Antiviral Research 226 (2024) 105896

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

The lack of HBsAg secretion does neither facilitate induction of antiviral T cell responses nor Hepatitis B Virus clearance in mice

Thomas Michler [a,b,1], Jakob Zillinger [a,1,2], Philipp Hagen [a], Fuwang Cheng [a], Julia Festag [a,c], Anna Kosinska [a,c], Ulrike Protzer [a,c,*]

[a] Institute of Virology, School of Medicine and Health, Technical University of Munich / Helmholtz Munich, Germany
[b] Institute of Laboratory Medicine, LMU University Hospital, LMU Munich, Germany
[c] German Center for Infection Research (DZIF), Munich Partner Site, Germany

ARTICLE INFO

Keywords:
HBV
Chronic hepatitis B
HBsAg
Immunotolerance
siRNA
Therapeutic vaccination

ABSTRACT

Immune tolerance to the hepatitis B virus (HBV) is crucial for developing chronic hepatitis B, and the HBV surface antigen (HBsAg) produced and secreted in high amounts is regarded as a key contributor. HBsAg is expressed in HBV-infected hepatocytes and those carrying an HBV integration. Whether either HBsAg secretion or the high antigen amount expressed in the liver determines its immunomodulatory properties, however, remains unclear. We, therefore, developed a novel HBV animal model that allowed us to study the role of secreted HBsAg. We introduced a previously described HBs mutation, C65S, abolishing HBsAg secretion into a replication-competent 1.3-overlength HBV genome and used adeno-associated virus vectors to deliver it to the mouse liver. The AAV-HBV established a carrier state of wildtype and C65S mutant HBV, respectively. We investigated antiviral B- and T-cell immunity in the HBV-carrier mice after therapeutic vaccination. Moreover, we compared the effect of a lacking HBsAg secretion with that of an antiviral siRNA. While missing HBsAg secretion allowed for higher levels of detectable anti-HBs antibodies after therapeutic vaccination, it did neither affect antiviral T-cell responses nor intrahepatic HBV gene expression, irrespective of the starting level. A treatment with HBV siRNA restricting viral antigen expression within hepatocytes, however, improved the antiviral efficacy of therapeutic vaccination, irrespective of the ability of HBV to secrete HBsAg. Our data indicate that clearing HBsAg from blood cannot significantly impact HBV persistence or T-cell immunity. This indicates that a restriction of hepatic viral antigen expression will be required to break HBV immunotolerance.

Dual-phase combinatorial treatment regimens that include a pre-treatment with a direct-acting antiviral drug followed by an immunostimulatory agent have emerged as one of the most promising strategies to cure chronic hepatitis B (CHB) (Lim et al., 2023). It is assumed that a crucial characteristic of the direct-acting antiviral should be to lower the high levels of viral antigens that are believed to cause immunotolerance to hepatitis B virus (HBV). While reverse-transcriptase inhibitors or core assembly modifiers primarily inhibit viral replication and only indirectly affect viral protein expression, monoclonal antibodies neutralize secreted hepatitis B surface antigen (HBsAg) (Lempp et al., 2023; Burm et al., 2023), nucleic acid polymers (NAPs) inhibit HBsAg secretion (Bazinet et al., 2020), and oligonucleotides such as short interfering (si) RNA (Yuen et al., 2023a; Gane et al., 2023) and antisense oligonucleotides (ASO) (Yuen et al., 2022) target HBV transcripts and prevent protein expression. However, none of these targets the HBV persistence form, the covalently closed circular DNA (cccDNA). Other strategies are trying to eliminate cccDNA (e.g. CRISPR/Cas, non-cytolytic effects of cytokines) or to kill virus-infected hepatocytes (e.g. bispecific antibodies, immunotoxins). While these approaches could theoretically achieve an HBV cure, they require that every single HBV-infected cell is targeted and all cccDNA can be successfully cleared. Thus, a curative therapy is still missing.

Immune-stimulatory approaches activate innate or adaptive immunity to control or even cure the HBV infection. Agents such as TLR agonists (Yuen et al., 2023b; Gane et al., 2021) or interferon α trigger the innate immune system but do not act in an HBV-specific fashion.

* Corresponding author. Institute of Virology, School of Medicine and Health, Technical University of Munich / Helmholtz Munich, Germany.
E-mail address: protzer@tum.de (U. Protzer).
[1] These authors contributed equally to the work.
[2] Current address: Department of Hematology, Oncology, and Stem Cell Transplantation, Freiburg University Medical Center.

https://doi.org/10.1016/j.antiviral.2024.105896
Received 31 January 2024; Received in revised form 9 April 2024; Accepted 23 April 2024
Available online 26 April 2024

Because T-cell responses strongly correlate with spontaneous HBV cure, alternative approaches are aiming at stimulating the adaptive immune system, such as PD-1/PD-L1 checkpoint inhibitors (Gilead Sciences, 2021; Bunse et al., 2022), antibodies with T cell stimulatory properties (Bournazos et al., 2020), adoptive T-cell therapy, or therapeutic vaccines (Brii et al., 2021; Cargill and Barnes, 2021).

