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Identification of hepatitis B virus-specific CTL epitopes presented by HLA-A*2402, the most common HLA class I allele in East Asia

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***Background/Aims*: The aim of this study was to identify and characterize hepatitis B virus (HBV)-specific cytotoxic T lymphocytes (CTL) epitopes presented by human leukocyte antigen (HLA)-A*2402, most common HLA class I allele in East Asia.**

***Methods*: HLA-A*2402-restricted CTL epitopes were identified by reverse immunogenetics. Immunogenecity of these epitopes was investigated using peripheral blood mononuclear cell (PBMC) from HLA-A24$^+$ patients with acute hepatitis B.**

***Results*: An HLA-A*2402 stabilization assay demonstrated that 36 of 63 HBV peptides carrying HLA-A*2402 anchor residues have high- and medium-HLA-A*2402 binding affinity. Two (C117–125 and P756–764) of the 36 peptides induced peptide-specific CTLs. CTL clones and lines specific for these peptides killed HBV recombinant vaccinia virus-infected target cells expressing HLA-A*2402, indicating that these two peptides are CTL epitopes presented by HLA-A*2402. These two peptides were able to induce specific CTLs in 7 and 11 of 12 HLA-A24$^+$ patients with acute hepatitis B, respectively.**

***Conclusions*: We identified two immunodominant CTL epitopes restricted by HLA-A*2402. Because HLA-A*2402 is the most common allele in East Asia, a region in which there are approximately 200 million HBV carriers, these epitopes will be useful for analysis of CTL responses in patients from East Asia.**

***Keywords*: Hepatitis B virus; HLA-A24; Cytotoxic T lymphocyte epitopes**

1. Introduction

Hepatitis B virus (HBV) is a hepatotropic, non-cytopathic, double-stranded DNA virus that causes acute and chronic liver disease [1]. Despite effective vaccine programmes, the number of chronic HBV carriers continues to increase. The World Health Organization predicted that the number of chronic HBV carriers would reach 400 million by the year 2000, with approximately 200 million of these individuals residing in East Asia [2].

HBV-specific cytotoxic T lymphocytes (CTLs) are believed to play a major role in both virus clearance and the pathogenesis of liver cell injury [3,4]. A vigorous CTL response specific for HBV-encoded proteins was detectable in peripheral blood from individuals with acute hepatitis B, who ultimately cleared the virus [5–9]. Conversely, only a weak CTL response specific for the HBV Core antigen was detectable in peripheral blood from patients with chronic hepatitis B [5,8,9]. However, chronically infected patients who experienced a spontaneous or interferon-induced remission developed a CTL response that was similar in

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strength and specificity to that of patients who recovered from acute hepatitis B [10]. These observations suggest that vigorous CTL responses to HBV are essential for viral clearance.

Identification of HBV-specific CTL epitopes is necessary for studies of immunopathogenesis in chronic hepatitis and for vaccine development. 16 of the 19 identified HBV-specific CTL epitopes are human leukocyte antigen (HLA)-A2-restricted [3,5–9], with only three epitopes being restricted by alleles other than HLA-A2 [11–13]. Because the immunodominancies of these three epitopes have not been analyzed, the HLA-A2 restricted epitopes have been mostly used for studies of HBV-specific CTLs in patients with hepatitis B [5–10,14]. In addition, development of a therapeutic T cell vaccine is now in progress using the most immunodominant HLA-A2-restricted CTL epitope, Core 18–27 [15–19]. However, approximately 75% of HBV-infected people reside in Asia and Africa [2,20], wherein HLA-A2 is not the most common HLA class I allele [21]. Therefore, identification and characterization of HBV-specific CTL epitopes restricted by alleles other then HLA-A2 is extremely important for the development of therapeutic vaccines. Although HLA-A24 is the most common HLA Class I allele in East Asian countries [21], no HLA-A24-restricted HBV specific CTL epitope has been identified.

We previously showed that multiple human immunodeficiency virus-1 (HIV-1)-specific CTL epitopes can be effectively identified using reverse immunogenetics, a strategy which identifies CTL epitopes using a panel of peptides matched to HLA class I binding peptide motifs [22,23]. In the present study, we employed reverse immunogenetics to identify HLA-A24-restricted HBV specific CTL epitopes. A previous HLA-genotype analysis in Japanese population showed that all 76 HLA-A24-positive Japanese have HLA-A*2402 [24]. We therefore used HLA-A*2402 peptide motif in the present study. Identified HBV epitopes were further characterized by investigating specific CTL responses in patients with acute hepatitis B.

