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Identification of hepatitis B virus-specific CTL epitopes presented by HLA-A*33:03 in peripheral blood mononuclear cells from patients and transgenic mice

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ABSTRACT

Cytotoxic T lymphocyte (CTL) epitopes in the HBV protein of hepatitis B virus (HBV) may play a key role in viral control and liver damage. The aim of this study was to identify and study the function of HLA-A*33:03-restricted CTL epitopes in HBV protein of the HBV genotypes B and C, which are epidemic in China. Sixteen HBV peptides were predicated by computational analysis, and synthesized peptides were examined for their affinity to HLA-A*33:03 using a stable cell line. After being analyzed by enzyme-linked immunospot and cytolytic activity assays, as well as the tetramers staining method using peripheral blood mononuclear cells isolated from HBV-infected patients, five peptides (Hbs245–253, HBs335–343, HBc119–127, HBc104–112, and HBp391–399) were chosen to further confirm their HLA_A*33:03 restriction in transgenic mice.

1. Introduction

Hepatitis B virus (HBV) infection is a global challenge, with an estimated worldwide distribution of millions of chronic carriers. All of these patients are at risk of developing adverse sequalae, including chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma [1]. Cellular immune responses play an important role in viral clearance and disease pathogenesis. The epitopes bound by the main type of HLA-A site, such as A2, A11, and A24, have been comprehensively investigated [2], but related data concerning HLA-A33 are almost completely lacking.

HLA-A33 is very rare, with a phenotypic frequency of 0–1% in Caucasian population. However, the phenotypic frequency of HLA-A33 is 6–23% in Asian populations, including Chinese (6–20.9%, $n = 133{,}046$), Japanese (7.7%, $n = 1024$), Korean (12.8%), Singaporean (9.3%, $n = 769$), and Malaysian peoples (12.9%, $n = 1445$). Further, hepatitis B patients in these areas account for larger proportion [3–5]. A*33:03 is dominant in Chinese, Korean, Singaporean, and Japanese populations. Identification of HLA-A*33:03-restricted HBV cytotoxic T lymphocyte (CTL) epitopes is therefore necessary to investigate the immmunopathogenesis of HBV in these areas and plan proper vaccine development.

2. Materials and methods

2.1. Patients

This study involved 322 HBV patient donors who all gave informed consent before blood donation. The diagnosis of chronic HBV infection was made according to the Chinese consensus criteria [6]. After blood was collected, genomic DNA was extracted using a Genomic DNA isolation kit (Promega), and HLA typing was performed using sequence-specific primers (S3; Protrans, Ketsch, Germany). Additionally, five chronic hepatitis B (CHB), four acute hepatitis B (AHB), and 15 liver cirrhosis patients were chosen for the experiment.

2.2. Cell lines

The HLA_A*33:03 gene was cloned into the pLentiLox3.7 (pLL3.7) viral vector using *Nhe*I and *Xho*I, and 15 μg of

Abbreviations: AHB, acute hepatitis B; CHB, chronic hepatitis B; CTL, cytotoxic T lymphocyte; ELISPOT, enzyme-linked immunospot; HBV, hepatitis B virus; HC, HLA heavy chain; HLA, human leukocyte antigen; IFN-γ, interferon-γ; PBMC, peripheral blood mononuclear cell; TH1, helper T cell type 1; WB, Western blotting; β2m, β 2microglobulin.

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pLL3.7_HLA*33:03 (GenBank ID: DQ327719.1) from a positive clone was co-transfected by Lipofectamine™ 2000 (Invitrogen, CA, USA) into HEK293T cells in a 10-cm dish with packaged plasmids VSVG, RSV-REV, and pMDLg/pRRE (5 μg each). Four hours after transfection, the culture medium was replaced with puromycin-containing medium, and 16 h later, transfected HEK293T cells were incubated at 32 °C overnight to increase the viral titer. Forty-eight hours after transfection, the lentiviral supernatant of the culture was collected, filtered through a 0.45-μm Millipore filter, and stored at −80 °C.

RMA-S cells or RD cells were infected using the lentiviral supernatant and selected with 4 μg/mL puromycin over five rounds of selection. Positive clones were identified using flow cytometry with a FITC-Flag antibody and named RMAS/A*33:03 and RD/A*33:03.

2.3. Peptides

The amino acid sequences of the S, C, X, and P proteins from HBV strains B and C were screened for HLA-A*33:03 binding peptide motifs (the detail is in Supplement). Candidate peptides were synthesized and shown to be 95% homogenous by high-performance liquid chromatography.

