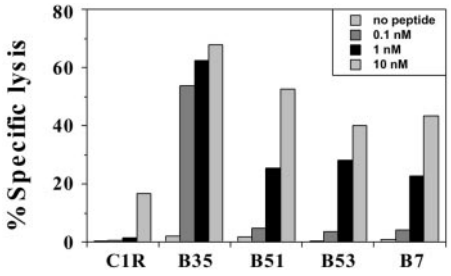

FIGURE 6. Cytotoxic activity of parental CTL clone against $Pol_{448-456}$-peptide-loaded target cells in the context of multiple HLA molecules. ^{51}Cr-labeled C1R cells and transfectants expressing the indicated HLA class I molecules (2×10^3 cells/well) were plated in a 96-well microtiter plate in the absence or presence of the indicated concentrations of $Pol_{448-456}$ peptide. CTL clone 589 (2×10^3 cells/well) was then added and the plate was incubated for 4 h at 37°C. Data are shown as the mean of duplicate assays.

B7. The CTL clone was most sensitive to target cells expressing B35 (Fig. 6), consistent with the finding that recognition of $Pol_{448-456}$ peptide by TG40/CD8/α12β5.6 cells was 50-fold more potent when the peptide was presented by B35 compared with when it was presented by B51 (Fig. 2*B*).

Discussion

The experiments presented in this study provide functional evidence that a single TCR complex has the ability to recognize a broad range of self and nonself HLA class I molecules while retaining fine specificity for a foreign antigenic peptide. The cross-reactivity of the TCR to the same peptide presented by multiple HLA class I molecules including B35, B51, B53, and B7 was confirmed using two different T cell systems and two different assay systems. In the first system, the Vα12.1/Vβ5.6 TCR was reconstructed on the surface of TG40/CD8 cells (a TCR-negative mouse T cell hybridoma cell line expressing human CD8α) and Ag recognition by the TCR was assessed by analyzing IL-2 release by the TCR-expressing TG40/CD8 cells. In the second system, the parental CTL clone was confirmed to exclusively express Vα12.1/Vβ5.6 TCR on its surface and Ag recognition by the TCR was assessed by determining the cytolytic activity of the CTL clone. In addition, modulation of peptide-binding affinity to different HLA class I molecules by introduction of mutant C-terminal anchor residues in the $Pol_{448-456}$ peptide revealed that peptide recognition by Vα12.1/Vβ5.6 TCR was comparable when the peptide was presented by different HLA class I molecules. A TCR with fine specificity for an HIV Ag but broad specificity to multiple HLA molecules may provide an advantage to the generation of allorestricted, peptide-specific T cells, and thus could be a potent candidate for immunotherapy against HIV infection based on TCR gene transfer technology (30–32).

As many as 30% of human T cells have been reported to coexpress two different TCR-α chains on their cell surface (9). These T cells may be involved in some forms of unconventional recognition, such as alloreactivity and autoimmunity (9). Alternatively, dual receptor T cells may provide a benefit to the immune system by extending the immune repertoire for foreign Ags (33). Dual specificity of these T cells may be via the two different $\alpha\beta$ TCR complexes each recognizing a different peptide-HLA complex. Indeed, in our previous study (7), a dual-specific CTL clone recognizing $Pol_{448-456}$ peptide presented by B35 and B51 was found to have one β and two inframe α TCR transcripts. However, using retroviral gene transfer of the two $\alpha\beta$ TCR complexes into TCR-negative TG40/CD8 cells, we showed that the dual specificity was not mediated through the two different $\alpha\beta$ TCR complexes. Instead, a single $\alpha\beta$ TCR complex bearing Vα12.1/Vβ5.6 exhibited multiple specificities, recognizing $Pol_{448-456}$ peptide presented by multiple HLA class I molecules.

It is of note that the parental CTL clone showed a >10-fold higher sensitivity to $Pol_{448-456}$ peptide presented by B35 compared with TG40/CD8 cells transduced with the Vα12.1/Vβ5.6 TCR. The latter recognized $Pol_{448-456}$ peptide at concentrations down to 1 nM, but the parent CTL clone exhibited significant cytotoxic activity even at a peptide concentration of 0.1 nM. It is conceivable that certain costimulatory molecules present on the surface of the CTL clone, but not on the mouse-derived TG40/CD8 cells, may enhance ligand recognition by the CTL clone, or that CTL clones have some intrinsic property that allowed them to achieve low-density ligand recognition. In fact, T cells have been shown to increase their affinity/avidity for an epitope following Ag stimulation through changes in their membrane organization and a redistribution of signaling molecules (reviewed in Ref. 34). We thus propose that the system used in this study involving reconstruction of the TCR complex on the surface of TG40/CD8 has a marked advantage in investigation of ligand recognition by TCRs because the effect(s) of costimulatory molecules or membrane architectures can be excluded.

