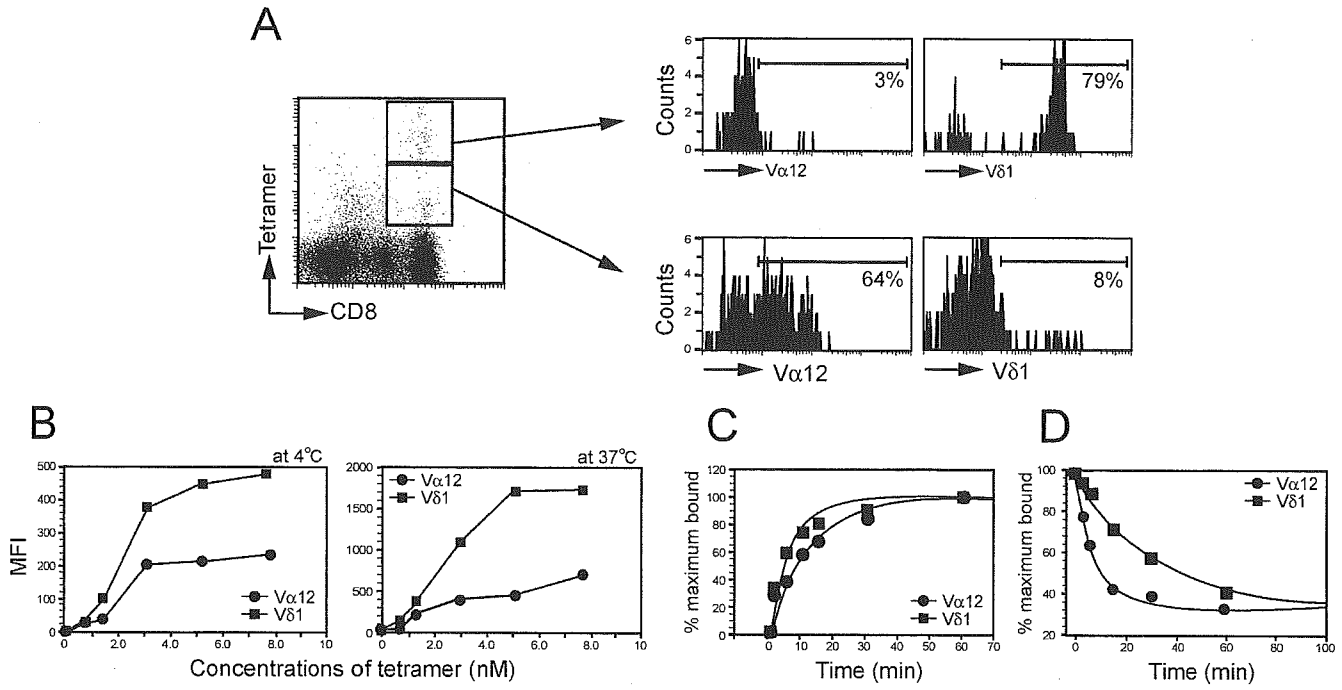


FIGURE 4. Flow cytometric analysis of kinetic interaction between the tetramer and CD8 T cell subsets. *A*, TCR analysis of tetramer^{high} and tetramer^{low} CD8 T cell subsets in Pol peptide-stimulated peripheral lymphocytes of the HIV-infected patient. *B*, Tetramer binding activities of V$\delta1^+$ and V$\alpha12^+$ CD8 T cell subsets determined at 4 or 37°C. *C* and *D*, Association (*C*) and dissociation (*D*) kinetic analysis of interaction between the tetramer and the V$\delta1^+$ and V$\alpha12^+$ CD8 T cell subsets at 4°C. Data are shown as the means of duplicate assays. An independent experiment gave similar results.

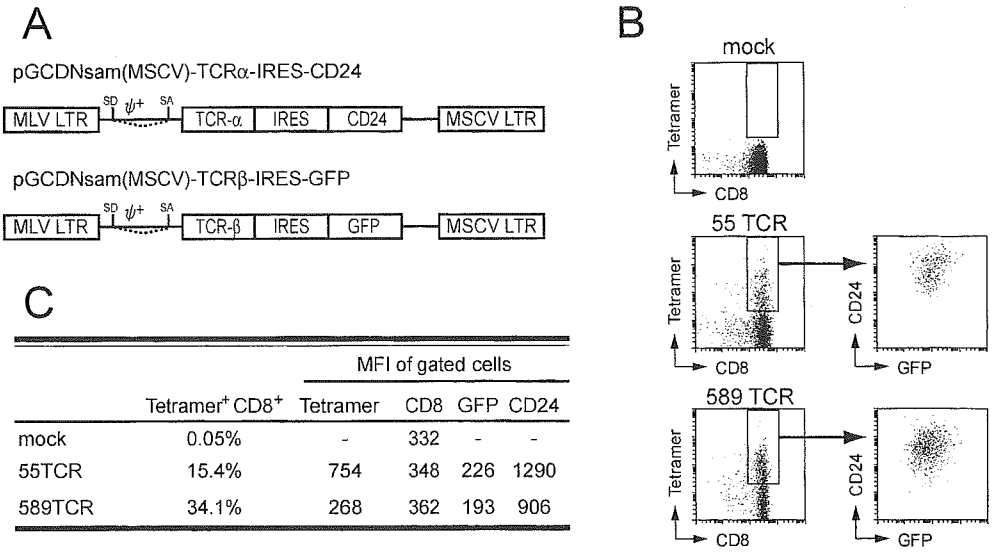
the V$\delta1^+$ and V$\alpha12^+$tetramer$^+$CD8 T cells were solely due to the difference in the TCR.

Discussion

We showed in this study that CD8 T cells with relatively high affinity TCR for an HIV-derived peptide were present in vivo and that these T cells had substantially diminished functional outcomes in response to cells infected with HIV. The difference in functional outcome of the two CD8$^+$tetramer$^+$ T cell subsets (V$\alpha12^+$ and V$\delta1^+$) was evaluated in terms of both the cytotoxic and cytokine production activities of T cell clones in vitro and their proliferation capacity, as assessed by direct ex vivo assays. The results revealed the impaired responsiveness of the higher affinity T cell subset (V$\delta1^+$) to virus-infected cells. In contrast, the V$\delta1^+$ T cells showed functional avidity to cells pulsed with the HIV-derived epitope peptide comparable to that of the other HIV-specific CD8 T cell subset (V$\alpha12^+$), which was competent for effector functions toward HIV-infected cells, indicating that the functional impairment observed in this study did not result from T cell anergy, skewed maturation of CD8 T cells in the periphery, or any defect in signal transduction machinery reported previously (1, 2, 6). There are a myriad of studies that focused on functional differences among Ag-specific (tetramer$^+$) CD8 T cells for HIV (6, 7, 28–30) as well as for other viruses and tumors (31–33), and such studies indicated functional heterogeneity of tetramer$^+$ CD8 T cells. In our study we demonstrated that the duration of TCR-pMHC interactions could have considerable effects on the antiviral effector functions and proliferation capacity of HIV-specific CD8 T cells.

It is known that the T cell is sensitive to subtle differences in the duration of TCR-ligand interactions and that, in general, a longer duration of TCR-pMHC interactions corresponds to higher T cell activation (14, 34, 35). However, Kalergis et al. (18) showed that T cell hybridoma cells transfected with a mutant TCR displayed impaired T cell activation and had an ~2-fold longer half-life for interaction with the tetramer than cells transfected with the wild-type TCR. The present study focused on the human primary CD8 T cells specific for an HIV Ag and showed that a >3-fold increased duration of the TCR-pMHC interaction resulted in impaired, rather than increased, T cell reactivity toward HIV-infected cells, supporting the findings by Kalergis et al. (18). It is likely that HIV-specific T cells have a means to improve their functional avidity to virus-infected cells because only limited numbers of the cognate epitope peptide could be presented on the surface of HIV-infected cells. A serial triggering model (36), based on the T cell integrating the number of TCRs that have interacted with pMHC, predicts that too long an interaction of TCR-pMHC slows the dissociation of TCR from pMHC, so that fewer TCRs are triggered; this may explain our observation. Indeed, we reproducibly observed the different cytotoxic activities of CTL 55 and 589 at low concentrations of the Pol peptide. If the amount of the Pol peptide presented on the surface of virus-infected cells was close to this range, their functional difference in contact with virus-infected cells could be simply explained by their different avidity to low density Ags. However, considering that CTL 55 and 589 showed significant difference in specific killing activity toward HIV-infected cells (<5 and >30%, respectively), the HIV-derived Ag would be preferentially presented at certain sites on the surface of HIV-infected cells with a local density sufficient to activate CTL 589, but not CTL 55. In this regard, it may be helpful to analyze the density and localization of the presenting epitopes on the surface of virus-infected and peptide-pulsed cells using a reagent such as a recombinant Fab specific to the peptide-MHC class I complex.