2. Materials and methods

2.1. Patients

Twelve HLA-A24$^+$ patients with acute hepatitis B and 11 HLA-A24$^+$ patients with chronic hepatitis B were investigated. Blood samples were obtained from Yokohama City University Hospital, Kumamoto University Hospital and Miyazaki Medical College Hospital. All blood samples were collected with an oral informed consent, and research protocols were approved by the institutional review boards of the participating hospitals. Acute HBV infection was diagnosed by a high level of serum transaminase activity, detection of hepatitis B surface antigen (HBsAg) and immunoglobulin M (IgM) anti-hepatitis B core antigen antibody (HBcAb) in the serum by enzyme immunoassay, histological examination of liver tissue in the recovery phase (for exclusion of chronic hepatitis), and the recent onset of jaundice and other symptoms typical of acute hepatitis. All patients were negative for antibodies to hepatitis C virus (HCV), hepatitis D virus (HDV) and HIV-1. All patients with acute hepatitis B completely recovered from the illness, exhibiting normalized serum transaminase levels and clearance of HBsAg and HBeAg from the serum within 6 months of the disease.

2.2. Cells

C1R cells expressing HLA-A*2402 (C1R-A*2402) and RMA-S cells expressing HLA-A*2402 (RMA-S-A*2402) were previously generated [25].

2.3. Peptides

HBV-derived peptides were synthesized using an automated peptide synthesizer (model PSSM-8, Shimazu Co., Kyoto, Japan), with F-moc strategy followed by cleavage. Peptide purity was determined by mass spectrometry and high pressure liquid chromatography.

2.4. Peptide binding assay

Binding of HBV-derived peptides to HLA-A*2402 was examined by an HLA-A*2402 stabilization assay using RMA-S-A*2402 cells as described previously [23,25]. Briefly, RMA-S-A*2402 cells were cultured for 16 h at 26°C and then were pulsed with peptides (10^{-3}–10^{-9} M) for 1 h at 26°C. After incubation for 3 h at 37°C, peptide-pulsed cells were stained with anti-HLA class I α 3 domain mAb TP25.99 [26] and the fluorescent-conjugated IgG fraction of sheep anti-mouse Ig (Silenius Laboratories, Hawthorn, Victoria, Australia). The mean fluorescence intensity (MFI) was measured using a FACS Calibur (Becton Dickinson, Mountain View, CA, USA). Peptides which at a concentration of 10^{-4} M gave more than 25% MFI of RMA-S-A*2402 cells [(MFI of RMA-S-A*2402 cells cultured at 26°C minus MFI of RMA-S-A*2402 cells cultured at 37°C) × 0.25] were evaluated as HLA-A*2402-binding peptides, which was employed in previous studies [25,27]. The peptide concentration which yielded the half-maximal MFI level (the BL_{50} value) was calculated as previously described [25].

2.5. Induction of peptide-specific CTL in PBMC from HLA-A24$^+$ patients with acute hepatitis B

Peripheral blood mononuclear cell (PBMC) were separated from HLA-A24$^+$ patients with acute hepatitis B within 1 week of disease onset and HLA-A24$^+$ patients with chronic hepatitis B. PBMC (2×10^6) were cultured with each or group of HLA-A*2402 binding peptides (10^{-6} M) in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) and 200 U/ml recombinant human IL2 (r-hIL2). On day 7, the cells were stimulated with 1×10^6 irradiated (50 Gy) PHA-induced autologous cells prepulsed with the corresponding peptide (10^{-5} M). The culture was maintained by changing half of the medium containing 200 U/ml r-hIL2 every 2 days. Peptide-specific CTL activity of cultured bulk cells was tested at effector: target (E:T) ratio of 20:1 using a standard 4 h ^{51}Cr release assay on day 14.

2.6. CTL assay

Cytotoxicity was measured using a standard 4 h ^{51}Cr release assay as previously described [22].

2.7. CTL assay for target cells infected with recombinant vaccinia virus

Recombinant vaccinia viruses containing the HBV pol and HBV core gene were kindly provided by F.V. Chisari (Scripps Research Institute, La Jolla, CA, USA) and T. Ishikawa (Aichi Medical University, Aichi, Japan), respectively. Targets were cultured with ten plaque-forming units recombinant or wild-type vaccinia virus per target cell for 18 h. The infected cells (5×10^5) were used as target cells for a standard 4 h ^{51}Cr release assay.

