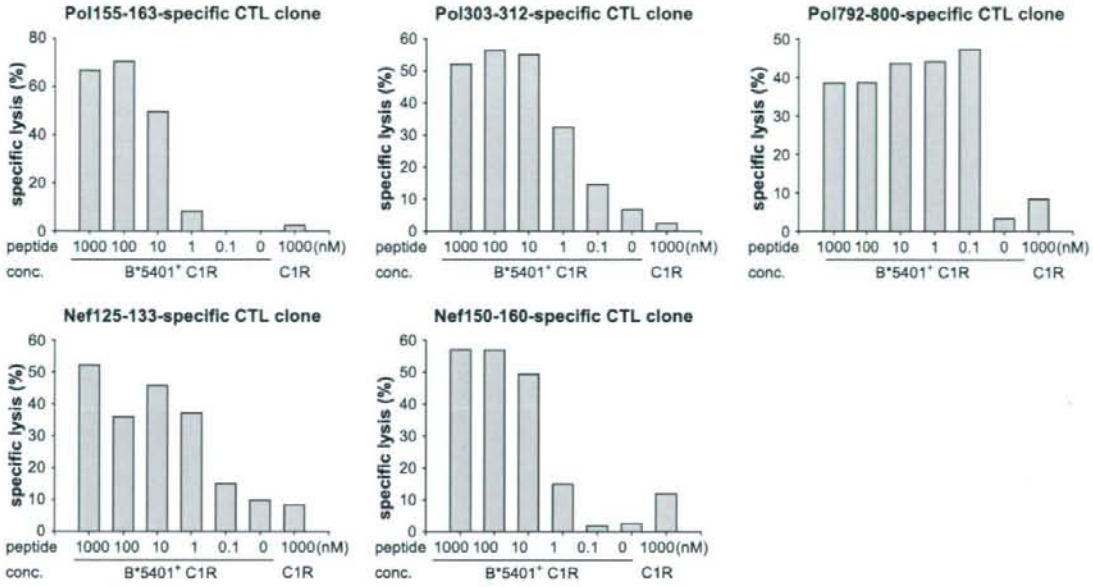

Fig. 5. Confirmation of HLA-B*5401-restricted recognition. Specific lysis of C1R-B*5401 cell lines by Pol155–163-specific, Pol303–312-specific, Pol792–800-specific, Nef125–133-specific, or Nef150–160-specific CTL clone was determined by performing the ^{51}Cr-release assay. The target cells were pulsed with each peptide at concentrations from 0.1 to 1000 nM, and the assays were performed at a 2:1 ratio of effector cells to target cells. The percentage of specific lysis is shown in each graph.

*3.6. Frequency of HLA-B*5401-restricted HIV-1-specific CD8$^+$ T cells in HIV-1-infected individuals with HLA-B*5401*

To clarify whether CD8$^+$ T cells specific for these epitopes were predominantly induced in chronically HIV-1-infected individuals bearing HLA-B*5401, we investigated the induction of the specific CD8$^+$ T cells in PBMCs from eight chronically HIV-1-infected HLA-B*5401-positive individuals by stimulating them with these epitope peptides. Pol155–163-specific CD8$^+$ T cells were found in four of the eight HIV-1-infected individuals. Pol792–800-, Nef125–133-, and Nef150–160-specific CD8$^+$ T cells were found in three individuals; and Pol303–312-specific CD8$^+$ T cells, in two of them (Table 1).