One of the CTL clones showing the higher affinity TCR-ligand interaction, CTL 55 demonstrated significant differences between their response to peptide-pulsed and virus-infected target cells in this study. It is conceivable that T cells bearing the high affinity TCR can be unduly susceptible to an inhibitory mechanism of HIV-infected cells. Alternatively, a longer duration of TCR-ligand



C

		MFI of gated cells			
	Tetramer$^+$CD8$^+$	Tetramer	CD8	GFP	CD24
mock	0.05%	-	332	-	-
55TCR	15.4%	754	348	226	1290
589TCR	34.1%	268	362	193	906

FIGURE 5. Retrovirus-mediated transduction of human primary CD8 T cells with TCR genes. *A*, Linear representations of the retroviral vector constructs used for transduction with α and β TCR genes. SD, splicing donor; SA, splicing acceptor; ψ^+, packaging signal; MLV, murine leukemia virus; MSCV, murine stem cell virus; LTR, long terminal repeat. *B*, Human primary CD8 T cells isolated from an HIV-negative donor, mock-transduced or transduced with 55 TCR or 589 TCR, and analyzed by flow cytometry. The tetramer$^+$CD8$^+$ subsets were gated, and their expression levels of GFP and CD24 were analyzed. *C*, Summary of frequencies and MFI values of the gated cells. Data shown are similar to those obtained in separate independent experiments.

interaction can lead to recruiting inhibitory receptors or molecules to the sites where the ligand-engaged TCR/CD3 signaling complexes were in action. However, similar different outcomes were observed in CTL 55 when the target cells were infected with vaccinia virus expressing HIV Pol protein, implying the existence of a general inhibitory mechanism of virus-infected cells, rather than an HIV-specific one. It is therefore interesting to see the differences in gene expression profiles of CTL 55 in contact with cells pulsed with the peptide and infected with viruses that had the epitope sequence.

We used an HLA class I tetramer for HIV-specific CD8 T cell subsets to analyze the relationship between affinity and cellular responses. The avidity of tetramer binding gives relative values that can be used as a surrogate for true affinity measurements, such as by surface plasmon resonance (19, 27). Tetramer binding more closely reflects the avidity of the T cell, rather than the intrinsic affinity of the TCR, because the tetramer binding to TCR is measured on the surface of the T cell in the presence of CD3 elements, coreceptors, and other molecules. In our study retrovirus-mediated transfer of TCR genes into human primary CD8 T cells showed that differences in the two CD8 T cells in terms of tetramer binding activity and effector functions toward HIV-infected cells solely depended on the TCR used for transduction. This experimental result clearly indicates that the affinity difference between the two TCRs interacting with the same pMHC ligand is the cause of their distinct responsiveness to HIV-infected cells.

It is reported that the Pol peptide was presented by both HLA-B*3501 and HLA-B*5101, and that CTL 589 cross-recognized the Pol peptide in complex with both HLA molecules (22). Although HLA-B*5101 bound the Pol peptide to a >50-fold lesser extent than HLA-B*3501, it is still possible that CTL 55 and 589 were differently restricted by either HLA-B*3501 or HLA-B*5101, yet specific to the same Pol peptide, because the patient has both *HLA-B*3501 and *HLA-B*5101. However, this is unlikely because CTL 55 did not respond to HLA-B*5101-expressing cells pulsed with the Pol peptide or to those infected with viruses expressing the epitope (H. Tomiyama and T. Ueno, unpublished observations), indicating that the different functional outcomes between CTL 55 and 589 were not due to the difference in their restriction elements.

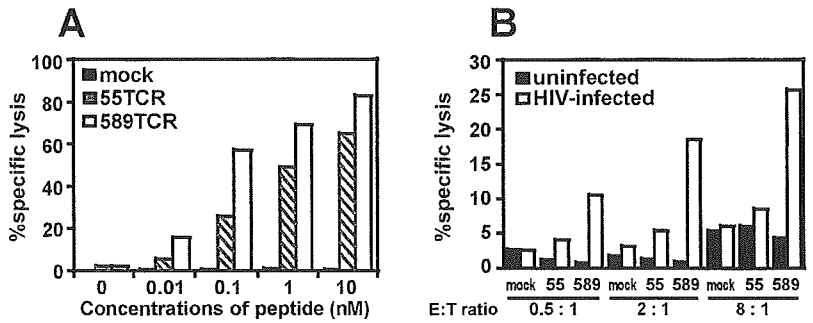


FIGURE 6. Cytotoxic activity of the 55 and 589 TCR-transduced CD8 T cells. The CD8$^+$tetramer$^+$ subsets of the 55 and 589 TCR-transduced cells were sorted (see Fig. 5) and analyzed for their cytotoxic activity toward C1R-B*3501 cells pulsed with the indicated concentrations of the Pol peptide at an E:T cell ratio of 1:1 (*A*). The same set of TCR-transduced cells was assessed for their cytotoxic activity toward C1R-B*3501 cells, infected or not with vesicular stomatitis virus envelope glycoprotein-pseudotyped HIV-1 HXB2D at the indicated E:T cell ratio (*B*). Data are shown as the means of duplicate assays for three independent experiments.

It remains unclear how such a high affinity TCR was generated in the periphery of the patient with chronic HIV infection. It was reported that T cells with high affinity TCR for foreign pMHC appear to be present in the thymus, but that these T cells are eliminated and not exported to the periphery (19). This scenario is most likely because TCRs with high affinity for foreign pMHC show considerable self-reactivity and therefore are negatively selected in the thymus (37). Considering the HIV-infected thymus, a significant increase in the expression of MHC class I molecules was reported, with this MHC class I up-regulation resulting in decreased surface expression of CD8 Ags on thymocytes (38, 39). This could lead to a decrease in the overall avidity of the TCR-CD8 signaling complex for self-pMHC ligands, allowing T cells with high affinity TCR to escape negative selection in the thymus and be exported to the periphery. Although we have only tested limited numbers of T cells, we provide evidence that the high affinity TCR-pMHC interactions can cause an additional level of functional defect in HIV-specific CD8 T cells. Also, it is strongly suggested that such defective CD8 T cells could undermine their control of HIV in vivo.

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Reconstitution of anti-HIV effector functions of primary human CD8 T lymphocytes by transfer of HIV-specific αβ TCR genes

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We redirected the antigen specificity of primary human CD8 T cells by retrovirus-mediated transduction of genes encoding αβ TCR specific to HIV-1 Pol protein. A large polyclonal population of TCR-transduced CD8 T cells showed substantial cytotoxic and cytokine production activities toward target cells either pulsed with the peptide or infected with HIV-1, and their functional activities were comparable to those of the parental CTL clone. Peptide fine-specificity and promiscuous recognition of HLA class I supertypes of the parental CTL clone were also preserved in the TCR-transduced cells. There were no signs of allogeneic responses in these cells, although hybrid TCR dimers consisting of transduced TCR and endogenous TCR were suspected to have been formed in these cells, as the effect of transgene expression on the surface expression of the desired TCR was limited. Moreover, the TCR-transduced cells showed potent inhibitory activity against HIV-1 replication *in vitro,* although the differential surface expression of the desired TCR resulted in differential functional avidity of individual TCR-transduced cells toward the peptide-pulsed target cells. These data suggest that the reconstitution of HIV-specific immunoreactive T cells engineered by genetic transfer of HIV-specific TCR is a potential alternative to immunotherapeutic applications against HIV infections.

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1 Introduction

The transfer of immunoreactive cells into patients, termed adoptive immunotherapy, for the control of viral infections as well as for the treatment of some tumors is an area of considerable interest both in basic research and clinical practice. Adoptive immunotherapy with $CD8^+$ cytotoxic T lymphocytes (CTL) had direct clinical impact on the management of patients at risk for cytomegalovirus and Epstein-Barr virus diseases [1–5]. However, the transfer of HIV-specific CTL lines and clones into HIV-infected patients, although safe, has so far produced only modest results [6–9], despite a line of evidence demonstrating that HIV-specific CTL have an important role in the immunopathogenesis of HIV infection [10].

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Abbreviations: GFP: Green fluorescent protein **IRES:** Internal ribosome entry site **MSCV:** Murine stem cell virus **MFI:** Mean fluorescence intensity

Such poor responses of autologous HIV-specific CTL in patients may be explained by recent findings showing that HIV-specific CTL taken *ex vivo* can have functional defects due to their impaired maturation in the periphery [10–13]. For example, whereas most HIV-specific CD8 T cells in patients with chronic HIV disease produced antiviral cytokines on contact with cognate antigen, these cells showed diminished perforin expression and capacity for proliferation compared with cytomegalovirus-specific T cells [14] and T cells in long-term nonprogressors [15], respectively. Moreover, the evidence that structured treatment interruption-mediated control of viral replication was associated with expansion of virus-specific CD8 T cells with a fully differentiated effector phenotype in patients with treated acute HIV-1 infection [16] strongly supports the importance of functionally mature CD8 T cells in the control of HIV-1 replication.

Redirecting the antigen specificity of T lymphocytes by the transfer of antigen-specific T cell receptor (TCR) genes to T lymphocytes has recently been described as

a potential method of generating large numbers of tumor antigen-reactive T lymphocytes with appropriate effector phenotypes [17, 18]. Using this approach, various investigators demonstrated that retroviral transfer of high-avidity TCR into T lymphocytes is accompanied by the maintenance of the parental T cell avidity and that TCR-transferred T cells preserved peptide fine-specificity in comparison to the parental CTL clone [19, 20]. Furthermore, in a murine model, T cells retrovirally transduced with gene-encoding TCR could expand *in vivo* upon antigen challenge, efficiently home into effector sites, and mediate the rejection of antigen-expressing tumors [21–24].

Although all of these studies were done using TCR specific for tumor antigens, adoptive transfer of TCR-transduced T cells into patients for the control of HIV infections could also have considerable potential. In this regard, it was reported that T cells transduced with the gene encoding a TCR specific for the p17 Gag peptide presented by HLA-A3 into T cells showed cytolytic activity toward both target cells pulsed with the epitope peptide and those infected with HIV-1 [25]. However, such cytolytic activity was observed only after cloning the transduced T cells having the highest surface expression of the transduced TCR [25]. In addition, analysis of the anti-HIV effector function of TCR-transduced T cells, including their cytokine secretion activity and antiviral replication capacity, has not yet been performed.