Five of the HLA class I alleles analyzed in this study, B35, B51, B52, B53, and B78, are members of a serologically cross-reacting group, whereas the sixth, B7, is a member of a different serological group. However, all except B52 preferentially bind peptides with (Pro at P2) in their binding peptides, with B52 preferentially binding peptides with Gln at P2 (14). There are only two amino acid differences between B51 and B52 alleles: Asn^{63} and Phe^{67} of B51 are replaced by Glu^{63} and Ser^{67} in B52 (19). Because structural analysis of the B51 molecule has shown that the Phe^{67} residue forms a part of the B pocket (35), the Phe to Ser substitution in B52 most likely accounts for its different peptide-binding preference. It is thus likely that the observed 10-fold reduced recognition by Vα12.1/Vβ5.6 TCR-bearing T cells of $Pol_{448-456}$ peptide presented by B52 compared with peptide presented by B51 is due to reduced binding of $Pol_{448-456}$ peptide to the B52 molecule.

Allelic difference in *HLA-B*3501* and *HLA-B*5301* stems five amino acid residues found in the α1 region associated with HLA-Bw4/Bw6 epitopes. Structural studies of B35 and B53 have shown that three of the five polymorphic residues form the part of F pocket and thereby attribute in part to the preference of these HLA molecules for particular C-terminal anchor residues in binding peptides (27, 28). By precise comparison of the data for T cell responsiveness and peptide-binding capacities, we show in this study that the Vα12.1/Vβ5.6 TCR interacted comparably with the $Pol_{448-456}$ peptide presented by both self B35 and nonself B53. The tolerance of the Vα12.1/Vβ5.6 TCR for polymorphic differences between different HLA class I molecules could be due to this TCR making relatively strong contacts with other regions of the antigenic surface of the peptide-HLA complex, such as charged interactions through the three Glu residues located at P5, P6, and P8 of the $Pol_{448-456}$ peptide. It is also likely that this TCR recognizes the peptide and shared structural features of several HLA-B molecules, as reflected in their common propensity to bind the same peptide, because crystal structures of several peptide/MHC class I complexes have consistently shown that 70–80% of the peptide surface is buried in the binding cleft of MHC, leaving only 20–30% of the peptide surface exposed for interaction with solvent or TCR (36).

A very high level of cross-reactivity is an intrinsic and necessary characteristic of ligand recognition of the TCR (37). For example, the murine 2C TCR recognizes the octapeptide LSPFPFDL with syngeneic K^b and also with allogeneic L^d (38, 39), and this TCR also recognizes another octapeptide EQYKFYSV with K^b and allogeneic K^{bm3} (1, 40). Cross-reactivity of TCR has often been explained by the molecular mimicry model, proposing that different peptide/MHC complexes may form antigenic surfaces which are similar in shape, charge, or both (41, 42). In contrast, Zhao et al. (43) have reported in their structural study that rather than simple molecular mimicry, unpredictable arrays of common and differential contacts on the different peptide/MHC complexes are used for their recognition by the xeno-reactive murine TCR AHIII12.2. In addition, a recent detailed mutational study focusing on 15 TCR contact sites on the HLA-A2 molecule recognized by an A6 TCR specific for the Tax peptide presented by HLA-A2 has shown that only three amino acids (Arg^{65}, Lys^{66}, and Ala^{69}) located on the α1 helix affect T cell recognition (44). However, in contrast, a study of mouse 2C TCR binding to the QL9 peptide presented by class I MHC H2-L^d estimated that 37% of the binding energy results from recognition of peptide, with 63% resulting from recognition of the MHC (45). Considering that different types

of interactions might be expected within different TCR/peptide/MHC complexes, mutational analysis and crystallographic structural analysis of the Vα12.1/Vβ5.6 TCR and the $Pol_{448-456}$ peptide in complex with self and nonself HLA class I molecules could provide further insights into the molecular details of how a single TCR recognizes different HLA class I molecules while retaining its peptide specificity.

