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Hepatitis B virus and Homo sapiens proteome-wide analysis: A profusion of viral peptide overlaps in neuron-specific human proteins

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Abstract: The primary amino acid sequence of the hepatitis B virus (HBV) proteome was searched for identity spots in the human proteome by using the Protein Information Resource database. We find that the HBV polyprotein shares sixty-five heptapeptides, one octapeptide, and one nonapeptide with the human proteins. The viral matches are disseminated among fundamental human proteins such as adhesion molecules, leukocyte differentiation antigens, enzymes, proteins associated with spermatogenesis, and transcription factors. As a datum of special interest, a number of peptide motifs are shared between the virus- and brain-specific antigens involved in neuronal protection. This study may help to evaluate the potential cross reactions and side effects of HBV antigen-based vaccines.

Keywords: HBV proteome, human proteome, similarity analysis, viral versus human proteome overlapping, vaccine-related cross-reactions

Introduction

Vaccination for infectious diseases may be associated with potential adverse events and possible long-term health disorders (see http://www.cdc.gov/vaccinesafety). Indeed, antigen-specific immunotherapy protocols may target not only the antigen from the infectious microorganism, but also host tissues expressing antigens that share sequences with the target.[1] In general, a vaccine produces a weak immune response; also auto-immune cross-reactions are extremely rare events.[2–5] Under normal non-stimulated conditions, immune system fails to make immune responses to protein vaccines, unless adjuvants are added.[6,7] Consequently, the active vaccine preparations currently in use contain adjuvants for obvious reasons of desired immunogenicity,[8,9] so intrinsically carrying a certain degree of inducing/enhancing a potential cross-reactivity risk.

In order to define quantitatively and qualitatively the molecular basis of active vaccine (auto)immunity, we are undertaking proteomic sequence-to-sequence profile analyses between microbial versus human proteins.[10] Here, the HBV polyprotein was examined for amino acid sequence similarity to the human proteome at the heptamer level. We describe a high level of sharing of heptapeptide motifs between HBV and human proteins, with numerous neuronal proteins involved in the viral versus human peptide overlapping.

Methods

The HBV polyprotein primary sequence (Taxonomic ID: 10407; EMBL Accession: X51970) was dissected into heptamers that were analyzed for exact sequence similarity to the human proteome using PIR perfect match program

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(http://pir.georgetown.edu/pirwww/search/peptide.shtml). The heptamers were offset by one residue, ie, overlapping by six residues: ie, MQLFHLC, QLFHLCL, LFHLCLI, FHLCLII, etc. The human proteome consisted of 36,103 proteins at the time of analysis. The function of the human proteins and potential disease associations were analyzed using the Universal Protein Resource (UniProt; see http://www.uniprot.org/uniprot). Repeated sequences, fragments, and uncharacterized entries were filtered out.

Results

HBV proteins were analysed for amino acid sequence identity to the human proteome using heptamers as scanning units. The theoretical probability of a sequence of 7 amino acids occurring at random in two proteins may be calculated as 20^{-7} or 1 in 1 280,000,000,[1] assuming that all amino acids occur with the same frequency. Moreover, to determine the number of times a given viral heptamer might occur at random in the human proteome, one must consider the size of the viral and human proteomes. The analyzed human proteome was formed by 36,103 proteins and 10,431,975 unique 7-mers, and the HBV polyprotein was formed by 1,586 unique 7-mers[10] Therefore, the number of times we would see a HBV 7-mer at random in the human proteome is 20^{-7} times the number of 7-mers in the two proteomes. This probability is 12.9. In contrast, Table 1 illustrates that HBV proteins actually share peptide sequences with the human proteome for a total of 65 heptamers. The table also shows that HBV and human proteomes also share one octamer (RLGLSRPL peptide, AA Pos 796-803 in the HBV polymerase protein) and one nonamer (SPRRRTPSP peptide, AA Pos 186–194 in the viral HBV core protein).

