Hepatitis B surface proteins

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Surface proteins have many tasks in the life cycle of viruses: attachment and penetration at the beginning of the infection process; envelopment, virus maturation and transport to the extracellular space at its end; and, in the case of persistent viruses, blocking of neutralizing or virus-eliminating immune reaction. In a virus like HBV which depends on persistent viremia for its propagation from person to person, surface proteins must allow for free circulation of the virus in the infected host, but nevertheless must attach reliably to target cells of a person who has become inoculated by HBV-containing material.

Attachment and Penetration

pHSA binding

The first structure on the HBV surface which was proposed to be an attachment site was the so-called receptor for polymerized human serum albumin (pHSA). Although after 15 years still no proof is available for that theory, some observations are in its favor: (i) HBV binds only human and other primate-derived serum albumin. This binding specificity corresponds to the narrow host range of HBV. (ii) pHSA binds to hepatocytes and this correlates with the organ-specificity of HBV. (iii) The pHSA-binding activity in serum of HBV carriers correlates to some extent with the infectivity of their blood. Low-viremic carriers do not have free pHSA-binding sites. (iv) pHSA binds to the preS2 domain of the middle surface protein of HBV. Antibodies against this domain are able to neutralize infectivity. (v) Anti-preS2 antibodies are a good prognostic sign in the course of acute or chronic HBV infections.

There is one point which does not yet allow the preS2 domain to be definitely considered as the specific attachment site: it has not yet been shown experimentally that pHSA mediated uptake of HBV to liver cells. It appears that hepatocyte cultures do not express a pHSA receptor in vitro. However, plasma membranes from surgically obtained human liver bind preS2-containing particles in a pHSA-dependent manner.

Another objection to the biological relevance of pHSA binding seems to be no longer valid. In most experiments it was necessary to artificially cross-link HSA by glutaraldehyde for efficient binding to preS2. Recent experiments showed, however, that a small subfraction of natural HSA is also capable of binding. One in approximately ten thousand HSA molecules is able to bind to preS2. On the average this is sufficient to saturate 10 μg of HBsAg/ml serum. Persons with higher HBsAg concentrations have free pHSA-binding sites. The circulating preS2-binding HSA is monomeric. One may speculate that naturally polymerized HSA is already bound in the liver and will substitute the monomeric HSA in case of a newly infected host, but there is no experimental evidence for this postulate. The binding site of pHSA and of natural HSA has not yet been precisely mapped, but preliminary data suggest that it is amino acid 19–29 of the preS2 domain. This region is well-conserved in the various HBV subtypes.

PreS2 glycan

There are other structural features of the middle-sized HBs protein which may be relevant for attachment. All preS2 domains of mammalian hepadnaviruses carry an *N*-linked glycan close to their amino end. At this site natural HBsAg particles contain a hybrid-type triantennary glycan with one mannose chain and two complex chains. This novel glycan seems to be liver specific and reacts with a surface structure of the human hepatoma cell HepG2. Certain monoclonal antibodies, e.g., F124 or Q19/10 react preferably with the preS2-

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peptidoglycan instead of the preS2 peptide alone. Remarkably, Q19/10 also reacts with a surface structure of HepG2 cells. One may speculate that the preS2 domain mimics a liver-specific surface structure which is recognized by a hypothetic liver specific adhesion molecule.

Fusion

A third structure of preS2 which may be involved in the early steps of HBV infection is the protease-hypersensitive sequence at its carboxy end. Cleavage at this site exposes the amino terminal part of the S domain. This sequence has homology to known 'fusion' peptides of other surface proteins of other viruses. Experimentally, fusion of HBV with cell membranes has not been shown, but we have found irreversible binding of HBsAg particles to all kinds of cellular membranes after proteolytic cleavage of the preS2 domain. The same putative 'fusion' sequence is present in the small surface protein, but here it seems to be trapped within the molecule.

PreS1 domain

A further attachment site of HBV has been found in the preS1 domain position 21–47. A receptor for this site is found on various types of human white blood cells and on human hepatocytes including hepatoma cells. Antibody against this attachment site is virus neutralizing, whereas antibodies against a second hydrophilic site (position 90–119) seem to be non-protective.

S domain

The role of the S domain in attachment and penetration is not yet understood. Experiments suggesting binding of S particles from yeast to various cell membranes were probably due to improper folding, thus causing a premature exposure of the 'fusion' sequence in the S domain.