Table 1
HLA-A*2402 binding HBV peptides with high and medium affinity[a]

Position	Peptide sequence	Binding affinity BL_{50} (M)
P 756–764	KYTSFPWLL	$< 1.0 \times 10^{-8}$
C 117–125	EYLVSFGVW	$< 1.0 \times 10^{-8}$
P 62–70	LYSSTVPVF	$< 1.0 \times 10^{-8}$
P 566–574	LYTSITNFL	3.4×10^{-8}
C 102–110	WFHISCLTF	1.7×10^{-7}
P 503–512	LYSHPIILGF	1.0×10^{-6}
P 115–123	FYPNLTKYL	2.1×10^{-6}
X 111–118	AYFKDCLF	2.4×10^{-6}
C 87–97	SYVNVNMGLKI	3.4×10^{-6}
C 117–126	EYLVSFGVWI	3.7×10^{-6}
S 205–213	LYNILSPFL	4.0×10^{-6}
C 87–95	SYVNVNMGL	4.6×10^{-6}
S 211–219	PFLPLLPIF	5.9×10^{-6}
X 132–142	IYVLGGCRHKL	8.0×10^{-6}
P 336–345	DYCLTHIVNL	1.2×10^{-5}
C 131–139	AYRPQNAPI	1.2×10^{-5}
C 131–140	AYRPQNAPIL	1.7×10^{-5}
S 199–208	WYWGPSLYNI	1.8×10^{-5}
X 132–142	FVLGGCRHKL	2.0×10^{-5}
X 111–121	AYFKDCLFKDW	2.2×10^{-5}
S 19–28	FFLLTRILTI	2.5×10^{-5}
P 4–11	SYQHFRKL	2.7×10^{-5}
P 566–573	LYAAVTNF	3.0×10^{-5}
C 22–31	DFFPSIRDLL	3.5×10^{-5}
P 756–763	KYTSFPWL	3.5×10^{-5}
P 797–804	LYRPLLRL	4.4×10^{-5}
C 17–24	SFLPSDFF	4.8×10^{-5}
P 548–555	SYMDDVVL	5.5×10^{-5}
P 140–148	HYFKTRHYL	5.5×10^{-5}
S 205–212	LYNILSPF	5.9×10^{-5}
P 650–657	GYPALMPL	7.4×10^{-5}
X 112–121	YFKDCLFKDW	7.6×10^{-5}
C 23–31	FFPSIRDLL	7.8×10^{-5}
S 178–186	PFVQWFVGL	9.6×10^{-5}
P 503–510	LYSHPIIL	9.9×10^{-5}
P 172–179	PYSWEQEL	1.0×10^{-4}

[a] BL_{50}: The half maximal binding level which is defined as the peptide concentration yielding the half maximal MFI was calculated.

2.8. Generation of CTL clones specific for HBV peptides

Peptide-specific CTL clones were generated from established HBV-specific bulk CTL cultures by seeding 0.8 cells/well into U-bottom 96 well microculture plates (Nunc, Roskilde, Denmark) together with 200 μl of cloning mixture (5×10^5 irradiated allogenic PBMC and 5×10^4 irradiated C1R-A*2402 cells prepulsed with the corresponding peptide at 10^{-6} M in RPMI1640 medium supplemented with 10% FCS and 200 U/ml r-hIL2). Wells which contained growing cells after 2–4 weeks were tested for specific CTL activity using the ^{51}Cr release assay. Established clones were used for analyzing cytolysis of target cells pulsed with the peptides or infected with the recombinant vaccinia viruses. CTL activity of CTL clone was tested at E/T ratio of 2:1.

3. Results

3.1. Binding of HBV derived peptides to HLA-A*2402

HLA-A*2402-binding peptides have two anchor residues, Tyr or Phe at position 2 and either Phe, Leu, Ile, or Trp at the C-terminus [28]. The amino acid sequences of Core, Pol, Env, and X proteins of HBV adr subtype present in the GenBank database were screened for 8- to 11-mer peptides carrying these anchor residues. The adr subtype was chosen as, with the exception of Vietnam, it is greatly predominant subtype in East and South-East Asia [29,30]. At total of 63 sequences were selected from regions which were highly conserved between at least 80% of the adr sequences in GenBank, and peptides corresponding to these sequences were synthesized.

The binding affinities of these peptides were tested in an HLA-A*2402 stabilization assay using RMA-S-A*2402 cells. 58 out of these peptides bound to HLA-A*2402 molecules. These peptides were divided in three categories according to their binding affinity: high ($BL_{50} \leq 10^{-5}$), medium ($10^{-5} < BL_{50} \leq 10^{-4}$) and low ($BL_{50} > 10^{-4}$) affinity peptides. Thirty-six peptides, comprising eleven 8-mer, thirteen 9-mer, eight 10-mer and four 11-mer peptides, had high or medium affinity for HLA-A*2402 (Table 1).