2.4. Temperature induction and peptide stabilization assays

HLA_A*33:03 surface expression with lowered temperature was performed as described [7]. RMAS/A*33:03 (10^6/mL) were incubated at 26 °C for 24 h, further incubated at 37 °C with or without 0.5 μM peptide and 1 nM human β 2microglobulin (hβ2m), and then examined by flow cytometry using a FITC-labeled W6/32 antibody at the indicated time points. FI values were calculated by the following formula: FI = (mean FITC fluorescence for a given peptide − mean FITC fluorescence without peptide)/(mean FITC fluorescence without peptide). Peptides with an FI > 2 were regarded as candidate epitopes with a capacity to bind to HLA-A*33:03 molecules.

2.5. Tetramer staining of virus-specific $CD8^+$ T cells

Soluble tetrameric human HLA-A*33:03 peptide complexes were constructed as previously reported [8].

2.6. Th1 polarization assay via the IFN-γ enzyme-linked immunospot (ELISPOT) method

Commercially available ELISPOT assays in IFN-γ kits (Dakewe biotech company, China) were used to assess type 1 cytotoxic T cell responses [9]. Briefly, A series of cell dilutions starting at 2.5×10^5 cells/well of PBMCs in 100 μl AIM-V medium (Life Technologies Inc., NY, USA) were co-cultured with individual HBV peptides (0.5 μM) for 18 h. The assay procedures then followed the manufacturer's instructions, and the spots were automatically counted using a Biospot counter (Cellular Technology, USA) and Immunocapture® software.

2.7. Generation of transgenic mice

Chimeric human-mouse class I cDNA contained the leader, α1, and α2 domains from HLA-A*33:03 (GenBank ID: DQ327719.1) fused to the murine H-$2K^d$ α3, transmembrane, and cytoplasmic domains (GenBank ID: KF831067.1). To easily trace the protein, StrepII and FLAG tags were added to the C-terminus. The chimeric gene sequence was optimized by the RNAfolding prediction program, and 10 silent mutations were made in the gene sequence. The class I hybrid construct was subsequently cloned into the pCAGG expression vector (encoding a CAG promoter and a polyadenylation signal) using *Nco*I and *Cla*I. Purified DNA was linearized with *Eco*RV and *Pst*I, and the 4.5-kb fragment containing the target gene was injected in fertilized C57BL/6 eggs to produce transgenic mice, as previously described [10]. The resulting progeny were screened by PCR, RT-PCR, Western blotting, and FACS using the B9.12.1 mAb (Beckman Coulter, CA, USA), which binds to the all HLA_A proteins [11].

2.8. CTL assays

RMAS-A*33:03 cells were incubated at 26 °C for 16 h, 10 μg/mL peptides and hβ2m were added, and then incubated for an additional 5 h. Then, the cells were labeled with 2.5 μmol/L PHK-26 (Sigma–Aldrich) and 2.5 μmol/L CFSE (Sigma–Aldrich) as target cells, as previously described [12]. The effect cells were added at different ratios: 10:1, 20:1, 40:1, or 80:1. After incubation for 6 h at 26 °C, the cells were counted by flow cytometry. Each assay was performed in triplicate. When target cells shifted to RD/A*33:03, the incubation temperature was adjusted to 37 °C.

2.9. Statistical analyses

Statistical analyses (analysis of variance, *t*-test, χ^2 analysis) were performed with SPSS software version 19. Differences with a p value <0.05 were considered statistically significant.

3. Results

3.1. Temperature induction and peptide stabilization assays

The RMAS/A*33:03 stable cell line presented the positive peptide P+ (FFVDGAANR, FI = 6.5) well compared to the negative peptide P− (FDVDGAANR, FI = 0.7), which only differ by one (the second) amino acid (Fig. 1A) [13]. Sixteen HBV type B and C candidate peptides were selected as described in the methods and screened in the same manner as P+ and P−. We found that five of them had strong affinity for HLA_A*33:03 (FI > 5). Representative flow cytometry results are shown in Fig. 1B, and the FI values >5 are shown in Table 1.

