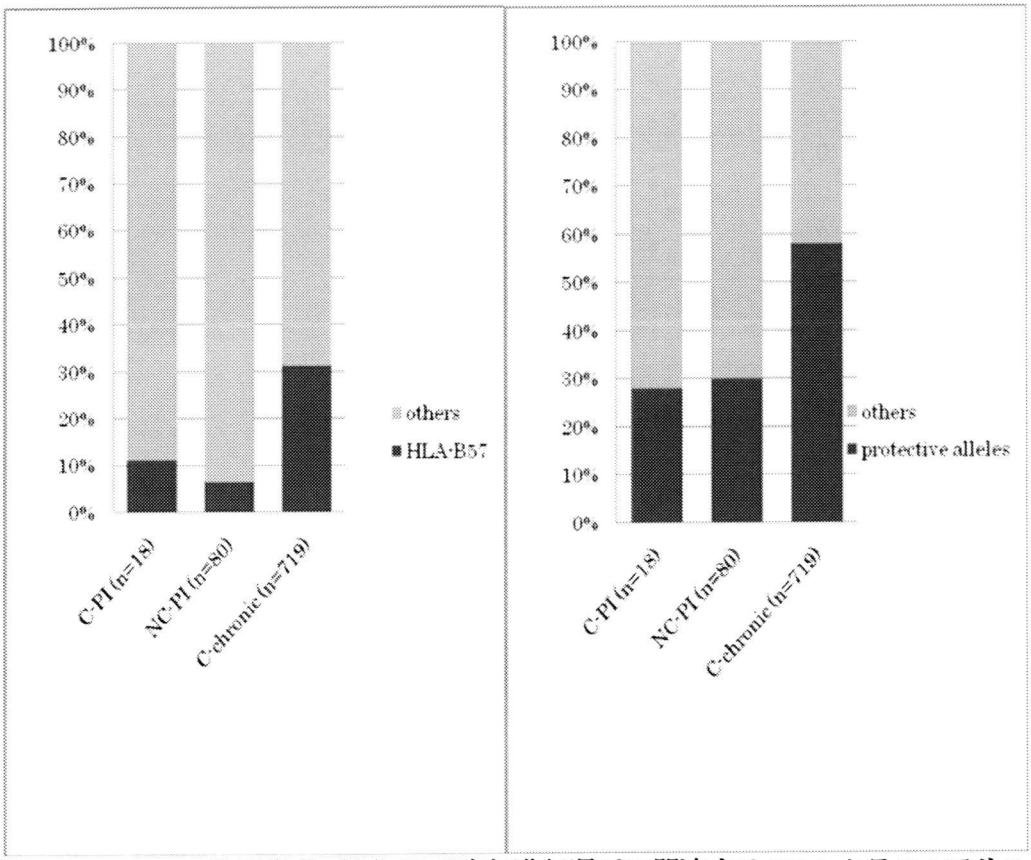

図１．HLA-B57 発現患者（左）及び病気進行遅延に関連する HLA クラス I アリル発現（右）の割合。C-PI (controllers after primary infection), NC-PI (non-controllers after primary infection), C-chronic (controlleres with chronic HIV infection)。

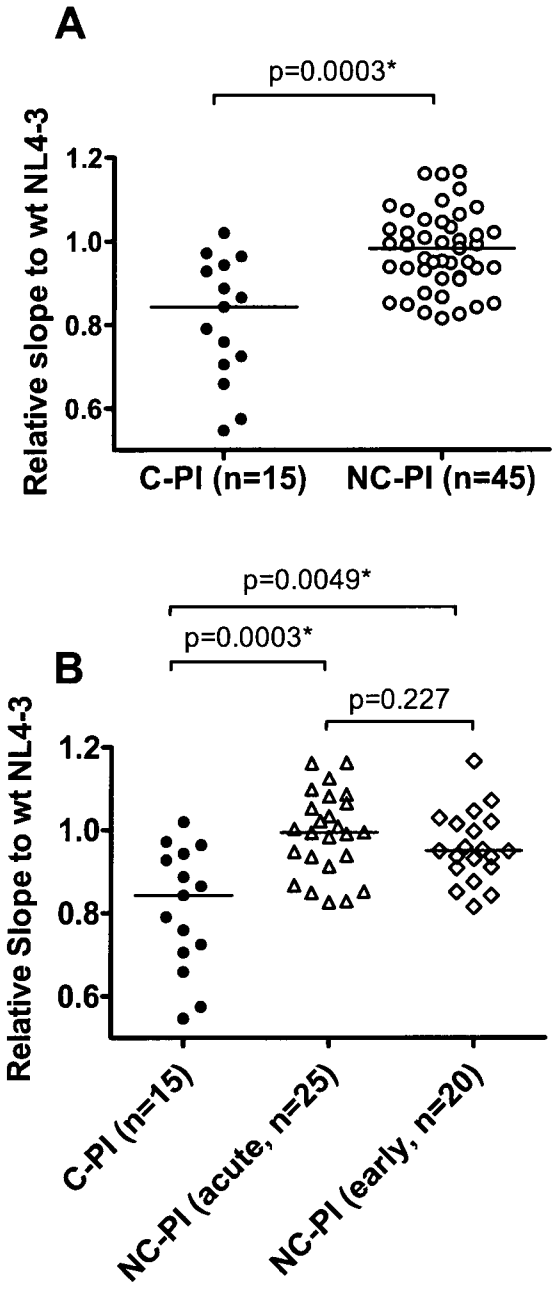

図２．臨床検体由来 Gag-protease を持つ NL4-3 の複製能の比較
C-PI (controllers after primary infection), NC-PI (non-controllers after primary infection)。A.全体の比較。B.NC-PI を急性感染期に診断された群（acute）と早期に診断された群（early）に層化した比較。