Moreover, Table 1 shows that the human proteins hosting heptapeptides from HBV proteome comprehend numerous critical antigens specifically (or, in a few instances, uniquely) expressed in the brain. The critical neuronal role exerted by the human molecules hosting viral motifs is illustrated by the following examples. RNF19 or E3 ubiquitin-protein ligase is involved in neuronal protection,[47] BSN or protein bassoon is exclusively expressed in brain and functions in the organization of the cytomatrix at the nerve terminals active zone which regulates neurotransmitter release,[51] CENG1 or phosphatidylinositol-3-kinase enhancer participates in the prevention of neuronal apoptosis,[23] and so on. Obviously, it is logical to postulate that immune cross-reactions with these neuronal antigens might carry a sequela of inflammatory brain lesions.

Furthermore, Table 1 shows that another set of human proteins hosting 7-mer viral motifs is represented by spliceosomal proteins.[18,21,25] This datum is worth noting in the light of the numerous reports on a possible link between splicing phenomena and neurodegenerative diseases. Indeed, (dysregulated) splicing has been implicated in the: 1) selection of the autoimmune T-cell repertoire in multiple sclerosis;[66] 2) reduction of the adenosine A1 receptor-β transcript in MS patients, that potentially leads to increased macrophage activation and central nervous system inflammation;[67] 3) expression of the citrullinated myelin basic protein isomer, an autoantigen in multiple sclerosis;[68] 4) generation of alternatively spliced transcripts of the gene for human Cu, Zn superoxide dismutase, a causative gene for autosomal dominant amyotrophic lateral sclerosis.[69] Moreover, a complex splicing pattern characterizes the human myelin/oligodendrocyte glycoprotein, an highly encephalitogenic autoantigen and a target for autoaggressive immune responses in CNS inflammatory demyelinating diseases.[70] Finally, aberrant splicing has been involved in the generation of an aberrant transcript of excitatory amino acid transporter 2 that has been associated with amyotrophic lateral sclerosis.[71] In this regard, it is also remarkable that the long viral nonamer motif, ie, the SPRRRTPSP peptide sequence (aa pos 186–194 in the viral HBV Core protein), is present in the human Ser/Arg repetitive matrix protein 1 (SRRM1), that is part of pre- and post-splicing multiprotein mRNP complexes.[21] SSRM1 is involved in a number of pre-mRNA processing events (see Table 1 for details). Again, it is quite logical to postulate that a cross-reaction with SRRM1 would alter a number of physiological functions.

Discussion

To our knowledge, this study is the first and most important of its kind in providing a clear-cut analysis of the identity platform linking HBV and Homo sapiens proteomes. Two considerations emerge from the data reported here. First, although the theoretical probability of sharing perfect identical heptapeptide fragments is relatively low, actually we find 65 perfect identical matches between the viral and human proteomes. Based on the need for five or six amino acids to induce a monoclonal antibody response,[1,72] the 65 heptapeptide overlaps might clearly induce autoimmune reactions. Second, the nature of the overlapping is also of interest since a number of viral motifs occur in human proteins that are crucially involved in the neuronal structure and functions.

Given the premises illustrated under the Introduction, these data warn against adverse side-effects of active vaccination using entire HBV antigens. In parallel, the present study might be useful for designing anti-HBV vaccines based on not-shared portions of the viral antigens. More

Table 1 Sharing of 7-mer motifs between HBV and human proteomes. Location in the viral protein and amino acid sequence of the heptapeptide motifs are reported. The human proteins sharing heptapeptides with the HBV proteome are characterized by accession number and available data on function, location, and disease association (www.uniprot.org/)