3.2. IFN-γ ELISPOT assays

The PBMCs from seven HLA-A*33 HBV patients were successfully obtained and measured. Two of the patients suffered from acute hepatitis, and five patients were in the CHB stage. The result shows that: (i) comparing AHB with CHB, the AHB patients had more HBV-specific CTL activity than CHB patients on peptide HBp391–399 ($p < 0.05$), though other peptides show higher CTL responses in AHB with no statistical difference ($p > 0.05$). (ii) Comparing the five candidate peptides in CHB patient samples with or without stimulation for 6 h, all five peptides generated higher specific CTL activity compare to the unstimulated state ($p < 0.05$). (iii) As for the difference between the peptides, combining the seven patients' data, HBp391–399 showed greater CTL activity ($x = 119.8$) than the other peptides.

3.3. Tetramer staining of virus-specific $CD8^+$ T cells

Based on the ELISPOT results, five positive peptides and one negative peptide HBp835–843 were used to generate tetramers, and five CHB patient samples were analyzed. Fig. 2A describes one typical PBMC sample that was stained by six HLA_A*33:03 tetramers (HBp835–843 is not a qualified tetramer; we apply it as a native control), and Fig. 2C shows the results from all five patients.

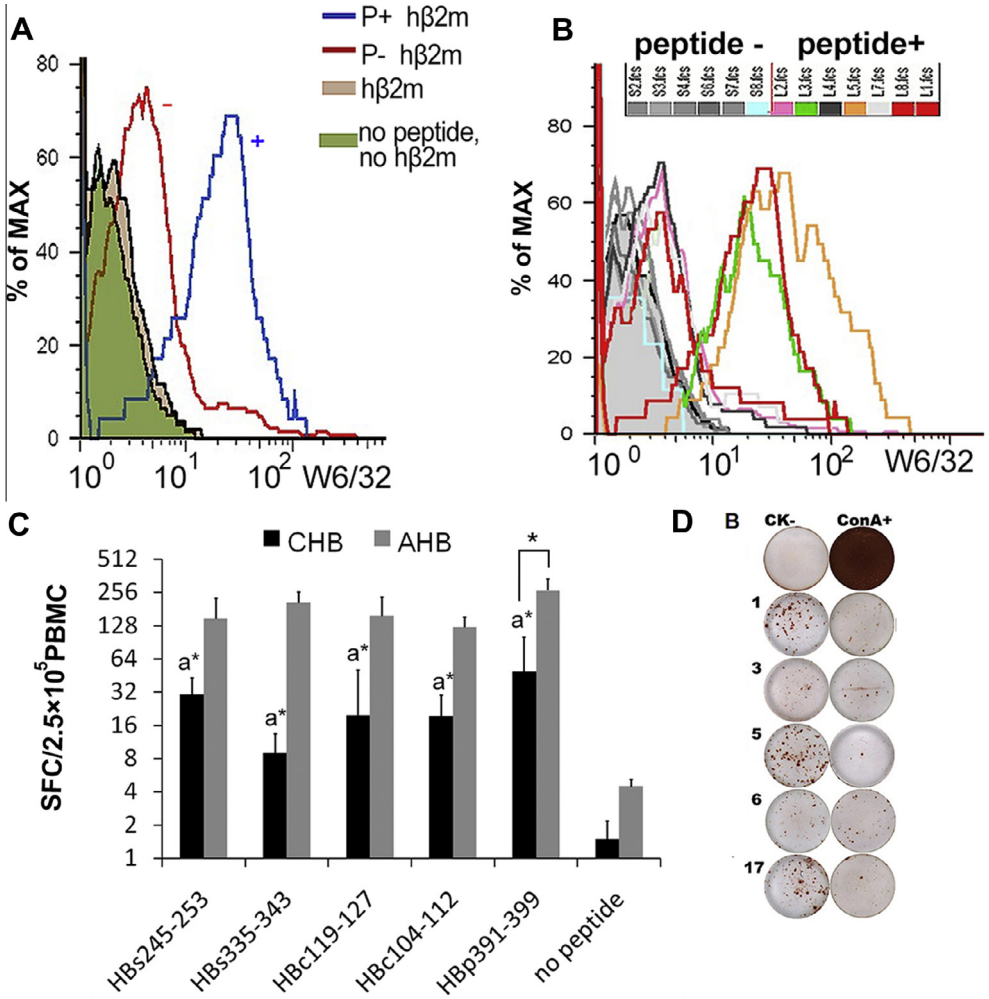

Fig. 1. Binding affinity of P+, P−, and candidate epitopes to HLA_A*33:03 and Assessment of the peptide-specific CD8+ T cell response of the five candidate epitopes in HBV patient PBMCs. (A) Flow cytometry results of peptide binding affinity for the positive epitope P+ (FFVDGAANR) and negative control P− (FDVDGAANR). (B) The binding affinity of the Hbs245–253, HBs335–343, HBc119–127, and HBp391–399 epitopes to RMAS/A*33:03 cell lines measured by flow cytometry. (C) ELISPOT results for all five peptides from freshly isolated PBMCs from HBV patients. Data were processed using SPSS software and multi-factor analysis of variance. * indicates that HBp391–399 displayed a difference between AHB to CHB ($p < 0.05$), and a* indicates the *t*-test results in CHB patients. (D) Representative sample of ELISPOT results in two CHB patients. CK- indicates no peptide stimulation. The numbers on the left correspond to the HBs245–253, HBs335–343, HBc119–127, HBc104–112, and HBp391–399 peptides. ConA+ is a positive control.

Table 1
Binding affinity of candidate epitopes.

No.	Name	Sequence	Protein of HBV	FI
1	Hbs245–253	GYRWMCLRR	S	6.7
3	HBs335–343	YLWEWASVR	S	6.0
5	HBc119–127	LVSFGVWIR	C	9.0
6	HBc104–112	HISCLTFGR	C	11.0
17	HBp391–399	VVDFSQFSR	P	6.8
	P+	FFVDGAANR		6.5
	P−	FDVDGAANR		0.7

To validate the tetramer staining results, we compare the peptides' *in vitro* refold efficiency ((heavy chain + β2m)/all refold protein) to the average tetramer staining results (Fig. 2B), and the correlation is 0.896 ($p = 0.039$), indicating that the tetramer staining results were statistically significant.

The tetramer stained CD8+ cell values were 0.71 ± 0.23, 0.51 ± 0.18, 2.02 ± 0.11, 1.61 ± 1.56, and 1.36 ± 0.87 for HBs245–253, HBs335–343, HBc119–127, HBc104–112, and HBp391–399, respectively. The CK- result was 0.02 ± 0.01. All five peptide staining values were greater than CK- ($p < 0.05$), indicating that these peptides can induce natural specific CD8+ cells responses in CHB patients.

Table 2
Summary of HBV patients in different HLA types and HBV progress.

HLA type	Liver cancer	Liver cirrhosis	CHB	Serious hepatitis
A02	25	56	33	5
A11	3	16	20	1
A24	10	30	13	1
A33	1	12	5	4*

* χ^2 test result with $p < 0.05$.

3.4. Cytotoxic T cells results

Next, to ensure that the tetramer-stained cells had CTL function, we use newly isolated PBMCs from three liver cirrhosis patients as effective cells, peptide-pulsed RMAS-A*33:03 stable cell line cells as target cells, and P+ peptide as a native control. Fig. 4A shows the scatter of mixed target and effective cells, and due to the clear boundary between them, it was easy to obtain repeatable results.

