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

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. In general, a vaccine produces a weak immune response; also autoimmune cross-reactions are extremely rare events. Under normal non-stimulated conditions, immune system fails to make immune responses to protein vaccines, unless adjuvants are added. Consequently, the active vaccine preparations currently in use contain adjuvants for obvious reasons of desired immunogenicity, 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. 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
The heptamers were offset by one residue, ie, overlapping by six residues: ie, MQLFHLCL, QLFHLCLL, LFHLCLLI, FHLLCLII, 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. 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 65 perfect identical matches between the viral and human proteomes. 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 (SPRRRTSP 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,92 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 anyotrophic lateral sclerosis.69

Moreover, a complex splicing pattern characterizes the human myelin/oligodendrocyte glycoprotein, an highly encephalitogenic autoantigen and a target for autoimmune 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 anyotrophic lateral sclerosis.71 In this regard, it is also remarkable that the long viral nonamer motif, ie, the SPRRRTSP 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-spooling multiprotein mRNP complexes.72 SRRM1 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,1,2 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/)

| Core protein: | Human proteins hosting heptapeptides from HBV proteome | Ref |
|--------------|--------------------------------------------------------|-----|
| **Aa Pos Sequence** | **HBV** | **Ref** |
| 44 | LLSFLPS | Q6ZNP3: CDNA FLJ27406 fs. | 11 |
| 53 | FPSVRDL | Q96IQ9: Zinc finger protein 414. | 12 |
| 60 | LTASAL | 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 | LPETTVV | Q6ZNJ7: 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 | RRRDRGR | PRPK4B: Serine/threonine-protein kinase PRP4 homolog. Has a role in pre-mRNA splicing. Identified in the spliceosome C complex, at least composed of AQR, ASCCL1, C19orf29, CDC40, CDC5L, CRNKL1, DDX23, DDX41, DDX48, DDX5, DGCRI4, DHX35, DHX38, EFTUD2, FRG1, GATC1, HRNPA1, HRNPC, HRNPK, HRNP, HNRP, LSM2, MAGOH, MORG1, PABPC1, RBM22, RBM5A, RBMX, SART1, SF3A1, SF3A2, SF3B1, SF3B2, SF3B3, SFRS1, SKIV2L, SNRP1, SNRPB, SNRPD1, SNRPD2, SNRPD3, SNRPE, SNRPF, SNRPG, SNW1, SRRM1, SRRM2, SYF2, SYNCRIP, TFIP11, THOC4, U2AF1, WDR57, XAB2, ZCCHC8, et cetera. | 17 |
| 179 | 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. | 18 |
| 180 | Q4VX62 | UCK1: Uridine-cytidine kinase 1. Phosphorylates uridine and cytidine to UMP and CMP. | 19 |
| 183 | LPRRTP | 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, ASCCL1, CDC40, CDC5L, CRNKL1, DDX23, DDX41, DDX48, DDX5, DGCRI4, DHX35, DHX38, EFTUD2, FRG1, GATC1, HRNPA1, HRNPC, HRNPK, HRNP, HNRP, LSM2, MAGOH, MORG1, PABPC1, RBM22, RBM5A, RBMX, SART1, SF3A1, SF3A2, SF3A3, SF3B1, SF3B2, SF3B3, SFRS1, SKIV2L, SNRP1, SNRPB, SNRPD1, SNRPD2, SNRPD3, SNRPE, SNRPF, SNRPG, SNW1, SRRM1, SRRM2, SYF2, SYNCRIP, TFIP11, THOC4, U2AF1, WDR57, XAB2, ZCCHC8, et cetera. | 20 |
| 186 | RRRDRGR | PRPK4B: Serine/threonine-protein kinase PRP4 homolog. Has a role in pre-mRNA splicing. Identified in the spliceosome C complex, at least composed of AQR, ASCCL1, CDC40, CDC5L, CRNKL1, DDX23, DDX41, DDX48, DDX5, DGCRI4, DHX35, DHX38, EFTUD2, FRG1, GATC1, HRNPA1, HRNPC, HRNPK, HRNP, HNRP, LSM2, MAGOH, MORG1, PABPC1, RBM22, RBM5A, RBMX, SART1, SF3A1, SF3A2, SF3A3, SF3B1, SF3B2, SF3B3, SFRS1, SKIV2L, SNRP1, SNRPB, SNRPD1, SNRPD2, SNRPD3, SNRPE, SNRPF, SNRPG, SNW1, SRRM1, SRRM2, SYF2, SYNCRIP, TFIP11, THOC4, U2AF1, WDR57, XAB2, ZCCHC8, et cetera. | 21 |
| 187 | RRRDRGR | Q4VX62: Putative uncharacterized protein C6orf99. | 22 |
| 188 | RRRRTP | 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, ASCCL1, CDC40, CDC5L, CRNKL1, DDX23, DDX41, DDX48, DDX5, DGCRI4, DHX35, DHX38, EFTUD2, FRG1, GATC1, HRNPA1, HRNPC, HRNPK, HRNP, HNRP, LSM2, MAGOH, MORG1, PABPC1, RBM22, RBM5A, RBMX, SART1, SF3A1, SF3A2, SF3A3, SF3B1, SF3B2, SF3B3, SFRS1, SKIV2L, SNRP1, SNRPB, SNRPD1, SNRPD2, SNRPD3, SNRPE, SNRPF, SNRPG, SNW1, SRRM1, SRRM2, SYF2, SYNCRIP, TFIP11, THOC4, U2AF1, WDR57, XAB2, ZCCHC8, et cetera. | 23 |
| 189 | RRRRTP | SRRM1 – see above. | 24 |
| 190 | RRRRTP | SRRM1 – see above. | 25 |
| 191 | TPSRRR | CENGI1: 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 metabolotropic glutamate receptor 1 to cytoplasmic PI3 kinase by interacting with Homer scaffolding proteins. Mediates anti-apoptotic effects of NGF by activating PI3 kinase. | 26 |
| 192 | PSRRR | Q6ZNH0: Tau-tubulin kinase 1. Ser/thr kinase which phosphorylates TAU. Induces aggregation of TAU. Expressed in cortical and hippocampal neurons. | 27 |
| 193 | PSRRR | Q6ZNH0: Tau-tubulin kinase 1. Ser/thr kinase which phosphorylates TAU. | 28 |
| 194 | PSRRR | Q6ZNH0: Tau-tubulin kinase 1. Ser/thr kinase which phosphorylates TAU. Expressed in cortical and hippocampal neurons. | 29 |
| 195 | PSRRR | Q6ZNH0: Tau-tubulin kinase 1. Ser/thr kinase which phosphorylates TAU. | 30 |
| 200 | PSRRR | Q6ZNH0: Tau-tubulin kinase 1. Ser/thr kinase which phosphorylates TAU. Expressed in cortical and hippocampal neurons. | 31 |
| 204 | PSRRR | Q6ZNH0: Tau-tubulin kinase 1. Ser/thr kinase which phosphorylates TAU. Expressed in cortical and hippocampal neurons. | 32 |