3.2. Identification of two HBV-specific CTL epitopes presented by HLA-A*2402

We attempted to induce HBV peptide-specific CTLs from three HLA-A24$^+$ patients with acute hepatitis B by stimulating their PBMC with the 36 identified high and medium affinity peptides. Cultures were stimulated with each peptide for 7 days, and re-stimulated with PHA-induced autologous cells pulsed with the corresponding peptide. The cytotoxic activity of each bulk culture against C1R-A*2402 cells pulsed with the corresponding peptide was tested 2 weeks later. Of the 36 HBV peptides, only C117–125 and P756–764 induced specific CTL responses (higher than 10% relative specific lysis) in two and three patients, respectively (Table 2). The sequences of C117–125 and P756–764 are derived from the HBV Core and Pol proteins, respectively.

Several CTL clones were established from the C117–125-specific cell culture to confirm specific recognition of CTLs for the peptide. We were unsuccessful in establishing CTL clones specific for peptide P756–764, and therefore used several P756–764-specific CTL lines in further studies. C117–125 specific CTL clone C117–125-52 and P756–764-specific CTL line P756–764–14 effectively lysed target cells pulsed with the corresponding peptides at concentrations between 10^{-6} and 10^{-10} M (Fig. 1). Similar results were observed for six other C117–125-specific CTL clones and one other P756–764-specific CTL line (data not shown).

Some peptide-induced CTLs lyse target cells pulsed with the corresponding peptide but fail to lyse those infected with virus containing the corresponding sequence. Such a peptide is not naturally processed peptide presented by HLA class I molecules. To clarify whether these peptides are processed and presented by HLA-A*2402 molecules, we investigated the killing of target cells infected with HBV recombinant vaccinia virus by CTL clone C117–125–65 and CTL line

Table 2
Induction of HBV-specific CTL by stimulating acute phase PBMC from HLA-A*2402$^+$ patients with acute hepatitis B

	Relative specific lysis(%)[a]		
Position	Pt.A16	Pt.A13	Pt.A10
High affinity peptides			
P 756–764	**19.2**	**26.8**	**23.4**
C 117–125	**10.3**	**18.9**	0.0
P 62–70	1.6	−4.2	−0.6
P 566–574	0.2	0.7	1.2
C 102–110	1.9	7.7	−1.9
P 503–512	3.2	0.7	−1.1
P 115–123	−2.7	2.9	−12.8
X 111–118	5.0	3.2	−0.8
C 87–97	−1.0	−3.5	0.4
C 117–126	6.9	−2.4	0.9
S 205–213	−3.9	−4.5	−4.3
C 87–95	0.1	−2.6	1.8
S 211–219	−2.5	2.3	7.4
X 132–142	0.1	−1.2	−0.8
Medium affinity peptides			
P 336–345	nt	−1.2[b]	−1.6
C 131–139	nt		
C 131–140	nt		
S 199–208	nt		
X 132–142	nt	−0.4	0.8
X 111–121	nt		
S 19–28	nt		
P 4–11	nt		
P 566–573	nt	−2.1	2.8
C 22–31	nt		
P 756–763	nt		
P 797–804	nt		
C 17–24	nt	1.6	−3.4
P 548–555	nt		
P 140–148	nt		
S 205–212	nt		
P 650–657	nt	−1.6	−4.0
X 112–121	nt		
C 23–31	nt		
S 178–186	nt	− 0.4	−0.2
P 503–510	nt		
P 172–179	nt		

[a] Relative specific lysis: % specific lysis of C1R-A*2402 cells loaded with 1 μM peptides minus % specific lysis of C1R-A*2402 cells loaded without peptide. The specific lysis was tested at an E/T ratio of 20:1. Bold shows positive response (more than 10% relative specific lysis).

[b] A mixture of three or four peptides with medium affinity (1 μM) were used to induce specific CTL. After 2 weeks culture, C1R-A*2402 pulsed with a mixture of the peptides were used as target cells at an E/T ratio of 20:1.