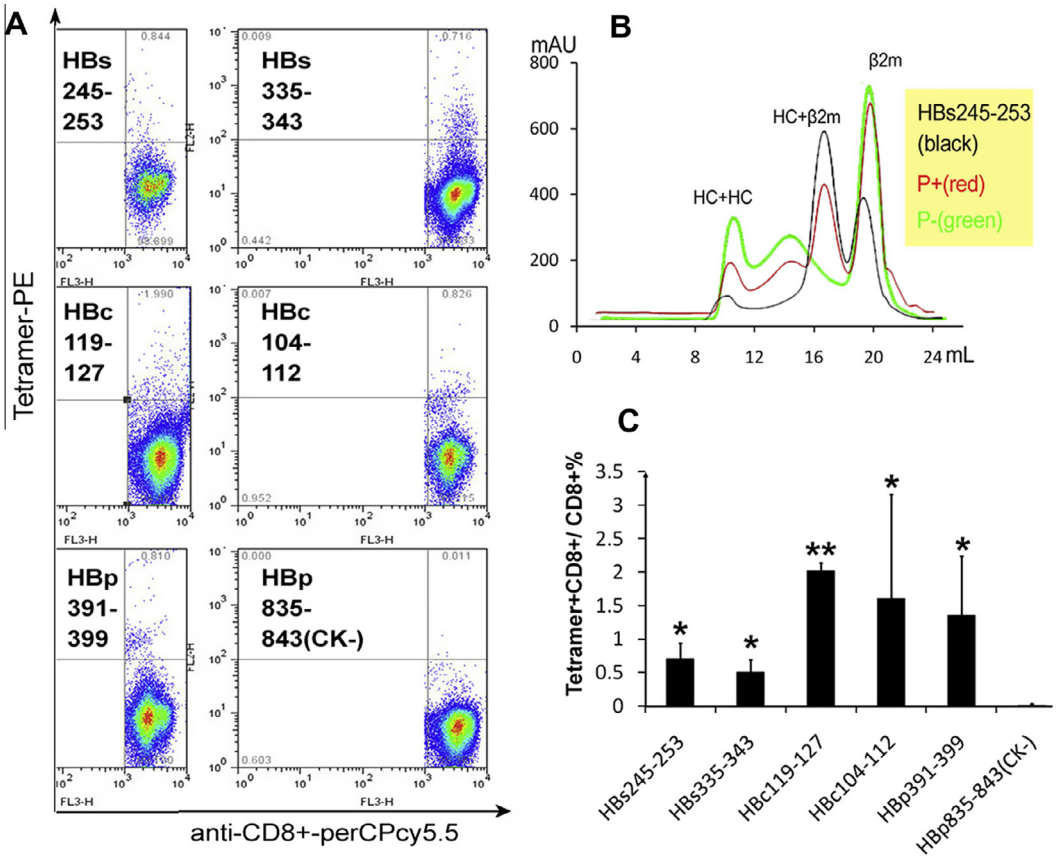

Fig. 2. Tetramer staining of peptide-specific CD8$^+$ T cell responses to the five candidate peptides. (A) Tetramer staining of different peptides to one HBV patient's PBMCs. (B) *In vitro* refolding of P+, P−, and HBs245–253 with HLA-A*3303 and hβ2m. (C) Comparison of the five CHB patients' tetramer staining results. *($p < 0.05$) **($p < 0.01$).

Fig. 3B shows that the average CTL results of the three patients to different peptides varied E:T, implying that: (i) All specific lysis due to peptides was greater than that due to the negative control, and (ii) as the number of effective cells increased, the specific lysis also increased. Notably, the lysis ability of HBc104–112 slowly increased when the ratio was >20:1.

3.5. Generation of HLA_A*33:03 transgenic mice

Eight transgenic mice founders were identified by PCR (A33-mice-FP: ATGGCAGCTCAGATCACCCAGCGCAAGT, A33-mice-RP: AGGCTCCCATCTCAGGGTTAAGGGCTCAG Ta = 72). After three generations, PCR using four pairs directed at different positions within the entire gene were performed, and the amplified DNA was sequenced. Two proper founders were identified by further RT-PCR and ELISPOT assays, but only one passed all tests (Fig 4D).

Fig. 4A and B shows the splenocyte staining results from FITC-B9.12.1. The curve is slightly shifted to the left compared to the unstained cells. Repeat experiments showed the same results in different mice. After Western blotting using an anti-StrepII antibody (recognizing the C-terminus of HLA-3303; Fig 4C), we found that HLA_A*33:03 is expressed in the liver, kidney, and spleen.

3.6. The five peptides generate specific CTL responses in HLA_A*33:03/k^d transgenic mice

Transgenic and wild type mice (5–7 weeks) were subcutaneously immunized with five doses of each peptide (100 μg/mouse for each immunization) on days 0, 7, 14, 21, and 28 in combination with complete Freund's adjuvant (first immunization) or incomplete Freund's adjuvant (the remaining immunizations). On day