We previously reported that combining an HBV-specific shRNA (Michler et al., 2016) or siRNA (Schlegel et al., 2022) followed by a therapeutic hepatitis B vaccine (*TherVacB*), developed in our group (Backes et al., 2016), achieved a cure in HBV-carrier mouse models (Michler et al., 2020). Two monthly injections of a liver-targeted siRNA that suppresses all HBV proteins preceded the immunization. A third siRNA injection was applied with the first vaccine injection. *TherVacB* consists of two i.m. injections of particulate, recombinant protein (HBsAg and HBcoreAg) formulated with ci-di-AMP as an adjuvant, followed by a Modified Vaccinia Virus Ankara (MVA) vector expressing HBs and HBcore (for further details, see Michler and Kosinska et al. (Michler et al., 2020)).

While the combination of an siRNA and a therapeutic vaccine is entering clinical evaluation (Brii et al., 2021), it remains unclear which drug classes would best synergize to break HBV immunotolerance. In this regard, key questions are (i) which viral protein the antiviral drug

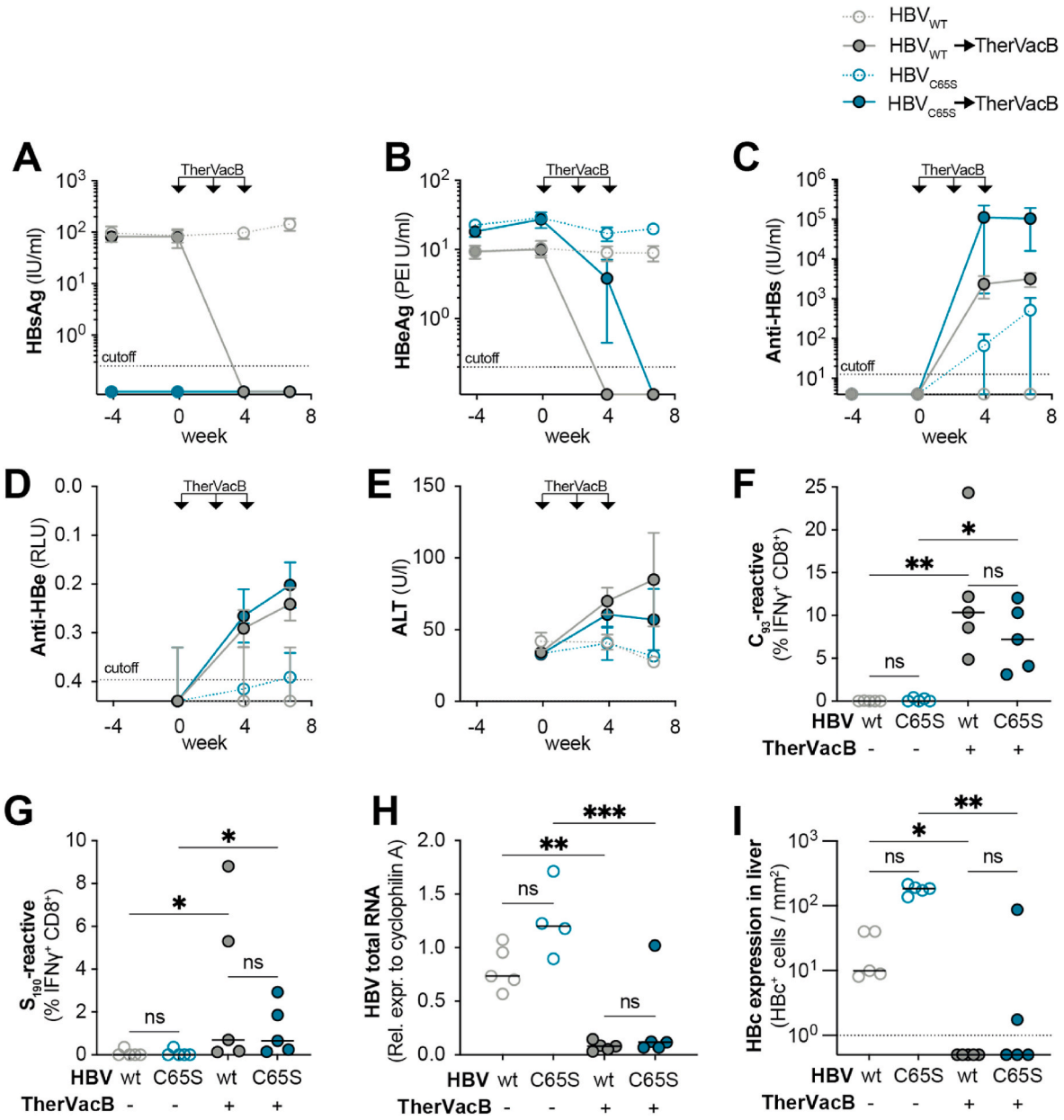

Fig. 1. Effect of HBsAg secretion on viral persistence and efficacy of TherVacB in mice expressing low levels of HBV. C57/BL6 mice were infected with 10e9 genome equivalents of Adeno-Associated Virus vectors carrying a 1.3 overlength genome of wildtype (HBV_{WT}) or C65S-mutant HBV (HBV_{C65S}). Eight weeks later, half of the mice (n = 5) from each group were immunized with TherVacB consisting of two bi-weekly intramuscular (i.m.) injections of ci-diAMP-adjuvanted HBsAg and HBcAg followed two weeks later by a boost with a recombinant Modified Vaccinia Ankara Virus expressing HBsAg and HBcAg. Course of HBV infection was evaluated by measuring serum (**A**) HBsAg, (**B**) HBeAg, (**C**) anti-HBs antibody, (**D**) anti-HBe antibody, and (**E**) ALT activity levels at indicated time points. (**F,G**) Intrahepatic virus-specific CD8 T cell responses were evaluated by ex vivo stimulation of liver-associated lymphocytes with (**F**) HBcAg derived C93 epitope, or (**G**) HBsAg derived S190 epitope and reactive CD8 T cells determined by flow cytometry following intra-cellular cytokine styining for IFNγ. Intrahepatic HBV gene expression was assessed by measuring (**H**) total HBV RNA from liver lysate, and (**I**) quantifying HBV-infected hepatocytes by immunohistochemical stainings for HBc. ns, non-significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

should target and (ii) in which compartment (i.e., liver or blood circulation) the respective viral protein must be reduced. The siRNAs used in our study targeted a common region of HBV transcripts and prevented the expression of all viral proteins, which were consequently absent from the liver and blood. Thus, it remains unclear which viral antigen caused immunotolerance and whether the local expression in the liver or the antigen secretion in large amounts was decisive.

To gain a better understanding of the role of secreted HBsAg, we developed a novel HBV-carrier mouse model in which HBsAg is expressed but not released from hepatocytes. For this, we used a previously described mutation that abolishes HBsAg secretion (Mangold and Streeck, 1993). Mangold et al. described that exchanging the cysteine at amino acid (aa) 65 of HBs to serin (C65S) completely prevented HBsAg secretion, while intracellular HBs protein levels remained unaffected.