It should also be noted that, in repeated experiments, TG40/CD8/TCRα12β5.6 cells recognized the $Pol_{448-456}$ peptide presented by B7 but not by B78. The failure to detect binding of $Pol_{448-456}$ peptide to B78 as assessed by an HLA stabilization assay appeared to directly correlate with a lack of responsiveness by TG40/CD8/TCRα12β5.6 cells to peptide-loaded C1R cells expressing B78. Consistent with these observations, the preferred binding peptide motif of B78 (Pro, Ala, or Gly at P2 and hydrophobic amino acid residues at P6) (46), does not fully match the $Pol_{448-456}$ peptide sequence (which has Glu at P6), whereas the preferred binding peptide motif of B7 (Pro at P2 and Leu or Phe at P9) (14) does match the $Pol_{448-456}$ peptide sequence. In addition, B78 and B51 have identical α2 helices and B78 and B35 differ by only one amino acid in the α1 helix at residue 74 (Asp and Tyr in B78 and B35, respectively), whereas B7 and B35 differ by 20 amino acids including the difference at residue 74 (Asp in B7 as well). It may be possible that the Tyr^{74} residue found in B35, B51, B52, and B53 plays an important role in binding to $Pol_{448-456}$ peptide and that the change to Asp^{74} is responsible for the loss of this binding in B78. However, because B7 also has an Asp^{74} residue, the other amino acid changes might contribute to compensating for the decreased peptide binding, TCR binding, or both. It is obvious that staining the CTL clones or the TCR-expressing TG40/CD8 cells using an HLA tetramer with B7, B51, or B53 could provide additional arguments on the ligand recognition of this cross-reactive TCR. However, we have not yet succeeded in refolding the $Pol_{448-456}$ peptide with these HLA molecules despite many attempts, probably because the HLA complex with a low-binding peptide is relatively unstable during the course of the refolding step.

In summary, by focusing on various HLA class I alleles that share similar peptide-binding motifs, we demonstrated that a single $\alpha\beta$ TCR complex (Vα12.1/Vβ5.6) has the ability to specifically recognize a foreign peptide presented by multiple self (B35 and B51) and nonself (B53 and B7) HLA class I molecules. Considering that this TCR was isolated from an HIV-infected patient (HLA-A24/26, B35/51, Cw3/−), T cells bearing this TCR should have been positively selected in the patient's thymus at least in the context of B35, B51, or both. However, the overall contribution of up to six different HLA class I alleles in the selection of one CD8 T cell is so far not clear. A recent report showed that self MHC shapes the repertoire of not only self-restricted, but also alloreactive, T cells, because the closer the foreign MHC molecule is related to the T cell's MHC, the higher the proportion of peptide-specific, alloreactive T cells vs T cells recognizing the foreign MHC molecule (47). Therefore, it is possible that, when an individual has two different HLA-B alleles with similar peptide-binding motifs (B35 and B51 in this study) after positive and negative thymic selection in the context of these HLA class I alleles, a portion of CD8 T cells would to some extent cross-react with nonself HLA-B alleles that have a similar peptide-binding motif (B53 and B7 in this study). It is also of significant interest that, through large genetic correlation studies using AIDS cohorts, Carrington and colleagues (48, 49) reported an association between certain subtypes of *HLA-B35* and *HLA-B53* and rapid progression to AIDS and an association between *HLA-B51* and slow progression to AIDS. Therefore, not only subtle differences in peptide-binding activity, but also the specific or cross-reactive nature of Ag-specific CTLs restricted by these *HLA-B* alleles will be helpful for understanding CD8 T cell-mediated immune defense in individuals with HIV infection.

Acknowledgments

We thank Drs. K. Tsumoto, K. Maenaka, S. Goda, I. Kumagai, and B. Rubin for valuable discussion, and M. Tokunaga and T. Matsuda for their excellent technical assistance in HLA tetramer preparation and cell sorting, respectively. We also thank Dr. Kitamura for providing retroviral vectors and a packaging cell, and Dr. Saitoh for providing TG40 cells.