In the present study, we redirected the antigen specificity of primary human CD8 T cells by sequential transduction of genes encoding αβ TCR specific for an HIV-1 Pol peptide (IPLTEEAEL) using a retrovirus vector system that allows highly sustained transgene expression in the transduced T cells. A large polyclonal population of TCR-transduced CD8 T cells showed peptide fine-specificity and promiscuous recognition of HLA class I supertypes as well as cytotoxic and cytokine production activities that were all comparable to those of the parental CTL clone. Moreover, although the single-cell-sorted TCR-transduced CD8 T cells had a distributed pattern of functional avidity toward the peptide-pulsed target cells, all of the TCR-transduced T cells tested showed comparably potent inhibitory activity toward HIV-1 replication *in vitro*.

2 Results and discussion

2.1 Creation of functional TCR retroviral vectors

CTL clone 589, specific for an HIV Pol peptide ($Pol_{448-456}$; IPLTEEAEL) presented by HLA-B*3501, was previously found to express one β (Vβ5.6) and two in-frame α (Vα10 and Vα12.1) transcripts of TCR [26]. In addition, the transfer of individual α and β chains of TCR to TCR-negative mouse T cell hybridoma cells by use of a Moloney murine leukemia virus (MLV)-based retroviral vector construct pMX [27] revealed that the complex of Vα12.1 TCR and Vβ5.6 was exclusively expressed on the cell surface and solely responsible for the peptide-specific responses [26]. In the present study, both α and β TCR genes of TCR 589 (Vα12.1/Vβ5.6) were cloned into a pGC-based retrovirus vector with the gene encoding mouse CD24 or green fluorescent protein (GFP) downstream of internal ribosome entry site (IRES) for bicistronic expression of α or β TCR genes, respectively (Fig. 1A), in order to facilitate monitoring the expression levels of transduced TCR genes without the need for anti-TCR antibodies. We used a retroviral vector, designated pGCsap(MSCV) [28], in which the 3′-long-terminal repeat (LTR) of pGCsap had been replaced with that of murine stem cell virus (MSCV), allowing sustained expression of the transgene in the transduced T cells [28].

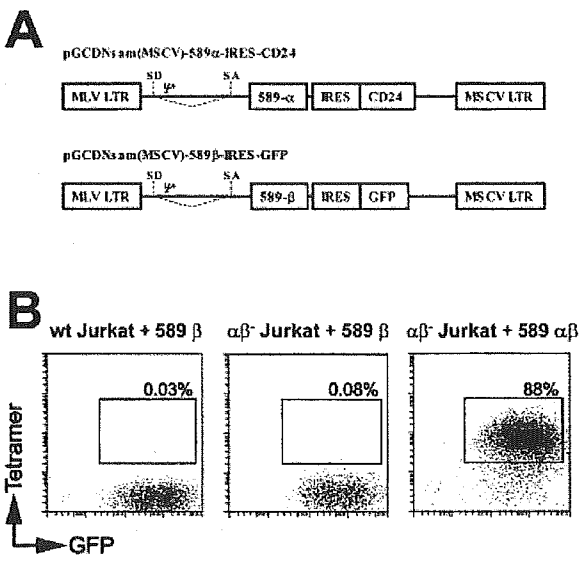

Fig. 1. Creation of functional retroviral vectors for TCR transduction. (A) Linear representation of retroviral vector constructs for transduction of αβ TCR 589 genes. SD and SA, splicing donor and acceptor, respectively; Ψ^+, packaging signal. (B) Wild-type (wt) and TCR-negative variant ($\alpha\beta^-$) of Jurkat cells were transduced with TCR 589 β-IRES-GFP alone, stained with HLA tetramer, and analyzed by flow cytometry. Other $\alpha\beta^-$ Jurkat cells that had been transduced with TCR 589 α-IRES-CD24 were further transduced with TCR 589 β-IRES-GFP and analyzed as above. GFP^+ $tetramer^+$ fractions were gated, and their frequencies within the total cell population are shown. The transduction efficiency for Jurkat cells was >90% as measured by the frequency of the GFP^+ fraction.

In order to confirm the potency of the retroviral vectors, we transduced wild-type Jurkat cells and a Jurkat variant that harbors neither α nor β TCR ($\alpha\beta^-$ Jurkat) with these constructs, stained them with HLA tetramer, and analyzed them by flow cytometry (Fig. 1B). The $\alpha\beta^-$ Jurkat variant transduced with both α and β TCR 589 genes appeared to be stained by the HLA tetramer, whereas the Jurkat cells and the $\alpha\beta^-$ Jurkat variant transduced with the β gene alone were not stained by the HLA tetramer (Fig. 1B). These results clearly indicate that the TCR 589 complex was expressed on the surface of the transduced cells and that the TCR had antigen specificity identical to that of the parental CTL 589.

2.2 TCR gene transfer to primary human CD8 T cells

Primary human CD8 T cells prepared from an HIV-negative donor carrying *HLA-B*3501* were activated by plate-coated OKT3 mAb and sequentially transduced with α and β genes of TCR 589. The transduced CD8 T cells were selected for their expression of GFP and CD24 antigen using fluorescence-activated cell sorting and stimulated again with the plate-coated OKT3 mAb. A fraction representing 9.5% of the resultant CD8 T cells bound the HLA tetramer in complex with the Pol peptide, whereas only 0.05% of the cells were stained by the tetramer in the case of the mock-transduced cells (Fig. 2A). We then stimulated these cells with the Pol peptide and cultured them for 10 days in the presence of rIL-2. Of great interest, the tetramer$^+$ fraction increased to 35% of the TCR-transduced cells, whereas the level of GFP and CD24 expression remained comparable (Fig. 2A), suggesting preferential proliferation of the tetramer$^+$ phenotype in response to antigen stimulation.

We then tested the TCR-transduced cells for their cytotoxic activity toward HLA-B*3501-expressing cells either pulsed with the Pol peptide or infected with HIV-1. The TCR-transduced cells showed substantial cytotoxic activity toward the peptide-loaded cells even at a peptide concentration down to 0.01 nM (Fig. 2B). Also, the cells showed cytotoxic activity toward HIV-infected cells, whereas they were not cytotoxic toward uninfected cells (Fig. 2C), indicating the antigen-specific and high-avidity nature of the TCR-transduced T cells.

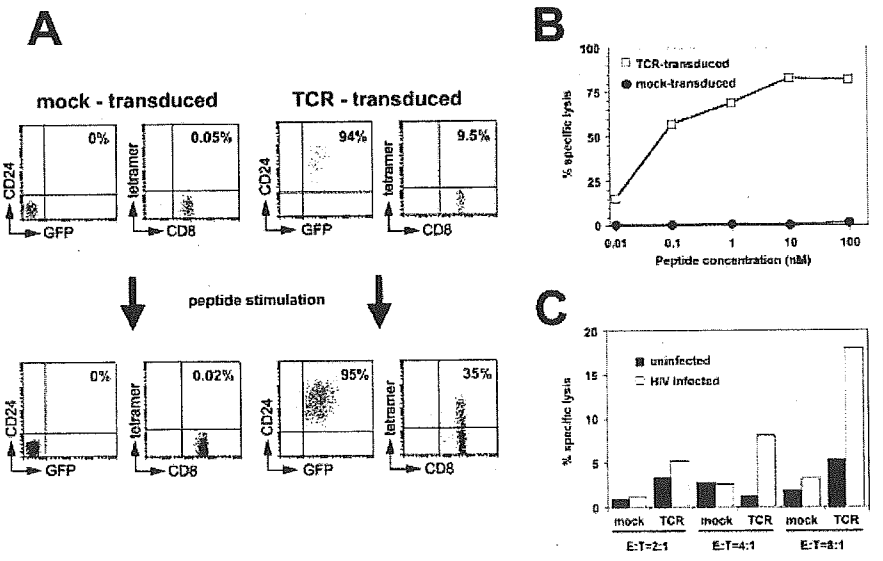

Fig. 2. Flow-cytometric and functional analysis of TCR-transduced primary CD8 T cells. (A) Primary human CD8 T cells that had been sorted into the GFP$^+$ CD24$^+$ fraction after transduction with TCR-encoded vectors were stained with anti-CD24-PE, anti-CD8-PerCP, and tetramer-allophycocyanin, and analyzed by flow cytometry before or after antigen stimulation. Mock-transduced cells were analyzed as above. Frequencies of GFP$^+$ CD24$^+$ or CD8$^+$ tetramer$^+$ fractions within the total cell population are indicated in the upper right corners of each dot plot. (B, C) The mock- or TCR-transduced cells were analyzed for their cytotoxic activity toward 221-CD4-B*3501 cells (2×10^4) either pulsed with the indicated concentrations of Pol peptide at an E/T ratio of 4:1 (B) or infected with HIV-1 LAI at the indicated E/T ratios (C). Data are shown as the means of duplicate assays in one experiment; an additional experiment gave similar results.

2.3 Maintenance of ligand specificity and functional avidity of TCR-transduced CD8 T cells

We next asked whether the ligand specificity and functional avidity of the parental CTL clone 589 would be preserved in the TCR-transduced T cells. The tetramer$^+$ subset of the TCR-transduced T cells was sorted and cultured with autologous feeder cells pulsed with the Pol peptide. A fraction comprising 82% of the resulting tetramer$^+$-sorted cells were CD8$^+$ tetramer$^+$ with a mean fluorescence intensity (MFI) value for the tetramer of 225; whereas >98% of the parental CTL 589 were CD8$^+$ tetramer$^+$ with an MFI for the tetramer of 411 (Fig. 3A).

Effector functions of the tetramer$^+$-sorted cells and parental CTL 589 appeared to be comparable as assessed by their cytokine production activity (Fig. 3B) and their cytotoxic activity (Fig. 3C) in response to the Pol peptide-pulsed target cells, indicating that the functional avidity of parental CTL 589 was maintained in the TCR-transduced T cells. In addition, the antigen specificity of both cells was tested using a series of naturally occurring mutations in the epitope region found in a database (http://www.hiv.lanl.gov/content/index). Both cells showed a similar pattern of reactivity toward the mutant peptides tested (Fig. 3C), indicating that the peptide fine-specificity of CTL 589 was preserved in the TCR-transduced T cells.