P756–764–24. CTL clone C117–125–65 lysed C1R-A*2402 cells infected with HBV recombinant vaccinia virus expressing HBV Core (pHBc102) but failed to lyse C1R cells infected with the recombinant virus or C1R-A*2402 cells infected with wild type vaccinia virus (Fig. 2A). These results were confirmed using 11 other C117–125-specific CTL clones (data not shown). The relative specific lysis of these CTL clones against pHBc102-infected target cells (obtained by subtracting the specific lysis of C1R-A*2402 cells infected with wild type vaccinia virus from the specific lysis of C1R-A*2402 cells infected with pHBc102) was 17.1–37.0%. These results indicate that C117–125 is naturally processed peptide presented by HLA-A*2402. Similarly, CTL line P756–764–24 lysed C1R-A*2402 cells infected with HBV recombinant vaccinia virus expressing HBV Pol (Vpol) (Fig.2B). Two other P756–764-specific CTL lines gave similar results (data not shown). The relative specific lysis of these P756–764-specific CTL lines against Vpol-infected target cells was 13.6–40.5%. These results demonstrate that P756–764 is also naturally processed peptides presented by HLA-A*2402.

The 9-mer peptide P756-764 contains an 8-mer sequence (KYTSFPWL) which also matches the HLA-A*2402 binding motif. To clarify whether this 8-mer peptide (P756-763) is the minimum required for CTL recognition, we examined the cytolytic activity of P756–764-specific CTL lines against target cells pulsed with P756–763. As shown in Fig.1B, these CTL lines failed to lyse C1R-A*2402 cells pulsed with P756–763. This 8-mer peptide also failed to induce specific CTLs in PBMC from two patients, Pt.A10 and Pt.A13 (Table 2). P756–763 could bind to HLA-A*2402 molecules on RMA-S-A*2402 cells but with a 1000-fold lower binding affinity than that of P756-764 (Fig. 3). This suggests that failure of CTLs to recognize P756-763 is mostly due to the low binding affinity of this peptide.

The 10-mer peptide C117–126 contains the C117–125 sequence. Consistent with the approximately 30-fold lower binding affinity of C117–126 to HLA-A*2402 as compared to C117–125 (Table 1), the 10-mer peptide failed to induce specific CTLs in PBMC from three patients with acute hepatitis B (Table 2). These results indicate that the 9-mer C117-125 is the minimum required for CTL recognition.

*3.3. Induction of CTLs specific for two HLA-A*2402-restricted epitopes in PBMC from HLA-A24$^+$ patients with acute and chronic hepatitis B.*

To investigate the frequency of C117–125- and P756–764-specific CTL responses in patients with acute hepatitis B, we examined CTL activities against these epitopes in 2-week old PBMC cultures established from 12 HLA-A24$^+$ patients with acute hepatitis B. C117–125- and P756–764-specific responses were detected in 7 and 11 of 12 patients, respectively (Fig. 4A). These peptides failed to induce specific CTLs (<10% relative specific lysis) in all 10 HBV-seronegative HLA-A24$^+$ individuals (Fig. 4C). Thus, CTLs specific for these epitopes can be frequently induced in HLA-A24$^+$ individuals with acute hepatitis B, indicating that they are immunodominant epitopes. Specific CTL was induced by P756–764 peptide in 2-week old PBMC cultures from only one of 11 patients with chronic hepatitis B while it was not induced by C117–125 peptide in those from these patients (Fig. 4B). These results indicate