References

1. Tallquist, M., T. Yun, and L. R. Pease. 1996. A single T cell receptor recognizes structurally distinct MHC/peptide complexes with high specificity. *J. Exp. Med. 184:1017.*
2. Smith, K., E. Huczko, V. Engelhard, Y. Li, and C. Lutz. 1997. Alloreactive cytotoxic T lymphocytes focus on specific major histocompatibility complex-bound peptides. *Transplantation 64:351.*
3. Aparicio, P., D. Jaraquemada, and J. Lopez de Castro. 1987. Alloreactive cytolytic T cell clones with dual recognition of HLA-B27 and HLA-DR2 antigens: selective involvement of CD8 in their class I-directed cytotoxicity. *J. Exp. Med. 165:428.*
4. Lopez, D., D. Barber, J. Villadangos, and J. Lopez de Castro. 1993. Cross-reactive T cell clones from unrelated individuals reveal similarities in peptide presentation between HLA-B27 and HLA-DR2. *J. Immunol. 150:2675.*
5. Udaka, K., K. Wiesmuller, H. Kienle, S. Jung, and P. Walden. 1996. Self-MHC-restricted peptides recognized by an alloreactive T lymphocyte clone. *J. Immunol. 157:670.*
6. Wang, R.-F., S. L. Johnston, S. Southwood, A. Sette, and S. A. Rosenberg. 1998. Recognition of an antigenic peptide derived from tyrosinase-related protein-2 by CTL in the context of HLA-A31 and -A33. *J. Immunol. 160:890.*
7. Tomiyama, H., N. Yamada, H. Komatsu, K. Hirayama, and M. Takiguchi. 2000. A single CTL clone can recognize a naturally processed HIV-1 epitope presented by two different HLA class I molecules. *Eur. J. Immunol. 30:2521.*
8. Buseyne, F., and Y. Riviere. 2001. The flexibility of the TCR allows recognition of a large set of naturally occurring epitope variants by HIV-specific cytotoxic T lymphocytes. *Int. Immunol. 13:941.*
9. Padovan, E., G. Casorati, P. Dellabona, S. Meyer, M. Brockhaus, and A. Lanzavecchia. 1993. Expression of two T cell receptor α chains: dual receptor T cells. *Science 262:422.*
10. Heemskerk, M. H. M., R. A. de Paus, E. G. Lurvink, F. Koning, A. Mulder, R. Willemze, J. J. van Rood, and J. H. Falkenburg. 2001. Dual HLA class I and class II restricted recognition of alloreactive T lymphocytes mediated by a single T cell receptor complex. *Proc. Natl. Acad. Sci. USA 98:6806.*
11. Sidney, J., M.-F. del Guercio, S. Southwood, V. H. Engelhard, E. Appella, H.-G. Rammensee, K. Falk, O. Rotzschke, M. Takiguchi, R. T. Kubo, et al. 1995. Several HLA alleles share overlapping peptide specificities. *J. Immunol. 154:247.*
12. Sidney, J., H. M. Grey, S. Southwood, E. Celis, P. A. Wentworth, M.-F. del Guercio, R. T. Kubo, R. W. Chesnut, and A. Sette. 1996. Definition of an HLA-A3-like supermotif demonstrates the overlapping peptide-binding repertoires of common HLA molecules. *Hum. Immunol. 45:79.*
13. Barbar, L. D., B. Gillece-Castro, L. Percival, X. Li, C. Clayberger, and P. Parham. 1995. Overlap in the repertoires of peptides bound in vivo by a group of related class I HLA-B allotypes. *Curr. Biol. 5:179.*
14. Rammensee, H.-G., T. Friede, and S. Stevanovic. 1995. MHC ligands and peptide motifs: first listing. *Immunogenetics 41:178.*
15. Cooper, S., H. Kowalski, A. Erickson, K. Arnett, A.-M. Little, C. Walker, and P. Parham. 1996. The presentation of a hepatitis C viral peptide by distinct major histocompatibility complex class I allotypes from two chimpanzee species. *J. Exp. Med. 183:663.*
16. Tomiyama, H., K. Miwa, H. Shiga, Y. I. Moore, S. Oka, A. Iwamoto, Y. Kaneko, and M. Takiguchi. 1997. Evidence of presentation of multiple HIV-1 cytotoxic T lymphocyte epitopes by HLA-B*3501 molecules that are associated with the accelerated progression of AIDS. *J. Immunol. 158:5026.*
17. Zumla, A., C. Marguerie, A. So, W. M. Yokoyama, T. Saito, J. R. Batchelor, and R. I. Lechler. 1992. Co-expression of human T cell receptor chains with mouse CD3 on the cell surface of a mouse T cell hybridoma. *J. Immunol. Methods 149:69.*
18. Matsumoto, K., J. Yamamoto, M. Hiraiwa, K. Kano, and M. Takiguchi. 1990. Discrimination of HLA-B5 cross reactive group antigen by human allospecific CTL clones. *Transplantation 49:1164.*
19. Hayashi, H., P. D. Ennis, H. Ariga, R. D. Salter, P. Parham, K. Kano, and M. Takiguchi. 1989. HLA-B51 and HLA-Bw52 differ only by two amino acids which are in the helical region of α1 domain. *J. Immunol. 142:306.*
20. Kitamura, T., M. Onishi, S. Kinoshita, A. Shibuya, A. Miyajima, and G. P. Nolan. 1995. Efficient screening of retroviral cDNA expression libraries. *Proc. Natl. Acad. Sci. USA 92:9146.*
21. Morita, S., T. Kojima, and T. Kitamura. 2000. Plat-E: an efficient and stable system for transient packaging of retroviruses. *Gene Ther. 7:1063.*
22. Sobao, Y., K. Miwa, and M. Takiguchi. 1999. The role of the amino acids associated with the HLA-Bw4/Bw6 epitope in peptide binding to HLA-B5, B35 CREG molecules. *Immunogenetics 49:819.*