We successfully introduced the T193A mutation in the S open reading frame, causing a C65S exchange in a plasmid carrying a 1.3-overlength genome of HBV genotype D by site-directed mutagenesis. We could confirm that HBsAg was expressed in hepatocytes but not secreted from cells that were either transfected with the HBV-expressing plasmid or transduced by an adeno-associated virus (AAV) vector carrying the mutated HBV genome (Suppl. Figs. S1A–C). We next evaluated how missing HBsAg secretion would impact viral persistence and immune-mediated HBV clearance in a setting of low-level HBV gene expression. The relatively low antigen level expressed results in a mild immunotolerance, which can be broken by therapeutic vaccination (Michler et al., 2020). Therefore, C57/BL6 mice were infected with a relatively low dose (10^9 genome equivalents [geq]) of AAV-HBV expressing either wildtype (HBV_{WT}) or C65S mutant (HBV_{C65S}) HBV. Animals carrying HBV_{WT} displayed serum HBsAg levels around 200 IU/ml and HBeAg levels of 10–15 PEI U/ml. As expected, no HBsAg was detected in the serum of mice carrying HBV_{C65S}, while HBeAg levels of 20–30 PEI U/ml indicated persistent HBV gene expression over the whole course (16 weeks) of the experiment (Fig. 1A and B). We confirmed the expression of HBsAg in the liver of these mice by immunohistochemical staining (Suppl.Fig. S1D).

To evaluate how missing HBsAg secretion would affect the efficacy of therapeutic vaccination, we immunized half of the mice with *TherVacB* starting eight weeks after the AAV-HBV injection. Vaccination resulted in a loss of serum HBsAg and anti-HBs seroconversion in all mice. Anti-HBs serum titers were approximately ten times higher in HBV_{C65S}-carrier mice, probably because HBsAg-antiHBs complexes in mice replicating HBV_{WT} reduced the detectable anti-HBs. *TherVacB* induced an anti-HBe seroconversion with loss of HBeAg irrespective of whether mice replicated HBV_{WT} or HBV_{C65S} (Fig. 1B–D). Immunized mice presented with mildly elevated ALT activities (Fig. 1E). These correlated with the induction of intrahepatic virus-specific CD8 T-cell responses detected in all vaccinated mice. S- and core-specific T-cell responses were similar in mice replicating HBV_{WT} and HBV_{C65S} (Fig. 1F and G).

We next assessed intrahepatic HBV gene expression after vaccination of HBV_{WT}- and HBV_{C65S}-carrier mice. HBsAg secretion did not affect intrahepatic HBV-RNA levels (Fig. 1H) or the number of HBc-expressing hepatocytes (Fig. 1I). *TherVacB* suppressed both intrahepatic viral RNA and HBc^+ hepatocytes to below the detection limit in almost all vaccinated mice, irrespective of which HBV variant the mice carried. In summary, this showed that HBsAg secretion had no significant impact on HBV persistence or virus-specific immune responses, neither in untreated nor in immunized mice carrying HBV at low to intermediate levels.

We next evaluated if inhibition of HBsAg secretion would benefit in a setting of high-level HBV gene expression in which *TherVacB* alone would not suffice to break immunotolerance. For this, we infected animals with a ten-fold higher dose of AAV-HBV (10^{10} geq). In addition, we directly compared the effects of missing HBsAg secretion and pretreatment with an antiviral siRNA reducing HBs expression on the efficacy of *TherVacB*.