We also tested the TCR-transduced T cells for their ligand specificity toward HLA class I molecules, since CTL clone 589 was previously found to exhibit dual specificity, recognizing the Pol peptide presented by both HLA-B*3501 and HLA-B*5101 [26]. The TCR-transduced T cells showed a peptide-specific response to cells expressing either HLA-B*3501 or HLA-B*5101, and their cytotoxic activities were comparable to those of parental CTL 589 (Fig. 3D), indicating that the TCR-transduced

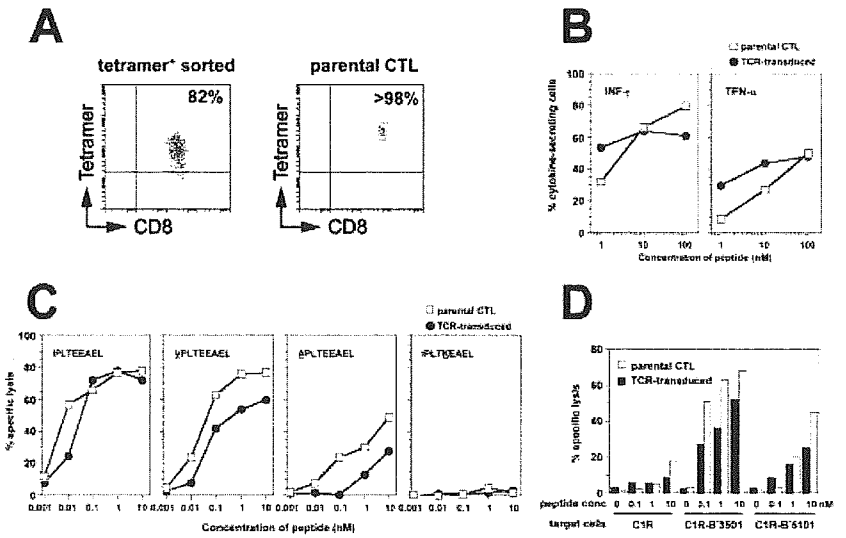

Fig. 3. Ligand specificity and functional avidity of TCR-transduced T cells compared with those of parental CTL 589. (A) The CD8$^+$ tetramer$^+$ subset of the TCR-transduced cells were sorted and cultured with autologous feeder cells pulsed with the Pol peptide. The resultant tetramer-sorted TCR-transduced cells and the parental CTL 589 were stained with anti-CD8-PerCP and tetramer-allophycocyanin and analyzed by flow cytometry. The frequency of the CD8$^+$ tetramer$^+$ subset within the total cell population is shown in the each dot plot. MFI values of tetramer-sorted TCR-transduced cells and the parental CTL 589 for the tetramer were 225 and 411, respectively. (B) Cytokine secretion activity of the tetramer-sorted TCR-transduced cells and the parental CTL 589 were analyzed in response to C1R-B*3501 cells pulsed with various concentrations of Pol peptide at an E/T ratio of 1:1. Percent cytokine-producing cells in response to C1R-B*3501 cells without pulsing the peptide was always <0.2%. Data shown are the means of duplicate assays. (C) Cytotoxic activity of the tetramer-sorted TCR-transduced cells and of the parental CTL 589 was analyzed in response to C1R-B*3501 cells pulsed with various concentrations of Pol peptide or a series of mutant peptides at an E/T ratio of 2:1. The sequences of the peptides tested are shown in each graph. The specific lysis activity of both cells in response to C1R-B*3501 cells without pulsing the peptide was always <5%. Data shown are the means of duplicate assays. (D) Cytotoxic activity of the tetramer-sorted TCR-transduced cells and of the parental CTL 589 was analyzed in response to C1R cells or transfectants expressing HLA-B*3501 or HLA-B*5101 pulsed with various concentrations of Pol peptide. The E/T ratio was 2:1. Data shown are the means of duplicate assays.

cells had promiscuous HLA restriction like their parental CTL 589. Moreover, there were virtually no signs of allogeneic responses in the TCR-transduced T cells toward target cells expressing these HLA molecules (Fig. 3D), despite the fact that the recipient T cells were derived from an individual who carries *HLA-B**3501 but not *HLA-B**5101.

These results indicate that the genetic transfer of HIV-specific TCR resulted in redirection of the antigen specificity of recipient T cells toward the HIV antigen, with activity of cytotoxicity and cytokine secretion (Fig. 3B, C) as well as specificity to peptides and HLA restriction elements (Fig. 3C, D) that were all comparable to those of the parental CTL clone. However, the tetramer binding level of the TCR-transduced T cells was slightly lower than that of the parental CTL clone (Fig. 3A).

2.4 Relationships between levels of tetramer binding and functional avidity of the TCR-transduced T cells

Ectopic expression of α and β chains of TCR in mature T cells will lead to the heterologous pairing of either protein with endogenous α and β chains of TCR. These 'hybrid' TCR would also result in decreased surface expression of the desired pair of TCR (*i.e.* ectopic chains) and may potentially lead to reduced avidity of the TCR-transduced T cells. In fact, TCR-transduced T cells obtained after sorting tetramer$^+$ fractions showed decreased tetramer binding activity compared to the parental CTL 589 (Fig. 3A). To examine this issue, we analyzed the relationships between HLA tetramer binding activity and functional capacity of the TCR-transduced T cells, since the tetramer binding activity of the TCR-transduced T cells could be dependent on the surface expression of the desired pair of TCR.

We first examined the effect of the expression level of transgenes on the surface expression of the desired TCR chains on the TCR-transduced T cells. The TCR-transduced T cells were gated in terms of tetramer$^+$ and tetramer$^-$ phenotypes, and each fraction was then analyzed for its expression levels of GFP and CD24 antigen (Fig. 4A). The tetramer$^+$ subset showed about twofold higher MFI values for both GFP and CD24 than the tetramer$^-$ subset (Fig. 4A), indicating that the difference in the levels of transgene expression contributed at some extent to the different tetramer binding activities of the TCR-transduced T cells. However, the tetramer$^+$ and tetramer$^-$ subsets showed markedly

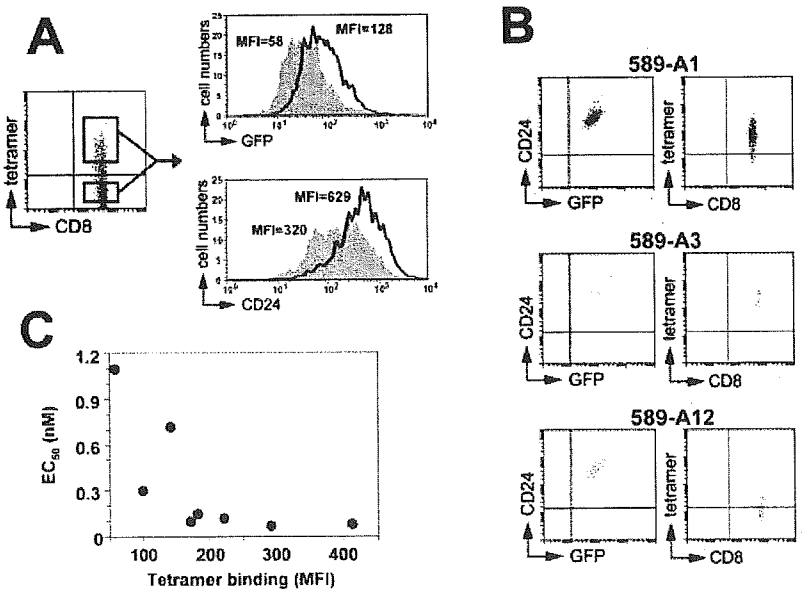

Fig. 4. Functional avidity versus tetramer binding activity of TCR-transduced cells. (A) The tetramer$^+$ (solid lines) and tetramer$^-$ (shaded areas) subsets of TCR-transduced, antigen-stimulated cells were gated and analyzed for their expression levels of GFP and CD24. The MFI values of each subset are indicated in the histograms. (B, C) The single-cell-sorted TCR-transduced cells were analyzed for their levels of tetramer binding and transgene expressions by flow cytometry. Representative dot plots are shown (B). Relationships between the tetramer binding levels and EC_{50} values of the TCR-transduced clones were analyzed (C). The EC_{50} values were determined from the cytolytic activities of these cells toward C1R-B*3501 cells pulsed with various concentrations of the Pol peptide at an E/T ratio of 2:1. The data are summarized in Table 1.

overlapped patterns in terms of both GFP and CD24 expression (Fig. 4A), suggesting that other factors, including heterodimer formation with endogenous TCR chains, influence the different surface expression of the desired TCR chains on the individual TCR-transduced T cells.

Next, the $CD8^+$ GFP^+ $CD24^+$ subset of transduced cells, including both $tetramer^+$ and $tetramer^-$ fractions, was single-cell sorted and cultured for 12 days in the presence of autologous feeder cells pulsed with the Pol peptide. Among the cells that had been positive for growth, eight clones were analyzed for their expression levels of CD8, GFP, and CD24 as well as for their levels of tetramer binding (Table 1, Fig. 4B). Tetramer binding by these cells resulted in MFI values ranging from approximately 50 to 400 (Table 1).