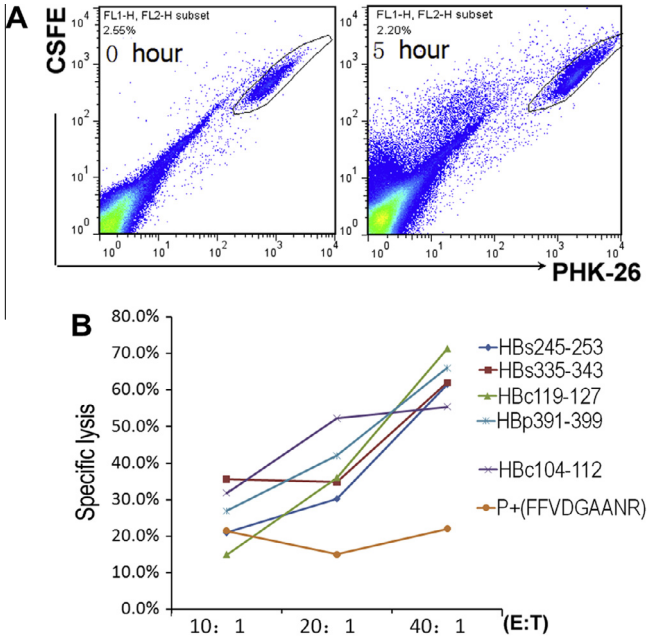

Fig. 3. Assessment of the killing effect of cytotoxic T cells in CHB patients by flow cytometry. (A) Scatter diagram showing how to distinguish target cells from effective cells. The PHK-26 and CFSE-double positive cells are located in the upper-right area, which is clearly distinct from the other cells. (B) Assessment of the five peptides' specific CTL, Target cells are RMAS/A*33:03.

35, splenocytes were isolated and cultured in RMPI-1640 supplemented with 10% FBS, 0.5 μmol/L hβ2m, and 0.5 μmol/L corresponding peptide used during the immunization, and the cells

