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An HBV mRNA vaccine with favorable virological suppression and superior immunity

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Abstract

Clinical management of chronic hepatitis B (CHB) virus infection remains a big challenge and urges the development of novel therapeutics to achieve long-term virological control and seroconversion. In this study, we report on the development and evaluation of a highly efficacious therapeutic mRNA vaccine encoding the full-length hepatitis B virus (HBV) surface antigen (HBsAg). In pAAV-HBV1.2 and rAAV8-HBV1.3-transduced CHB mouse models, the HBV mRNA vaccine demonstrated potent therapeutic efficacy indicated by a complete serum viral clearance, a remarkable decline in intrahepatic HBcAg, viral DNA and RNA copies, as well as the induction of robust levels of anti-HBs antibodies, virus-specific T cells and memory B cells. In addition, the HBV mRNA vaccine induced strong innate immune activation, represented by the maturation of $CD8\alpha^+$ and $CD103^+$ cDC1, $CD11b^+$ cDC2, monocytes and neutrophils. Taken together, the HBV mRNA vaccine is a promising therapeutic candidate holding prospect for further development and clinical investigation.

Results and Discussion

CHB infection is one major cause of liver fibrosis, cirrhosis, and hepatocellular carcinoma and poses a major public health threat. Current CHB treatments such as nucleoside/nucleotide- and interferon (IFN)-based therapy, were unable to achieve sufficient viral clearance. In addition, multiple approaches to HBV therapeutic vaccines have been developed intensively, including recombinant protein-based subunit, adenoviral vectored, and DNA-based vaccines, but yet achieved efficient seroclearance of HBsAg and seroconversion of anti-HBs antibody (Ab) [1,2]. mRNA vaccines that contain antigen-encoding mRNAs encapsulated into for example lipid nanoparticle (LNP) have shown superior immunogenicity in eliciting both Ab and cellular-mediated immune responses over other types of vaccines. Several mRNA-based prophylactic or therapeutic vaccines for infections, malignancies or other diseases are currently being evaluated in clinical trials [3-6].

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In this study, we developed an HBV mRNA vaccine using our well-established platform [7]. The HBsAg-encoded mRNA sequence was designed using a proprietary artificial intelligence-based algorithm to ensure a high translation efficiency[8]. Methyl-pseudouridine-modified mRNA was synthesized using in-vitro transcription (IVT) procedure and showed enhanced antigen expression upon transfection into HEK-293T cells (Supplementary Figure 1). HBV mRNA vaccine formulation was prepared by encapsulating mRNAs into an ionizable lipid-based LNP system using a microfluidics-based procedure.

To evaluate the therapeutic efficacy of vaccine, rAAV-HBV1.2-transduced HBV-carrier mice were immunized with three doses of 5-μg or 10-μg HBV mRNA vaccine intramuscularly at a 1-week interval (Figure 1A). Compared to PBS-treated mice with a high serum HBsAg level, HBV mRNA vaccine-treated mice showed a rapid decline of serum HBsAg upon vaccination, which was even undetectable 7 days after the 3rd dose (Figure 1B). Clinical management of CHB remains challenging largely due to the failure to achieve seroconversion of anti-HBs Ab and the resulting HBV recurrence[4]. Magnitude of anti-HBs Ab response was therefore evaluated longitudinally (Figure 1C). HBV mRNA vaccination elicited robust anti-HBs Ab levels on day 21 and 35 after the treatment initiation, which offered a remarkable advantage for the vaccine since conventional therapy failed to achieve Ab seroconversion (Figure 1C). To assess whether the vaccination could induce sustained memory responses against HBV recurrence, mice were re-challenged with HBV on day 60 after the treatment initiation. As expected, PBS-treated mice demonstrated enhanced serum HBsAg level. While all HBV mRNA-vaccinated mice were fully protected against infection showing no detectable level of serum HBsAg (Figure 1D), which was accompanied by further augmented anti-HBs Ab titer upon viral challenge (Figure 1E). Moreover, intrahepatic HBV cccDNA, HBV DNA, HBV RNA, and HBcAg in hepatocytes from vaccinated mice were almost undetectable (Figure 1F&G). In addition, serum ALT remained at baseline levels during treatment,

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suggesting a safety profile of the mRNA vaccine without causing liver damage (Supplementary Figure 2). We also assessed the therapeutic efficacy of vaccine in rAAV8-HBV1.3 mouse model, which shows more efficient and homogeneous HBV transduction than models initiated by hydrodynamic injection [9]. Again, we found that the HBV mRNA-vaccinated mice demonstrated a remarkable decline in serum HBsAg level relative to control mice, and the anti-viral effect was maintained for at least 4 weeks (Figure 1H). The HBV mRNA vaccine showed more potent efficacy when compared to recently reported novel therapeutic ferritin nanoparticle-preS1 vaccine or anti-PDL1-IFN-α heterodimer combined with HBsAg vaccine tested using same animal model [10,11].