HBV		Human proteins hosting heptapeptides from HBV proteome	Ref
Core protein:			
Aa Pos	**Sequence**		
44	LLSFLPS	Q6ZNP3: CDNA FLJ27406 fis.	11
53	FPSVRDL	Q96IQ9: Zinc finger protein 414.	12
60	LDTASAL	SEM3F: Semaphorin 3F variant.	13
66	LYREALE	NARG1: NMDA receptor-regulated protein 1. Involved in vascular and neuronal growth and development. Controls retinal neovascularization. Found in brain (corpus callosum).	14
70	ALESPEH	LYAM2: E-selectin or endothelial leukocyte adhesion molecule. Involved in the adhesion of blood neutrophils in cytokine-activated endothelium, and in capillary morphogenesis.	15
132	FRISYLT	Q6ZN37: Flap endonuclease GEN homolog 1. Cleaves flap structures at the junction between single-stranded DNA and double-stranded DNA. Specific for 5'-overhanging flap structures in which the 5'-upstream of the flap is completely double-stranded.	16
172	LPETTVV	UCK1: Uridine-cytidine kinase 1. Phosphorylates uridine and cytidine to UMP and CMP.	17
179	RRRDRGR	PRP4B: Serine/threonine-protein kinase PRP4 homolog. Has a role in pre-mRNA splicing. Identified in the spliceosome C complex, at least composed of AQR, ASCC3L1, C19orf29, CDC40, CDC5L, CRNKL1, DDX23, DDX41, DDX48, DDX5, DGCR14, DHX35, DHX38, DHX8, EFTUD2, FRG1, GPATC1, HNRPA1, HNRPC, HNRPF, HNRPM, HNRPR, LSM2, MAGOH, MORG1, PABPC1, RBM22, RBM8A, RBMX, SART1, SF3A1, SF3A2, SF3A3, SF3B1, SF3B2, SF3B3, SFRS1, SKIV2L2, SNRPA1, SNRPB, SNRPD1, SNRPD2, SNRPD3, SNRPE, SNRPF, SNRPG, SNW1, SRRM1, SRRM2, SYF2, SYNCRIP, TFIP11, THOC4, U2AF1, WDR57, XAB2, ZCCHC8, et cetera.	18
180	RRDRGRS	Q4VX62: Putative uncharacterized protein C6orf99.	19
183	RGRSPRR	Q5TZA2: Rootlet coiled-coil protein. Contributes to centrosome cohesion before mitosis.	20
186	SPRRRTP	SRRM1: Ser/Arg repetitive matrix protein 1. Part of pre- and post-splicing multiprotein mRNP complexes. Involved in pre-mRNA processing events. Identified in the spliceosome C complex, at least composed of AQR, ASCC3L1, CDC40, CDC5L, CRNKL1, DDX23, DDX41, DDX48, DDX5, DGCR14, DHX35, DHX8, EFTUD2, FRG1, GPATC1, HNRPA1, HNRPC, HNRPF, HNRPH1, HNRPK, HNRPM, HNRPR, HNRPU, LSM2, MAGOH, MORG1, PABPC1, PLRG1, PNN, PPIE, PPIL1, PPWD1, PRPF19, PRPF4B, PRPF6, PRPF8, RALY, RBM22, RBMX, SART1, SF3A1, SKIV2L2, SNRPA1, SNRPB, SNRPD1, SNRPD2, SNRPD3, SNRPE, SNRPF, SNRPG, SNW1, SRRM1, SRRM2, SYF2, SYNCRIP, TFIP11, THOC4, U2AF1, WDR57, XAB2, ZCCHC8, et cetera.	21
187	PRRRTPS	SRRM1 – see above.	21
188	RRRTPSP	SRRM1 – see above.	21
		Q8WZ42: Titin. Connectin. Rhabdomyosarcoma antigen MU-RMS-40.14 Key component in the functioning of vertebrate striated muscles. Contributes to the fine balance of forces between the two halves of the sarcomere. In non-muscle cells, seems to play a role in chromosome condensation and chromosome segregation during mitosis.	22
191	TPSPRRR	CENG1: Centaurin-γ-1 or Phosphatidylinositol-3-kinase enhancer. Participates in the prevention of neuronal apoptosis by enhancing PI3 kinase activity. Involved in the coupling of metabotropic glutamate receptor 1 to cytoplasmic PI3 kinase by interacting with Homer scaffolding proteins. Mediates anti-apoptotic effects of NGF by activating PI3 kinase.	23
192	PSPRRRR	Q6ZNH0: Tau-tubulin kinase 1. Ser/thr kinase which phosphorylates TAU. Induces aggregation of TAU. Expressed in cortical and hippocampal neurons.	24
		Q8N8X8 cDNA FLJ38717 fis, clone KIDNE2009647.	16
193	SPRRRRS	SFR11 Splicing factor, arginine/serine-rich 11.	25
196	RRRSQSP	BOP1: Ribosome biogenesis protein BOP1. Required for maturation of the 25S and 5.8S ribosomal RNAs.	26
201	SPRRRRS	SFR11: Splicing factor, Arg/Ser-rich 11 that localizes with spliceosome components.	25
Polymerase protein:			
Aa Pos	**Sequence**		
20	AGPLEEE	BIEA: Biliverdin reductase A Reduces the γ-methene bridge of the open tetrapyrrole, biliverdin IX α, to bilirubin with oxidation of a NAD(P)H cofactor.	27
24	EEELPRL	CHD5 Chromodomain-helicase-DNA-binding protein 5. May play a role in the development of the nervous system and the pathogenesis of neural tumors. Preferentially expressed in total brain, fetal brain, and cerebellum.	28
198	SFCSQPS	BARD1: BRCA1-associated RING domain protein 1 Implicated in BRCA1-mediated tumor suppression. Functions in the response to DNA damage.	29
204	SGILSRS	RCOR2: REST corepressor 2.	30

