Hepatitis B virus X protein: TRIMming antiviral defences in hepatocytes

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Humans and viruses have coevolved over thousands of years; however, very few viruses are able to manifest as chronic infections with most being cleared after an acute course. Consequently, success of the human species has relied on a functional immune system that is capable of fighting off most viral pathogens. The heptatitis B virus (HBV) is one of the most successful viruses to establish chronic infection in man with over 300 million individuals currently infected worldwide and over 2 billion humans having been infected by this virus.[1] These numbers underscore the tremendous ability of HBV to thwart the human immune system and establish chronic infection. In this issue of *Gut*, Lim *et al*[2] have provided evidence for a new mechanism through which HBV is able to establish and maintain chronic infection.

They specifically examine the role of the enigmatic HBx protein[3] in the regulation of TRIM22, a protein that has increasingly become associated with innate antiviral responses.[4 5] Previous studies have demonstrated that HBx, a virally encoded protein that plays unusual roles in its life cycle, can

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block the antiviral response in addition to other interactions with hepatocyte proteins.[6] However, the authors now propose a new model where the HBx protein blocks TRIM22 transcription leading to a decrease in interferon (IFN)-induced TRIM22 expression. IFNs are potent broadly acting antiviral effector molecules mainly produced by infected hepatocytes[7] and liver-infiltrating HBV-specific T cells.[8] TRIM22 is one of many antiviral genes induced by IFN as part of its multipronged antiviral defence.[9]

The authors demonstrated that this mechanism was specific for the HBx protein and not due to other viral factors (eg, HBV core and polymerase proteins). In addition, the effect of HBx is targeted to TRIM22 and not directly on other IFN-stimulated genes that they evaluated. Furthermore, downregulation of TRIM22 facilitated HBV replication and viral protein expression in vitro. These results imply that the inhibitory effect of HBx is specific to TRIM22 and this is a mechanism where HBV can increase its ability to infect and subsist in hepatocytes.

The authors also examined the underlying mechanism through which the HBx protein carries out this effect. They found that of the four CpG sites in the promoter region of TRIM22, all were methylated with HBx expression but only the third (position 71) was sensitive to 5′-aza-2′-deoxycytidine (5AzaC) treatment, a DNA methylation inhibitor, implying that this site was actively methylated by HBx during infection. This would result in a decrease in promoter activity and impaired TRIM22 expression. This was also confirmed using mutant constructs in the various CpG sites. They subsequently demonstrated that methylation at this site impaired IRF1 binding, an important transcription factor in the antiviral response,[10] which could be restored in the presence of 5AzaC treatment. The data demonstrate that only the third CpG (+71) site is responsible for both promoter activity and the HBx-mediated suppression of TRIM22. The implication of the HBx protein control of gene and protein expression has broad implications. Recently, several studies have demonstrated an interaction between the SMC5/6 complex and HBx that promotes HBV gene expression.[11 12] The intersection of this previously published mechanism and that involving HBx control of DNA methylation discussed here could be significant for the viral life cycle, establishing chronic infection and mechanism of hepatocarcinogenesis.

Importantly, this study validated many of their findings using several distinct models of HBV infection including HepG2-NTCP cells, the HepaRG cell line, primary human hepatocytes and, finally, human liver tissues.[13] Furthermore, this viral strategy to block immunity occurs at different levels immunity. The IFNs are central players in antiviral defence and are also used as an exogenous antiviral treatment for HBV. The authors have demonstrated that this mechanism can block antiviral activity of both type I and II IFNs through TRIM22 regulation. Since IFNγ, type II IFN, mainly mediates the antiviral effect of HBV-specific cytotoxic T lymphocytes, the results suggest a mechanism of how HBV evades the adaptive and innate immune system. Similarly, since HBx also suppressed IFNα-induced TRIM22 expression, their results also suggest a mechanism of why the response to pegylated IFNα treatment is not as effective as would be predicted. The IFNs continue to be used in therapy against HBV with innovative combinations of IFN and nucleosides/nucleotides being pursued to facilitate cure.[14] Additional clinical studies to study the interaction between the HBx protein and TRIM22 in patients undergoing IFN therapy would be useful to further validate these findings.

Several exciting questions remain in the field based on this work. First, is this mechanism present in other organisms that are susceptible to chronic infection with HBV-related viruses (eg, woodchuck, ducks)? In addition, although the authors demonstrate an antiviral effect of TRIM22 on HBV replication in vitro, the biological relevance of this on HBV replication in humans is unknown; however, additional data from in vivo models using HBV-related viruses could be used to study this further. Another area that is left unanswered is when is this pathway most important during infection? Specifically, is it in effect during all four of the known clinical phases of HBV infection (eg, immune tolerant, immune active)? Lastly, can this mechanism be targeted to aid in the development of novel therapeutic strategies to facilitate a durable cure for chronic HBV infection?[15] In summary, Lim *et al* have put forth a novel and exciting mechanism for HBV persistence and suppression of host antiviral responses, involving the HBx protein, that may be amenable to future therapeutic intervention.

Contributors ET has written the submitted commentary.

Competing interests None declared.

Provenance and peer review Commissioned; internally peer reviewed.

To cite Thomas E. *Gut* 2018;**67**:3–5.

Received 3 April 2017
Revised 10 April 2017
Accepted 11 April 2017
Published Online First 5 May 2017

► http://dx.doi.org/10.1136/gutjnl-2016-312742

Gut 2018;**67**:3–5.
doi:10.1136/gutjnl-2017-314013

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