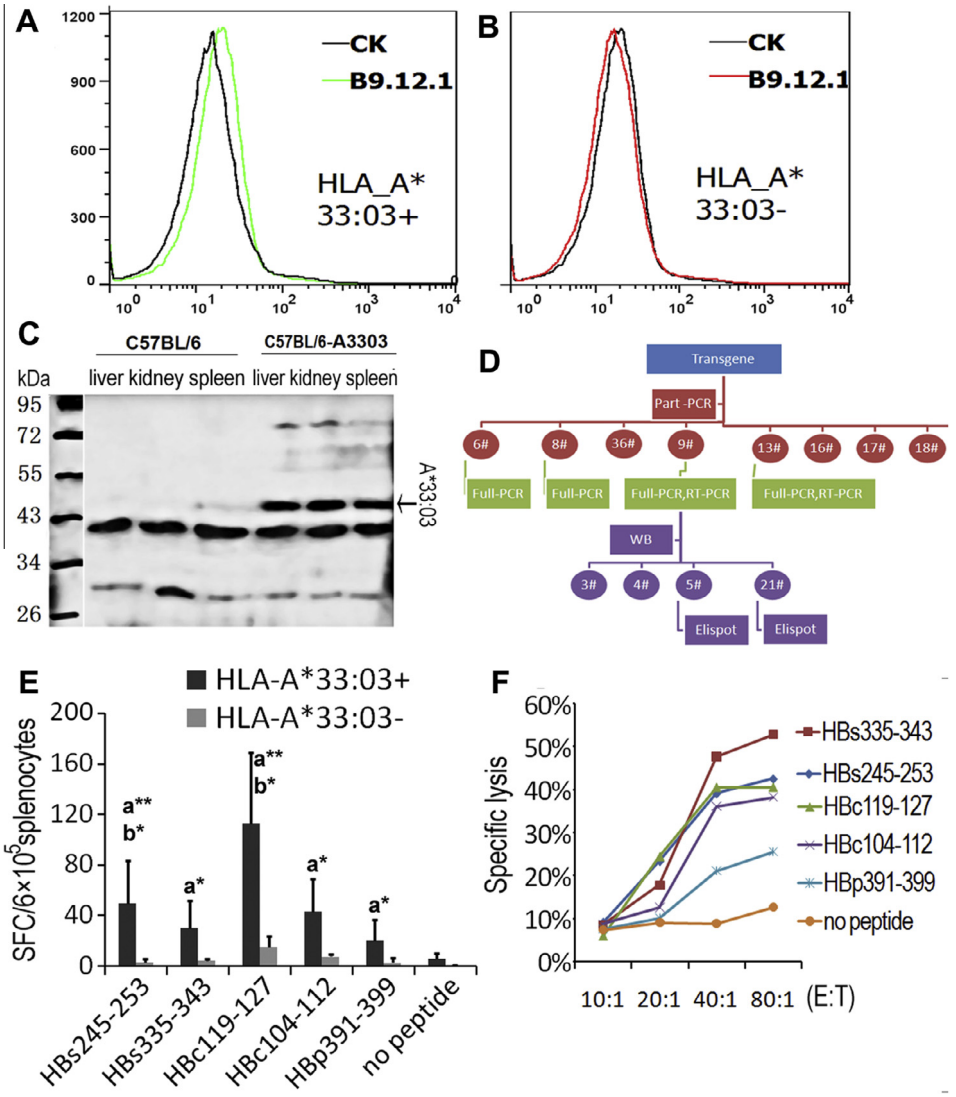

Fig. 4. Phenotypic characterization of HLA_A*33:03 transgenic mice and detection of the peptide-specific $CD8^+$ T cell responses to the five candidate peptides in HBV transgenic mice. (A and B) Flow cytometric analyses were performed on spleen lymphocytes from transgenic and wild type mice using a FITC-B9.12.1 mAb. The results are expressed in fluorescence intensity (*X*-axis, log scale) and relative cell number (*y*-axis.) (C) Western blot analysis of HLA_A*33:03 expression in the liver, kidney, and spleen. The primary antibody was a rabbit anti-StrepII mAb. (D) Tree diagram showing the positive selection process for mice founders. Each number represents a founder or sub-founder. (E) Peptide-specific $CD8^+$ T cells were measured by ELISOPT assays. (character 'a' indicates *t*-test between HLA_A*33:03+ and HLA_A*33:03- mice immunized with same peptide; character 'b' indicates difference between different peptide in HLA_A*33:03+ mice; '*' means $p < 0.05$; '**' means $p < 0.01$), (F) cytotoxic T cells were detected by flow cytometry.

were further culture at 26 °C for 1 week. A mouse ELISPOT kit was used to measure peptide-specific T cells. As for the CTL detection, RD/A*33:03 were the target cells. The negative control was no peptide pulsed RD/A*33:03 cells.

Fig. 4E shows the ELISPOT results; it is clear that all peptides elicited specific responses ($p < 0.05$, $n = 10$). Further, HBs245–253 and Hbc119–127 induced higher IFN-γ polarization than the other peptides. This phenomenon was not the same as in HBV patients. The CTL results displayed the same trend, except with peptide HBp391–399 (Fig 4F).

4. Discussion

In this study, we identified five new HLA_A*33:03-restricted $CD8^+$ T cell epitopes: HBs245–253, HBs335–343, HBc119–127, HBc104–112, and HBp391–399. All five elicited TH1 cell IFN-γ polarization, propagated HLA_A*33:03-specific clones, and killed HLA_A*33:03 target cells in both HBV patients and transgenic mice.