4. Discussion

In the present study, we could identify 5 HLA-B*5401-restricted epitopes in HIV-1 Pol and Nef by using 17-mer overlapping peptides. A previous study had shown that Pro at position 2 is the primary anchor residue and that Phe, Met, Arg, Tyr or Asp at position 3, and Ala at position 9 is the secondary anchor residue for HLA-B*5401 [29]. However, CTL epitopes are not always consistent with the peptide-binding motif of HLA [20,21]. In fact, out of the five HLA-B*5401-restricted HIV-1 epitopes, one does not have Pro at position 2. Pol792–800 epitope (HVASGYIEA) has Ala residue at position 3. This Ala is a candidate of the anchor at position 2 because Ala has similar characteristics as Pro, but the specific

Table 1
Induction of epitope-specific CD8$^+$ T cells among PBMCs from HLA-B*5401$^+$ HIV-1-infected individuals

Patients[a]	Viral load[b]	CD4[c]	CD8[c]	Percentage of IFN-γ-producing cells in CD8$^+$ T cells				
				Pol155–163	Pol303–312	Pol792–800	Nef125–133	Nef150–160
KI-119	3.0×10^3	536	1268	16.1	58.0	39.8	26.3	21.1
KI-160	3.5×10^4	360	831	3.9	0.0	0.5	0.0	0.0
KI-172	1.8×10^4	512	558	0.0	0.0	0.0	8.1	0.0
KI-115	5.3×10^3	264	721	19.9	7.0	0.0	0.1	2.1
KI-150	2.4×10^4	307	1411	0.0	0.0	3.5	0.0	0.0
KI-167	4.2×10^4	281	1055	0.0	0.0	0.0	48.5	0.0
KI-141	1.7×10^5	578	1414	0.8	0.0	0.0	0.4	0.0
KI-201	<50	518	374	7.2	0.0	12.8	0.0	3.5

[a] HIV-1-infected individuals with HLA-B*5401.
[b] Copies/ml.
[c] Cells/μl.

$CD8^+$ T cells failed to recognize three truncated peptides carrying Ala at position 2. Thus, this epitope is not consistent with the HLA-B*5401 peptide motif. We note that the Pol792–800 epitope cannot be identified by reverse immunogenetics. Interestingly, the Pol792–800-specific CTL clone showed high cytotoxicity toward B*5401-transfected C1R cells pulsed with peptides at low concentrations (Fig. 5), thus suggesting that the Pol792–800 peptide may be a high-affinity HLA-B*5401-binding peptide.

In the present study, we used 17-mer overlapping peptides to identify HIV-1-specific CTLs, because the cost of making shorter peptides is much cheaper than that for the longer ones. The optimal length of epitope peptides presented by HLA class I molecules is thought to be 8–11 amino acid residues [30]. Therefore, the affinity of 17-mer peptides toward HLA class I molecules is thought to be low. This suggests that some epitopes are not identified by this approach using 17-mer overlapping peptides.

Interestingly, Gag-specific epitopes were not identified in the present study, although Pol- and Nef-specific CTL ones were. We used PBMCs from only HIV-1-infected individual KI-119. Therefore, we speculate that this individual does not have any ability to elicit CTL specific for Gag. However, KI-119 showed strong HLA-A*0206-restricted or HLA-B*4801-restricted $CD8^+$ T cells responses to Gag (data not shown), suggesting that HLA-B*5401-restricted Gag-specific T cell responses are hardly induced. This suggests the possibility that Gag does not include a high-affinity peptide carrying HLA-B*5401 motif. Recent studies reported that Gag-specific CTLs play a critical role in the control of viral replication, because their frequency was correlated with viral loads in HIV-1-infected individuals [31]. If this is also the case in Japanese and other Asian populations, HLA-B*5401 may be associated with rapid progression to AIDS. The role of these HLA-B*5401-restricted CTLs still remains unknown. Further analysis of these CTLs will be required to clarify the role of HLA-B*5401-restricted CTLs in Asian populations.

When we examined the frequency of these five epitope-specific CTL in eight chronically HIV-1-infected individuals, these CTLs were detected in two to four of eight chronically HIV-1-infected individuals with HLA-B*5401 (Table 1), indicating that these five epitopes were relatively recognized ones in chronically HIV-1-infected individuals. These epitopes except Nef150–160 are relatively conserved in clade B (approximately more than 80% of clade B has consensus sequences: Los Alamos National Laboratory HIV Molecular Immunology Database, http://www.hiv.lanl.gov/content/immunology/maps/ctl/ctl.pdf). In contrast, many substitutions are found in Nef150–160. They include D at position 4, D at position 6, Q/E/R at position 8, I at position 8, and K at position 10. These results imply that these CTLs play an important role in the control of HIV-1. Further analysis of these epitopes such as escape mutants is now under investigation.

