BASIC STUDIES

Interleukin-15 suppresses hepatitis B virus replication via IFN-β production in a C57BL/6 mouse model

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Keywords

Abstract
Background: Interleukin-15 (IL-15) is a pleiotropic cytokine known to modulate both innate and adaptive immunity. It is suggested that IL-15 may play an important role in the regulation of immune response to hepatitis B virus (HBV). Aims: We investigated whether IL-15 could modulate the immune response to HBV. Methods: A mouse model for HBV tolerance was established by hydrodynamical injection of pAAV/HBV1.2 plasmid into C57BL/6 mice. This HBV-carrier mouse was simultaneously hydrodynamically injected with either an IL-15-expression plasmid pLIVE-IL-15 or a mock control vector pLIVE-EGFP. The serum levels of HBsAg and HBeAg were measured by radioimmunoassay. Results: Hydrodynamic injection of the plasmid pLIVE-IL-15 resulted in sustained high level of IL-15 in mouse serum, along with the markedly decreased serum HBsAg and HBeAg titres and liver HBV DNA levels. IL-15 also induced anti-HBV activity in T cell- and B cell-deficient Rag1−/− mice. Interestingly, despite an increase in NK cell numbers in both spleen and liver of IL-15 treated mice, the anti-HBV effect of IL-15 was neither dependent on presence of NK cells nor on production of IFN-γ. Furthermore, IL-15 could exert anti-HBV function independent of the common IL-2γR. Lastly, we found that IFN-β expression in the liver and serum was significantly up-regulated by liver expression of IL-15, and blockade of IFN-β function abrogated the anti-HBV activity of IL-15. Conclusions: Liver over-expression of IL-15 may suppress HBV replication in an IFN-β-dependent manner.

The hepatitis B virus (HBV) is a noncytopathic, hepatotropic virus that causes acute and chronic hepatitis and can increase the risk of hepatocellular carcinoma (1). During HBV infection, the immune system plays a pivotal role in eradication of the infection. Surprisingly, HBV appears to act as a ‘stealth virus’ in the early phase of infection (2) and sometimes seems not to induce a protective immune response (3). Although some papers have reported that activation of innate immunity (4, 5) and adoptively transferred HBV specific CD8+ T cells (6) could inhibit HBV replication in HBV transgenic mice, it remains largely unknown how immune system responds to HBV infection.

Interleukin-15 is a γ-common cytokine that is produced by a variety of cells such as dendritic cells, macrophages, epithelial and fibroblast cells (7). IL-15 is a pleiotropic cytokine with multiple effects on cells of the innate and adaptive immune system (7, 8). The published records suggest that IL-15 might be used as an immunomodulator in bone marrow transplantation (9), tumour immunotherapy (10) and anti-HIV therapies (7, 11). It has been reported that the innate and adaptive immune response to HBV is defective in patients with chronic HBV infection (12–14). Thus, it is conceivable that IL-15 might play an important role in modulating immune response to HBV.

Until recently, most in vivo studies on the mechanisms of HBV tolerance have been performed by using HBV transgenic mice (15, 16); however, HBV transgenic mice are inherently tolerant to HBV virus and have limitations in addressing the factors at the onset of infection that influence the final outcomes of chronic HBV infection. Huang et al. have created a nontransgenic mouse model of persistent HBV infection by hydrodynamic injection of pAAV/HBV1.2 plasmid into C57BL/6 mice. This HBV-carrier mouse was simultaneously hydrodynamically injected with either an IL-15-expression plasmid pLIVE-IL-15 or a mock control vector pLIVE-EGFP. The serum levels of HBsAg and HBeAg were measured by radioimmunoassay. Results: Hydrodynamic injection of the plasmid pLIVE-IL-15 resulted in sustained high level of IL-15 in mouse serum, along with the markedly decreased serum HBsAg and HBeAg titres and liver HBV DNA levels. IL-15 also induced anti-HBV activity in T cell- and B cell-deficient Rag1−/− mice. Interestingly, despite an increase in NK cell numbers in both spleen and liver of IL-15 treated mice, the anti-HBV effect of IL-15 was neither dependent on presence of NK cells nor on production of IFN-γ. Furthermore, IL-15 could exert anti-HBV function independent of the common IL-2γR. Lastly, we found that IFN-β expression in the liver and serum was significantly up-regulated by liver expression of IL-15, and blockade of IFN-β function abrogated the anti-HBV activity of IL-15. Conclusions: Liver over-expression of IL-15 may suppress HBV replication in an IFN-β-dependent manner.
injection of the plasmid pAAV/HBV1.2 into C57BL/6 mice (17). The characteristics of this mouse model for HBV tolerance are analogous to those of human chronic HBV infections. In the current study, we used this model to examine the effects of IL-15 on immune response to HBV.