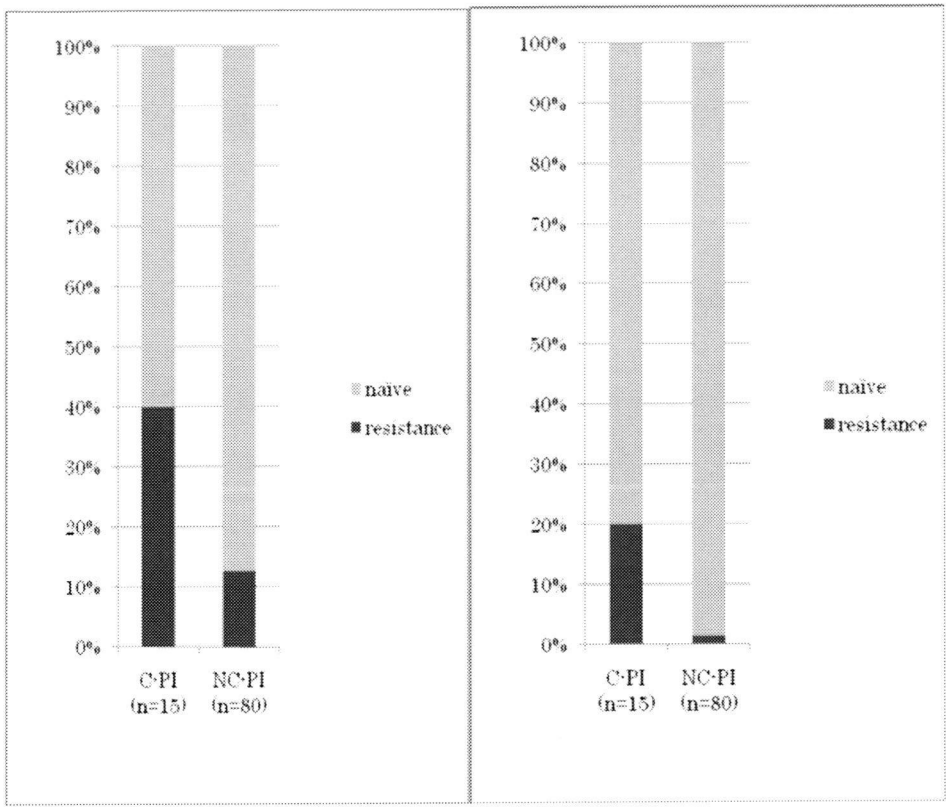

図３．薬剤耐性変異を持つウイルス感染者の割合

C-PI (controllers after primary infection), NC-PI (non-controllers after primary infection)。左：major drug resistance mutations を持つ人の割合。右：multi-class drug resistance mutations を持つ人の割合。

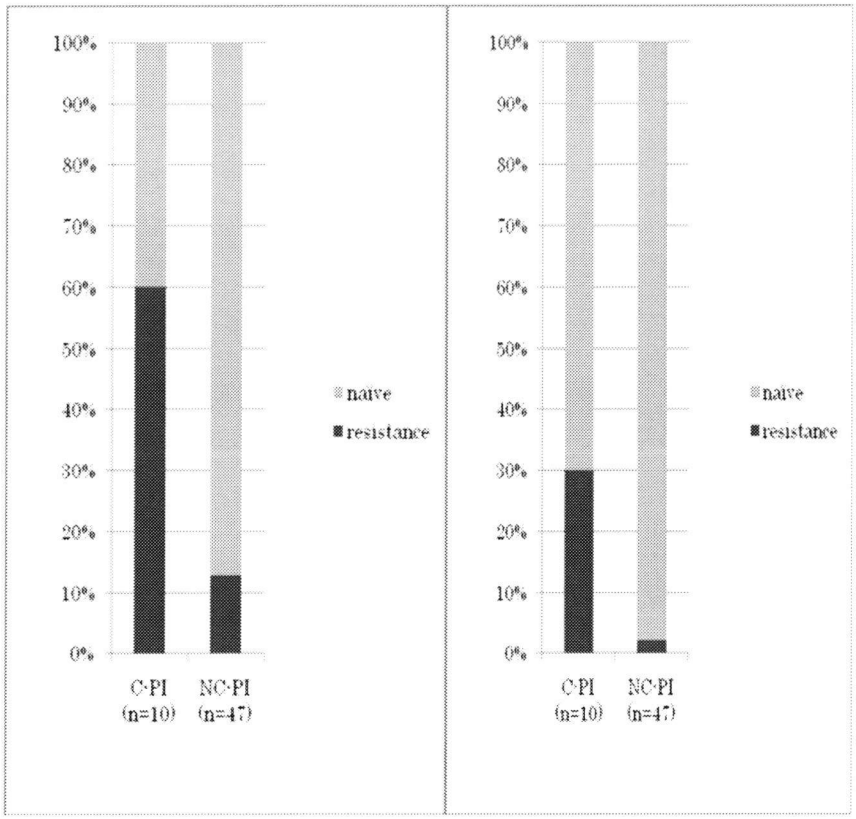

図４．Protective な HLA クラス I アリルを発現していない患者群における薬剤耐性変異ウイルス感染の割合

C-PI (controllers after primary infection), NC-PI (non-controllers after primary infection)。左：major drug resistance mutations を持つ人の割合。右：multi-class drug resistance mutations を持つ人の割合。

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Highly restricted T-cell receptor repertoire in the $CD8^+$ T-cell response against an HIV-1 epitope with a stereotypic amino acid substitution

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Objective: In peripheral blood mononuclear cells (PBMCs) from HIV-1-positive patients, we sought to identify $CD8^+$ T-cell populations and the corresponding T-cell receptor (TCR) repertoires that react to an immunogenic cytotoxic T lymphocyte (CTL) epitope with or without an escape mutation.

