## **T Cell Epitopes from the Proteome of HBV**

Subjects: Infectious Diseases Contributor: yandan wu

T cell epitopes functionally validated from HBV antigens during the past 33 years; the human leukocyte antigen (HLA) supertypes to present these epitopes, and the methods to screen and identify T cell epitopes.

hepatitis B virus T cell epitope HLA restriction

## 1. Introduction

Hepatitis B virus (HBV) infection still disseminates across the world and causes the most common and fatal liver diseases including acute liver failure, chronic hepatitis, liver cirrhosis (LC), and hepatocellular carcinoma (HCC) <sup>[1]</sup> <sup>[2]</sup>. Nucleoside analogs and/or interferon are widely utilized antiviral drugs, which can effectively suppress virus replication, decrease serum HBV DNA to undetectable levels, mitigate liver fibrosis, and reduce HCC risk <sup>[3][4][5]</sup>, however cannot eliminate the virus in patients. Recurrence after therapy discontinuation is emerging to be a common etiology of morbidity and mortality in patients with chronic HBV infection<sup>[6]</sup>.

Numerous researches have demonstrated the important influence of HBV-specific T cell responses on virus clearance<sup>[Z]</sup>, disease progression<sup>[B][9][10]</sup>, antiviral efficacy<sup>[11][12]</sup>, and recurrence<sup>[13][14][15]</sup>, particularly the CD8 T cells, which act as vital effector cells to kill virus-infected hepatocytes and secret cytokines. Patients with acute-resolving HBV infection show robust HBV-specific CD8 T cell responses, while the patients with chronic HBV infection present a phenomenon termed CD8 T cell functional exhaustion with multifactorial heterogeneity<sup>[9]</sup>, and differs depending on the targeted antigen for HLA-A02 restricted epitopes located in the core antigen versus polymerase<sup>[16]</sup>. Furthermore, the heterogeneity of HBV-specific T cells also responds differently to therapeutic stimuli. Therefore, T cells specific for HBV not only are the potential markers for monitoring the effects of antiviral therapy and predicting the recurrence <sup>[17]</sup>, but also are the promising modulators in specific T cell immunotherapy. Identifying the T cell epitopes as many as possible from HBV antigens will greatly contribute to the design and development of epitope-based and T cell-based therapies and the detection of host HBV-specific T cell immunity. Although a systematic review of T cell epitopes in HBV antigens was reported in 2008<sup>[18]</sup>, an updated map of the T cell epitopes is urgently needed.<sup>+++</sup>

## 2. HBV Proteome and the Approaches Identifying T Cell Epitopes

HBV is one of the smallest viruses with a genome length of 3.2 Kb <sup>[19]</sup>. Its genome contains four open reading frames (ORFs) coding four partially overlapping proteins as displayed in **Figure 1**: (1) preS/S ORF encodes large

(L), middle (M), and small (S) surface antigens (HBsAg). HBsAg is being widely investigated in clinical fields and quantified as a diagnostic marker of HBV infection as it can reflect the level of covalently closed circular DNA (cccDNA) and intrahepatic HBV DNA in chronic infection<sup>[20][21]</sup>. (2) Pre-core/core ORF encodes hepatitis B e antigen (HBeAg), core antigen (HBcAg) or in combination core-related antigen (HBcrAg). HBeAg has long been advocated as a serum marker for guiding the clinical practice of chronic hepatitis B virus <sup>[22][23]</sup>. HBcrAg has been demonstrated more recently as a potential surrogate marker of cccDNA<sup>[24]</sup>. (3) X ORF encodes HBx antigen (HBxAg), which plays an important role in virus genome transcription and is correlated with liver cancer. The expression of HBxAg in HBV-associated HCC patients is significantly higher than other viral proteins <sup>[25]</sup>. (4) P ORF encodes the viral DNA polymerase (HBpol), which is responsible for the replication of the viral genome and is an effective target for the therapeutic intervention of chronic HBV infection <sup>[26]</sup>. Human HBV strains occur in nine genotypes A-I, and its major HBV surface antigen (HBsAg) has several immune protective conformational B cell epitopes a, d or y, w1–4 or r <sup>[27]</sup>. The entire amino acid sequences of each protein from different genotypes were obtained from the UniProt database and aligned in **Figure 2**.