An optimal therapeutic vaccine for CHB treatment should have the potentials to trigger sufficient innate immune activation leading to robust antigen presentation and the resulting generation of HBV-specific cellular and humoral immunity [12,13]. To this end, we also monitored the innate immune responses induced at early time point (12 h) after the prime dose. Following vaccination, an increased infiltration of major dendritic cell (DC) subsets ($CD8\alpha^+$ cDC1s, $CD103^+$ cDC1s and $CD11b^+$ cDC2s) [14] into spleen was detected, accompanied by strong cell maturation (Figure 2A-C). Other innate cell subsets including macrophages, monocytes and neutrophils also showed phenotypic maturation, indicated by elevated expression of the costimulatory molecules (Figure 2D, Supplementary Figure 3).

We next evaluated the frequencies of class-switched memory B cells (MBCs) [15] and T cells specific to HBsAg. As expected, HBV mRNA vaccination induced a significantly expanded $HBsAg^+$ MBCs in bone marrow, which was more prominent in mice receiving 10-μg dose of vaccine (Figure 2E, Supplementary Figure 3). T cell exhaustion is one major hallmark of CHB infection and is characterized by decreased secretion of Th1-type cytokines (IFN-γ, IL-2, and TNF-α) and impaired cytotoxic and proliferative capacities[16,17]. The restored HBV-specific $CD8^+$ T cell function has been well-acknowledged to predict the efficacy of therapeutic vaccines for HBV. We

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therefore determined whether the vaccination could improve HBV-specific T cell responses. Following vaccination, we detected a significant increase in the proportion of HBV-specific $CD11a^{hi}CD8\alpha^{lo}$ cells [12,18] in the liver (Figure 2F), as well as a higher level of $CD8^+$ T cells in the spleen producing IFN-γ, TNF-α and IL-2 (Figure 2G), which suggested a clearly augmented HBV-specific $CD8^+$ T cell response.

Collectively, our study reported a HBV mRNA vaccine candidate with potent therapeutic efficacy. To our knowledge, this is the first reported mRNA vaccine candidate for CHB treatment. Three doses of HBV mRNA vaccines could efficiently clear HBV and achieve seroconversion of anti-HBs Ab. The strong innate immune activation and generation of functional HBV-specific $CD8^+$ T cells and class-switched $HBsAg^+$ MBCs by the mRNA vaccine may hold prospects of functional cure of CHB and prevention of HBV recurrence.

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Figure 1.