Methods: PBMCs from HLA-A*2402(A24)-positive patients were stimulated with peptides representing a wild-type CTL epitope in the HIV-1 Nef protein [Nef138-10(wt)] or an escape mutant with a Y to F (Y139F) substitution at the second position [Nef138-10(2F)]. Cultured PBMCs were stained with peptide-major histocompatibility complex tetramers containing Nef138-10(wt) or Nef138-10(2F) sequences. After in-vitro stimulation of PBMCs with cognate peptides, the $CD8^+$ T-cell population was sorted into different fractions: positive only to the wild-type tetramer (wt-positive), positive only to the mutant tetramer (2F-positive), and positive to both wt-tetramers and mutant-tetramers (dual-positive). TCR repertoires of sorted epitope-specific $CD8^+$ T-cell populations were determined by sequencing.

Results: A 2F-positive population was rarely observed under our culture and staining conditions. The wt-positive $CD8^+$ T-cell populations had a diverse TCR repertoire, but the TCR repertoires in dual-positive $CD8^+$ populations were highly restricted. In the dual-positive $CD8^+$ T-cell populations, most clonotypes used the TRBV4-1 and TRBJ2-7 gene segments for the TCR β-chain and the TRAV8-3 and TRAJ40-1 for the TCR α-chain. The CDR3 region of the TCR β-chain showed little variation.

Conclusion: These results provide an example of restricted TCR repertoire in a specific CTL response against the escaping epitope. We speculate that impairment of antigen presentation in escaping viruses may underlie the restricted repertoire.

AIDS 2009, **23**:651–660

Keywords: $CD8^+$ T cells, HIV infection, human leukocyte antigen, T-cell epitope, T-cell receptor repertoire

Introduction

Cytotoxic T lymphocytes (CTLs) play a very important role in counteracting HIV-1 infection [1–3]. However, the hallmark of HIV-1 infection is the incomplete response of CTLs. It is crucial to understand the molecular mechanisms of antigen presentation and recognition in the context of immunopathogenesis of HIV-1.

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CTLs use T-cell receptors (TCRs) to recognize peptide-major histocompatibility complex (MHC) (pMHC) complexes presented on the surface of the infected cells. Error-prone reverse transcription of HIV-1 can result in amino acid substitutions in the cognate peptides. Mutated viruses may acquire selective advantage against CTLs and become dominant escape variants [4,5]. Substitution of amino acid residues critical for binding to MHC molecules [6,7] or TCR recognition [8,9] can result in escape mutants. Even substitution of the amino acids flanking the cognate peptides can result in escape mutants by altering the peptide processing and decreasing the number of pMHC molecules that are recognized by CTLs [10–14].

Amino acid substitutions in the HIV-1 escape mutants may be stereotypic in different individuals sharing the same MHC haplotypes [12,15]. We previously reported that HIV-1 with a stereotypic substitution from Y [Nef138-10(wt)] to F [Nef138-10(2F)] at the second position in an immunodominant HLA-A*2402(A24)-restricted CTL epitope in the Nef protein (Nef138-10) has a strong selective advantage in A24-positive patients [12]. There is a high prevalence of A24 in the Japanese population, and unprotected sexual contact has transmitted the 2F substitution among A24-positive individuals throughout Japan. How HIV-1 with the Nef138-10(2F) substitution could have a selective advantage in A24-positive patients remains an enigma, as we detected vigorous $CD8^+$positive T-cell ($CD8^+$) responses not only against Nef138-10(wt) but also against Nef138-10(2F) in PBMCs from A24-positive patients [12].

In this study to explore the effector side, we stimulated cultured $CD8^+$ T cells obtained from A24-positive, HIV-1-infected patients and stained them simultaneously with two A24 tetramers that presented either Nef138-10(wt) or Nef138-10(2F). We then sorted the epitope-specific $CD8^+$ T cells that recognized wild-type or 2F or both and analyzed the TCR repertoire.

Materials and methods

Study patients

We analyzed CTL response in peripheral blood mononuclear cells (PBMCs) from seven patients who were HIV-1 infected and HLA-A*2402 positive. Patients were randomly selected among patients participating in an ongoing HIV-1-immunopathogenesis study at an HIV outpatient clinic affiliated with the Institute of Medical Science, the University of Tokyo. All but one of the seven subjects (S15) were antiretroviral therapy naive. The study was approved by the internal review board of the Institute of the Medical Science of the University of Tokyo (No. 11-2), and all patients provided informed consent.

Cell media and study reagents

Culture media and supplements were purchased from Sigma (St Louis, Missouri, USA) except as otherwise noted. R(−) medium consisted of RPMI 1640 supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 10 mmol/l 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 2 mmol/l L-glutamine. R10 medium was R(−) medium supplemented with 10% heat-inactivated fetal calf serum (FCS).

Synthetic peptides Nef138-10(wt) (RYPLTFGWCF), Nef138-10(2F) (RFPLTFGWCF) were purchased from Sigma-Genosys (Ishikari-shi, Hokkaido, Japan).