In summary, we identified five novel HLA-B*5401-restricted HIV-1 epitopes in HIV-1-infected individuals by using 17-mer overlapping peptides derived from HIV-1 Gag, Pol, and Nef. In addition, one of them, Pol792–800, did not have an amino acid sequence matching the HLA-B*5401 peptide motif. These epitopes identified by using 17-mer overlapping peptides will be useful to clarify immune response toward HIV-1 and to develop a population-based AIDS vaccine.

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References

[1] R.A. Koup, J.T. Safrit, Y. Cao, C.A. Andrews, G. McLeod, W. Borkowsky, C. Farthing, D.D. Ho, Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome, J. Virol. 68 (1994) 4650–4655.

[2] G. Pantaleo, J.F. Demarest, H. Soudeyns, C. Graziosi, F. Denis, J.W. Adelsberger, P. Borrow, M.S. Saag, G.M. Shaw, P.S. Sekaly, A.S. Fauci, Major expansion of CD8+ T cells with a predominant Vβ usage during the primary immune response to HIV, Nature 370 (1994) 463–467.

[3] P.A. Moss, S.L. Rowland-Jones, P.M. Frodsham, S. McAdam, P. Giangrande, A.J. McMichael, J. Bell, Persistent high frequency of human immunodeficiency virus-specific cytotoxic T cells in peripheral blood of infected donors, Proc. Natl. Acad. Sci. U. S. A. 192 (1995) 5773–5777.

[4] S. Rowland-Jones, J. Sutton, K. Ariyoshi, T. Dong, F. Gotch, S. McAdam, D. Whitby, S. Sabally, A. Gallimore, T. Corrah, M. Takiguchi, T. Schultz, A. McMichael, H. Whittle, HIV-1 specific cytotoxic T cells in HIV-exposed but uninfected Gambian women, Nat. Med. 1 (1995) 59–64.

[5] C.M. Walker, D.J. Moody, D.P. Stites, J.A. Levy, CD8+ lymphocytes can control HIV infection in vitro by suppressing virus replication, Science 234 (1986) 1563–1566.

[6] O.O. Yang, B.D. Walker, CD8+ cells in human immunodeficiency virus type I pathogenesis: cytolytic and noncytolytic inhibition of viral replication, Adv. Immunol. 66 (1997) 273–311.

[7] T. Matano, R. Shibata, C. Siemon, M. Connors, H.C. Lane, M.A. Martin, Administration of an anti-CD8 monoclonal antibody interferes with the clearance of chimeric simian/human immunodeficiency virus during primary infections of rhesus macaques, J. Virol. 72 (1998) 164–169.

[8] P. Borrow, H. Lewicki, X. Wei, M.S. Horwitz, N. Peffer, H. Meyers, J.A. Nelson, J.E. Gairin, B.H. Hahn, M.B. Oldstone, G.M. Shaw, Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus, Nat. Med. 3 (1997) 205–211.

[9] P.J. Goulder, R.E. Philips, R.A. Colbert, S. McAdam, G. Ogg, M.A. Nowak, P. Giangrande, G. Luzzi, B. Morgan, A. Edwards, A.J. McMichael, S. Rowland-Jones, Late escape from an immunodominant cytotoxic T-lymphocyte response associated with progression to AIDS, Nat. Med. 3 (1997) 212–217.

[10] A.J. Frater, H. Brown, A. Oxenius, H.F. Günthard, B. Hirschel, N. Robinson, A.J. Leslie, R. Payne, H. Crawford, A. Prendergast, C. Brander, P. Kiepiela, B.D. Walker, P.J. Goulder, A. McLean, R.E. Phillips, Effective T-cell responses select human immunodeficiency virus mutants and slow disease progression, J. Virol. 81 (2007) 6742–6751.