High-level HBV_{WT}-carrier mice displayed HBsAg levels above 1.000 IU/ml. At the same time, HBsAg remained negative in HBV_{C65S}-carriers (Fig. 2A). Both groups of mice presented HBeAg levels around 200 PEI U/ml (Fig. 2B). Eight weeks after AAV-HBV injection, we initiated a combinatorial siRNA and vaccination treatment. Mice received three monthly subcutaneous (s.c.) injections of either an HBV-specific siRNA (siHBV) or a control siRNA (siCtrl) followed by immunization with *TherVacB*. Treatment with siHBV reduced serum HBeAg by 1.5 $\log_{10}$ and serum HBsAg in HBV_{WT}-carrier mice by almost 3 $\log_{10}$ (Fig. 2A and B). As described before (Michler et al., 2020), siHBV-pretreated animals responded better to *TherVacB*. This was illustrated by the loss of serum HBsAg in HBV_{WT}-infected mice and an additional decline of HBeAg levels in HBV_{C65S} and HBV_{WT}-infected mice that had received siHBV before *TherVacB*. Similar to our first experiment, anti-HBs titers were 10-fold higher in HBV_{C65S}-infected mice, with no difference between siCtrl- and siHBV-pretreated groups (Fig. 2C). Two of the siHBV-pretreated mice (one replicating HBV_{C65S} and the other HBV_{WT}) seroconverted to anti-HBe (Fig. 2D).

Animals from siHBV-treated groups developed mild hepatitis, which was independent of the type of HBV they carried (Fig. 2E). This correlated with increased HBc-specific CD8 T-cell responses in siHBV-pretreated mice, which was statistically significant in HBV_{WT} and a clear trend in HBV_{C65S}-infected animals. No differences were seen in the HBs-specific CD8 T cell responses between both treatment groups (Fig. 2G). When assessing viral gene expression in the liver, we once again found that HBsAg secretion did not affect the clearance of HBV. Pre-treatment with siHBV, however, significantly suppressed HBV gene expression, irrespective of whether HBsAg was secreted or not. This was illustrated by nearly equal levels of intrahepatic HBV RNA (Fig. 2H) and HBc^+ hepatocytes (Fig. 2I) in both HBV_{C65S} and HBV_{WT} carrier mice.

In summary, this experiment showed that in a setting of high-level HBV gene expression where *TherVacB* alone did not suffice to control HBV, blocking HBsAg secretion had no significant impact on the efficacy of therapeutic vaccination while reducing HBV protein expression improved vaccine efficacy.

Extensive efforts are currently being made by academia and industry to develop a curative, finite treatment for CHB that enables sustained HBV control after the stoppage of antiviral therapy. Directly acting antivirals neither eliminate HBV cccDNA nor activate the endogenous immune response. Therefore, they could only achieve a cure if cccDNA was spontaneously lost (e.g., through cell division) or by a spontaneous reconstitution of antiviral immunity. Immunotherapies, in contrast, aim at reconstituting antiviral immunity or inducing new B- and T-cell responses. However, immunotherapies face HBV-induced immunotolerance, which prevents the generation of curative immune responses, especially under high-level HBV antigen production persisting for a long time (Iannacone and Guidotti, 2022) supported by the integration of defective HBV genomes (Wooddell et al., 2017). Consequently, dual-phase combinatorial therapies that first reduce viral antigens followed by an immunostimulatory approach are in focus.

In our study, we evaluated how HBsAg secretion affects the course of HBV infection alone or in the context of therapeutic vaccination. Because there are no drugs available that block HBsAg secretion in rodents, we generated a genetic model that prevents HBsAg secretion by HBV but does not affect antigen expression in the liver (Mangold and Streeck, 1993). We compared the effect of a lack of HBsAg secretion with an antiviral siRNA and assessed possible synergistic effects of inhibiting HBsAg secretion and reducing HBV antigens by an antiviral siRNA. While missing HBsAg secretion led to higher levels of detectable anti-HBs antibodies, it did not affect virus-specific T-cell responses, intrahepatic HBV persistence, or the efficacy of therapeutic vaccination. This finding was independent of the level of HBV infection or whether mice were treated with a therapeutic vaccine alone or combined with an antiviral siRNA.

Several approaches are being developed to reduce the induction of immune tolerance by high levels of HBV antigens. ASOs and siRNAs