We then analyzed their cytotoxic activities toward C1R-B*3501 cells pulsed with various concentrations of the Pol peptide and determined their functional avidities as the 50% effective concentration of the Pol peptide (EC_{50}). The EC_{50} values of the TCR-transduced clones were also distributed widely, ranging from 0.07 to 1.1 nM (Table 1). A set of clones with the higher tetramer binding activity (MFI>200) showed relatively constant EC_{50} values (average EC_{50} of 0.09±0.026 nM), and these values were comparable to the value for the parental CTL 589 (EC_{50} of 0.07 nM) (Fig. 4C). In contrast, another set of clones with the lower tetramer binding activity (MFI<200) showed low functional avidity (average EC_{50} of 0.47±0.43 nM) and their EC_{50} values varied, mostly depending on their tetramer binding activity (Fig. 4C). These results are in good agreement with the data showing that the tetramer-sorted TCR-transduced T cells and the parental CTL 589 had comparable functional avidity (Fig. 3B, C) and yet had different tetramer binding activity (Fig. 3A).

Thus we concluded that although both the level of transgene expression and the extent of 'hybrid' TCR formation can differentially affect the surface expression levels of desired TCR chains in the individual TCR-transduced T cells, the fraction of TCR-transduced T cells with the surface expression of desired TCR chains above a certain threshold (*i.e.* MFI>200 for the tetramer under the assay condition tested in this study) had virtually identical functional avidities toward peptide-pulsed target cells.

2.5 Antiviral replication activity of TCR-transduced CD8 T cells

We further examined the ability of TCR-transduced cells to inhibit HIV-1 replication *in vitro*. CD4-expressing 221-B*3501 cells were infected with HIV-1 HXB2D and co-cultured with the TCR-transduced clones for 6 days. We determined the amounts of p24 Gag antigen production in the culture supernatant by an enzyme-linked immunosorbent assay (Fig. 5A), and the frequency of $p24^+$ cells, by flow cytometric analysis (Fig. 5B). In both assays, all of the TCR-transduced clones showed substantial suppression activity toward HIV-1 replication, whereas virtually no suppression activity was found in the case of mock-transduced cells (Fig. 5). It is of interest that all of the tested clones showed similar inhibitory activity against HIV replication (Fig. 5), although the functional avidity of these clones toward peptide-pulsed target cells showed a broad distribution, with EC_{50} values ranging

Table 1. Summary of functional and phenotypic characterization of mock- or TCR-transduced cells

	EC_{50} (nM)	MFI			
		Tetramer	CD8	GFP	CD24
A1	0.10	172	453	143	769
A3	0.08	412	584	286	1,405
A6	0.72	143	434	55.5	1,353
A11	0.15	182	311	220	161
A12	1.10	57.5	529	106	633
A14	0.07	292	453	288	809
A16	0.12	221	422	650	1,239
A23	0.30	100	331	50.7	1,101
Mock	>100	3.8	303	3.3	2.7

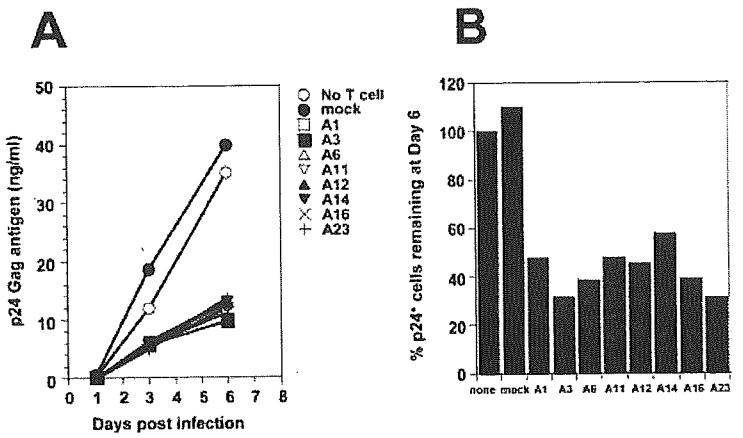

Fig. 5. Inhibitory potency of TCR-transduced clones toward HIV-1 replication *in vitro*. (A) The 221-CD4-B*3501 cells (2×10^4 cells/well) infected with HIV-1 HXB2D were co-cultured or not with the mock- or TCR-transduced clones at an E/T ratio of 0.5:1. On days 1, 3, and 6 post-infection, a portion of the culture supernatant was collected and the amounts of p24 antigen produced by the HIV-infected 221 cells were then determined. (B) The HIV-infected 221 cells were collected on day 6 and intracellularly stained with FITC-conjugated mAb specific for the p24 Gag antigen for flow cytometry. The relative frequency of $p24^+$ cells at day 6 in the co-cultures with the indicated TCR-transduced T cells is shown. The data are given as the means of duplicate assays in one experiment. These results are representative of those of two additional independent experiments.

from 0.07 to 1.1 nM (Table 1). Particularly, clone A12 showed potent suppression activity for HIV replication (Fig. 5) despite its modest functional avidity and tetramer binding activity (Table 1). This finding appears to be consistent with the recently reported study demonstrating that epitope specificity of CTL, rather than functional avidity of CTL, is a key factor in the ability of CTL to control HIV replication, and that the process of epitope presentation on HIV-infected cells greatly influences CTL efficiency *in vivo* [29].

It should be noted that we used an Epstein-Barr virus-transformed B cell line as an HIV-infected target cell. Since kinetics of HIV replication is generally variable dependent on cell types as well as HIV strains, it may be possible that inhibition potency of HIV-specific CD8 T cells toward HIV replication is also variable, dependent on host cell types both *in vitro* and *in vivo*. In this regard, suppression activity of HIV-specific CD8 T cells toward HIV replication is intriguing as assessed using primary human CD4 T cells and macrophages as HIV-infected target cells.

The introduction of a chimeric TCR containing human CD4 or HIV-specific immunoglobulin sequences linked to the signaling domain of the TCR ζ chain (universal TCR) into CD8 T cells for targeting of HIV-infected cells has been reported [30–32]. In comparison with chimeric receptor approaches, the transfer of native TCR may have distinct advantages. In a chimeric receptor approach, high-affinity receptor-ligand interaction will most likely result in incomplete T cell activation owing to omission of immunoreceptor tyrosine-based activation motifs normally present in the CD3 complex. More importantly, it is unclear whether or not chimeric receptor-ligand engagement results in functional maturation of transduced T cells to a fully differentiated effector phenotype, which character of CD8 T cells has been shown to be important to control HIV replication *in vivo* [16].

In summary, the data presented in this study suggest that the reconstitution of HIV-specific immunoreactive T cells engineered by genetic transfer of the native form of αβ TCR into primary CD8 T cells is a viable strategy to suppress HIV replication. This approach represents a potential alternative to other types of immunotherapy for HIV infection, although further studies to elucidate the phenotypic and functional properties of such engineered HIV-specific CD8 T cells *in vivo* are required.

3 Materials and methods

3.1 T cell culture

The Jurkat variant that lacked expression of either α or β TCR was kindly provided by Bent Rubin (Unite de Physiopathologie Cellulaire et Moleculaire, CNRS, France) and maintained in RPMI 1640 and 10% fetal bovine serum. CTL clone 589 cells were generated before [33] from an HIV-infected patient (*HLA-A*2402/A*2601*, *HLA-B*3501/B*5101*) following stimulation of peripheral lymphocytes with an HLA-B*3501-restricted and HIV Pol-derived epitope peptide (HIV $Pol_{448-456}$; IPLTEEAEL). CTL clones were maintained in

RPMI 1640 and 10% human serum supplemented with 200 U/ml rIL-2, and were stimulated weekly with irradiated C1R-B*3501 cells or autologous PBMC pulsed with 100 nM Pol peptide as needed.

3.2 Construction of retroviral vectors and gene transfer

The genes encoding full-length α and β TCR of CTL 589 (DDBJ accession numbers AB164620 and AB164621, respectively) were subcloned into the pGC-based retroviral vector pGCDNsap(MSCV) [28]. The genes encoding a mouse heat-stable antigen (CD24) or GFP were also incorporated into the constructs along with an IRES following the α or β TCR gene (Fig. 1A) to facilitate monitoring the expression of α or β TCR genes, respectively, in the transduced cells. Amphotropic retrovirus containing each of these constructs was then prepared, essentially as described previously [28].

Human primary CD8 T cells were isolated from PBMC of an HIV-negative healthy donor (*HLA-A*0201/A*2402, HLA-B*3501/B*4002*) using anti-CD8 magnetic beads (Miltenyi Biotech, Bergisch Gladbach, Germany). The resultant CD8 T cells (>90% of the cells were $CD8^+$) were activated by plate-coated anti-CD3 mAb (OKT3) for 3 days, plated on recombinant fibronectin-coated plates (Takara Shuzo, Otsu, Japan), and incubated with the retrovirus supernatant containing the TCR 589 α-IRES-CD24 gene for 72 h, during which interval the cells were exposed to fresh retrovirus supernatant every 12 h. Transduced CD8 T cells expressing CD24 antigens were isolated by use of PE-labeled anti-CD24 mAb (PharMingen, San Diego, CA) and anti-PE magnetic beads (Miltenyi Biotech). The isolated cells were subsequently transduced by the construct containing TCR 589 β-IRES-GFP, as above. The fraction of transduced CD8 T cells that were positive for GFP, CD24, and CD8 molecules was obtained by sorting with a FacsVantage (BD Biosciences, San Jose, CA). The sorted T cells were propagated by stimulation with OKT3 mAb and subsequently by irradiated autologous PBMC pulsed with 100 nM Pol peptide. These T cells were maintained in RPMI 1640 and 10% human autologous serum supplemented with 200 U/ml rIL-2.

3.3 HLA-tetramer analysis

The HLA-B*3501-tetramer complex with allophycocyanin-conjugated avidin (Molecular Probes, Inc., Eugene, OR) was prepared as previously described [26]. T cells were first stained by the tetramer for 15 min at 37°C, and then stained for 20 min at 4°C with other antibodies, such as anti-CD8 mAb and anti-mouse CD24 mAb conjugated to peridinin chlorophyll protein (PerCP) and PE (BD Biosciences), respectively. They were then washed twice with a washing buffer (2% newborn bovine serum in phosphate-buffered saline) and fixed in a 1% paraformaldehyde solution. The resultant cells were analyzed by flow cytometry.