(Continued)
### Table 1 (Continued)

| Human proteins hosting heptapeptides from HBV proteome | Ref |
|------------------------------------------------------|-----|

#### Polymerase protein:

| Aa | Pos | Sequence | Ref |
|----|-----|----------|-----|
| 264 | SGHVDP | 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 NFκB and IRF activation, and to induce apoptosis. Ubiquitously expressed. | 32 |
| 315 | RPQSQGS | Q8N7I0: Tigger transposable element derived I-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 |
| 444 | GFAAPFT | COQ10A Protein COQ10 A, mitochondrial. | 41 |
| 467 | QAFTFSP | Q6QU7 Mitogen-activated protein kinase 7. Plays a role in various cellular processes such as proliferation, differentiation and cell survival. | 42 |
| 476 | RLGLSRPl | Q8N5F4 IGL@ protein. | 46 |
| 490 | LNLNSLNN | Q9UFD9 RIMS-binding protein 3A. | 50 |
| 504 | LNLNPENT | Q53EF6 Tigger transposable element-derived protein 5. | 54 |

#### Large Envelope protein:

| Aa | Pos | Sequence | Ref |
|----|-----|----------|-----|
| 89 | STIPPAPA | VMAT1: Chromaffin granule amine transporter. Vesicular transport of biogenic amines. | 44 |
| 142 | PAGSSG | 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. Ubiquitously. | 46 |
| 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 |
| 227 | SRSPTSN | Q8BEX29: Lipolysis-stimulated lipoprotein receptor. | 52 |
| 254 | FIILFLI | 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 | K1L2: Killer cell immunoglobulin-like receptor 3DL2. Inhibits the activity of NK cells thus preventing cell lysis. | 54 |
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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