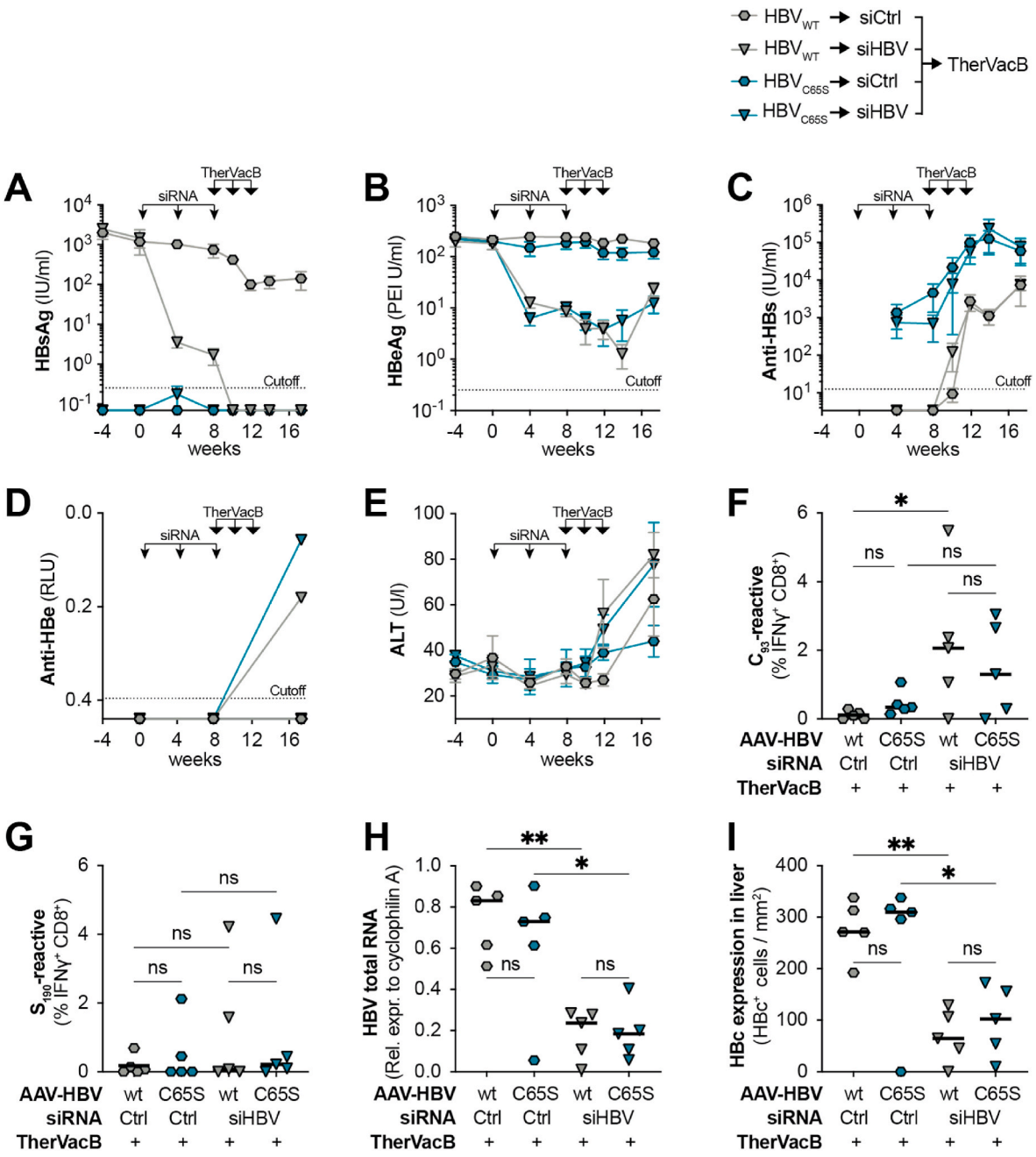

Fig. 2. Comparison of the effects of inhibition of HBsAg secretion with antiviral siRNA on efficacy of therapeutic vaccination for CHB. C57/BL6 mice were infected with 10e10 genome equivalents of Adeno-Associated Virus vectors carrying a 1.3 overlength genome of wildtype (HBV_{WT}) or C65S-mutant HBV (HBV_{C65S}). Starting eight weeks later, mice were treated with three monthly subcutaneous injections of an HBV-specific siRNA (siHBV) with half of mice (n = 5) receiving a control siRNA (siCtrl). Together with the last siRNA application, immunization with TherVacB was initiated in all mice. Course of HBV infection was evaluated by measuring serum levels of (**A**) HBsAg, (**B**) HBeAg, (**C**) anti-HBs antibodies, (**D**) anti-HBe antibodies, and (**E**) ALT activity. (**F,G**) Intrahepatic virus-specific CD8 T cell responses were evaluated by ex vivo stimulation of liver-associated lymphocytes with (**F**) HBcAg derived C93 epitope, or (**G**) HBsAg derived S190 epitope and the fraction reactive CD8 cells determined by flow cytometry following intra-cellular cytokine staining for IFNγ. Intrahepatic HBV gene expression was assessed by measuring (**H**) total HBV RNA from liver lysate, and (**I**) quantifying HBV-infected hepatocytes by immunohistochemical stainings for HBc. ns, non-significant; *, $p < 0.05$; **, $p < 0.01$.

reduce HBV proteins in the liver and blood; monoclonal antibodies or HBsAg secretion inhibitors mainly affect serum HBsAg levels. Mouse studies in which neutralizing antibodies were used to lower serum HBsAg levels reported conflicting data regarding their effect on HBV immunotolerance. While an initial study reported that pretreatment with a neutralizing antibody enhanced the efficacy of a therapeutic vaccine (Zhu et al., 2016), another study reported that HBsAg clearance had minimal impact on antiviral CD8 T cell responses (Fumagalli et al., 2020). A drug class whose effect on HBV immunotolerance is unclear is NAPs. NAPs are thought to act primarily as HBsAg secretion inhibitors. As they lack activity in preclinical animal models (Schoneweis et al., 2018), knowledge of their mode of action and their immune-stimulatory properties is scarce.