3.4 Cytotoxicity assay

The cytotoxic activity of the CTL clone 589 and the TCR-transduced T cells was determined by a standard ^{51}Cr-release assay as described [26]. For peptide-pulsed target cells, ^{51}Cr-labeled C1R-B*3501 cells (2×10^3 cells/well) were pulsed with various concentrations of the peptide for 1 h and incubated with the effector T cells for an additional 4 h at 37°C. For virus-infected target cells, C1R-B*3501 cells or 221-B*3501 cells expressing human CD4 antigen (2×10^3 cells/well) were infected with HIV-1 HXB2D or HIV-1 LAI. These viruses have the same epitope sequence as was used for the synthetic Pol peptide. The cells were subsequently labeled with ^{51}Cr and incubated with the effector T cells for 6 h at 37°C. Note that >80% cells were positive for intracellular p24 Gag antigen when HIV-infected cells were used for CTL assays.

3.5 Cytokine secretion assay

CTL clone and TCR-transduced T cells were co-cultured with 221-CD4-B*3501 cells pulsed with various concentrations of the Pol peptide for 2 h at 37°C. Brefeldin A (10 μg/ml) was then added, and the cultures were continued for an additional 4 h. Cells were permeabilized and stained with anti-IFN-γ and anti-TNF-α mAb conjugated to PE and allophycocyanin, respectively (PharMingen), as previously described [34]. Frequencies of IFN-γ^+ and TNF-α^+ $CD8^+$ cells were determined by flow cytometry.

3.6 Antiviral replication assay

The 221-CD4-B*3501 cells were infected with HIV-1 HXB2D at 10 $TCID_{50}$/ml for 6 h at 37°C and washed with culture medium. The resultant infected cells were plated in a 96-well U-bottom plate at 2×10^4 cells/well, and then the mock- or TCR-transduced $CD8^+$ T cells were added to each well at an E/T ratio of 0.5:1. The culture medium contained RPMI 1640 and 10% human serum supplemented with 200 U/ml rIL-2. On days 1, 3, and 6 post-infection, a portion of the culture supernatant was collected and the amounts of p24 antigen produced by HIV-infected 221 cells were determined by use of an enzyme-linked immunosorbent assay (ZeptoMetrix Corporation, New York, NY). In addition, on day 6, cells were collected, intracellularly stained with FITC-conjugated mAb specific for p24 Gag antigen (KC57; Coulter Immunology, Hialeah, FL), and analyzed by flow cytometry.

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Lecture

解　説

T細胞によるHIV-1の増殖制御*

滝口雅文**

Key Words：HIV-1, CTL, viral suppression, escape, HLA

はじめに

HIV-1感染症では，細胞性免疫，液性免疫が誘導されるにもかかわらず，これらの免疫系により生体からHIV-1は排除されることはなく，いずれ免疫系組織を破壊し，エイズを発症させる．しかし多くの研究がなされているにもかかわらず，免疫系がなぜHIVを排除できないか，いまだ明らかになっていない．最近のHIV-1の細胞性免疫の研究分野に関する研究の進展を紹介する．

HIV感染に対する生体の免疫反応

ウイルスが感染すると抗原提示細胞を介してヘルパーT細胞が認識し，細胞傷害性T細胞(CTL)の誘導と特異的抗体の産生が起こる．普通は感染してから少なくとも1週間後にはCTLの誘導が起きており，その活性を測定することができる．一方この時にはすでに抗体の産生もみられており，多くのウイルス感染症においては2週間でその産生はピークに達する．CTLはウイルスが感染した細胞を認識し，その細胞を殺すことによりウイルス増殖の場をなくす．一方抗体，とくに中和抗体は血中のウイルス粒子そのものに結合し，ウイルスを不活性化させる．そのほかNK細胞などもウイルス感染細胞を認識して細胞傷害活性を示すと考えられている．これらの免疫系の反応によってウイルスは完全に生体から排除される．

一方HIV-1の感染では，CD4陽性T細胞がその標的細胞であるため，感染初期にその数が一時的に減少する．しかし，CTLによってこのHIV-1感染CD4陽性細胞は排除され，ほとんどの例でCD4陽性細胞は正常まで回復する．抗体の産生は，やや遅れてみられることがあり，とくに感染してから1～2か月間その産生がみられないこともある．他のウイルス感染と異なっているのは，HIVは免疫系によって完全に排除される事はきわめて稀であり，生体内に残り続ける．数年後にウイルス量は増大し，エイズを発症する[1]．低レベルのウイルス量が持続することは，おそらく生体内で免疫系がウイルスの増殖を抑えこんでいるのでないと考えられている．しかしなぜ免疫系がHIV-1を完全に排除できないのか，またある期間を経ってからHIVがなぜ増殖しはじめて来るのかその機序は十分には明らかになっていない．

細胞傷害性T細胞(CTL)による HIV-1抗原認識とHIV-1の増殖抑制

ウイルスが感染した細胞では，細胞質内で合成されたウイルス蛋白は，細胞質内のプロテアゾームに運ばれて，ペプチド化される．さらにペプチドは，粗面小胞体の膜(ER)にあるトランスポーターと呼ばれるレセプターを介してER内に取り込まれる．ER内に運ばれたペプチドはMHCクラスI抗原の重鎖およびβ2マイクログロブリン(β2m)とともに結合して安定したクラスI分子を

* Suppression of HIV-1 replication by HIV-1-specific CD8 T cells.

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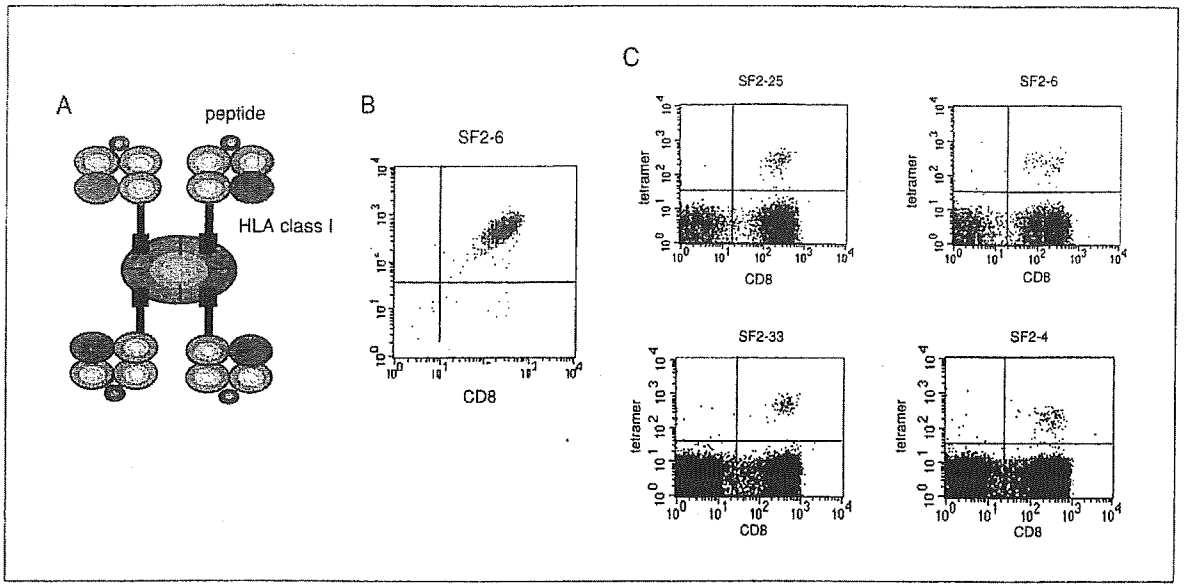

図1　HLAクラスⅠテトラマーによるCTLクローンへの特異的結合

A：HLAクラスⅠテトラマー．C末端をビオチン化したHLAクラスⅠ分子に目的のエピトープペプチドとβ_2ミクログロブリンを結合させる．この分子を4つ標識したアビチンに結合させることで，TCRに結合できるテトラマーができる．

B：テトラマーのCTLクローンへの特異的結合．HLA-B*3501拘束性エピトープSF2-6(RPQVPLRPMTY)特異的CTLクローンへの特異的ペプチドの結合．

C：HLAクラステトラマーを用いたHIV-1感染者末梢血中の特異的$CD8^+$ T細胞の解析．HLA-B*3501拘束性エピトープを結合させたHLA-B*3501テトラマー(4種類)と抗$CD8^+$抗体を用いたHIV-1感染者末梢血単核球中のHIV-1特異的$CD8^+$ T細胞の解析．SF2-25(EPIVGAETF)，SF2-6(RPQVPLRPMTY)，SF2-33(DPNPQEVVL)，SF2-24(IPLTEEAEL)．

形成する．このMHCクラスⅠ抗原はさらに膜輸送にて，細胞表面へと運ばれる．細胞表面へと運ばれたMHCクラスⅠ分子は，T細胞レセプター(TCR)によって認識される．HIV-1抗原に特異的なCTLは，TCRを介してMHCクラスⅠ分子に結合した特異的なウイルス蛋白由来のペプチド(エピトープ)を認識する．TCRはMHC分子とそれに結合したペプチドのアミノ酸残基に結合し，シグナルを細胞内に伝達してその機能を発現させると考えられている．HIV-1感染細胞においても同様な機序で，HIV-1エピトープペプチドはMHCクラスⅠ分子に結合し，細胞表面に運ばれ特異的CD8T細胞に認識されると考えられる．

