662

Soluble Plasma HLA Peptidome as a Potential Source for CHB Biomarkers

Diana Chen,[1] Ruidong Li,[1] Eustache Paramithiotis,[2] Gwen Pottiez,[2] Ross Martin,[1] Xinan Liu,[1] Jenny Svarovskaia,[1] Hongmei Mo,[1] Li Li,[1] Anuj Gaggar,[1] Jeffrey Wallin[1]

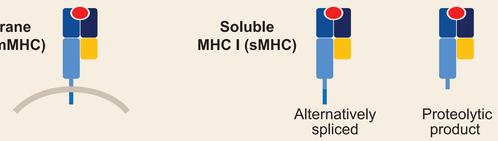
[1]Gilead Sciences, Inc., Foster City, CA; [2]Caprion Biosciences, Montréal, Québec, Canada

Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404
800-445-3235

Introduction

- Viral peptide epitopes are presented by major histocompatibility complex (MHC) I on the surface of hepatitis B virus (HBV)–infected hepatocytes for recognition by HBV antigen-specific cluster of differentiation 8 (CD8) T cells and this mechanism is critical to the ultimate control of chronic HBV (CHB)[1]
- MHC has both membrane and soluble forms; liver is the largest contributor to soluble MHC, which is increased in cancer patients and those with active infections[2]
- An understanding of MHC I–presented peptides will be important for the design and evaluation of immunotherapies designed to treat CHB

Major Histocompatibility Complex I



Objective

- To determine whether peptides eluted from sMHC could serve as biomarkers for monitoring liver immunopathogenesis and HBV epitope presentation in CHB infection

Methods

- 9 CHB (4 with matching liver tissues) and 4 healthy volunteer (HV) plasma samples were used
- Peptide-MHC I molecules from liver tissue homogenates and plasma were immunoaffinity-purified with human leukocyte antigen (HLA)–ABC monoclonal antibody W6/32–bound columns, followed by peptide elution and capillary liquid chromatography–tandem mass spectrometry analysis
- Peptides were identified using SWISS-PROT, UniProt, and National Center for Biotechnology Information databases, as well as Gilead's HBV sequence database of patients with CHB
- Tissue sources of the identified peptides were determined using Human Protein Atlas and Human Proteome Project[3] databases
- Prediction of MHC I motifs was determined using the GibbsCluster server and predicted motifs were compared with published motifs in the Immune Epitope Database (IEDB)
- Peptide-MHC I–binding prediction was determined using NetMHC 4.0 software

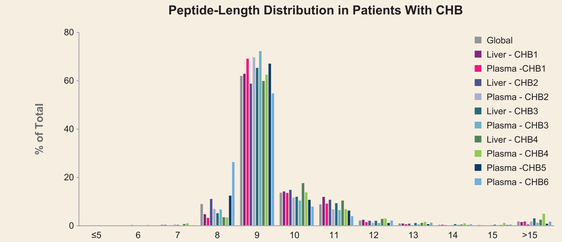
Samples Analyzed

Sample ID	Sample Type	Age, y	Sex	Sample Volume/Weight	HLA-I Alleles	Viral Load, IU/mL	HBsAg, ng/mL	HBeAg, ng/mL
CHB1	Liver	47	M	1.21 g	HLA-A*30:01/11:01, HLA-B*07:02/38:02, HLA-C*07:02/07:02	N/A	208.675	0.217
	Plasma			6.0 mL				
CHB2	Liver	52	M	0.94 g	N/A	N/A	2.336	5.139
	Plasma			7.6 mL				
CHB3	Liver	N/A	F	0.24 g	HLA-A*33:03/ 02:06, HLA-B*13:01/15:25, HLA-C*03:02/07:02	N/A	127.438	0.891
	Plasma			4.5 mL				
CHB4	Liver	N/A	M	0.44 g	HLA-A*11:01/ 02:03, HLA-B*27:06/40:01, HLA-C*03:04/07:02	N/A	29.509	0.135
	Plasma			4.5 mL				
CHB5	Plasma	64	M	8.5 mL	N/A	800,000	37.560	0.038
CHB6	Plasma	60	M	8.5 mL	N/A	1140	195.165	0.239
CHB7	Plasma	32	M	>5 mL	N/A	1170	N/A	N/A
CHB8	Plasma	63	M	>5 mL	N/A	230	N/A	N/A
CHB9	Plasma	37	M	>5 mL	N/A	684	N/A	N/A

F, female; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; M, male; N/A, not available.