Based on the above data, we can draw the following conclusions (though there are some details that require further scrutiny). First, according to previous reports, HBV ELISPOT and tetramer staining-positive results are not easily to detect in CHB patients due to weak T cell responses [2]. However, in this experiment, PBMCs (from CHB patients) that were not pre-stimulated for a long time yielded detectable results. Comparing the HLA type to HBV disease pathogenesis data in the 322 patients that we enrolled, we found that serious hepatitis patients were enriched for the HLA-A33 genotype ($p < 0.05$) (Table 2). There are also two reports that HLA_A33 is more prevalent in patients with persistent infections than in those that recover (9.37 *vs.* 0%, $p < 0.008$ in Iran [14] and 18.9 *vs.* 12.8% $p \leqslant 0.004$ in Korean [15]). These reports, combined with our findings, imply that the HLA_A33 T cell response maybe more vigorous and sustained during HBV infection.

Second, no HLA_A33 transgenic mice have ever been formally reported. Are the mice that we generated as good the models

previously reported for HLA_A*02:01, HLA_A*11:01, and HLA_A*24:01 [16–19]? We found that the HLA_A*33:03 protein was expressed in the liver, kidney, and spleen of the mice and also elicited a specific T cell response. However, the expression was weaker than we expected. After we refolded the chimeric heavy chain and full human heavy chain with mouse β2m and different peptides, we found that there was huge refolding efficiency difference between the chimera and original. Indeed, the chimeric heavy chain easily to formed polymer, and the HC + β2m + peptide proportion was lower than that with the original human heavy chain (The average value of polymer/(HC + β2m + peptide) in chimeric heavy chain is 1.55 ± 0.59% and in original human heavy chain is 8.47 ± 1.86%, $p < 0.01$). We hypothesize that much of the HLA_A*33:03 protein did not fold correctly and was thus not transported to the cell surface in mice. Although the HLA_A*33:03 levels were relatively low, it was enough to form a T cell response as in another A2 transgenic mouse expressing HLA A2 [18].

Author contributions

Po Tien and Xuwen Pan designed the study and wrote the manuscript; Xuyu Zhou manipulated transgenic mice. Huiguo Ding communicated with HBV patients and signed the informed consent.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.05.001.