A crucial question is whether viral antigens expressed within hepatocytes, in contrast to secreted antigens, cause HBV-specific T-cell tolerance. We found that circulating HBsAg did not impact CD8 T cell

responses induced by therapeutic vaccination or its antiviral activity. Reducing intrahepatic antigen levels by siRNA, in contrast, led to an improved HBcore-specific T-cell response and an improved antiviral effect. Our data are in accordance with Fumagalli et al. (2020), who reported that reducing circulating HBsAg by neutralizing antibodies has only a minimal effect on the expansion of HBV-specific naive CD8 T cells undergoing intrahepatic priming. The current study alone, however, does not allow to completely disentangle the roles of secreted compared to locally expressed HBV antigens, as the siRNA did not only restrict the expression of HBsAg but also of all other viral proteins. Due to the highly condensed HBV genome with largely overlapping open reading frames, it is not possible to target the HBs-encoding transcripts selectively. Thus, the expression of HBV core and polymerase, and the secretion of HBeAg, are also affected by a siRNA, so we cannot exclude that they mediate the antiviral effect. In a prior study, however, we have already demonstrated that HBeAg expression does not affect the efficacy of therapeutic vaccination (Kosinska et al., 2021). HBeAg-positive and HBeAg-negative HBV carrier mice responded equally well. We, therefore, reason that viral proteins within hepatocytes crucially contribute to HBV immunotolerance.

A consequence of the small size of the HBV genome and its condensed organization is that the mutation, which we introduced in the S open reading frame, not only results in the desired exchange of cysteine to serine at position 65 of HBsAg but also inserts a stop codon at position 408 of the polymerase open reading frame. However, this was not a major confounding factor in the context of our study because both changes prevent the secretion of mature virions. While HBV transcripts are transcribed by the cellular DNA-depended RNA Polymerase II (Rall et al., 1983), the HBV polymerase reversely transcribes HBV pregenomic RNA to relaxed circular DNA within newly assembled capsids. Thus, the main effect of a lacking HBV polymerase function would be a higher rate of RNA-containing, immature capsids and no secretion of mature virions. The mutated HBV genome persisted in hepatocytes as long as the *wild-type* HBV genome and T-cell responses targeting both variants were very comparable. Thus, we concluded that the defective polymerase is not relevant to the conclusions drawn from our study.

While our study was only designed to determine the role of secreted HBsAg, our data fits with numerous other studies that show that reducing viral antigens within the liver may be key to breaking HBV immunotolerance. First, exhaustion of virus-specific CD8 T cells by high viral antigen levels is a well-known phenomenon affecting even viruses that do not secrete antigens (Utzschneider et al., 2016). The influence of a high-antigen load on CD8 T cell fate has been confirmed for hepatocyte-primed CD8 T cells (Tay et al., 2014). However, the liver has a unique and important role in inducing immunotolerance, particularly in viral hepatitis (Knolle and Thimme, 2014). Interestingly, tolerance to an exogenous antigen occurs especially if antigens are presented to CD8 T cells by hepatocytes (Morimoto et al., 2007; Wiegard et al., 2007). Such hepatocyte-primed T cells are unable to differentiate into effector cells (Benechet et al., 2019) and become highly prone to apoptosis (Lopes et al., 2008), reducing their survival (Bertolino et al., 1998). Moreover, hepatocytes can also directly kill T cells through emperiopolesis (Benseler et al., 2011). HBV proteins were reported to reduce MHC-I expression on hepatocytes (Chen et al., 2006), manipulate the innate immune system (Kuipery et al., 2020), and induce the immunosuppressive cytokine TGFβ-1 (Yoo et al., 1996), all of which may contribute to immunotolerance.

However, our model does not completely reflect the properties of drugs targeting HBsAg secretion or neutralizing serum HBsAg. For instance, neutralizing monoclonal antibodies have immunostimulatory properties by stimulating T cells via their Fc region (Bournazos et al., 2020). It cannot be excluded that NAPs affect cellular processes that promote HBV clearance. Furthermore, Bulivertide, which inhibits viral entry and spread, will likely lower the intrahepatic HBV antigen load in the long run by reducing the number of HBV-infected hepatocytes and may induce immunity against the HBV preS. Thus, clinical studies investigating respective drugs must thoroughly evaluate whether the treatment affects antiviral T-cell immunity by applying elaborate assays.

Another conclusion from our study is that for antiviral drugs with novel modes of action, the predictive power of established diagnostic markers might lose prognostic value. For example, serum HBsAg and even anti-HBs seroconversion in our study neither reflected intrahepatic HBV replication nor the state of immunotolerance. With the repertoire of HBV drugs becoming more complex, the role of novel and individually applied diagnostic markers will become more important.

In summary, we present a novel animal model for CHB, which allowed us to study the effects of HBsAg secretion on viral persistence, antiviral immunity, and efficacy of therapeutic vaccination. Our data indicate that clearing HBsAg from serum alone has no impact on HBV persistence or the ability to induce HBV-specific immunity with therapeutic vaccination. It further indicates that restriction of hepatic viral antigen expression may be key to breaking HBV immunotolerance and developing curative combinatorial therapies for CHB.