Enzyme-linked immunosorbent spot assay

Enzyme-linked immunosorbent spot (ELISPOT) assay was performed using freshly prepared PBMCs (5×10^4 cells) as previously described [16].

In-vitro stimulation with Nef138-10 peptides

PBMCs were divided into two aliquots and prepared for in-vitro stimulation with Nef138-10 peptides as previously described [17]. To prepare antigen-presenting cells, PBMCs of 5×10^5 patients were pulsed with 10 nmol/l Nef138-10(wt) or Nef138-10(2F) at 37°C for 1 h. Cells were washed twice with R10, then cultured in R10 with 1×10^6 fresh autologous PBMCs and 4×10^6 irradiated (3300 rads) PBMCs from healthy individuals. After 4 days, recombinant human IL-2 (rIL-2; Wako, Osaka, Japan) was added to 50 U/ml. The culture was continued for 2 weeks, with medium changed every 3–4 days (R10 with 50 U/ml rIL-2).

Preparation of major histocompatibility complex-class I tetramers presenting Nef138-10(wt) or Nef138-10(2F)

Soluble forms of pMHC molecules were produced in CV-1 cells using a Sendai virus (SeV) vector expression system and purified from the supernatant as described previously [18]. After affinity purification, pMHC molecules were biotinylated with BirA enzyme (Avidity, Aurora, Colorado, USA) and purified by gel filtration chromatography with a Superdex 200 column (GE Healthcare, Piscataway, New Jersey, USA).

Biotinylated Nef138-10(wt)/HLA-A24 (Nef138-10(wt)/A24) or Nef138-10(2F)/HLA-A24 (Nef138-10(2F)/A24) complexes were tetramerized with allophycocyanin (APC)-labeled or phycoerythrin-labelled streptavidins (Invitrogen, Eugene, Oregon, USA), respectively

Flow cytometry and sorting of cytotoxic T lymphocytes

Stimulated PBMCs were incubated at 37°C for 15 min in the presence of Nef138-10(wt)/A24-APC or Nef138-10(2F)/A24-PE or both. The final concentrations of Nef138-10(wt)/A24-APC and Nef138-10(2F)-PE in monomer pMHC were 11 and 8 μg/ml, respectively.

Cells were washed with fluorescence-activated cell sorter (FACS) buffer (PBS supplemented with 2% FCS and 0.02% NaN_3) and further stained with either fluorescein isothiocyanate (FITC)-labeled or Pacific Blue-labeled anti-CD8 T-cell antibodies (BD Pharmingen, San Jose, California, USA) or anti-TCR β-chain V gene 4-1 antibodies (TRBV4-1) (Beckman Coulter, Fullerton, California, USA) at 4°C for 30 min. Cells were then washed with FACS buffer and fixed by a 20-min incubation at reverse transcriptase in the dark in PBS containing 1% paraformaldehyde.

Flow cytometry was performed using a FACS Calibur (Beckton Dickinson, Franklin Lakes, New Jersey, USA) and FACS Aria (Beckton Dickinson). Flowjo ver. 6.4.7 (Tree Star, Ashland, Oregon, USA) was used for the analysis. For cell sorting, the cells were stained in R10 medium instead of FACS buffer, stained with propidium iodide to remove dead cells, and sorted without fixation using a FACS Aria.

T-cell receptor repertoire determination

Total RNA was extracted from sorted T cells using an RNeasy Micro Kit (Qiagen, Venlo, The Netherlands). We synthesized full-length cDNA by anchored RT-PCR using the super switching mechanism at 5′-end of the RNA transcript (SMART) PCR cDNA synthesis kit (TakaraBio, Otsu, Shiga, Japan), according to the manufacturer's protocol, with the switching mechanism at the 5′ end of RNA transcript. To amplify the variable, diversity, and joining regions of the TCR genes, the second PCR was done with the first primer and the reverse primers specific for the TCR α or β constant region: the 3′ T-cell receptor α chain constant region (TRAC) primer (5′-GGCAGACAGACTTGTCACTG GATTTAGAG-3′) or the 3′ T-cell receptor β chain constant region (TRBC) primer (5′-TGACCC CACTGTGCACCTC-3′), respectively. Reaction conditions in the second PCR were as follows: 94°C for 1 min; 25 extension cycles of 94°C for 30 s, 55°C for 30s, 72°C for 1 min; and a final extension at 72°C for 7 min. Reaction products from the second PCR were purified with Wizard PCR preps DNA purification System (Promega, Madison, Wisconsin, USA) and subcloned into pGEM-T East vector (Promega).

DNA sequencing was performed using an ABI Prism dye terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, California, USA) on a Perkin-Elmer ABI-377 sequencer. Designation of TCR genes follows the international immunogenetics (IMGT) nomenclature [19]. We defined the CDR3 region of the TCR β-chain as the region from aa 104 in T-cell receptor Vβ (TRBV) to aa 7 in TRBJ and the CDR3 region of the TCR α-chain as the region from aa 105 in TRAV to aa 11 in TRAJ.

Results

Nef138-10-specific response in HLA-A*2402+ patients

We analyzed PBMCs from seven A24-positive patients with chronic HIV-1 infection. All patients except one (S15) were naive to antiretroviral treatment. The median viral load was 6700 copies/ml (range, 120–24000 copies/ml), and the median CD4 T-cell count was 437 cells/μl (range, 278–807 cells/μl).

Using the plasma samples obtained closest to the following analyses, we confirmed that plasma viruses had stereotypically Nef138-10(2F) in all the patients analyzed (Fig. 1). Nef138-10-specific responses of $CD8^+$ T cells were analyzed by IFN-γ ELISPOT assay. Although the magnitude of specific response varied substantially among the samples, all showed a response to Nef138-10(wt) and Nef138-10(2F) (Fig. 1).