(*Continued*)

Table 1 (*Continued*)

HBV		Human proteins hosting heptapeptides from HBV proteome	Ref
Polymerase protein:			
Aa Pos	**Sequence**		
264	SGHVDPS	TAF1C: TATA box-binding protein-associated factor RNApol I subunit C.	31
308	CLPPSSA	TICAM-1 or Toll-Interleukin 1 receptor domain-containing adapter protein inducing INF-β. Involved in innate immunity against invading pathogens. Adapter used by TLR3 and TLR4 to mediate NFkB and IRF activation, and to induce apoptosis. Ubiquitously expressed.	32
315	RPQSQGS	Q8N7I0: Tigger transposable element derived 1-like 2.	33
363	RIPRTPA	ZDHC1: Probable palmitoyltransferase ZDHHC1.	34
372	TGGVFLV	Q2TBD6: Urea transporter, kidney specialized low-affinity vasopressin-regulated urea transporter. Has a role in the urinary concentrating mechanism.	35
385	TAESRLV	Q15751: E3 ubiquitin-protein ligase. Binds phosphatidylinositol-4,5-bisphosphate, which is required for GEF activity. Acts as a E3 ubiquitin-protein ligase which accepts ubiquitin from an E2 ubiquitin-conjugating enzyme and then transfers the ubiquitin to targeted substrates.	36
417	LTNLLSS	Q68DA7: Formin-1, Limb deformity protein homolog. Plays a role in the formation of adherens junction and the polymerization of linear actin cables.	37
537	SVVRRAF	FFAR2: Free fatty acid receptor 2. The rank order of potency for agonists of this receptor is acetate = propionate = butyrate > pentanoate = formate.	38
575	FLLSLGI	XCR1: Chemokine XC receptor 1. Receptor for chemokines SCYC1 and SCYC2. Subsequently transduces a signal by increasing the intracellular calcium ions level.	39
583	LNPNKTK	CD68: MacrosialinCD_antigen. CD68. Plays a role in phagocytic activities of tissue macrophages. Binds to tissue- and organ-specific lectins or selectins, allowing homing of macrophage subsets to particular sites. Highly expressed by monocytes and macrophages.	40
643	GFAAPFT	CQ10A Protein COQ10 A, mitochondrial.	41
667	QAFTFSP	Q6QLU7 Mitogen-activated protein kinase 7. Plays a role in various cellular processes such as proliferation, differentiation and cell survival.	42
796	RLGLSRPI	Q5JSZ5 Protein BAT2-like or HLA-B-associated transcript 2-like.	16
797	LGLSRPL	Q5JSZ5 as above.	16
798	GLSRPLL	Q9UFD9 RIMS-binding protein 3A.	43
799	LSRPLLR	Q53EQ6 Tigger transposable element-derived protein 5.	16
811	TTGRTSL	ECT2 Epithelial cell-transforming sequence 2 oncogene.	16
Large Envelope protein:			
Aa Pos	**Sequence**		
89	STIPPPA	VMAT1: Chromaffin granule amine transporter. Vesicular transport of biogenic amines.	44
142	PAGGSSS	NRG2: Pro-neuregulin-2. Neural- and thymus-derived activator for ERBB kinases.	45
143	AGGSSSG	CCNL1: Cyclin-L1. Transcriptional regulator of the pre-mRNA splicing process. May be a candidate protooncogene in head and neck squamous cell carcinomas. Ubiquitous.	46
		RNF19: E3 ubiquitin-protein ligase. Transfers ubiquitin to targeted substrates. Specifically ubiquitinates pathogenic SOD1 variants (not wild-type SOD1) which cause amyotrophic lateral sclerosis, so leading to their proteasomal degradation and to neuronal protection. Present in the hyaline bodies found in motor neurons from amyotrophic lateral sclerosis patients. Present in the Lewy bodies found in neurons from Parkinson disease patients.	47
144	GGSSSGT	CCNL1: see above.	46
145	GSSSGTV	Q8N5F4 IGL@ protein.	48
185	PLPVLQA	PO210: Nuclear pore membrane glycoprotein 210. Essential for nuclear pore assembly fusion, and spacing. Recognized by antinuclear autoantibodies in primary biliary cirrhosis.	49
186	LPVLQAG	Q6NSZ9: Zinc finger protein 498.	50
217	GGSPVCL	BSN: Protein bassoon. Involved in the organization of the cytomatrix at the nerve terminals active zone. Regulates neurotransmitter release from a subset of brain glutamatergic synapses. Localized to the active zone of presynaptic density Exclusively expressed in brain.	51
227	SRSPTSN	Q86X29: Lipolysis-stimulated lipoprotein receptor.	52
254	FIIFLFI	GIMA5: GTPase IMAP family member 5 Immunity-associated nucleotide 4-like 1 protein. Required for mitochondrial integrity and T-cell survival. Widely expressed.	53
256	IFLFILL	KI3L2: Killer cell immunoglobulin-like receptor 3DL2. Inhibits the activity of NK cells thus preventing cell lysis.	54