CTL活性の測定は，^{51}Cr releasing assayによってほとんどの場合おこなわれてきた．この方法はアイソトープが使える施設に限られること，effector : target ratioやtarget細胞の種類によって得られるデーターに差が出ること，実験日や実験者間における誤差などさまざまな問題がある．これらの問題を解決するために，エピトープペプチドを結合させたHLAクラスⅠ分子を標識し，これをTCRに結合させて，CTLをフローサイトメトリーで測定する方法が考えられた．しかし，HLAクラスⅠ分子とTCRの結合能は，抗原と抗体の結合と比べて1000分の1以下であり，今まではこの方法による測定は不可能であった．Altmanら[2]，CTLエピトープペプチドを結合させたHLAクラスⅠ分子を4つ結合させたAvidin(テトラマー)を用いることにより，特異的TCRをもったT細胞をフローサイトメトリーで解析できることを可能にした(図1A, B)．この方法は凍結させておいたサンプルをそのまま解凍しても測定でき，抗体と組み合わせて多色染色することにより多くの情報を得ることができ，きわめて有効な手段である．このテトラマーを用いたHIV-1感染者の末梢血の解析で，HIV-1感染者は感染初期から多数のHIV-1特異的$CD8^+$ T細胞が誘導されており[3]，慢性感染期でもこの特異的$CD8^+$ T細胞は維持されていることが明らかになった(図1C)．

HIV-1特異的CD8T細胞は，主に2つの機序で

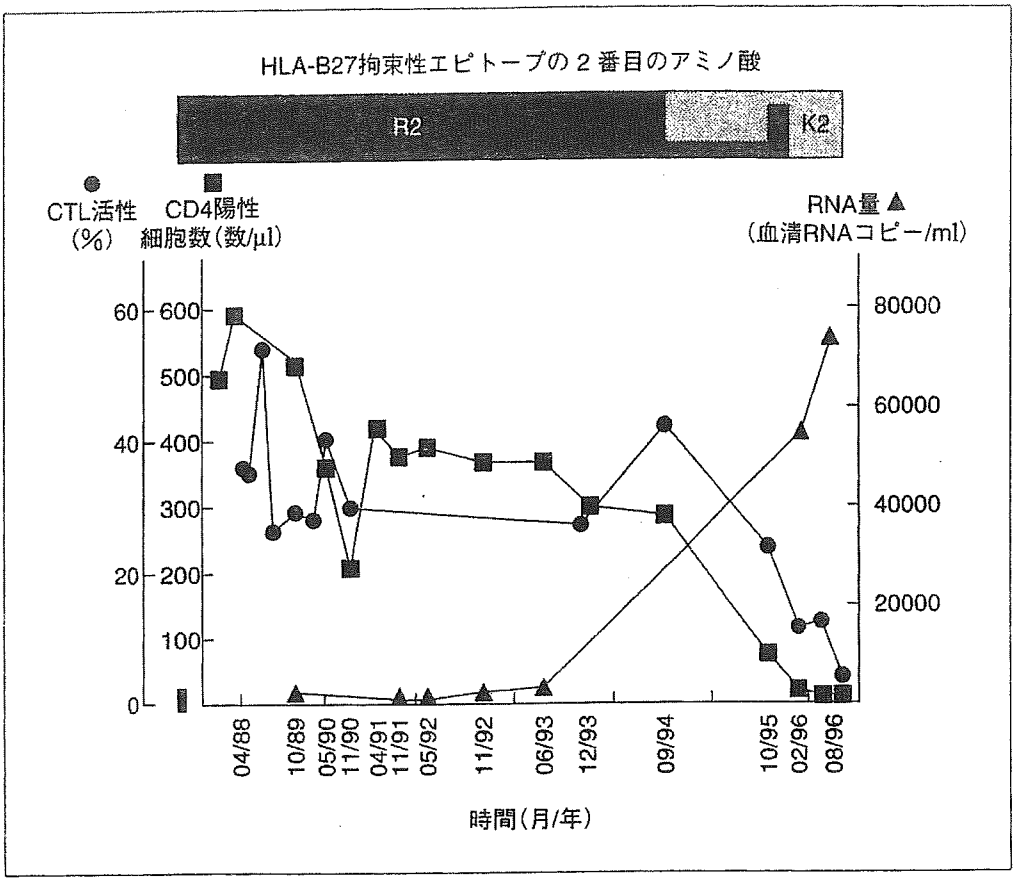

図２ HLA-B27拘束性CTLエピトープ上に生じたエスケープミュータントによるAIDSの発症
唯一のB27エピトープKPWIMGLNK上の２番目のアミノ酸ArgがLysに変異したウイルスが出現すると血中CD4陽性Ｔ細胞が低下し，AIDSが発症した．KPWIMGLNKに特異的CTLはこのミュータントエピトープを認識できない．

HIV-1の増殖を抑制していると考えられる．第一に，抗原認識によりTCRから刺激が入ると，perforinやgranzymeを放出し，これによりHIV-1感染細胞を傷害してHIV-1の増殖の場を奪うことにより，生体内でのHIV-1増殖を抑制しようとするものである．もう１つはTCRから刺激が入るとHIV-1特異的CD8T細胞からサイトカインを産生し，これらのサイトカインによってHIV-1の細胞内への侵入を阻止したり，HIV-1自体の増殖を抑制するものである．前者には，MIP-1やRANTESのようなケモカインがあり，後者にはIFN-γなどがある．主にこれらの２つの機序により，HIV-1の増殖が抑制されると考えられる．実際長期未発症者ではHIV-1に対する高いCTL活性が維持されているという事例[4]やハイリスク集団の中にHIVの感染から免れているが高いHIV特異的なCTL活性が認められるケース[5]があり，またサルのSIVでは，CD8T細胞を取り除くとエイズが発症することが知られている[6]．これらのことから，CTLがHIV-1の増殖抑制に大きな役割を果たしていることは明らかである．

HIVのCTLからの逃避機序

それでは，どうしてHIV-1はCTLからの逃避が可能であるのであろう．この質問の答えを示唆する研究が報告された．その１つは，HIVに感染後，きわめて短時間(1年以内)にエイズを発症した患者のケースである[7]．この患者では，感染してすぐにEnv蛋白質上のたった１つのエピトープに対して強いCTL活性がみられた．しかし約１か月後にこのエピトープ上にCTLが認識できなくなってしまう変異をもったウイルスが出現すると，CTLからの攻撃を逃れることができるためウイルスは急速に増殖して，患者の免疫系を破壊してしまったのである．もう１つの例は，長期未発症生存者の例である[8]．この患者はHLA-B27によっ

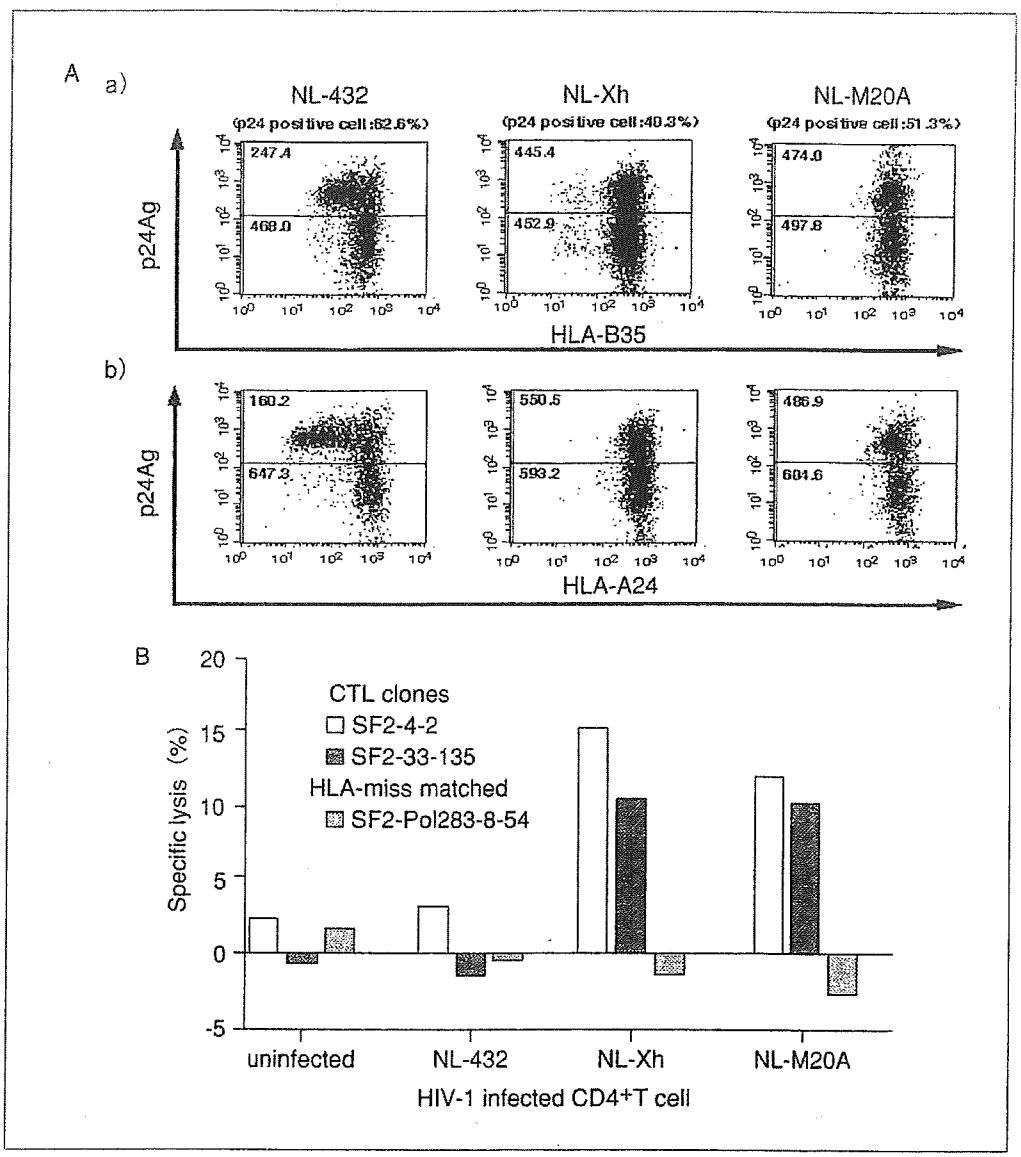

図3　HIV-1感染細胞でのHLAクラスⅠ抗原の細胞表面のdown-regulation

A：HIV-1 Nef欠損株(NL-Xh)およびNefの変異株(NL-M20A)感染CD4T細胞では，(a)HLA-B35および(b)HLA-A24抗原の細胞表面の発現低下はわずかしか起きない．一方Nefを欠損していないHIV-1 NL4-3株を感染させた細胞では，HLA-B35およびHLA-A24の著しい細胞表面上の発現の低下がみられた．