[11] S.J. O'Brien, X. Gao, M. Carrington, HLA and AIDS: a cautionary tale, Trends Mol. Med. 7 (2001) 379–381.

[12] M. Altfeld, E.T. Kalife, Y. Qi, H. Streeck, M. Lichterfeld, M.N. Johnston, N. Burgett, M.E. Swartz, A. Yang, G. Alter, X.G. Yu, A. Meier, J.K. Rockstroh, T.M. Allen, H. Jessen, E.S. Rosenberg, M. Carrington, B.D. Walker, HLA alleles associated with delayed progression to

AIDS contribute strongly to the initial CD8+ T cell response against HIV-1, PLoS Med. 3 (2006) e403.
[13] Y. Ikeda-Moore, H. Tomiyama, K. Miwa, S. Oka, A. Iwamoto, Y. Kaneko, M. Takiguchi, Identification and characterization of multiple HLA-A24-restricted HIV-1 CTL epitopes: strong epitopes are derived from V regions of HIV-1, J. Immunol. 159 (1997) 6242–6252.
[14] K. Fukada, H. Tomiyama, Y. Chujoh, K. Miwa, Y. Kaneko, S. Oka, M. Takiguchi, HLA-A*1101-restricted cytotoxic T lymphocyte recognition for a novel epitope derived from the HIV-1 Env protein, AIDS 13 (1999) 2597–2599.
[15] H. Tomiyama, T. Sakaguchi, K. Miwa, S. Oka, A. Iwamoto, Y. Kaneko, M. Takiguchi, Identification of multiple HIV-1 CTL epitopes presented by HLA-B*5101 molecules, Hum. Immunol. 60 (1999) 177–186.
[16] M.S. Hossain, H. Tomiyama, T. Inagawa, S. Ida, S. Oka, M. Takiguchi, Identification and characterization of HLA-A*3303-restricted, HIV type 1 Pol- and Gag-derived cytotoxic T cell epitopes, AIDS Res. Hum. Retroviruses 19 (2003) 503–510.
[17] M. Satoh, Y. Takamiya, S. Oka, K. Tokunaga, M. Takiguchi, Identification and characterization of HIV-1-specific CD8+ T cell epitopes presented by HLA-A*2601, Vaccine 23 (2005) 3783–3790.
[18] Y. Kawashima, M. Satoh, S. Oka, M. Takiguchi, Identification and characterization of HIV-1 epitopes presented by HLA-A*2603: comparison between HIV-1 epitopes presented by A*2601 and A*2603, Hum. Immunol. 66 (2005) 1155–1166.
[19] M.A. Borghan, S. Oka, M. Takiguchi, Identification of HLA-A*3101-restricted cytotoxic T-lymphocyte response to human immunodeficiency virus type 1 (HIV-1) in patients with chronic HIV-1 infection, Tissue Antigens 66 (2005) 305–313.
[20] T. Dong, D. Boyd, W. Rosenberg, N. Alp, M. Takiguchi, A. McMichael, S. Rowland-Jones, An HLA-B35-restricted epitope modified at an anchor residue results in an antagonist peptide, Eur. J. Immunol. 26 (1996) 335–339.
[21] P.J. Goulder, S.W. Reid, D.A. Price, C.A. O'Callaghan, A.J. McMichael, R.E. Phillips, E.Y. Jones, Combined structural and immunological refinement of HIV-1 HLA-B8-restricted cytotoxic T lymphocyte epitopes, Eur. J. Immunol. 27 (1997) 1515–1521.
[22] M. Altfeld, M.M. Addo, R.L. Eldridge, X.G. Yu, S. Thomas, A. Khatri, D. Strick, M.N. Phillips, G.B. Cohen, S.A. Islam, S.A. Kalams, C. Brander, P.J. Goulder, E.S. Rosenberg, B.D. WalkerHIV Study Collaboration, Vpr is preferentially targeted by CTL during HIV-1 infection, J. Immunol. 167 (2001) 2743–2752.