Escape mutant

The existence of neutralizing antibodies against S particles and the development of an escape mutant with a mutation at position 145 suggest that this site participates in viral attachment or entry. The paper of Harrison et al. (1) is a further example that generation of this escape mutation very often occurs in vivo after vaccination with S particles. The frequent occurrence of the escape mutant suggests widening the antigenic spectrum of the hepatitis B vaccines. Inclusion of neutralizing epitopes from preS1 and preS2 domains would be the most obvious step. Simultaneous generation of three escape mutations in one genome is far more improbable than mutation at one neutralizing epitope.

Virus maturation and secretion

It has long been known that the small HBs protein is sufficient for the generation of 20 nm particles. HBs filaments, however, are only formed if a sufficient proportion of large HBs protein is present in the cell. Recently, it was shown that the large HBs protein is also necessary for the morphogenesis and/or secretion of core particles within an HBs envelope. The middle HBs protein is obviously not required for morphogenesis of HBs filaments or virions. Furthermore, if one considers that the large HBs protein contains both the preS1 and preS2 domain, the middle protein could possibly be a non-essential HBV protein like HBeAg. In keeping with this hypothesis is the recent finding of Will (2) of an HBV variant where the start codon of the middle protein is mutated away. This mutant caused high viremia in an HBV-infected liver transplant recipient and, thus, seems to be infective. Infectivity in normal hosts has, however, not yet been analysed. A mutant with even more severe mutations in the preS region was found by Gerken et al. (3) in a hepatoma patient. Although help by wild-type virus cannot be excluded in this case, it appears that this mutant was also infectious for hepatocytes within a given host. The attachment sites of the preS1 and S domain were not affected by mutation.

Role of middle HBs

In view of the fact that the middle HBs protein is conversed in the three well-studied mammalian hepadnaviruses one has to ask what the advantage of this protein in addition to the large HBs protein is. In native HBs particles or virions, pHSA cannot bind well to the preS2 domain of the large protein. The proteolysis site of preS2 is less accessible in the large, than the middle, protein. Most importantly the glycosylation site of preS2 is not used in the large protein. In this respect it is noteworthy that the MHBs-variants of Will seem to have restored the glycosylation of the preS2 domain.

HBs negative variants

Most enigmatic are HBV variants from patients where not HBsAg, but HBV-DNA, is detectable in the serum. Several possibilities can explain this finding. (i) The HBsAg may be complexed by anti-HBs. This has been repeatedly reported. (ii) The HBs epitopes may be mutated in such a way that normal HBsAg assays can no longer detect them. Systematic mutational analysis like the one suggested by Vyas et al. (4) would help to recognize the potential gaps of current serological tests. The increasing use of monoclonal anti-HBs for HBsAg ELISAs makes these assays more vulnerable to mutations of

major epitopes. The escape variants with the arginine at position 145 of the S domain still react with HBsAg ELISAs, but much weaker than the normotype of HBsAg. (iii) HBsAg is not synthesized at all. In this case it would be necessary to assume secretion and circulation of naked core particles. We found recently that free core particles do not circulate in anti-HBc-negative HBV carriers. Another possibility would be lysis of HBV-infected cells, whereby either naked core particle, episomal DNA or even chromosomal fragments of integrated HBV-DNA could be released. (iv) Low level synthesis may not allow detection of normal HBsAg.

No matter what the explanation is, it appears that HBsAg assay is not as reliable a tool for the detection of productive HBV infection (e.g., in blood donation services) as it may have appeared some years ago.

Systemic interactions between host and HBV

Both mutational analysis and detection of natural HBV variants suggest that several of the HBV proteins may be non-essential for viral replication. HBeAg is obviously such a protein, and the recent data on deletions which cause absence of middle HBs protein, suggest that this protein may be also dispensible. HBx protein is, at least in transfected cell cultures, dispensible. Given the small size of the HBV genome and its extremely compact genome organization, these so-called non-essential gene products must have an essential role for the spread and maintenance of the virus within the susceptible population. It appears typical that the variants with deleted HBe or middle HBs protein are isolated from chronically infected persons with either impaired immune system or severe disease activity. Long-term survival in the population of a liver-specific, blood-transmitted virus, however, requires per se long-term, nearly healthy carriers. It may be the preferential task of these non-essential proteins to establish both high level viremia and suppression of the immune response against the circulating antigens and the virus-infected cells. While the role of HBe protein remains presently enigmatic, for middle HBs protein one might postulate that binding of HSA is indeed a factor stabilizing circulation in the blood and suppressing antiviral immune responses. Enhancement of infectivity would be a further task.

References

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