Figure 1. The circular (A) and linear (B) diagram of HBV genome.

HBsAg-C (P31868) HBsAg-A (P31873) HBsAg-B (Q67926) HBsAg-D (P03139)	10    MGGWSSKPRQGMGT AK 	20    NLSVPNPLGFF	30 PDHQLDPAF	40    GANSNNPDWDI KE1 RT	50 FNPNKDHWPEA	60    ANQVGVGTFGE A KAI	70    PGFTPPHGGLL	80   .GWSPQ S.
HBsAg-C (P31868) HBsAg-A (P31873) HBsAg-B (Q67926) HBsAg-D (P03139)	90    AQGILTTVPAAPPE T.HV L MQ.LN	100    ASTNRQSGRQP T	110   TPISPPLRD: 	120    SHPQAMQWNS T TH	130    TTFHQALLDPI .A.QQ L.T.Q.S T.Q	140     RVRGLYFPAGC F AL	150    ;SSSGTVNPVP A. S.AQ	160 •TTASP NIH •N.V.A T
HBsAg-C (P31868) HBsAg-A (P31873) HBsAg-B (Q67926) HBsAg-D (P03139)	170    ISSIFSRTGDPAPN S.KV I.L.	180    MENTTSGFLGP IH IAL.H	190   PLLVLQAGFF: 	200    LLTRILTIPQ:  SK	210	220    FLGGAPTCPGQ S.V.L T.A	230    NSQSPTSNHS QI.S	240   PTSCP 
HBsAg-C (P31868) HBsAg-A (P31873) HBsAg-B (Q67926) HBsAg-D (P03139)	250   PICPGYRWMCLRRF 	260    IIFLFILLLCL	270 	280    QGMLPVCPLLJ I T	290    PGTSTTSTGPO ST STS	300     CKTCTTPAQGT 	310 SMFPSCCCTK	320   IPSDGN .T .T
HBsAg-C (P31868) HBsAg-A (P31873) HBsAg-B (Q67926) HBsAg-D (P03139)	330 CTCIPIPSSWAFAR 	340    FLWEWASVRFS Y YGA	350   WLSLLVPFV(	360    QWFAGLSPTVI V VI.	370	380    WGPSLYNILSE R. S	390    PFLPLLPIFFC .I	400   :LWVYI  
HBeAg-C (P0C6H5) HBeAg-A (Q91C37) HBeAg-B (P0C6G7) HBeAg-D (P0C573)	10    MQLFHLCLIISCSC T.	20    PTVQASKLCLG	30   WLWGMDIDP? 	40     YKEFGASVELI T	50 SFLPSDFFPS	60    SIRDLLDTASA V	70    LYREALESPE	80 HCSPH
HBeAg-C (P0C6H5) HBeAg-A (Q91C37) HBeAg-B (P0C6G7) HBeAg-D (P0C573)	90    HTALRQAILCWGEL ET	100    MNLATWVGSNL .TN .TV	110 EDPASRELVV	120     VSYVNVNMGLF .NT T	130    KIRQLLWFHIS	140    SCLTFGRETVI	150   EYLVSFGVWI	160   RTPPA 
HBeAg-C (P0C6H5) HBeAg-A (Q91C37) HBeAg-B (P0C6G7) HBeAg-D (P0C573)	170 ••••• <b>YRPPNAPILSTLPE</b> ••••••	180    TTVVRRRGRSP D.GR	190 RRRTPSPRRI SP.RRT.SP	200     RRSQSPRRRS RR.QSPF	210 SQSRESQC RR.QSRESQC			
HBx-C(P0C686) HBx-A(Q91C38) HBx-B(Q67923) HBx-D(Q9QMI3)	10 MAARVCCQLDPARDVL LY.SS	20    CLRPVGAESRG	30 RPVSGPFGP LAL.A LPL.T L.S	40 LPSPSSSAVP .SP .PA.PP .S.S.P	50 ADHGARLSLR SH FH FH	60 	70 	80 •••  •RME •C•••
HBx-C(P0C686) HBx-A(Q91C38) HBx-B(Q67923) HBx-D(Q9QMI3)	90 TTVNAHQVLPKVLHKR GN	100   . TLGLSAMSTTD P T	110 LEAYFKDCLI	120 FKDWEELGEE	130 IRLMVFVLGG K K	140 CRHKLVCSPAI	150 	