(*Continued*)

Table 1 (*Continued*)

HBV		Human proteins hosting heptapeptides from HBV proteome	Ref
Large Envelope protein:			
Aa Pos	**Sequence**		
257	FLFILLL	KI3L2 as above.	54
262	LLCLIFL	Q6ZVP3 cDNA FLJ42265 fis, clone TKIDN2014771.	55
263	LCLIFLL	Q6GU65 HHIP-like protein 2.	56
286	GSTTTST	LPHN3: Latrophilin-3. Calcium-independent α-latrotoxin receptor 3.	57
287	STTTSTG	UBQL2: Ubiquilin-2. Increases the half-life of proteins destined to be degraded by the proteasome. Interacts with the 19S proteasome subunit.	58
343	RFSWLSL	Q6ZUX8: cDNA FLJ43235 fis, clone HCHON2004007.	59
X protein:			
Aa Pos	**Sequence**		
36	TLSSPSP	ZN583: Zinc finger protein 583 May be involved in transcriptional regulation.	16
37	LSSPSPS	OXER1: Oxoeicosanoid receptor 1. Receptor for eicosanoids and polyunsaturated fatty acids such as 5-OXO-ETE, 5(S)-HPETE, arachidonic acid.	60
38	SSPSPS A	CUL4B: Cullin-4B. Core component of E3 ubiquitin-protein ligase complexes which mediate the ubiquitination and subsequent proteasomal degradation of target proteins. Required for histone H3 and H4 ubiquitination in response to UV. Defects in CUL4B cause mental retardation-hypotonic facies syndrome type 2, microcephaly, short stature, macrostomia, patulous lips, difficulty in speech, micrognathia, short thumbs and little fingers, hypotonia at age less than 10 years, hypertonia, restlessness, and seizures. IQ: from 40 to 57.	61
		Q8NEN5: Sperm-specific antigen 2. Involved in early cleavage of the fertilized oocyte.	62
96	RTLGLPA	115RA: Interleukin-15 receptor α. Expressed in fetal brain (mainly in the hippocampus).	63
104	STTDLEA	Q5JTZ9: mitochondria alanyl-tRNA synthetase.	64
121	EELGEEI	MYH8: Myosin heavy chain 8. Defects cause Carney syndrome characterized by spotty skin pigmentation, myxomas, endocrine tumors, and psammomatous melanotic schwannomas.	65

in general, the data reported in this study define a practicable procedure to define possible cross-reactions potentially associated with active vaccines.

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