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Biological and clinical implications of HBV infection in peripheral blood mononuclear cells

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ABSTRACT

The liver is the main site of HBV replication, however extrahepatic organs, such as the lymphoid system, are an important reservoir of the virus. Viral DNA into different mononuclear cell subsets has been mainly detected in monocytes and B lymphocytes. The attachment site of the virus has been identified in the preS1 encoded protein of the virus envelope, the same involved in hepatocyte infection. The risk of HBV transmission by infected lymphocytes has been clearly documented in the setting of liver transplantation where *de novo* HBV infection has been found in up to about 80% of liver grafts from HBsAg negative but anti-HBc positive donors. In the hemodialysis setting the percentage of HBV DNA detection in mononuclear cells of HBsAg negative patients has been described in up to 54% of the cases. Vertical transmission studies indicate that HBV-infected mononuclear cells of the mother may result in viral infection of mononuclear cells of the newborns and possible HBV vaccine response failure. HBV can also infect bone marrow cells and *in vitro* studies demonstrate a block of hematopoiesis by HBV, supporting clinical observations of isolate cases of aplastic anemia associated to the infection.

Contents

1. Introduction

Viral hepatitis is a diffuse inflammatory reaction of the liver caused by different hepatotropic viruses. Among them, only hepatitis B virus (HBV) and hepatitis C virus (HCV) are

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able to persist in the host and cause immune-mediated necroinflammation with progressive liver disease. The HBV replication cycle is not directly cytotoxic, in agreement with the fact that many HBV carriers are asymptomatic and have minimal liver injury, despite ongoing virus replication. HBV is also associated with extrahepatic manifestations of disease that can be mediated by virus-specific immune-complex injury and they include arthritis, vasculitis and glomerulonephritis [1,2].

2. Molecular characteristics of HBV

Hepatitis B virus is the prototype member of the Hepadnaviridae (hepatotropic DNA virus) family. HBV virions are double-shelled particles of 40–42 nm in diameter, with an outer lipoprotein envelope that contains three related envelope glycoproteins (surface antigens) [3]. The viral nucleocapsid [4] encloses the viral genome, a relaxed-circular, partially duplex DNA of 3.2 kb and a polymerase that is responsible for the synthesis of viral DNA in infected cells [5]. In addition to virions, HBV-infected cells produce two distinct subviral lipoprotein particles: 20-nm spheres and filamentous forms of similar diameter. These HBsAg particles contain only envelope glycoproteins and host-derived lipids [4].

HBV DNA contains four open reading frames, which encodes major structural and non-structural viral proteins. These include the polymerase gene region for polymerase, the preS-S (preS1, preS2 and S) region of the genome for the three viral surface antigen proteins (large, middle and small HBsAg), the precore and core gene regions for hepatitis B core antigen (HBcAg) and hepatitis B e antigen (HBeAg), as well as the X gene region for hepatitis B X protein [6,7].

3. Immunologic features in chronic HBV infection

3.1. Innate immune responses

Microarray analysis of serial liver biopsies of experimentally infected chimpanzees has revealed that HBV does not induce any significant change in the expression of intrahepatic genes during the first week of infection. These findings seem to exclude the induction of a strong innate immune response in the early phase of HBV infection, despite its high recovery rate [8]. However, a role for the innate immune response in the control of early HBV replication should not be dismissed and expression of immune-response genes might occur below the detection level. The fact that most of HBV DNA can be cleared from experimentally infected chimpanzees before a detectable adaptive immune response in the liver supports this hypothesis [9]. In transgenic mice, the production of antiviral cytokines, such as IFN-α and IFN-β has been associated to the inhibition of new HBV capsids formation, destabilization of the existing capsids and degradation of pre-formed HBV RNA [10]. In addition, down-regulation of HBV replication can be mediated by IFN-γ, produced by activated Natural Killer T cells (NKT) and T cells [11–13].

3.2. Adaptive cellular immune responses

Non-cytolitic down-regulation of viral replication seems to have a peculiar role in HBV infection, because most HBV DNA can be cleared from experimentally infected chimpanzees before any detectable T cell infiltration and liver injury. A series of studies using transgenic mouse models showed that CD8+ T cells have the ability to clear HBV from infected hepatocytes in a non-cytolitic manner [13]. Down-regulation of HBV replication has been directly linked to IFN-γ production, since it can be obtained after adoptive transfer of CD8+ T cells, despite the lack of other cytokines, as perforin and CD95 ligand (FAS ligand) [10]. Single-stranded and relaxed-circular replicative DNA intermediates are removed from the cytoplasm and nucleus by a post-transcriptional mechanism [14,15].

Although clinical recovery from acute hepatitis B is associated with lifelong protective immunity, trace amounts of virus persist in the blood of recovered patients and are controlled by cellular and humoral immune responses. Replicative forms of HBV are found not only in the liver but also in extrahepatic sites which indicate that immunoprivileged sites might contribute to low-level HBV persistence [3]. Conversely, trace amounts of persisting virus might be essential for the maintenance of HBV-specific immunity [16].

3.3. Viral escaping and chronic hepatitis

HBV establishes chronic hepatitis mainly by vertical transmission from HBsAg-positive and HBeAg-positive mothers to neonates, as the immune system of the neonates has not yet fully developed. The immunomodulatory effects of HBeAg might play a relevant role in this setting, inducing tolerance at T cell level, as shown in transgenic mice [17]. In addition, the same mechanisms that have been described to mediate down-regulation of HBV replication might also facilitate viral persistence if antigen expression and presentation are reduced to levels undetectable by T cells. Another candidate mechanism, the development of viral escape mutations, seems to be more relevant for escape from vaccine-induced humoral immune responses than for escape from cellular immune responses. In chronic hepatitis B, T cell escape mutants are not common [18], which are consistent with a weak HBV-specific T cell response.

4. HBV in peripheral blood mononuclear cells

HBV is not strictly hepatotropic and early observations have shown that this virus can infect peripheral blood mononuclear cells (PBMC) [19], as well as bone marrow cells [20] and lymphoblastoid cell lines [21]. In the early eighties we have demonstrated that the DNA of the virus is detectable in PBMC of the majority of the patient with chronic HBV infection, but not in normal controls, as assessed by Southern blot hybridization [22]. The typical patterns of the agarose gel electrophoresis, after restriction enzyme digestion, allowed to differentiate free viral episomic genomes from HBV DNA integrated into the host genome. Free virus DNA was usually associated with ongoing viral replication, while integrated profile was characteristic of inactive viral infection.

Studies in animal models have further confirmed that viral DNA replicative intermediates, as well as viral transcript proteins, can be detected in PBMC under certain conditions. Molecular hybridization studies have described the presence