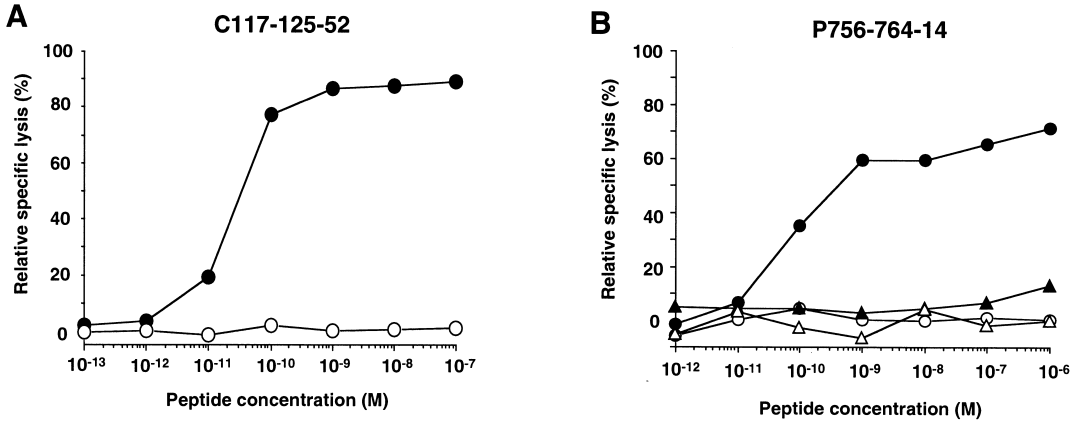

Fig. 1. Recognition of C117–125 and P756–764 peptides by HLA-A24-restricted CTL clones and lines. (A) Cytolysis of C117–125-specific CTL clones for peptide-pulsed target cells. Cytolysis of the CTL clone for target cells (C1R cells and C1R-A*2402 cells) pulsed with various concentrations of peptide C117–125 was tested at an effector:target (E/T) ratio of 2:1. The results are represented as relative specific lysis (percent specific lysis of target cells pulsed with the peptide minus percent specific lysis of target cells without the peptide) of C1R cells (○) and C1R-A*2402 cells (●). One representative result obtained from a CTL clone (C117–125–52) is presented. (B) Cytolysis of P756–764-specific CTL lines for peptide-pulsed target cells. Cytolysis of CTL lines for target cells (C1R and C1R-A*2402) pulsed with various peptide concentrations was tested at an E:T ratio of 2:1. The results are represented as relative specific lysis of C1R cells (○, △) and C1R-A*2402 cells (●, ▲) pulsed with the peptide KYTSFPWLL(○, ●) and the peptide KYTSFPWL(△, ▲). One representative result obtained from a CTL line (P756–764–14) is presented.

that there is low number of CTL specific for these epitopes in PBMC from patients with chronic hepatitis B.

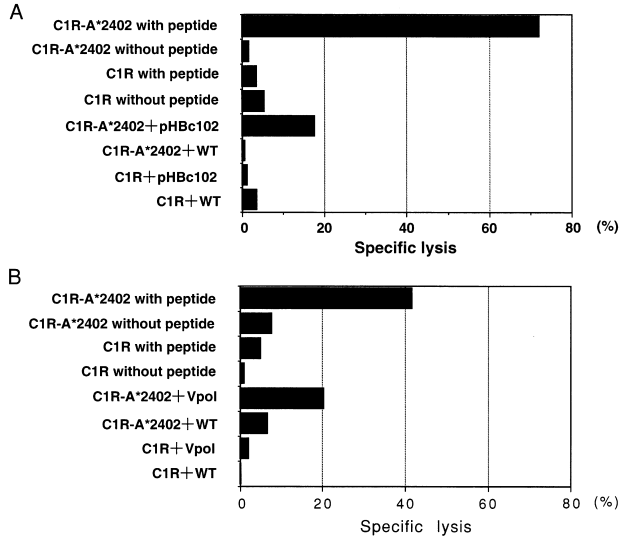

Fig. 2. Cytolysis of target cells infected with HBV recombinant vaccinia by C117–125-specific CTL clones and P756–764-specific CTL lines. Cytolysis of target cells by C117-125-specific CTL clones (A) or P756-764 specific CTL lines (B). Target cells (C1R-A*2402 and C1R) were pulsed with the corresponding peptide (1 μM) or infected with wild type vaccinia (WT) or recombinant vaccinia containing Core (pHBc102) or Pol (V pol). The cytolytic activity of these target cells was tested at an E/T ratio of 2:1. Results of one representative CTL clone (clone C117–125–65; A) and CTL line (line P756–764–24; B) are presented.

4. Discussion

Of the 63 HBV-derived peptides containing HLA-A*2402 peptide binding motifs, only two, C117–125 and P756–764 could induce specific CTLs. This contrasts with a previous study using the same strategy, in which 11 HLA-A24 restricted HIV-1 epitopes capable of inducing specific CTLs were identified from 59 peptides containing the HLA-A*2402 binding motifs [23]. Thus, reverse immunogenetics identified fewer HBV epitopes than HIV-1 epitopes. This was also observed when reverse immunogenetics was used to identify HLA-B*3501 restricted HCV [31] and HIV-1 epitopes [22,32]. One explanation for this could be that in the present study we used PBMC from patients with acute infection whereas HLA-A24-restricted HIV-1 epitopes were identified using PBMC from chronically infected patients.