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HBpol - C(POC688)	FPNTHLOED	TINRCOOVV	GPLTVNEKR	RLKLTMPARE	PNT.TKYT.PLI	KGTKPYYPE	AVNHYFKTR	HYTHTTWKAC	TT.Y
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HBpol-B(Q67925)	s	.VDK.F.	N.		V		/I.DQ		
HBpol-D(Q9QMI1)	HQ.	K.E.F.		Q	F		.LQ		
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HBpol-A(Q02314)			H	IKQ	PP	S	F	нртя	SQP.
HBpol-B(Q67925)	s		.D	кк	PPI	2sQ	PKP	AQGF	RQQ.
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HBpol-C(P0C688)	RSGSIWARV	HSTTRRSFG	VEPSGSGHII	DNSASSASSCI	LYQSAVRKTAY	SHLSTSKRQ	SSSGHAVELH	NIPPSCARS	SEG
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HBpol-B(Q67925)	.VP	E	s	I	RT				
HBpol-D(Q9QMI1)	.VFP	к	s		н		A.		
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HBpol-A(Q02314) HBpol-B(Q67925) HBpol-D(Q9QMI1)	R N.R NYR				. L		N. 	.IN.NI .IN.N .IFDH	.N. .N.
HBpol-A(Q02314) HBpol-B(Q67925) HBpol-D(Q9QMI1)	R N.R NYR				.L	Is	N. H. N.	.IN.NI .IN.N .IFDH	.N. .N.
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HBpol-A(Q02314) HBpol-B(Q67925) HBpol-D(Q9QMI1)	R N.R NYR 4	90	500	510	.L		N. 	. IN.NI . IN.N . IFDH 550	560
HBpol-A(Q02314) HBpol-B(Q67925) HBpol-D(Q9QMI1) HBpol-C(P0C688)	R N.R NYR 4   HDSCSRNLY	90 VSLLLLYKT	500 -	510 511 HPIILGFRKI	520 .L	530 	N. 	.IN.NI .IN.N .IFDH 550 	560 
HBpol-A(Q02314) HBpol-B(Q67925) HBpol-D(Q9QMI1) HBpol-C(P0C688) HBpol-A(002314)	R N.R NYR HDSCSRNLY	90    VSLLLLYKT M	500 	510 	520 520 PMGVGLSPFLI		540 VVRRAFPHCL	. IN.NI . IN.N . IFDH 550 	.N. .N. .560 
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**Figure 2.** Homologous analysis of HBsAg, HBeAg, HBx and HBpol proteins from HBV C, A, B, and D genotypes. The entire amino acid sequences of each protein from different HBV genotypes were obtained from the UniProt database, aligned and used for in silico prediction of HBV antigen T cell epitopes presented by HLA-A allotypes.

The process of T cell epitope identification begins with the selection of candidate epitope peptides. The first strategy is using overlapping peptides (OLPs) spanning the entire proteome or selected antigens of interest (peptide scanning). Chen et al. expanded HBV-specific T cells in vitro by co-culturing the overlapping peptide pools spanning the entire sequence of HBV genotypes B and C and the peripheral blood mononuclear cells (PBMCs) from patients with chronic HBV infection, followed by the detection of T cell response in each co-culture using IFNy enzyme-linked immunospot (IFN-y ELISpot) assay, IFN-y intracellular staining and flow cytometry [28]. However, peptide scanning is a high-cost and laborious method due to a large amount of OLPs spanning overall HBV proteins. For CD8 T cell epitopes, HBsAg, HBeAg, HBx and HBpol contain 131, 68, 49, and 279 OLPs, respectively, when overlapping 6 amino acids in each 9-mer peptide. An alternative strategy is to focus on the in silico predicted T cell epitopes binding to the indicated HLA supertypes as calculated by multiple epitope prediction tools and algorithms. Brinck-Jensen et al. predicted 20 HBV-specific epitopes using combined in silico methods and evaluated for the immunogenicity of these epitopes through exposure to patients' PBMCs by IFN-y ELISpot <sup>[29]</sup>. More recently, a similar in silico approach was also employed to assess all previously verified HBx- and HBpolderived epitopes and to predict novel HLA-binding peptides for 6 HLA supertypes. Then, a part of reported epitopes were chosen for experimental validation. A total of 13 HLA binders derived from HBx and 33 binders from HBpol were described across HLA subtypes by this strategy<sup>[30]</sup>. Predicted epitopes are based on the indicated HLA restrictions and limit the number of research objects with diverse HLA subtypes to a reasonable range, yet the inaccuracy of theoretical prediction may omit some real-world epitopes.<sup>+</sup>

