Hepatology Snapshot

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Occult Hepatitis B Infection

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Key facts

Occult hepatitis B virus (HBV) infection (OBI) is recognized as one of the possible phases in the natural history of chronic HBV infection [1]. OBI defines the persistence of HBV genomes in the hepatocytes of individuals testing negative for HBV surface antigen (HBsAg) and, usually, also for serum HBV DNA [2]. Apart from some cases in which the lack of HBsAg detection is attributable to the HBV genetic heterogeneity (i.e., infection with replication-defective variants or with S-escape mutants producing a modified HBsAg undetectable by diagnostic kits), in most cases OBI is related to replication-competent viruses that are strongly suppressed in their activities (replicative and transcriptional) by the host's defense mechanisms. Very importantly, this suppression (a) does not have an absolute effect and residual, low-levels of replication and transcription may persist over time, and (b) may be reversible in particular circumstances leading to viral reactivation and development of a typical HBsAg-positive (namely, "overt") infection [3].

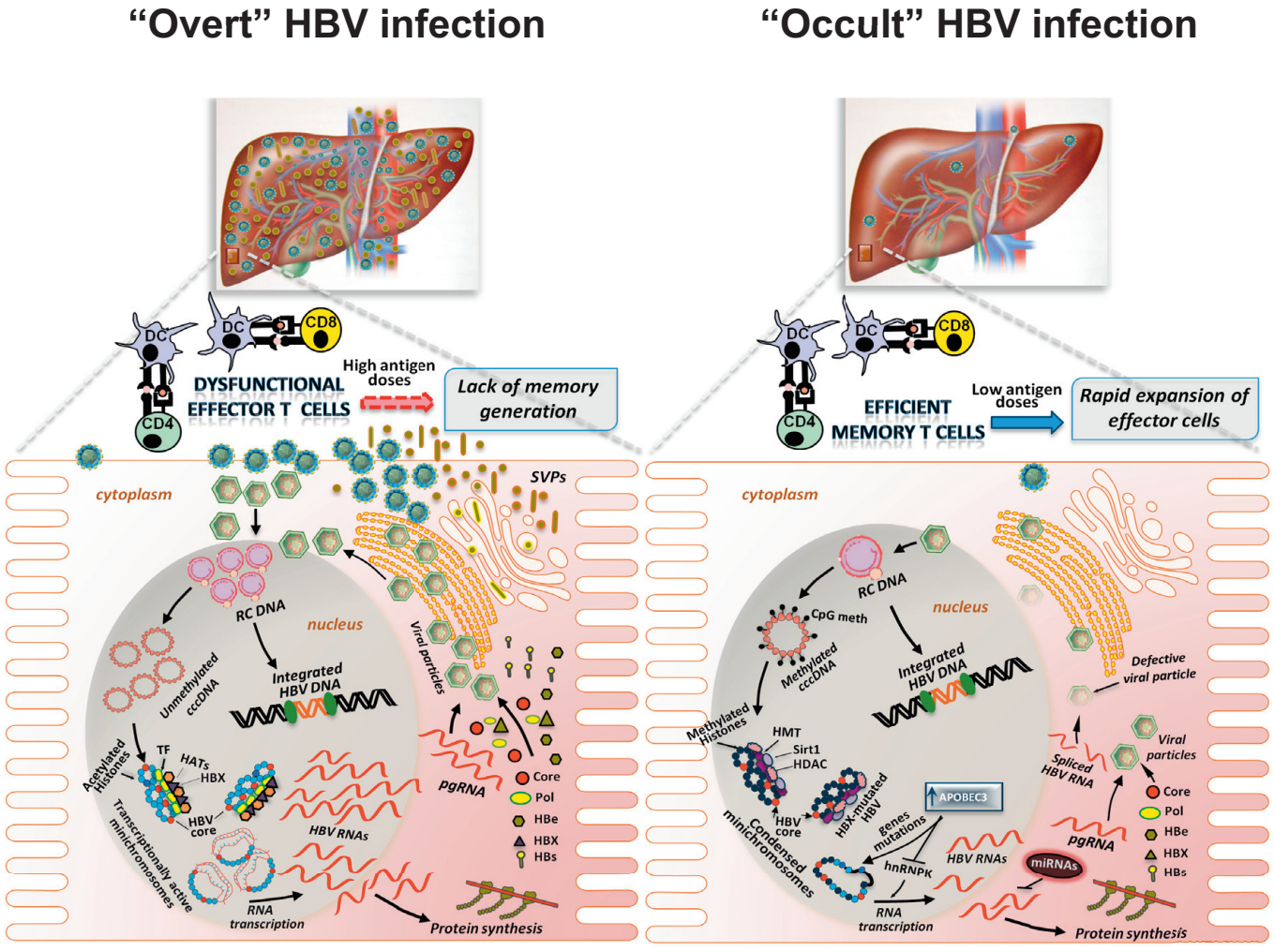

Fig. 1 Schematic comparison of overt and occult HBV infection. Mechanisms potentially involved in HBV inhibition and OBI induction are summarized. In particular, immunological (presence of functionally efficient central/effector memory T cells), genetic (HBV genomic variability, APOBEC hyperediting), epigenetic (methylation of CpG-rich regions within the HBV genome as well as acetylation/methylation of cccDNA-bound histones and recruitment of chromatin modifying enzymes onto the viral minichromosome) and co-/posttranscriptional (HBV RNA splicing, HBV replication inhibition by cellular miRNAs and by editing-independent functions of the cellular APOBEC3 proteins) mechanisms are represented. Differences in nucleosomal packaging and transcriptional activity of HBV minichromosomes as well as in amounts of total viral DNA, transcripts, proteins and virion formation and secretion are also displayed. DC, Dendritic Cell; CD4, CD4+ T cell; CD8, CD8+ T cell; RC DNA, Relaxed Circular DNA; cccDNA, covalently closed circular DNA; pgRNA, pregenomic RNA; HBs, envelope proteins; SVPs, subviral particles; TF, cellular transcription factors; HATs, histone acetyl transferases; HMT, histone methyltransferases; APOBEC3, apo B mRNA editing enzyme catalytic polypeptide; hnRNP K, heterogeneous nuclear ribonucleoprotein K; miRNAs, microRNAs; CpG meth, methylated CpG islands.

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Key Points

- The molecular basis of the occult infection is closely related to the peculiar life cycle of the HBV, and in particular to the long-lasting persistence of free viral genomes — such as HBV cccDNA chromatinized episomes — in the nucleus of the infected cells. The stability and long-term persistence of cccDNA molecules together with the long half-life of hepatocytes imply that HBV infection, once it has occurred, may possibly continue for life even in conditions of strong inhibition of viral functions [4]

- HBV DNA can integrate in the host's genome. Integration has no role in the replicative cycle of HBV and it involves only segments of the viral DNA. Integrated HBV may persist forever in the liver cells of infected individuals, independently of the HBsAg positive/negative status. The presence of integrated viral DNA in HBsAg-negative subjects does not per se have to be identified as an occult infection, since OBI is essentially related to the intrahepatic persistence of entire, episomal, replication-competent HBV genomes

- A not negligible portion of OBI cases are negative for all HBV serum markers (OBI seronegative): they might have either progressively lost the anti-HBV antibodies or might be HBV antibody negative since the beginning, as a consequence of a very limited number of virions in the infecting inoculums, in analogy to what was observed in the woodchuck model of hepadnavirus infection [5]