Results

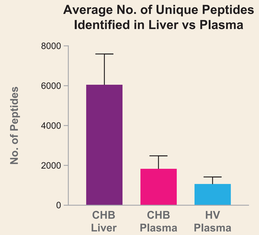
Majority of Eluted Peptides From sMHC and mMHC Were 8–11 Amino Acids in Length



	≤5	6	7	8	9	10	11	12	13	14	15	>15
Liver - CHB1	0	0.1	0.4	4.8	62.9	14.2	12.0	2.5	0.9	0.4	0.2	1.6
Plasma - CHB1	0	0.1	0.2	3.3	69.2	13.6	9.2	1.5	0.7	0.3	0.2	1.8

- Peptide-length distributions were similar between paired CHB liver and plasma samples
- Similar peptide-length distribution was also observed in HV samples
- Peptide lengths and frequencies matched known properties of MHC I–presented peptides

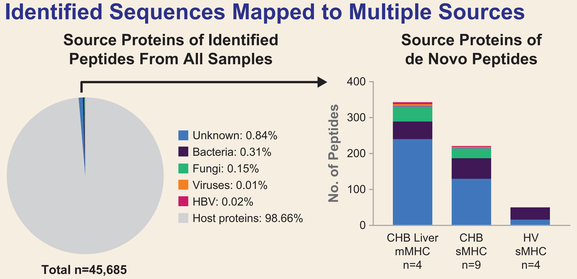
sMHC Presentome Correlated Well With mMHC Presentome in Liver



Patient ID	Tissues	No. of Peptides Identified	No. of Common Peptides	% Common
CHB1	Liver	6507	1964	30
	Plasma	2757		71
CHB2	Liver	7957	807	10
	Plasma	1063		76
CHB3	Liver	4341	1263	29
	Plasma	1704		74
CHB4	Liver	5387	1047	19
	Plasma	1904		55

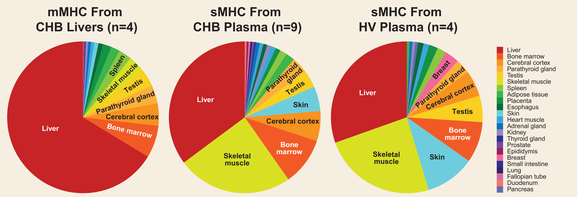
- ~55–76% of plasma sMHC peptides mapped to liver mMHC peptides

Identified Sequences Mapped to Multiple Sources



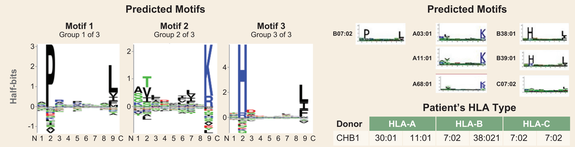
- Most blasted peptides mapped to human proteins (>98%)
- <2% of peptides did not match to any human sequence in the interrogated databases
- De novo peptide sequences were blasted against nonhuman databases
- Proteins for ~50% of de novo peptide sequences have not been identified

Tissue Source of Presentome After Removal of Housekeeping Proteins



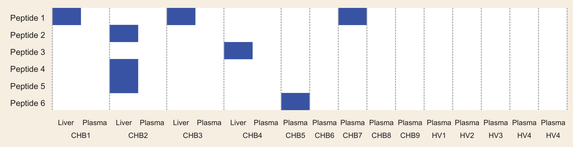
- ~68% of host peptides were derived from housekeeping proteins, which could be expressed by any tissues
- The largest tissue source of host peptides was liver after removal of housekeeping proteins

Deciphering MHC Motifs Across Peptidomes



- 1334 9-amino-acid–long peptides shared between liver and plasma of sample CHB1 were analyzed for their motifs
- 3 predicted HLA-I motifs were compared with published motifs in IEDB
- Predicted HLA alleles matched to patient's HLA-I allotypes
- A similar strategy was applied to samples that did not have HLA information