[23] M.M. Addo, M. Altfeld, E.S. Rosenberg, R.L. Eldridge, M.N. Philips, K. Habeeb, A. Khatri, C. Brander, G.K. Robbins, G.P. Mazzara, P.J. Goulder, B.D. WalkerHIV Controller Study Collaboration, The HIV-1 regulatory proteins Tat and Rev are frequently targeted by cytotoxic T lymphocytes derived from HIV-1-infected individuals, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 1781–1786.
[24] P.J. Goulder, M.M. Addo, M.A. Altfeld, E.S. Rosenberg, Y. Tang, U. Govender, N. Mngqundaniso, K. Annamalai, T.U. Vogel, M. Hammond, M. Bunce, H.M. Coovadia, B.D. Walker, Rapid definition of five novel HLA-A*3002-restricted human immunodeficiency virus-specific cytotoxic T-lymphocyte epitopes by elispot and intracellular cytokine staining assays, J. Virol 75 (2001) 1339–1347.
[25] R. Draenert, M. Altfeld, C. Brander, N. Basgoz, C. Corcoran, A.G. Wurcel, D.R. Stone, S.A. Kalams, A. Trocha, M.M. Addo, P.J. Goulder, B.D. Walker, Comparison of overlapping peptide sets for detection of antiviral CD8 and CD4 T cell responses, J. Immunol. Methods 275 (2003) 19–29.
[26] H.T. Maecker, H.S. Dunn, M.A. Suni, E. Khatamzas, C.J. Pitcher, T. Bunde, N. Persaud, W. Trigona, T.M. Fu, E. Sinclair, B.M. Bredt, J.M. McCune, V.C. Maino, F. Kern, L.J. Picker, Use of overlapping peptide mixtures as antigens for cytokine flow cytometry, J. Immunol. Methods 255 (2001) 27–40.
[27] T. Imanishi, T. Akaza, A. Kimura, K. Tokunaga, T. Gojobori, in: K. Tsuji, M. Aizava, T. Sasazuki (Eds.), Allele and Haplotype Frequencies for HLA and Complement Loci in Various Ethnic Groups, HLA 1991: Proceedings of the Eleventh International Histocompatibility Workshop and Conference, Oxford University Press, Oxford, 1992.
[28] A.D. Blagoveshchenskaya, L. Thomas, S.F. Feliciangeli, C.-H. Hung, G. Thomas, HIV-1 Nef downregulates MHC-1 by a PACS-1- and PI3K-regulated ARF6 endocytic pathway, Cell 111 (2002) 853–866.
[29] L.D. Barber, B. Gillece-Castro, L. Percival, X. Li, C. Clayberger, P. Parham, Overlap in the repertoires of peptides bound in vivo by a group of related class I HLA-B allotypes, Curr. Biol. 1 (1995) 179–190.
[30] J.W. Yewdell, J.R. Bennink, Immunodominance in major histocompatibility complex class I-restricted T lymphocyte responses, Annu. Rev. Immunol. 17 (1999) 51–88.
[31] P. Kiepiela, K. Ngumbela, C. Thobakgale, D. Ramduth, I. Honeyborne, E. Moodley, S. Reddy, C. de Pierres, Z. Mncube, N. Mkhwanazi, K. Bishop, M. van der Stok, K. Nair, N. Khan, H. Crawford, R. Payne, A. Leslie, J. Prado, A. Prendergast, J. Frater, N. McCarthy, C. Brander, G.H. Learn, D. Nickle, C. Rousseau, H. Coovadia, J.I. Mullins, D. Heckerman, B.D. Walker, P. Goulder, CD8+ T-cell responses to different HIV proteins have discordant associations with viral load, Nat. Med. 13 (2007) 46–53.