- Growing evidence indicates that genetic (i.e., HBV genomic variability, APOBEC deamination-dependent activity), epigenetic (i.e., methylation of CpG-rich regions within the HBV genome as well as acetylation/methylation of cccDNA-bound histones and recruitment of chromatin modifying enzymes onto the viral minichromosome) and co-/post-transcriptional mechanisms (i.e., viral RNA splicing, miRNAs effects, APOBEC deamination-independent activity) may be involved in the control of HBV replication and gene expression, and thus in the OBI occurrence [3,6] (Fig. 1). Most of these mechanisms might be triggered by the host's immune response. Indeed, OBI subjects show long-lasting, potent, and multispecific HBV T cell responses (although with a profile that is different between OBI-seropositive and OBI-seronegative individuals) [7,8]. Based upon the present knowledge of HBV immunopathogenesis, it may be proposed that in overt chronic HBV infection anti-viral T cell dysfunction is maintained by different mechanisms, including the persistent exposure to high antigen loads, that precludes the generation of functionally efficient memory T cells. In occult infection, traces of virus are tightly controlled by the presence of functionally efficient central/effector memory T cells that are able to rapidly differentiate into effector cells. Moreover, also the innate immune response might be involved in suppression of HBV replication through non-cytolytic, immune-mediated mechanisms [9]

- *In vitro* studies showed that the replication, transcription, and protein synthesis capabilities of HBV isolates from liver of OBI individuals can be fully restored once the viruses are taken out of the host's microenvironment [10]. In analogy, the profound changes of the host's immune surveillance mechanisms observed mainly in patients with hematological malignancies undergoing immune- and/or chemo-therapies have been shown to lead to OBI reactivation with consequent development of acute hepatitis showing the typical serological profile of acute hepatitis B (HBsAg (re)-appearance and even HBeAg positivity) [3]. Finally, a similar scenario may occur in HBV-naïve patients undergoing liver transplantation: if the donor is OBI positive, the recipient may develop typical hepatitis B (when a proper anti-HBV prophylaxis is not performed) [3]

Key Points *(continued)*

- Longitudinal evaluation of serum HBV DNA in OBI patients shows phases of absent viremia alternating with phases of very low but detectable viral load. These episodes of transient, partial viral reactivation may be associated with a slight increase of transaminase levels [11]. Various studies indicated that OBI is associated with the most severe forms of chronic hepatitis (particularly in HCV-infected patients), thus suggesting that it might favor or accelerate the progression toward cirrhosis of patients with various causes of liver disease [3,12,13]

- Many studies indicate that OBI is an important risk factor for hepatocellular carcinoma development [3,13,14]. In fact, OBI may maintain most of the pro-oncogenic properties of the overt HBV infection, including the capacity to integrate into the host's genome, to produce proteins with transforming properties (even if at low levels) and a mild but persisting necroinflammation

- Reliable OBI diagnosis can at present be performed only in highly specialized laboratories. The development in the near future of valid and commercially available assays (i.e., real time PCR on DNA extracts from liver tissue fine-needle aspiration) allowing the detection of OBI in all cases, in which its presence might be of clinical relevance, appears of great importance

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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