23. SantAngero, D. B., P. Cresswell, C. A. J. Janeway, and L. K. Denzin. 2001. Maintenance of TCR clonality in T cells expressing genes for two TCR heterodimers. *Proc. Natl. Acad. Sci. USA 98:6824.*
24. Hill, A. M., M. Takiguchi, and A. McMichael. 1993. Different rates of HLA class I molecule assembly which are determined by amino acid sequence in the α2 domain. *Immunogenetics 37:95.*
25. Zemmour, J., A.-M. Little, D. J. Schendel, and P. Parham. 1992. The HLA-A, B "negative" mutant cell line C1R expresses a novel HLA-B35 allele, which also has a point mutation in the translation initiation codon. *J. Immunol. 148:1941.*
26. Brodsky, F. M., and P. Parham. 1982. Monomorphic anti-HLA-A, B, C monoclonal antibodies detecting molecular subunits and combinatorial determinants. *J. Immunol. 128:129.*
27. Smith, K., S. Reid, K. Harlos, A. McMichael, D. Stuart, J. Bell, and E. Jones. 1996. Bound water structure and polymorphic amino acids act together to allow the binding of different peptides to MHC class I HLA-B53. *Immunity 4:215.*
28. Smith, K., S. Reid, D. Stuart, A. McMichael, E. Jones, and J. Bell. 1996. An altered position of the α2 helix of MHC class I is revealed by the crystal structure of HLA-B*3501. *Immunity 4:203.*
29. Sakaguchi, T., M. Ibe, K. Miwa, S. Yokota, K. Tanaka, C. Schoenbach, and M. Takiguchi. 1997. Predominant role of N-terminal residue of nonamer peptides in their binding to HLA-B*5101 molecules. *Immunogenetics 46:245.*
30. Stanislawski, T., R.-H. Voss, C. Lotz, E. Sadovnikova, R. A. Willemsen, J. Kuball, T. Ruppert, R. L. H. Bolhuis, C. J. Melief, C. Huber, et al. 2001. Circumventing tolerance to a human MDM2-derived tumor antigen by TCR gene transfer. *Nat. Immunol. 2:962.*
31. Fujio, K., Y. Misaki, K. Setoguchi, S. Morita, K. Kawahata, I. Kato, T. Nosaka, K. Yamamoto, and T. Kitamura. 2000. Functional reconstitution of class II MHC-restricted T cell immunity mediated by retroviral transfer of the $\alpha\beta$ TCR complex. *J. Immunol. 165:528.*
32. Cooper, L. J. N., M. Kalos, D. A. Lewinsohn, S. R. Riddell, and P. D. Greenberg. 2000. Transfer of specificity for human immunodeficiency virus type 1 into primary human T lymphocytes by introduction of T-cell receptor genes. *J. Virol. 74:8207.*
33. He, X., C. Janeway Jr., M. Levine, E. Robinson, P. Preston-Hurlburt, C. Viret, and K. Bottomly. 2002. Dual receptor T cells extend the immune repertoire for foreign antigens. *Nat. Immunol. 3:127.*
34. Bromley, S., W. Burack, K. Johnson, K. Somersalo, T. Sims, C. Sumen, M. Davis, A. Shaw, P. Allen, and M. Dustin. 2001. The immunological synapse. *Annu. Rev. Immunol. 19:375.*
35. Maenaka, K., T. Maenaka, H. Tomiyama, M. Takiguchi, D. I. Stuart, and E. Y. Jones. 2000. Nonstandard peptide binding revealed by crystal structures of HLA-B*5101 complexed with HIV immunodominant epitopes. *J. Immunol. 165: 3260.*
