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Hepatitis B Virus Does Not Interfere With Innate Immune Responses in the Human Liver

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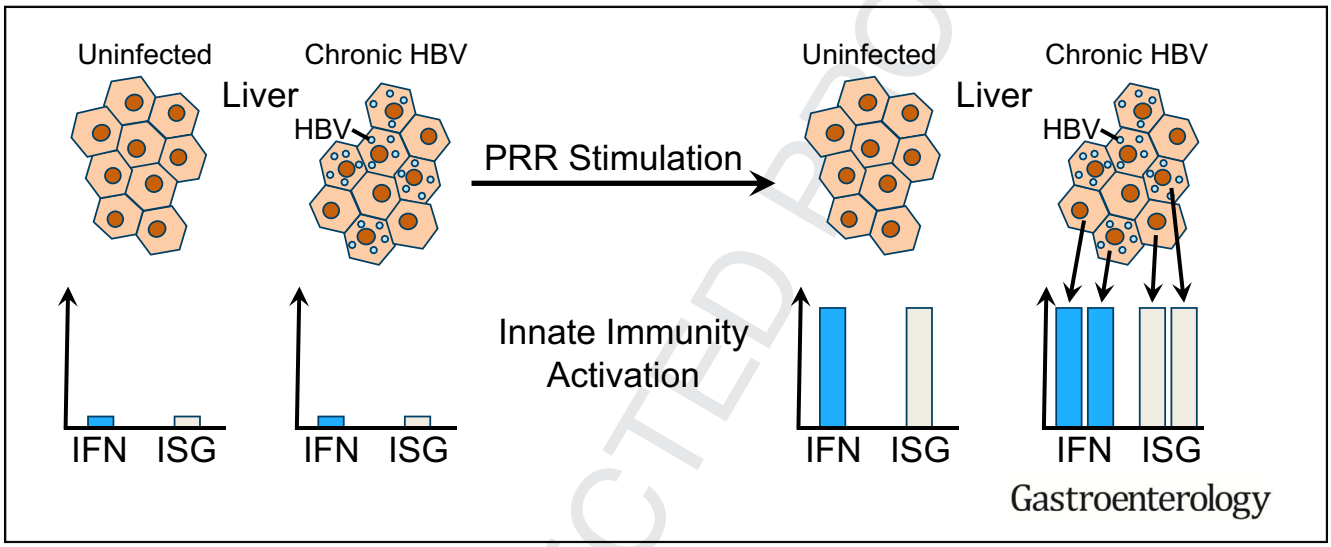

BACKGROUND & AIMS: Most viruses are detected at early stages of cell infection and induce an innate immune response mediated by production interferons (IFNs). IFNs induce expression of hundreds of IFN-stimulated genes (ISGs). Infection of chimpanzees with hepatitis C virus, but not hepatitis B virus (HBV), induces ISG expression in the liver. HBV might not induce an innate immune response because it is not detected by pattern recognition receptors (the stealth properties of HBV) or because HBV suppresses IFN production or signaling despite detection by pattern recognition receptors. We studied innate immune signaling in liver biopsies from patients with different stages of chronic HBV infection and uninfected individuals (controls). **METHODS:** We obtained liver within 10 minutes after collection from 30 patients with chronic HBV infection (hepatitis B e antigen-positive or -negative, with or without hepatitis) and 42 controls (most with fatty liver disease). The liver tissues were analyzed by histology, immunohistochemistry, quantitative reverse-transcription polymerase chain reaction, in situ hybridization, HBV RNA quantification, and HBV genotyping; some specimens were incubated with toll-like receptor (TLR) ligands (polyinosinic-polycytidylic acid) or infected with Sendai virus and then analyzed. **RESULTS:** Liver specimens from patients with HBV infection were not expressing more IFN or ISGs than those from control patients, indicating that chronic HBV infection did not activate an innate immune response. However, liver specimens from patients with HBV infection did produce IFN and induce expression of ISGs following activation of TLR3 with poly(I:C) or Sendai virus infections, so the innate immune response is not suppressed in these tissues. **CONCLUSION:** Liver tissues from patients with chronic HBV infection do not have induction of an innate immune response, but this response can be activated by other factors (TLR3 binding, Sendai virus infection) in HBV-infected liver tissue. These findings support the hypothesis that HBV is invisible to pattern recognition receptors.

Keywords: PRR; Virus Immune Evasion; PAMP; Ex Vivo.

[§]Authors share co-senior authorship.