Tetramer dual-staining of Nef138-10-specific $CD8^+$ T cells

After 2-week culture in the presence of 10 nmol/l Nef138-10(wt) or Nef138-10(2F) peptides, PBMCs were stained with Nef138-10(wt)/A24-APC [Tet(wt)] or Nef138-10(2F)/A24-PE [Tet(2F)] or both. Using cultured cells from patient S15, we examined whether dual staining fractionated the Nef138-10-specific cells more precisely than single staining. Single stainings with Tet(wt) and Tet(2F) stained 9.8% (Fig. 2a) and 6.2% (Fig. 2b) of S15 $CD8^+$ T cells, respectively, after stimulation with Nef138-10(wt). In dual staining with the two tetramers, 7.2% of S15 $CD8^+$ T cells were in the Tet(wt)-positive/Tet(2F)-positive (dual-positive) fraction and 4.2% were in the Tet(wt)-positive/Tet(2F)-negative (wt-positive) fraction (Fig. 2c). Similarly, after Nef138-10(2F) stimulation, single staining of $CD8^+$ T cells from patient S15 with Tet(wt) and Tet(2F) stained 6.0 (Fig. 2d) and 6.1% of cells, respectively (Fig. 2e). With dual staining, 3.3% of S15 $CD8^+$ T cells stimulated with Nef138-10(2F) were dual-positive, and 2.2% were wt-positive.

These preliminary pilot experiments suggested that dual staining could fractionate dual-positive and wt-positive cells regardless of the peptide used for stimulation. Under the culture and staining conditions we used, the Tet(wt)-negative/Tet(2F)-positive (2F-positive) population was minimal. As we could fractionate dual-positive and wt-positive cells under these conditions, we inferred that the $CD8^+$ T cells in each fraction had different affinities for pMHC, that is, Tet(wt) or Tet(2F).

To examine the TCR repertoire in different fractions, we cultured PBMCs from seven patients and stained the cells with both Tet(wt) and Tet(2F) (Fig. 2g–m). In cells from patient O14, only Tet(wt)-positive cells were more than

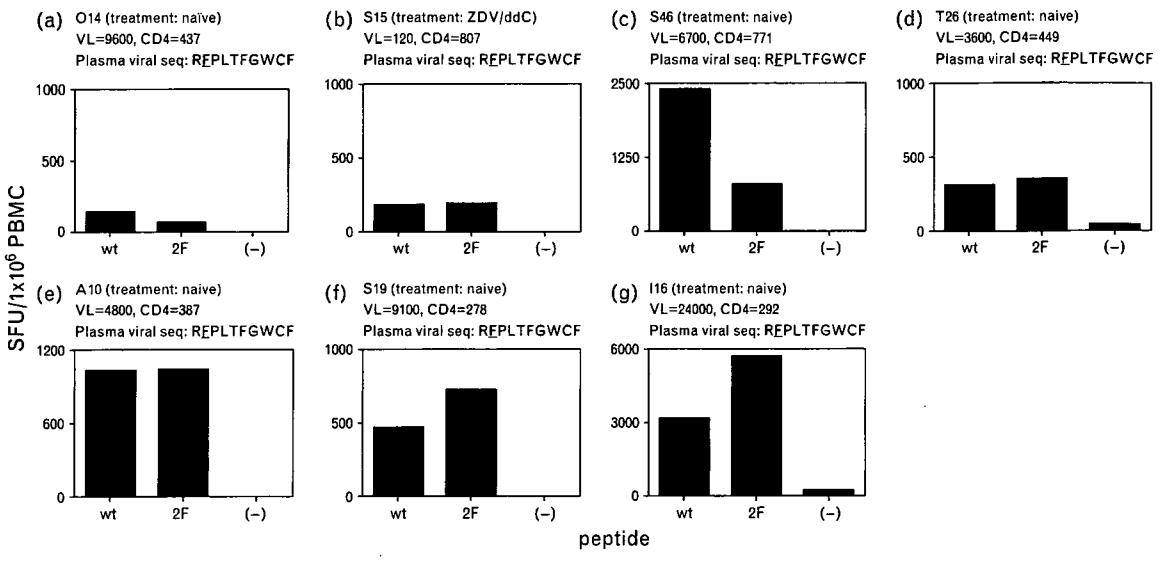

Fig. 1. Study patients and Nef138-10-specific responses. Peripheral blood mononuclear cells (PBMCs) from seven patients were stimulated with Nef138-10(wt) (RYPLTFGWCF), Nef138-10(2F) or mock. Spot-forming units (SFUs)/1 $\times 10^6$ PBMCs after stimulation are shown in subparts a–g. The viral load (VL) and CD4 T-cell count (CD4) of each patient are shown. All patients had HLA-A*2402 and their plasma viruses had Nef138-10(2F) mutation (RFPLTFGWCF).