Summary of Identified HBV Peptides



- Peptides mapped to different viral proteins
- More HBV peptides were identified in liver vs plasma
- Very few peptides were identified in peripheral blood

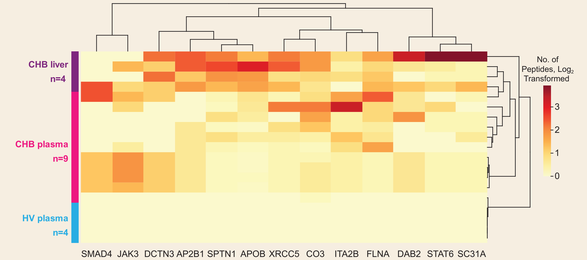
Frequency of Identified HBV Peptides Across Different HBV Genotypes and Predicted MHC I Restriction of the Peptides

HBV Protein		Genotype, n A (862)	B (1642)	C (2555)	D (1276)	E (256)	F (223)	G (35)	H (28)	In Silico Prediction of HLA Restriction
Pol	Peptide 1	0	0	0	5	0	0	0	0	A*68:23; B*35:03/42:01/83:01
S	Peptide 2	1	14	0	0	0	0	0	0	B*18:01
Pol	Peptide 3	4	1155	804	0	0	2	0	0	A*02:03/11:01; B*27:06/40:1; C*07:02/03:04
S	Peptide 4	0	1263	0	0	0	0	0	0	B*07:02
S	Peptide 5	0	102	1	0	0	0	0	0	A*24:02/26:01; B*15:01/15:02/35:01/58:01
S	Peptide 6	0	0	0	1	0	0	0	0	A*02:12/02:11; B*15:17/40:13/58:01

Pol, polymerase; S, HBsAg.

- 6 peptides were identified with exact match to HBV
- Matched peptides came from different HBV proteins
- Peptide 3 was present in 29% of all HBV sequences and peptide 4 in 77% of genotype B sequences in Gilead's HBV sequence database

sMHC Identified Increased Presentation of Host Immune Proteins in CHB Infection



AP2B1, adaptor-related protein complex 2 subunit-β1; APOB, apolipoprotein B; CO3, cytochrome C oxidase III; DAB2, DAB adaptor protein 2; DCTN3, dynactin subunit 3; FLNA, filamin A; ITA2B, integrin-α2b; JAK3, Janus kinase 3; SPTAN1, spectrin-α, nonerythrocytic 1; STAT6, signal transducer and activator of transcription 6; XRCC5, X-ray repair cross-complementing 5.

- Increased presentation of proteins involved in type I interferon, transforming growth factor-β signaling, and MHC II antigen presentation, as well as in cytoskeleton and metabolism in patients with CHB

Conclusions

- Peptides presented in plasma sMHC and liver mMHC can be sequenced using an immunoprecipitation/mass spectrometry approach
- A large proportion of peptides presented in liver mMHC were detected in plasma sMHC
- HBV peptides identified in CHB samples were predicted to bind to a number of different MHC-I alleles; these peptide-MHC I complexes have not been reported previously
- Increased presentation of host immune proteins was observed in patients with CHB, suggesting an ongoing immune response in liver
- **Next step:** evaluate presentome in longitudinal clinical samples to investigate the utility of the approach in biomarkers/diagnostic discovery

References: **1.** Rehermann B. Nat Med 2013;19:859-68; **2.** Bassani-Sternberg M, et al. Proc Natl Acad Sci U S A 2010;107:18769-76; **3.** Kim MS, et al. Nature 2014;509:575-81.

Acknowledgments: We extend our thanks to the patients, subjects, and all participating investigators. This research was funded by Gilead Sciences, Inc. **Disclosures:** **D. Chen, R. Martin, X. Liu, R. Li, J. Svarovskaia, H. Mo, A. Gaggar,** and **J. Wallin:** Gilead; **E. Paramithiotis** and **G. Pottiez:** Caprion.