36. Eisen, H. N., Y. Sykulev, and T. J. Tsomides. 1996. Antigen-specific T-cell receptors and their reactions with complexes formed by peptides with major histocompatibility complex proteins. *Adv. Protein Chem. 49:1.*
37. Mason, D. 1998. A very high level of crossreactivity is an essential feature of the T-cell receptor. *Immunol. Today 19:395.*
38. Udaka K, T. J. Tsomides, and H. N. Eisen. 1992. A naturally occurring peptide recognized by alloreactive $CD8^+$ cytotoxic T lymphocytes in association with a class I MHC protein. *Cell 69:989.*
39. Dutz J. P, T. J. Tsomides, S. Kageyama, M. H. Rasmussen, and H. N. Eisen. 1994. A cytotoxic T lymphocyte clone can recognize the same naturally occurring self peptide in association with a self and nonself class I MHC protein. *Mol. Immunol. 31:967.*
40. Tallquist M. D., and L. R. Pease. 1995. Alloreactive 2C T cells recognize a self peptide in the context of the mutant K^{bm3} molecule. *J. Immunol. 155:2419.*
41. Wucherpfennig K. W., and J. L. Strominger. 1995. Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. *Cell 80:695.*
42. Maverakis, E., P. van den Elzen, and E. E. Sercarz. 2001. Self-reactive T cells and degeneracy of T cell recognition: evolving concepts-from sequence homology to shape mimicry and TCR flexibility. *J. Autoimmun. 16:201.*
43. Zhao, R., D. J. Loftus, E. Appella, and E. J. Collins. 1999. Structural evidence of T cell xeno-reactivity in the absence of molecular mimicry. *J. Exp. Med. 18:359.*
44. Baker, B. M., R. V. Turner, S. J. Gagnon, D. C. Wiley, and W. E. Biddison. 2001. Identification of a crucial energetic footprint on the α1 helix of human histocompatibility leukocyte antigen (HLA)-A2 that provides functional interactions for recognition by Tax peptide/HLA-A2-specific T cell receptors. *J. Exp. Med. 193:551.*
45. Manning, T., C. Schlueter, T. Brodnick, E. Parke, J. Speir, K. Garcia, L. Teyton, I. Wilson, and D. M. Krantz. 1998. Alanine scanning mutagenesis of an $\alpha\beta$ T cell receptor: mapping the energy of antigen recognition. *Immunity 8:413.*
46. Falk, K., O. Roetzschke, M. Takiguchi, V. Gnau, S. Stevanovic, G. Jung, and H.-G. Rammensee. 1994. Peptide motifs of HLA-B51, -B52 and -B78 molecules, and implications for Behcet's disease. *Int. Immunol. 7:223.*
47. Obst, R., N. Netuschil, K. Klopfer, S. Stevanovic, and H. Rammensee. 2000. The role of peptides in T cell alloreactivity is determined by self-major histocompatibility complex molecules. *J. Exp. Med. 191:805.*
48. Gao, X., G. W. Nelson, P. Karacki, M. P. Martin, J. Phair, R. Kaslow, J. J. Goedert, S. Buchbinder, K. Hoots, D. Vlahov, et al. 2001. Effect of a single amino acid change in MHC class I molecules on the rate of progression to AIDS. *N. Engl. J. Med. 344:1668.*
49. O'Brien, S. J., X. Gao, and M. Carrington. 2001. HLA and AIDS: a cautionary tale. *Trends Mol. Med. 7:379.*