CRediT authorship contribution statement

Thomas Michler: Writing – original draft, Visualization, Validation, Project administration, Investigation, Formal analysis, Data curation. **Jakob Zillinger:** Writing – review & editing, Investigation, Formal analysis. **Philipp Hagen:** Investigation, Data curation. **Fuwang Cheng:** Investigation. **Julia Festag:** Investigation. **Anna Kosinska:** Investigation. **Ulrike Protzer:** Writing – review & editing, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2024.105896.

References

Backes, S., et al., 2016. Protein-prime/modified vaccinia virus Ankara vector-boost vaccination overcomes tolerance in high-antigenemic HBV-transgenic mice. Vaccine 34, 923–932. https://doi.org/10.1016/j.vaccine.2015.12.060.

Bazinet, M., et al., 2020. Safety and efficacy of 48 Weeks REP 2139 or REP 2165, tenofovir disoproxil, and pegylated interferon alfa-2a in patients with chronic HBV infection naive to nucleos(t)ide therapy. Gastroenterology 158, 2180–2194. https://doi.org/10.1053/j.gastro.2020.02.058.

Benechet, A.P., et al., 2019. Dynamics and genomic landscape of CD8(+) T cells undergoing hepatic priming. Nature 574, 200–205. https://doi.org/10.1038/s41586-019-1620-6.

Benseler, V., et al., 2011. Hepatocyte entry leads to degradation of autoreactive CD8 T cells. Proc. Natl. Acad. Sci. U. S. A. 108, 16735–16740. https://doi.org/10.1073/pnas.1112251108.

Bertolino, P., Trescol-Biemont, M.C., Rabourdin-Combe, C., 1998. Hepatocytes induce functional activation of naive CD8+ T lymphocytes but fail to promote survival. Eur. J. Immunol. 28, 221–236. https://doi.org/10.1002/(SICI)1521-4141(199801)28:01<221::AID-IMMU221>3.0.CO;2-F.

Bournazos, S., Corti, D., Virgin, H.W., Ravetch, J.V., 2020. Fc-optimized antibodies elicit CD8 immunity to viral respiratory infection. Nature 588, 485–490. https://doi.org/10.1038/s41586-020-2838-z.

Brii, B., Vir Biotechnology, I. & Inc, V. B. I. V. Brii Biosciences, 2021. Vir Biotechnology, and VBI Vaccines Announce Initiation of Phase 2 Clinical Trial of BRII-835 (VIR-2218) in Combination with BRII-179 (VBI-2601) for the Treatment of Hepatitis B | Vir. Press release.

Bunse, T., Kosinska, A.D., Michler, T., Protzer, U., 2022. PD-L1 silencing in liver using siRNAs enhances efficacy of therapeutic vaccination for chronic hepatitis B. Biomolecules 12. https://doi.org/10.3390/biom12030470.