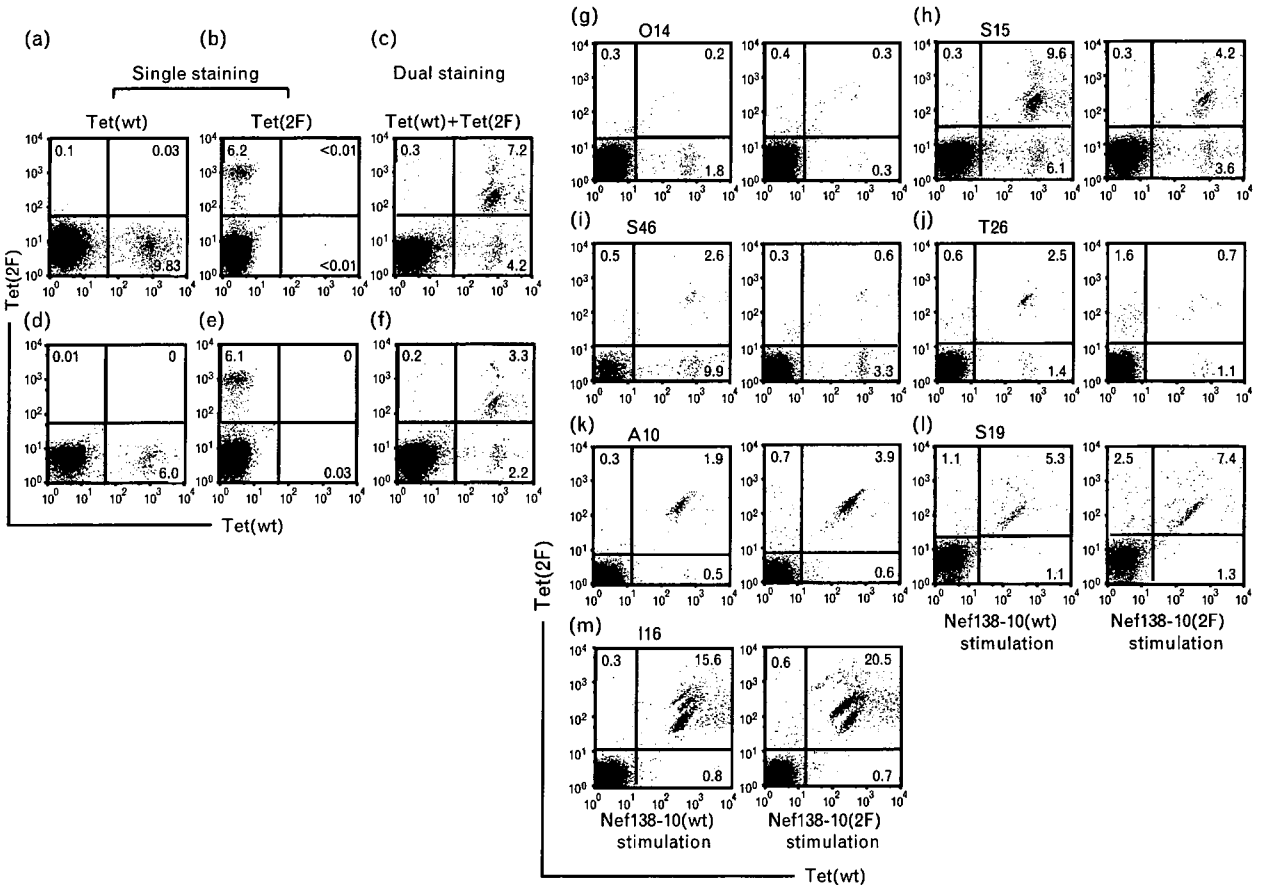

Fig. 2. Tetramer staining of peripheral blood mononuclear cells stimulated with Nef138-10(wt) or Nef138-10(2F). Peripheral blood mononuclear cells (PBMCs) from patient S15 were stimulated with Nef138-10(wt) (a, b, c) or Nef138-10(2F) peptides (d, e, f). Cells were stained with Nef138-10(wt)/A24-APC-tetramer [Tet(wt)] (a, d) or Nef138-10(2F)/A24-PE-tetramer [Tet(2F)] (b, e) or both (c, f) as described in 'Materials and Methods'. Cultured PBMCs from seven patients were stained with both Tet(wt) and Tet(2F) (g–m) including an S15 sample from a different culture (h). Dot plots are gated on $CD8^+$ T cells. Numbers refer to the percentages of gated cells in each quadrant.

1% of the population after Nef138-10(wt) stimulation (Fig. 2g). Nef138-10(2F) failed to induce specific $CD8^+$ T cells in cultures derived from patient O14. In cultures from patients S15, S46, and T26, both dual-positive and wt-positive $CD8^+$ T-cell populations were detected (Fig. 2h–j). Although Nef138-10-specific $CD8^+$ T cells were induced with both Nef138-10(wt) and Nef138-10(2F), the former induced higher expansion of cells derived from patients S15, S46, and T26. However, in cultures from patients in A10, S19, and I16, higher expansion of Nef138-10-specific $CD8^+$ T cells was induced with Nef138-10(2F) stimulation than with Nef138-10(wt) stimulation (Fig. 2k–m). In cultures derived from these three patients, the great majority of cells after stimulation were dual-positive.

Only in patient T26 was the 2F-positive population distinguished clearly from the dual-positive population, though the 2F-positive population showed lower fluorescent intensity (phycoerythrin) than did the dual-positive population (Fig. 2j). With a 1000-fold increase, from 10 nmol/l to 10 μmol/l, in concentration of Nef138-10(wt) and Nef138-10(2F) peptides used for stimulation, we observed expansion of 2F-positive $CD8^+$ T cells from other patients (data not shown).