References

[1] Y. Zhang, H. Zhang, et al., Epidemiology of hepatitis B and associated liver diseases in china, Chin. Med. Sci. J. 27 (4) (2013) 243–248.
[2] C.P. Desmond, A. Bartholomeusz, et al., A systematic review of T-cell epitopes in hepatitis B virus: identification, genotypic variation and relevance to antiviral therapeutics, Antivir. Ther. 13 (2) (2008) 161–175.
[3] D. Middleton, L. Menchaca, H. Rood, R. Komerofsky, New allele frequency database: http://www.allelefrequencies.net, Tissue Antigens 61(5) (2003): 403–407.
[4] C.K. Shaw, L.L. Chen, A. Lee, T.D. Lee, Distribution of HLA gene and haplotype frequencies in Taiwan: a comparative study among Min-nan, Hakka, Aborigines and Mainland Chinese, Tissue Antigens 53 (1) (1999) 51–64.
[5] J.S. Dhaliwal, M. Shahnaz, C.L. Too, et al., HLA-A, -B and -DR allele and haplotype frequencies in Malays, Asian Pac. J. Allergy Immunol. 25 (1) (2007) 47–51.
[6] K.L. Rock, C. Gramm, et al., Low temperature and peptides favor the formation of class I heterodimers on RMA-S cells at the cell surface, Proc. Natl. Acad. Sci. U.S.A. 88 (10) (1991) 4200–4204.
[7] Chinese Society of Hepatology and Chinese Society of Infectious Diseases, Chinese Medical Association, The guideline of prevention and treatment for chronic hepatitis B, Chin. J. Hepatol. 3 (2005) 881–889.
[8] T.H. Lee, Y.L. Chuang, S.L. Tsai, Y.F. Liaw, Effects of lamivudine on the hepatitis B virus specific $CD8^+$ cytotoxic T lymphocyte response via peptide-MHC tetrameric complexes assay, Lett. Pept. Sci. 8 (2002) 179–185.
[9] T.R. Mosmann, S. Sad, The expanding universe of T-cell subsets: Th1, Th2 and more, Immunol. Today 17 (1996) 138–146.
[10] G. Hogan, F. Constantini, E. Lacy, Manipulating the Mouse Embyo: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Habor, NY, 1986.
[11] B. Malissen, N. Rebai, Structural and genetic analyses of HLA class I molecules using monoclonal xenoantibodies, Tissue Antigens 22 (1983) 107.
[12] M.E. Sheehy, A.B. McDermott, et al., A novel technique for the fluorometric assessment of T lymphocyte antigen specific lysis, J. Immunol. Methods 249 (1–2) (2001) 99–110.
[13] M. Takiguchi, T. Matsuda, et al., Analysis of three HLA-A*3303 binding peptide anchors using an HLA-A*3303 stabilization assay, Tissue Antigens 55 (4) (2000) 296–302.
[14] A. Ramezani, A. Aghakhani, et al., HLA-A *3303* and *3301 predispose patients to persistent hepatitis B infection, J. Gastrointestin. Liver Dis. 18 (1) (2009) 117–118.
[15] S.H. Hwang, Y.H. Sohn, et al., Human leukocyte antigen alleles and haplotypes associated with chronicity of hepatitis B virus infection in Koreans, Arch. Pathol. Lab. Med. 131 (1) (2007) 117–121.
[16] A.X. Le, E.J. Bernhard, et al., Cytotoxic T cell responses in HLA-A2.1 transgenic mice. Recognition of HLA alloantigens and utilization of HLA-A2.1 as a restriction element, J. Immunol. 142 (4) (1989) 1366–1371.
[17] J. Alexander, C. Oseroff, et al., Derivation of HLA-A11/Kb transgenic mice: functional CTL repertoire and recognition of human A11-restricted CTL epitopes, J. Immunol. 159 (10) (1997) 4753–4761.
[18] L. BenMohamed, R. Krishnan, et al., Induction of CTL response by a minimal epitope vaccine in HLA A*0201/DR1 transgenic mice: dependence on HLA class II restricted T(H) response, Hum. Immunol. 61 (8) (2000) 764–779.
[19] R. Boucherma, H. Kridane-Miledi, et al., HLA-A*01:03, HLA-A*24:02, HLA-B*08:01, HLA-B*27:05, HLA-B*35:01, HLA-B*44:02, and HLA-C*07:01 monochain transgenic/H-2 class I null mice: novel versatile preclinical models of human T cell responses, J. Immunol. 191 (2) (2013) 583–593.