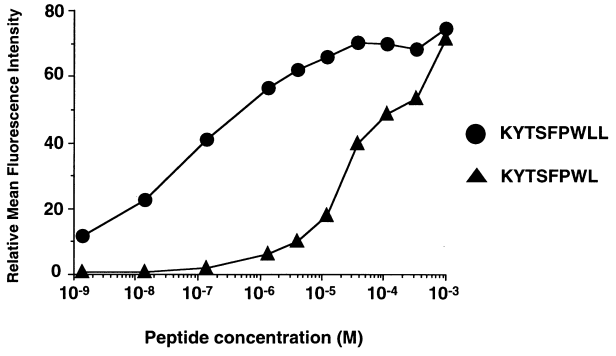

Fig. 3. Binding of the 9-mer P756–764 and the 8-mer P756–763 peptides to HLA-A*2402. Binding of P756–764 (KYTSFPWLL) (●) and P756–763 (KYTSFPWL) (▲) to HLA-A*2402 was measured using RMA-S-A*2402 cells.

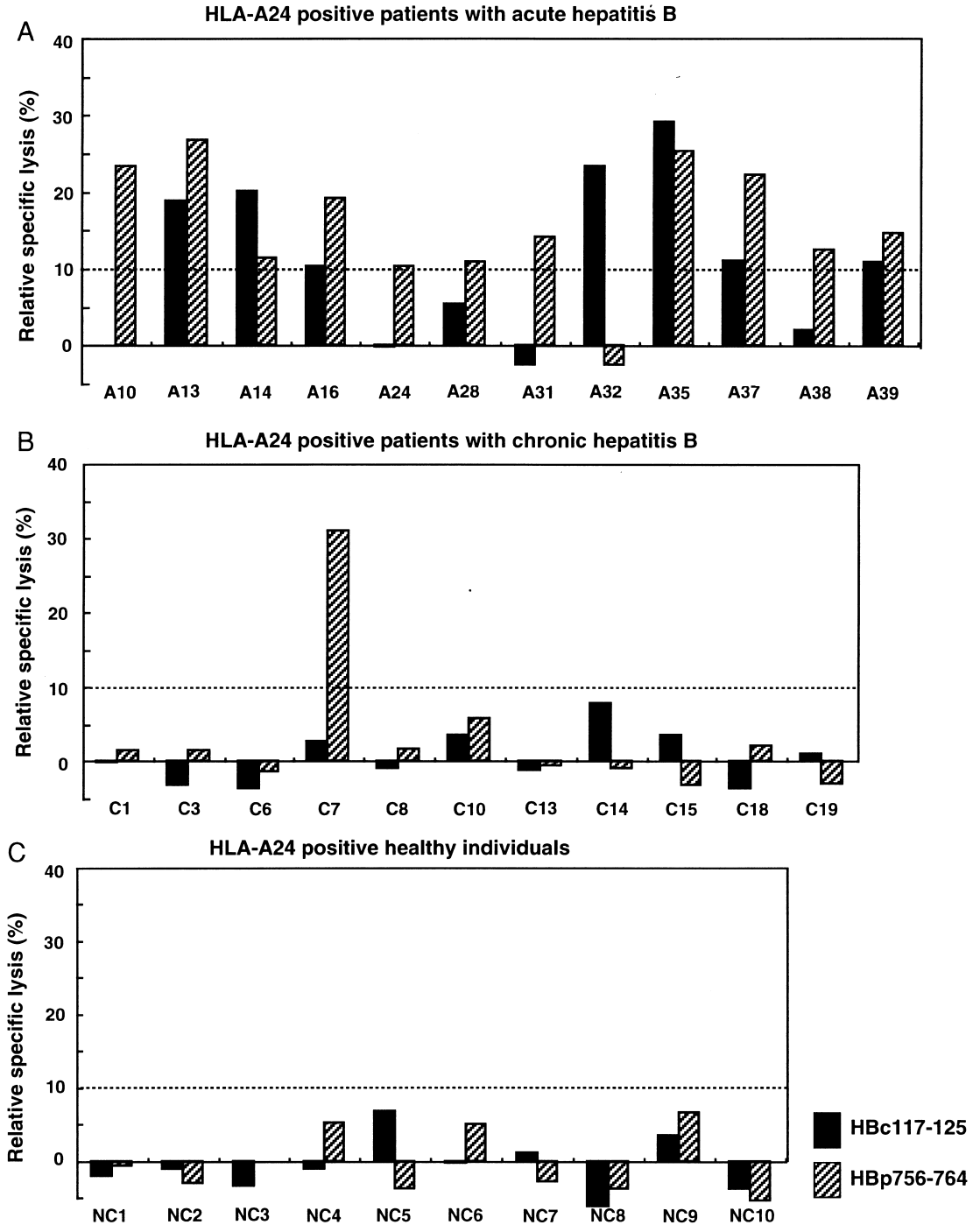

Fig. 4. In vitro induction of CTLs specific for two HLA-A*2402-restricted HBV epitopes in PBMC from patients with acute and chronic hepatitis B. PBMC from 12 HLA-A24$^+$ patients with acute hepatitis B (A), from 11 HLA-A24$^+$ patients with chronic hepatitis B (B) and from 10 HBV seronegative HLA-A24$^+$ individuals (C) were stimulated with C117–125 or P756–764 peptide and cultured for 2 weeks. Relative specific lysis of target cells (specific lysis of C1R-A*2402 pulsed with the corresponding peptide minus specific lysis of C1R-A*2402 without the peptide) was measured at an E/T ratio of 20:1.

However, a previous study showed that the number of HLA-A2 restricted HBV epitopes and the frequency of HBV-specific CTLs were lower in chronic compared to acute HBV patients [33]. The present study also demonstrated low frequency of two HLA-A*2402-restricted, HBV-specific CTLs in peripheral blood of patients with chronic hepatitis B. Thus, these results suggest that the frequency of hepatitis virus-specific peripheral CTLs is lower than that

of HIV-1-specific peripheral CTLs. These results also suggest that a high number of HIV-1 specific CTLs migrate from lymph nodes (the site of active HIV-1 infection) while only a low number of HBV-specific CTLs migrate from the liver (the site of HBV infection).

As no HBV peptide with medium-affinity induced a CTL response (Table 2), we did not test the ability of low affinity peptides to induce specific CTLs. Of the 63 peptides tested in this study, C117–125 and P756–764 had the highest affinity for HLA-A*2402. In a previous study using 53 peptides, only that with the second highest binding affinity was identified as an HLA-B*3501-restricted HCV epitope [31]. Furthermore, eight of nine HLA-A*0201-restricted HBV epitopes also had high binding affinities for HLA-A*0201 [34]. Thus, it appears that peptides with a high binding affinity for HLA class I molecules are preferentially recognized by T cells in acute hepatitis. In support of this, T cells specific for C117–125 and P756–764 can recognize target cells pulsed with only a low concentration (10^{-10} M) of peptide (Fig. 1).