T-cell receptor repertoire of wt-positive and dual-positive $CD8^+$ T-cell populations

Cell sorting showed substantial diversity among the study patients in the TCR repertoire of the wt-positive population of CD8 cells induced with Nef138-10(wt) (Fig. 3). The TCR repertoire also varied slightly according to the peptides used for stimulation. We observed 7.0 ± 2.2 different TCR β-chain clonotypes per individual after stimulation with Nef138-10(wt) peptides, compared with 6.3 ± 3.5 different clonotypes per individual after stimulation with Nef138-10(2F) peptides. Patients differed in whom β-chain V gene (TRBV) was most frequently used after Nef138-10(wt) stimulation. After Nef138-10(2F) stimulation, TRBV7-9 was the most common TCR β-chain clonotype seen in each of the three patients analyzed (patients S15, S46, and T26) (Fig. 4a).

In the dual-positive population, we observed only 3.3 ± 1.5 clonotypes per patient following Nef138-10(wt) stimulation and 1.8 ± 1.0 different clonotypes per patient following Nef138-10(2F) stimulation (Fig. 3). There were significantly fewer clonotypes in the dual-positive populations than in the wt-positive populations, regardless of stimulation conditions. Following Nef138-10(wt) stimulation, the mean number of clonotypes observed in the dual-positive population was 3.3 ± 1.6, compared with 7.0 ± 2.2 in the wt-positive population ($P = 0.01$, Mann–Whitney *U*-test). Following Nef138-10(2F) stimulation, the mean numbers of clonotypes in the dual-positive and wt-positive populations were 1.8 ± 1.0 and 6.5 ± 3.5, respectively ($P = 0.048$).

Notably, TRBV usage was highly restricted in the dual-positive population in all patients examined. TRBV4-1 was used in 84% of the analyzed clones, irrespective of stimulating peptides, and was the major TRBV in all clones except in those from patient I16 (Figs 3 and 4a). At the time of our analysis, the major TCR β-chain gene segments in patient I16 were TRBV15 and TRBV10-3; however, further studies using frozen PBMCs obtained from this patient 2 years earlier showed TRBV4-1/TRBJ2-7 to be the most frequently used clonotype (data not shown).

TCR β-chain joining gene (TRBJ) usage was more restricted in the dual-positive population compared with the wt-positive population, irrespective of the peptides used for stimulation (Fig. 3). Ninety-four percent of the dual-positive population in the analyzed clones used TRBJ2-7, whereas in the wt-positive population, TRBJ usage was more diverse (Fig. 4a). The CDR3 TCR β-chain region was also conserved in the dual-positive $CD8^+$ T-cell population. The length of the CDR3 region ranged from 12–16 amino acids in the dual-positive population, compared with 10–19 amino acids in the wt-positive population (Fig. 3). The CDR3 region had a length of 13 amino acids in 63% of the dual-positive population induced by Nef138-10(wt) stimulation and in 68% stimulated with Nef138-10(2F). More than 70% of the dual-positive clones using TRBV4-1/TRBJ2-7 conserved proline at the variable–diversity junction (fifth position of CDR3) and glycine and isoleucine at the diversity–joining junction. Surprisingly, the most frequent CDR3 amino acid sequence was identical in clones from three patients (S15, S46, and S19) (Fig. 4b). However, the nucleotide sequences were not identical, and distinctive clones were isolated from patients S15 and S46 (Fig. 4b), clearly indicating that the same TCR β-chain arose from different recombination events.

To examine the TCR α-chain diversity in a population with a highly restricted TCR β-chain repertoire, we analyzed TCR α-chain sequences in the dual-positive population after Nef138-10(2F) stimulation in PBMCs from patients S46, A10, and S19. In these three patients, the dual-positive population used TCR α-chain variable gene (TRAV) 8-3 and joining gene (TRAJ) 40 (Fig. 4c). The CDR3 region was highly conserved in length, ranging from 14 to 15 amino acids and clones using TRAV8-3/TRAJ40-conserved proline at the sixth position. Thus, the dual-positive $CD8^+$ T-cell population showed a highly restricted repertoire in both TCR α and β chains, including the CDR3 sequence.

T-cell receptor Vβ usage in the dual-positive $CD8^+$ T-cell population *in vivo*

The analyses described above showed that dual-positive $CD8^+$ T-cell populations expanded *in vitro* had a highly restricted TCR repertoire; that is, TRBV4-1/TRBJ2-7 and TRAV8-3/TRAJ40. We hypothesized that selection