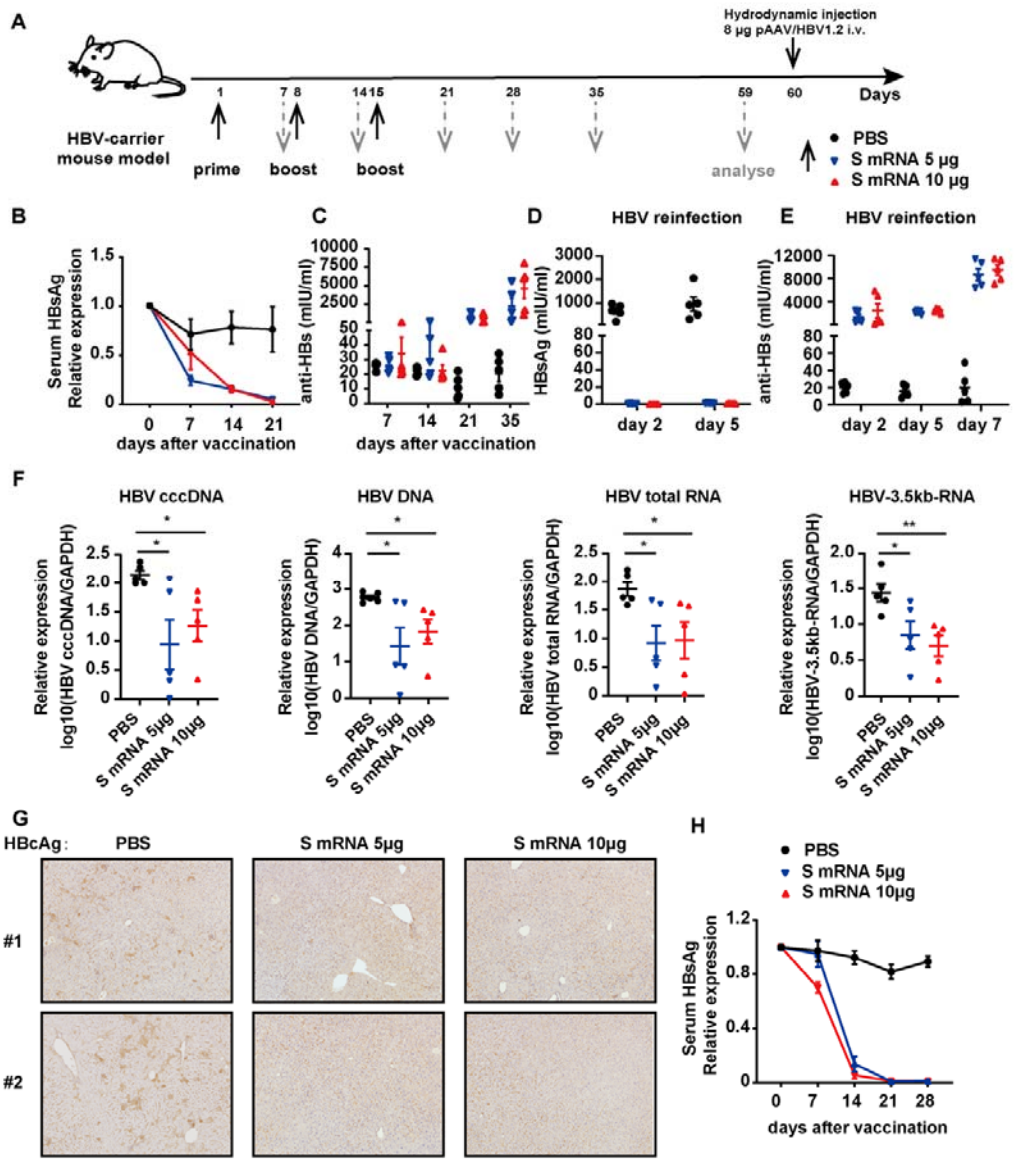

Figure 1. HBV mRNA vaccine efficiently eliminated HBV and achieved HBsAg seroconversion in AAV-HBV-transduced CHB mice.

(A) Study design. pAAV-HBV1.2-transduced HBV-carrier mice were immunized i.m. with HBV mRNA vaccine three times at a 1-week interval. At day 60, mice were re-challenged with 8 μg pAAV/HBV. PBS-treated mice were used as control. (B) Relative levels of serum HBsAg in mice were detected by CLIA. (C) Serum anti-HBs Ab levels were measured by ELISA. (D) Serum HBsAg and (E) anti-HBs levels after HBV re-challenge were evaluated by CLIA and ELISA, respectively. (F) Intrahepatic

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HBV intermediate products 3.5-kb RNA, HBV total RNA, HBV cccDNA, and HBV DNA were analyzed by qPCR and normalized against GAPDH expression on day 7 after re-challenge, and data were shown on log10 scale. (G) HBcAg expression in hepatocytes on day 7 after re-challenge was determined by IHC staining (magnification, × 200; scale bar, 100 μm). (H) rAAV8-HBV1.3-transduced HBV-carrier mice were immunized i.m. with HBV mRNA vaccine three times at a 1-week interval, with control mice administered with PBS. Serum HBsAg levels were detected by CLIA after treatment. Data represent the mean ± SEM (n ≥ 5). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ vs. controls. GAPDH, glyceraldehyde 3-phosphate dehydrogenase; SEM, standard error of the mean.

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Figure 2.