The methodologies to validate the immunogenicity of candidate epitope peptides have been improved remarkably over the last two decades. Different assays are utilized for the detection of peptide-induced T cell response or peptide-specific T cells with individual advantages and disadvantages in terms of practicability, cost, sensitivity, function evaluation. The following approaches are currently widely used, such as cytotoxicity assay, proliferation assay, intracellular cytokine staining (ICS), ELISpot/FluoroSpot, and peptide-MHC multimers staining (tetramers, pentamers, or dextramers). The cytotoxicity assay was initially performed to validate CD8 T cell epitopes by co-culturing patients' PBMCs with target cells labeled with Chromium-51, after the PBMCs were stimulated by the indicated candidate epitope peptides<sup>[31]</sup>. Additionally, lymphocyte proliferation assay is mostly applied to CD4 T cell epitopes validation. The PBMCs from HBV-infected or HBV-vaccinated individuals were co-cultured with HBV-derived peptides for several days and <sup>++3</sup>H-thymidine pulses were administered eventually followed by quantifying the incorporated radioactivity<sup>[32]</sup>. One more common approach currently utilized is ICS or ELISpot/FluoroSpot. Patient's PBMCs are in vitro or ex vivo stimulated with the candidate epitope peptides and simultaneously cytokine release is blocked followed by ICS and flow cytometry to define whether CD4 T cells or CD8 T cells activation<sup>[33]</sup>. The ELISpot or FluoroSpot technology enables the detection of single activated cells among one million PBMCs. The accuracy, sensitivity, reproducibility and durability have led to its widespread applications in researches and

the broad prospects in the clinical detection of antigen-specific T cells<sup>[34][35]</sup>. An issue encountered with ELISpot, FluoroSpot, ICS, and related assays is that they may ignore T cells that produce different cytokines or trace cytokines during the window of time of the assay (e.g., Follicular helper CD4 T cells generally produce very low amounts of cytokines). Peptide-MHC tetramer staining has been the gold standard to quantify antigen-specific T cells with high sensitivity and precision, thus is often used to identify T cell epitopes in many researches. However, the preparation of peptide-MHC tetramers or multimers is high-cost, complicated, and time-consumption<sup>[36][37]</sup>. A pioneering study focused on all possible peptides of the entire HBV genome and 484 unique HLA-A1101-restricted epitopes predicted by NetMHC algorithms were validated using mass cytometry and multiplex peptide-tetramers staining<sup>[38]</sup>. Many researchers also have established a transgenic mouse model to map HLA-restricted epitopes. Ru et al. developed and immunized HLA-A2/DP4 mice with epitopes derived from HBsAg to identify four new HLA-DP4-restricted epitopes<sup>[39]</sup>. Besides cellular functional experiments, peptide-HLA molecule binding and stabilization assays were commonly used to identify epitopes. Pan et al. defined 16 HBV epitopes by analyzing the different binding affinities of candidate epitope peptides with HLA-A3303 using RMA-S cells binding and stability assay. More recently, Ferretti et al. used a high-throughput genome-wide screening technology to identify the target cells expressing candidate epitopes productively recognized by T cells (T-Scan) and determined 29 epitopes in SARS-CoV-2 for the six most prevalent HLA types<sup>[40]</sup>. Chikata et al. employed immunocapture and liquid chromatography mass spectrometry (LC-MS) subsequent to pre-treatment of the target protein to disrupt its three-dimensional structure to characterize HIV-1 epitope peptides on a large scale presented by HLA-C1202<sup>[41]</sup>. A variety of epitope assay strategies have been utilized with their own features and potential. +++

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