wt-positive CD8+T cells

	Nef138-10(wt)-stimulation						Nef138-10(2F)-stimulation					
	TRBV	CDR3	TRBJ	Freq. (%)	No.Seq		TRBV	CDR3	TRBJ	Freq. (%)	No.Seq	
O14	27	CASS TGG EQF	2-1	(38)	104clones							
	11-2	CASS SDTGTGGLI NGYT	1-2	(35)								
	19	CASS IGAGTGT PTLY	1-3	(9)								
	5-5	CASS SEVL AEAF	1-1	(7)								
	6-1	CASS DIGRGV HEQY	2-7	(6)								
	6-1	CAS RIDGSSY NEQF	2-1	(2)								
	7-2	CASS LGGGGT DTQY	2-3	(1)								
	7-9	CASS LVEGTG WEQF	2-1	(1)								
	2	CA RRTSGRE TQYF	2-5	(1)								
S15	10-2	CASS ESIGGRDNN QPQH	1-5	(43)	23clones		7-9	CASS SYYRSG NTIY	1-3	(22)	27clones	
	28	CASSLSTGRGT YEQY	2-7	(26)			28	CASS LSTGRGT YEQY	2-7	(22)		
	7-9	CASS SYYRSG NTIY	1-3	(9)			10-2	CASS ESIGGRDNN QPQH	1-5	(19)		
	4-1	CASS QGWFAASV QPQH	1-5	(4)			6-5	CTSV PAVRSQRDRGQ ETQY	2-5	(7)		
	4-1	CASS PTAGI YEQY	2-7	(4)			5-4	CASS LTG ETQY	2-5	(7)		
	6-1	CASS DIGQGAI EQY	2-7	(4)			12-3	CASS LGQGE ETQY	2-5	(7)		
	30	CAW GNPGLNT GELF	2-2	(4)			10-1	CAKR AGGAT DTQY	2-3	(3)		
	11-2	CASS YDRS YEQY	2-7	(4)			6-1	CASS DIGQGA IEQY	2-7	(3)		
							7-9	CASS LDETGG YEQY	2-7	(3)		
							7-9	CASS ARAGTSGA GELF	2-2	(3)		
S46	5-5	CASS LEQFT DEQY	2-7	(32)	25clones		7-9	CASS LRDSVP ETQY	2-5	(49)	19clones	
	7-9	CASS LRDSVP ETQY	2-5	(24)			5-5	CASS LEQFTD EQY	2-7	(42)		
	7-9	CASS SWDTG ELF	2-2	(20)			25-1	CAS SASGQQP YEQY	2-7	(10)		
	6-1	CASS DAQTGTVLN YGYT	1-2	(8)								
	25-1	CAS SASGQQP YEQY	2-7	(8)								
	5-1	CASS LELST GELF	2-2	(4)								
	4-2	CASS PPGL NTQY	2-3	(4)								
T26	7-9	CASS LRDRVP ETQY	2-5	(57)	30clones		7-9	CASS LRDRVP ETQY	2-5	(50)	30clones	
	6-1	CASS DPGQGD EAF	1-1	(23)			6-1	CASS DPGQGD EAF	1-1	(20)		
	21	CATW DMSSYN SPLH	1-6	(12)			6-1	CASS FRPGLAV TGELF	2-2	(10)		
	6-1	CASS FRPGLAV TGELF	2-2	(6)			7-8	CASS LIVQGW YEQY	2-7	(10)		
							21	CATW DMSSYN SPLH	1-6	(6)		
							27	CASS AGYN EQF	2-1	(3)		

Dual-positive CD8+T cells

	Nef138-10(wt)-stimulation						Nef138-10(2F)-stimulation					
	TRBV	CDR3	TRBJ	Freq. (%)	No.Seq		TRBV	CDR3	TRBJ	Freq. (%)	No.Seq	
S15	4-1	CASS PGQGI YEQY	2-7	(28)	29clones		4-1	CASS PGQGI YEQY	2-7	(53)	30clones	
	4-1	CASS QLAGV YEQF	2-1	(28)			4-1	CASS QLAGV YEQF	2-1	(40)		
	4-1	CASS RTSGPS YEQY	2-7	(18)			4-1	CASS QLSGPT YEQY	2-7	(7)		
	4-1	CASS PTAGI YEQY	2-7	(14)								
	4-1	CASS PTSGPT YEQY	2-7	(10)								
	4-1	CASS QLSGPT YEQY	2-7	(3)								
S46	4-1	CASS PGQGI YEQY	2-7	(92)	24clones		4-1	CASS PGQGI YEQY	2-7	(100)	26clones	
	5-1	CASS LELST GELF	2-2	(8)								
T26	4-1	CASS GTGI YEQY	2-7	(90)	31clones		4-1	CASS QTSGPT YEQY	2-7	(100)	31clones	
	4-1	CASS QTSGPT YEQY	2-7	(6)								
	20	CSAG RTSGI YEQY	2-7	(4)								
A10	4-1	CASS PTQGI YEQY	2-7	(98)	45clones		4-1	CASS PTQGI YEQY	2-7	(100)	50clones	
	24	CATS DPDREVE ETQY	2-5	(2)								
S19	4-1	CASS PGQGI YEQY	2-7	(90)	48clones		4-1	CASS PGQGI YEQY	2-7	(95)	55clones	
	5-4	CASS FGSNL YEQY	2-7	(6)			4-1	CASS HTTGL YEQY	2-7	(5)		
	4-1	CASS HTTGL YEQY	2-7	(4)								
I16	15	CATS RASGPT YEQY	2-7	(68)	31clones		10-3	CAIS ESTGLAVF YEQY	2-7	(77)	30clones	
	10-3	CAIS ESTGLAVF YEQY	2-7	(20)			4-1	CASS PQKGI YEQY	2-7	(16)		
	4-1	CASS PQKGI YEQY	2-7	(10)			15	CATS RASGPT YEQY	2-7	(7)		
	28	CASS LMKAGDG YGYT	1-2	(2)								

Fig. 3. T-cell receptor β-chain repertoire of Nef138-10/A24-tetramer wt-positive and dual-positive CD8$^+$ T-cell populations. CDR3 amino acid sequence, TRBV and TRBJ usage and relative frequency of Nef138-10-specific CD8$^+$ T cells stimulated with Nef138-10(wt) or Nef138-10(2F) are shown. Data from Tet(wt)+/Tet(2F)−(wt-positive) population are shown in the upper column, and data from Tet(wt)+/Tet(2F)+ (dual-positive) are shown in the lower column. Numbers in parentheses indicate frequency (%) of each clonotype. TCRs detected in both Nef138-10(wt) and Nef138-10(2F) stimulation in the same patient are highlighted with gray bars. The TCR clonotype conserved among patients is highlighted with yellow bars. The consensus amino acid sequences, occurring in more than 70% clones in TRBV4-1/TRBJ2-7, are indicated with red. The designation of TRBV and TRBJ follows Folch's nomenclature [19].