Abbreviations used in this paper: **CHB, chronic hepatitis B; CTRL, uninfected control; dsRNA, double-stranded RNA; HBcAg, HBV core protein; HBeAg, hepatitis B e antigen; HBsAg, HBV surface protein; HBV, hepatitis B virus; IFN, interferon; ISG, interferon-stimulated gene; ISH, in situ hybridization; JAK-STAT, Janus-associated kinase–Signal Transducer and Activator of Transcription; Mx1, Interferon-induced GTP-binding protein Mx1; OCT, optimum cutting temperature compound; poly(I:C), polyinosinic-polycytidylic acid; PRR, pattern recognition receptor; pSTAT1, phosphorylated STAT1; RIG-I, retinoic acid–inducible gene I; RLR, RIG-I-like receptor; SeV, Sendai virus; ssRNA, single-stranded RNA; TLR, Toll-like receptor.**

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EDITOR'S NOTES

BACKGROUND AND CONTEXT

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NEW FINDINGS

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LIMITATIONS

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IMPACT

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Most viruses activate the innate immune system in the cells they infect, because they bring along, or generate so-called pathogen-associated molecular patterns (PAMPs) (typically viral genomes or replication intermediates) that the host cell recognizes as foreign.[1] Cells detect those PAMPs using pattern recognition receptors (PRRs), such as cytoplasmic retinoic acid–inducible gene I (RIG-I)-like receptors (RLR) that specifically detect 5′-triphosphate-containing RNA and double-stranded RNA (dsRNA) in the cytoplasm of infected cells, and endosomal toll-like receptors (TLRs) that detect incoming dsRNA (TLR3), single-stranded RNA (ssRNA) (TLR7/8), or CpG motif-containing unmethylated DNA (TLR9).[1] The activation of these sensory pathways results in production of interferons (IFNs) and expression of interferon-stimulated genes (ISGs) that limit viral replication and spread.[2] During evolution, viruses have developed numerous strategies to escape from the host innate immune system, often involving active suppression of corresponding sensory pathways.[3]

Hepatitis B virus (HBV) is a small hepatotropic, noncytopathic DNA virus infecting humans and chimpanzees.[4] On primary infection, HBV spreads throughout the liver infecting up to 100% of hepatocytes and producing very high virus titers (up to $\sim 10^9$ – 10^{10} particles per mL of serum) until after 6 to 10 weeks the adaptive immune response takes control over the virus, which happens in approximately 90% of immunocompetent adults.[4,5] Approximately 5% to 10% of adult HBV infections and virtually all mother-to-infant transmissions result in chronic infection. Chronic hepatitis B (CHB) can lead to cirrhosis and liver cancer. It is estimated that HBV infections cause up to approximately 800,000 liver-related deaths per year worldwide.[6,7]

In vivo studies with experimentally infected chimpanzees showed that HBV does not induce an IFN/ISG response in the infected hepatocytes when it spreads through the liver.[8] In agreement with that, no induction of type I/III IFN was detected in the serum of human patients with acute hepatitis B infection.[9] These results suggested that the virus might not be detected by PRRs in infected cells, leading to the concept of HBV behaving like a "stealth virus."[10] Alternatively, HBV could actively interfere with downstream sensory pathways and suppress IFN induction despite being detected by PRRs. Evidence for such a transient activation followed by viral suppression of sensory pathways comes from recent work in cell culture.[11–14] Of note, one report described that early after HBV infection cells lose their ability to induce IFN-β in response to stimulation with poly(I/C) or Sendai virus (SeV) infection.[12] Finally, HBV could also block IFN-stimulated signal transduction through the JAK-STAT (Janus-associated kinase–Signal Transducer and Activator of Transcription) pathway to inhibit ISG induction in the liver. It is well known that efficient ISG induction depends on the amplification of the initial danger signal through autocrine stimulation of the IFN receptors followed by JAK-STAT signaling.[15] Inhibition of IFN signaling by HBV infection or overexpression of viral proteins has been demonstrated in cell culture work[16–18] and more recently in a humanized mouse model.[19]

Despite this substantial evidence for an active role of HBV in suppressing innate immunity, our knowledge of the innate immune response to HBV is still hampered by technical limitations. HBV in vitro model systems do not accurately reflect the situation of in vivo HBV infection, as they are typically conducted with much higher virus and subviral particle concentrations than those achieved during natural HBV infection in humans or chimpanzees. Data from early infection states in humans are very sparse because of the difficulty in recruiting patients at the earliest presymptomatic stages of HBV infections. Experiments with chimpanzees are limited by ethical constraints and high costs.

In the present work, we developed and validated an ex vivo method using freshly obtained liver biopsies from patients with different stages of chronic HBV infection and from controls. Although we could not investigate patients with early acute HBV infection, we reasoned that inhibition of innate immunity by HBV should by detectable in ex vivo liver tissue when liver cells would be stimulated with TLR agonists or by productive viral infections.

The ex vivo analysis of liver tissue turned out to be a robust and highly informative experimental system. Freshly obtained human liver biopsies could be cultured for a least 24 hours without significant cell death or RNA degradation. The samples could be treated with TLR agonists and infected with SeV. Induction of IFNs and ISGs was readily detectable and quantifiable. Comparing liver biopsies from HBV-infected patients with uninfected controls, we could not detect any inhibition of innate responses by HBV. In situ hybridization (ISH) and immunostaining techniques allowed confirmation of this finding at the cellular level. Collectively, our data unequivocally demonstrate that the cell-autonomous innate immune system in HBV-infected human liver is intact, and support the hypothesis that HBV behaves like a "stealth" virus in vivo.