Burm, R., et al., 2023. A human monoclonal antibody against HBsAg for the prevention and treatment of chronic HBV and HDV infection. JHEP Rep 5, 100646. https://doi.org/10.1016/j.jhepr.2022.100646.
Cargill, T., Barnes, E., 2021. Therapeutic vaccination for treatment of chronic hepatitis B. Clin. Exp. Immunol. 205, 106–118. https://doi.org/10.1111/cei.13614.
Chen, Y., Cheng, M., Tian, Z., 2006. Hepatitis B virus down-regulates expressions of MHC class I molecules on hepatoplastoma cell line. Cell. Mol. Immunol. 3, 373–378.
Fumagalli, V., et al., 2020. Serum HBsAg clearance has minimal impact on CD8+ T cell responses in mouse models of HBV infection. J. Exp. Med. 217 https://doi.org/10.1084/jem.20200298.
Gane, E.J., et al., 2021. Safety, pharmacokinetics, and pharmacodynamics of the oral TLR8 agonist selgantolimod in chronic hepatitis B. Hepatology 74, 1737–1749. https://doi.org/10.1002/hep.31795.
Gane, E., et al., 2023. Evaluation of RNAi therapeutics VIR-2218 and ALN-HBV for chronic hepatitis B: results from randomized clinical trials. J. Hepatol. 79, 924–932. https://doi.org/10.1016/j.jhep.2023.05.023.
Gilead Sciences, I., 2021. Gilead and Vir Biotechnology Establish Clinical Collaboration to Explore Combination Strategies for Functional Cure for Chronic Hepatitis B Virus. Press release.
Iannacone, M., Guidotti, L.G., 2022. Immunobiology and pathogenesis of hepatitis B virus infection. Nat. Rev. Immunol. 22, 19–32. https://doi.org/10.1038/s41577-021-00549-4.
Knolle, P.A., Thimme, R., 2014. Hepatic immune regulation and its involvement in viral hepatitis infection. Gastroenterology 146, 1193–1207. https://doi.org/10.1053/j.gastro.2013.12.036.
Kosinska, A.D., et al., 2021. Immunogenicity and antiviral response of therapeutic hepatitis B vaccination in a mouse model of HBeAg-negative, persistent HBV infection. Vaccines (Basel) 9. https://doi.org/10.3390/vaccines9080841.
Kuipery, A., Gehring, A.J., Isogawa, M., 2020. Mechanisms of HBV immune evasion. Antivir. Res. 179, 104816 https://doi.org/10.1016/j.antiviral.2020.104816.
Lempp, F.A., et al., 2023. Potent broadly neutralizing antibody VIR-3434 controls hepatitis B and D virus infection and reduces HBsAg in humanized mice. J. Hepatol. https://doi.org/10.1016/j.jhep.2023.07.003.
Lim, S.G., et al., 2023. The scientific basis of combination therapy for chronic hepatitis B functional cure. Nat. Rev. Gastroenterol. Hepatol. 20, 238–253. https://doi.org/10.1038/s41575-022-00724-5.
Lopes, A.R., et al., 2008. Bim-mediated deletion of antigen-specific CD8 T cells in patients unable to control HBV infection. J. Clin. Invest. 118, 1835–1845. https://doi.org/10.1172/JCI33402.
Mangold, C.M., Streeck, R.E., 1993. Mutational analysis of the cysteine residues in the hepatitis B virus small envelope protein. J. Virol. 67, 4588–4597. https://doi.org/10.1128/JVI.67.8.4588-4597.1993.
Michler, T., et al., 2016. Blocking sense-strand activity improves potency, safety and specificity of anti-hepatitis B virus short hairpin RNA. EMBO Mol. Med. https://doi.org/10.15252/emmm.201506172.
Michler, T., et al., 2020. Knockdown of virus antigen expression increases therapeutic vaccine efficacy in high-titer hepatitis B virus carrier mice. Gastroenterology 158, 1762–1775 e1769. https://doi.org/10.1053/j.gastro.2020.01.032.
Morimoto, J., Tan, X., Teague, R.M., Ohlen, C., Greenberg, P.D., 2007. Induction of tolerance in CD8+ T cells to a transgenic autoantigen expressed in the liver does not require cross-presentation. J. Immunol. 178, 6849–6860. https://doi.org/10.4049/jimmunol.178.11.6849.
Rall, L.B., Standring, D.N., Laub, O., Rutter, W.J., 1983. Transcription of hepatitis B virus by RNA polymerase II. Mol. Cell Biol. 3, 1766–1773. https://doi.org/10.1128/mcb.3.10.1766-1773.1983.
Schlegel, M.K., et al., 2022. From bench to bedside: improving the clinical safety of GalNAc-siRNA conjugates using seed-pairing destabilization. Nucleic Acids Res. 50, 6656–6670. https://doi.org/10.1093/nar/gkac539.
Schoneweis, K., et al., 2018. Activity of nucleic acid polymers in rodent models of HBV infection. Antivir. Res. 149, 26–33. https://doi.org/10.1016/j.antiviral.2017.10.022.
Tay, S.S., et al., 2014. Antigen expression level threshold tunes the fate of CD8 T cells during primary hepatic immune responses. Proc. Natl. Acad. Sci. U. S. A. 111, E2540–E2549. https://doi.org/10.1073/pnas.1406674111.
Utzschneider, D.T., et al., 2016. High antigen levels induce an exhausted phenotype in a chronic infection without impairing T cell expansion and survival. J. Exp. Med. 213, 1819–1834. https://doi.org/10.1084/jem.20150598.
Wiegard, C., et al., 2007. Defective T helper response of hepatocyte-stimulated CD4 T cells impairs antiviral CD8 response and viral clearance. Gastroenterology 133, 2010–2018. https://doi.org/10.1053/j.gastro.2007.09.007.
Wooddell, C.I., et al., 2017. RNAi-based treatment of chronically infected patients and chimpanzees reveals that integrated hepatitis B virus DNA is a source of HBsAg. Sci. Transl. Med. 9 https://doi.org/10.1126/scitranslmed.aan0241.
Yoo, Y.D., et al., 1996. Regulation of transforming growth factor-beta 1 expression by the hepatitis B virus (HBV) X transactivator. Role in HBV pathogenesis. J. Clin. Invest. 97, 388–395. https://doi.org/10.1172/JCI118427.
Yuen, M.F., et al., 2022. Efficacy and safety of bepirovirsen in chronic hepatitis B infection. N. Engl. J. Med. 387, 1957–1968. https://doi.org/10.1056/NEJMoa2210027.
Yuen, M.F., et al., 2023a. Efficacy and safety of the siRNA JNJ-73763989 and the capsid assembly modulator JNJ-56136379 (bersacapavir) with nucleos(t)ide analogues for the treatment of chronic hepatitis B virus infection (REEF-1): a multicentre, double-blind, active-controlled, randomised, phase 2b trial. Lancet Gastroenterol Hepatol 8, 790–802. https://doi.org/10.1016/S2468-1253(23)00148-6.
Yuen, M.F., et al., 2023b. TLR7 agonist RO7020531 versus placebo in healthy volunteers and patients with chronic hepatitis B virus infection: a randomised, observer-blind, placebo-controlled, phase 1 trial. Lancet Infect. Dis. 23, 496–507. https://doi.org/10.1016/S1473-3099(22)00727-7.
Zhu, D., et al., 2016. Clearing persistent extracellular antigen of hepatitis B virus: an immunomodulatory strategy to reverse tolerance for an effective therapeutic vaccination. J. Immunol. 196, 3079–3087. https://doi.org/10.4049/jimmunol.1502061.