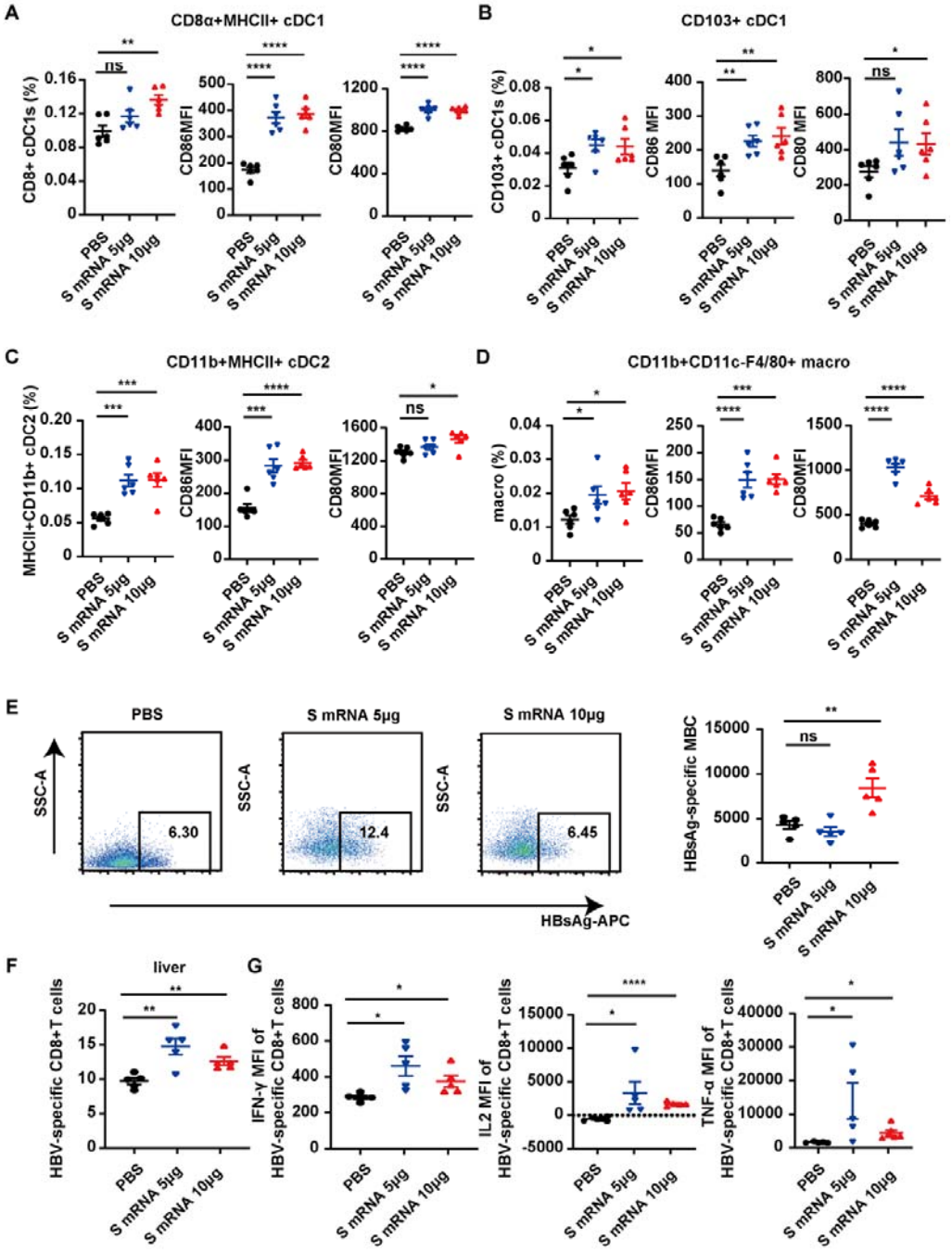

Figure 2. HBV mRNA vaccine induced strong innate immune activation and stimulated HBV-specific T cell and MBC responses.

(A-D) pAAV-HBV1.2-transduced HBV-carrier mice were immunized i.m. with HBV mRNA vaccine three times at a 1-week interval. At day 60, mice were re-challenged

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with 8 μg pAAV/HBV. PBS-treated mice were used as control. Splenic MNCs were harvested 12 hours after prime vaccination. Frequencies of $CD8\alpha^+CD11c^+$ DCs ($CD8\alpha^+$ cDC1s, A), $CD103^+$ $CD11c^+$ DCs ($CD103^+$ cDC1s, B) $CD11b^+$ $CD11c^+$ DCs (cDC2s, C) and $CD11b^+$ $CD11c^-$ $F4/80^+$ Macrophages (Macro, D), and expression of CD80 and CD86 on these cells were analyzed by flow cytometry. (E) Bone marrow-derived MNCs were harvested from HBV-carrier mice on day 7 after re-challenge. Frequencies of class-switched $HBsAg^+$ MBC in bone marrow (BM) were assessed (n=5). (F-G) Hepatic MNCs were harvested from HBV-carrier mice on day 7 after re-challenge. (F) The frequency of $CD11a^{hi}$ $CD8^{lo}$ cells among hepatic $CD8^+$ T cells. (G) Hepatic MNCs (2×10^6) were stimulated with PMA/ionomycin in vitro for 4 h in the presence of BFA (5 μg/mL), and the production of IFN-γ, TNF-α, IL-2 by HBV-specific $CD11a^{hi}$ $CD8^{lo}$ cells was analyzed by flow cytometry. Data represent the mean ± SEM (n ≥ 5). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$ vs. controls.

Author Contributions

A.L., H.Z., Y.Y. and J.Z. designed the project. H.Z., Y.Y., Z.L., L.H., A.Y., L.J., Z.W., Y.W., C.B., G.W., D.W., Q.J. and A.L performed experiments and analysis; A.L. and H.Z. discussed the data; H.Z., A.L. Y.Y. and J.Z wrote the manuscript.

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Competing Interests

The authors declare no competing interests.