A previous study reported that several peptides with HLA-A3- and HLA-B7 supertype binding motifs could induce peptide-specific CTL responses in PBMC from patients with acute hepatitis B, however, it was not demonstrated whether these are naturally processed peptides [35]. Excluding these candidate HBV epitopes, 19 HBV-specific CTL epitopes have been identified [5–9,11–13]. Of these epitopes, only three are restricted by alleles other than HLA-A2. One epitope, derived from the Core protein (C141-151: STLPETTVVRR), is restricted by HLA-A31 and HLA-A68 [11], the second, derived from the PreS1 (PreS1 10–17: PLGFFPDH), is restricted by HLA-A11 [12], and the third, derived from PreS2 proteins (PreS2 109–123: MQWNSTALHQALQDP), is restricted by HLA-A3 [13]. The PreS2 109–123 peptide is not a minimal epitope since it consists of 15 amino acids. A previous study reported that the C141–151 peptide induced a response in PBMC from four of seven patients (57%) with acute hepatitis B [35]. However, no information is available about the immunodominancy of PreS1 10–17 or Pre S2 109–123. Three (Core18–27, Env 335–343 and Pol 575–583) of 16 HLA-A2 restricted epitopes are recognized with a relatively high frequency (45–67%) in patients with acute hepatitis B [8,9,32] and therefore can be considered as immunodominant epitopes. In the present study, CTLs specific for C117–125 and P756–764 were found in 7 (58%) and 11 (92%) of 12 patients with acute hepatitis B, respectively, indicating that both are immunodominant epitopes. Thus, the present study provides two additional immunodominant HBV epitopes.

The amino acid sequences of these epitopes and their flanking regions are highly conserved not only in the adr subtypes but also in all HBV subtypes. Based on the sequences of 50 HBV isolates including all subtypes available in GenBank, 49 of 50 (98%) and 47 of 50 (94%) HBV isolates have no substitution within the C117–125 and P756–764 sequence, respectively. Our findings suggest that these epitopes will be useful for analysis of CTL responses in HLA-A24^{+} patients with hepatitis B. This is of particular relevance as HLA-A*2402 is the most common HLA class I allele found in East Asia, a region in which approximately 50% of the world's HBV-infected patients live. Therefore, the C117–125 and P756–764 epitopes identified in the present study will be extremely useful for studies of HBV specific CTLs in HBV patients in this area.

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