B：NL-Xh, NL-M20AおよびNL4-3株を感染させたCD4T細胞に対する特異的CTLクローン(SF2-4-2, SF2-33-135)の細胞傷害活性．NL-Xh, NL-M20Aを感染させたCD4 T細胞に対しては細胞傷害活性を示したが，NL4-3株を感染させたCD4 T細胞には細胞傷害活性を示さなかった．

て提示される唯一のCTLエピトープに対して強い活性を保っており，血中のウイルス量も低く押さえられていた．しかし，このエピトープ上にCTLが認識できなくなるような変異を生じたウイルスが出現すると，この変異ウイルスは生体内で増殖してやはり患者の免疫系を破壊し，エイズを発症させたのである(図2)．さらに同様なケースがHLA-B57をもった患者でも報告されている[9]．

以上のケースはHIV-1に感染した人の中ではきわめて稀なケースといえる．なぜなら一般的には，生体内に強く認識されるCTLエピトープは1つでなく，少なくとも何種類もあるはずである．しかし，これらのケースは，CTLエピトープ上の変異の蓄積が，CTLからの攻撃を逃れるウイルスの出現を生み出すことを示唆している．この結果数年という期間を経てCTLエピトープを多数もっ

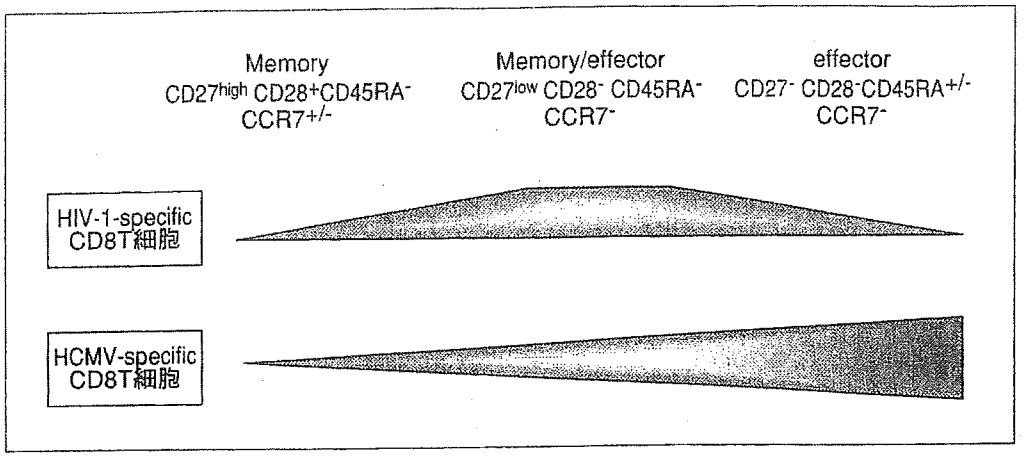

図4 HIV-1特異的CD8T細胞とヒトサイトメガロウイルス(HCMV)特異的CD8T細胞の分化・成熟度の違い

ているような感染者でもCTLが認識できないようなウイルスが出現し，エイズを発症すると考えられる．今後このようなCTLエスケープミュータントの蓄積が多くのHIV-1感染者で起こっているのか検討することが重要であると考えられる．

一方Nef蛋白が欠損したHIV-1に感染した細胞では，HLAクラスⅠ抗原の発現は感染していない細胞と比べても変わらないが，Nef蛋白が欠損していないHIV-1に感染した細胞ではHLAクラスⅠ抗原の発現が低下することが知られている(図3A)．HIV-1の感染によってHLAクラスⅠ抗原が低下することにより，HIV-1の抗原提示が低下し，これによりCTLから認識をHIV-1は逃避するという研究が報告されている(図3B)[10][11]．しかしHIV-1感染によるHLAクラスⅠ抗原の低下はHIV-1が細胞に感染してから相当の時間がたってから起こる現象と考えられ，実際に生体内でどれほどの影響を与えるかはまだ明らかでない．

最近，HIV-1慢性感染者では，HIV-1特異的CD8T細胞はeffectorまで分化しておらず(図4)，分化・成熟異常があるため十分その機能を示せないのではないかという報告がされている[12]．しかしその証拠は十分とはいえず，今後の解析が期待される．

おわりに

免疫がなぜHIV-1を完全に排除できないのか，また免疫系とHIV-1との間で長い間バランスをとっていた状態が突然変わりHIV-1が増殖してしまうのか，依然これらの質問には十分にまだ答えられていない．今後さらなるこの分野の研究によって，HIV-1の免疫逃避機序の解明ができることが期待される．これらの機序の解明は，免疫を用いた治療の開発につながる．実際，化学療法の限界がみえてきており，治療ワクチンの開発が強く望まれている．

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特集：HIVと免疫

HIVに対する細胞傷害性T細胞の免疫応答

Cytotoxic T Lymphocyte-mediated Immune Responses to HIV

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はじめに

HIVの感染防御およびHIVに感染後の病態制御では，CD8陽性の細胞傷害性T細胞（$CD8^+$ cytotoxic T lymphocyte；CTL）の働きが重要であることが，さまざまな側面から明らかにされてきた。しかしながらCTLを中心とする免疫応答では，HIVを排除することはできず，慢性持続感染が成立して病態が進行する。HIVによるCTL免疫応答からの逃避機序の解明は重要な課題であるばかりでなく，その成果は有効なワクチンの開発研究にとって極めて有用である。本編では，HIV特異的なCTLの性質について，特にHIV感染制御にCTLとはどのようなものかという点に焦点を絞って，最近の知見を中心に述べる。

1. HIV感染症の病態進行とHLA遺伝子型

HIV感染症の病態進行には個体差が認められる。遺伝学的な解析から*CCR5*の変異を始め，さまざまな遺伝学的要因が報告されている[1]。このうち獲得免疫系に関わる要因はHLA遺伝子型との相関である。

HIVに感染した細胞では，HIV由来の蛋白質はプロテアソームを始めとする蛋白質分解系によって消化されて，15アミノ酸程度の短いペプチド断片となる。こうしたペプチド断片の一部は，小胞体でMHC（ヒトの場合はHLA）クラスI分子と結合して，やがて細胞表面に運ばれ，CTLの抗原として提示される。HLAクラスIは多型性の著しい分子（A, B, C合わせて1,000種類以上）で，それぞれのHLA分子は異なった構造を持ったペプチドと結合する。たとえば*HLA-A**02を持つ人と*HLA-A**24を持つ人では，たとえHIVの同じ蛋白質に由来しても，異なったペプチドが抗原として提示されてCTLに認識される。したがって，HIV抗原に応答するCTLのレパートリーは，その人がどのHLAクラスIを持っているかに依存する。

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HLA遺伝子型とHIV感染症の病態進行の相関を表1にまとめた。ヒトのHLAクラスIは*HLA-A, B, C*と大きく3種類のアリルで構成され，それぞれが二対ずつあるため最大6種類の異なったHLAアリルを持つ。この種類が多いほど，提示する抗原ペプチドの構造多様性が大きく，より広範なCTL応答が期待できるため，宿主免疫応答にとっては優位であると考えられている。しかしながら，ときに*HLA-A**24や*HLA-A**02など同一のHLAを両染色体に持つ人（homozygote）がいるが，こうしたhomozygoteでは異なったHLAアリルを持つ人（heterozygote）に比べて，エイズへの病態進行が統計学的に早いことが報告されている（表1）。このことは，HLA分子が仲介するCTL応答がHIV感染症の制御に大きな役割を担うことを示す。また，*HLA-B**57が病態進行の遅延と*HLA-B**35が病態の早期進行と相関するなど個別のHLAアリルが病態進行に影響することは，提示される抗原ペプチドの構造や種類によってHIV特異的なCTL応答の有効性が異なることを示唆している。我々は，病態進行の遅延と相関するHLAのうち，日本人に比較的頻度の高いHLA-B*51に拘束性のCTLによる抗ウイルス免疫応答の解析から，HIV感染症の病態進行にかかわるHLAあるいは免疫系の役割の解明を目指している。

最近HLAクラスIアリルの抗HIV免疫応答の関与について興味深い報告がなされた。大規模なHIV感染者のコホートを対象として，HLA遺伝子型と病態進行の集団遺伝学的解析と，HIVに対するCTL応答の機能的解析を組み合わせたところ，*HLA-A, B, C*アリルのうち*HLA-B*アリルに拘束されるCTL応答が最もよく病態進行の遅延と関連するという報告である[9]。実際，アリル多型性では*HLA-B*アリルが600種類以上の多型性を持っていて最大である。HLAがアリル多型性を得る進化学的要因は，数多くの病原体による選択圧だと考えられており，このことはHIV感染症で*HLA-B*アリルが最も重要であるという結果と矛盾しない。しかし，この統計解析の結果は，*HLA-B**27や*HLA-B**57など個別のアリルで有意にHIV感染症の病