Accumulating evidences demonstrate that hydrodynamic-based gene delivery is a highly efficient method for studying the biological activities of cytokines in vivo in the absence of a need for large amounts of purified proteins (18, 19). With the aim to investigate the immunomodulatory effects of IL-15 in vivo, we constructed a gene transfer vector to endogenously overexpress IL-15 in mice. We found that hydrodynamic injection of the plasmid pLIVE-IL-15 resulted in a sustained concentration of IL-15 in serum. IL-15 liver gene transfer induced enhanced immune responses to HBV in a mouse model for HBV tolerance. The anti-HBV effect of IL-15 was not dependent on adaptive immune cells, NK cells, IL-2Rc-related immune cells or even IFN-γ but absolutely required IFN-β.

Materials and methods

Animals

Male C57BL/6 mice (6–8 weeks old) were purchased from the Shanghai Experimental Animal Center (Shanghai, China). Rag-1−/− mice were purchased from Model Animal Research Center (Nanjing, China). IL-2Rc−/− mice were purchased from the Jackson Laboratory (Bar Harbor, ME). IFN-γ−/− mice were kindly provided by Dr. Shaobo Su (Shantou University, Shantou, China). All mice were housed under specific pathogen-free conditions and used according to the regulations of animal care of University of Science and Technology of China.

Plasmids

pAAV/HBV1.2 plasmid was kindly provided by Dr. Pei-Jer Chen (17). The optimized DNA sequences encoding mouse IL-15 (20), which were provided kindly by Dr. Barbara K. Felbe, were inserted into the pLIVE-vector (Mirus Corporation, Madison, WI, USA). pLIVE-EGFP plasmid was used as a control plasmid for pLIVE-IL-15. All the plasmids were isolated by using an endotoxin-free kit (Qiagen, Inc., Valencia, CA, USA). Hydrodynamic injection of the pAAV/HBV1.2, pLIVE-IL-15 or pLIVE-EGFP plasmids into mice was performed as described (21).

Detection of HBV antigen and liver transaminase activities

Serum levels of HBsAg, HBeAg were determined with commercially available radioimmunoassay (RIA) kits (Beijing North Institute of Biological Technology, Beijing, China). Serum enzyme activities of alanine aminotransferase (ALT) were measured using commercially available kit (Rong Sheng, Shanghai, China).

Analysis of HBV replication by Southern blotting

A piece of liver tissue was digested overnight in lysis buffer (10 mM Tris-Cl (pH 8.0), 100 mM EDTA (pH 8.0), 100 mM NaCl, 2 mg/ml RNase A, 0.5% SDS, 0.5 mg/ml proteinase K) at 55°C, and the DNA was phenol-chloroform extracted. Thirty micrograms of DNA were digested with HindIII enzyme, electrophoresed on a 1% agarose gel and transferred to nylon membrane (Hybond-N; Amersham Biosciences, Piscataway, NJ, USA). After UV cross-linking, the membrane was hybridized with a DIG (Digoxigenin)-labelled HBV DNA probe generated by the PCR DIG Probe Synthesis Kit (Roche Diagnostics GmbH, Germany) using the pAAV/HBV1.2 plasmid as a template. The oligonucleotide sequences for the DIG-labelled HBV probe corresponding to the HB×Ag sequence were 5′-ATGCTGCTAGGCTGTAC-3′ and 5′-TTAGGCGAAGTGGAAAAAG-3′. The blotting signals were visualized by chemiluminescence and imaged with Image Quant LAS 4000 mini imaging system (GE Healthcare, Uppsala, Sweden).

ELISA for serum cytokine detection

The serum samples were kept at −20°C until ready for cytokine measurement. Levels of IL-15, IFN-α and IFN-β were measured by using commercially available ELISA kits (Cusabio, Wuhan, China).

Immunohistochemistry

Liver tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. Five-micron sections were affixed to slides, deparaffinized. Intrahepatic HBcAg was visualized by immunohistochemical staining of liver sections with rabbit anti-HBc antibodies (DAKO, Carpinteria, CA, USA) and the liver sections were also stained with haematoxylin.

Cell preparations and fluorometric analysis

Splenocytes and Liver mononuclear cells (MNCs) were isolated essentially as described previously (22). The phenotype of the cells was analysed using fluorescence-labelled mAbs. The mAb used in this study included FITC-anti-CD69, PE-anti-NK1.1, PE-anti-IFN-γ, PerCP/CY5.5-anti-CD3, PE/CY7-anti-NK1.1, FITC Armenian Hamster IgG1, λ isotype control, PE Rat immunoglobulin G1k (IgG1, k) isotype control (all of above from BD Pharmingen, San Diego, CA, USA). Cells were stained with indicated fluorescence-labelled mAbs for surface antigens according to a standard protocol. For the intracellular cytokine assay, Mononuclear cells were stimulated with PMA (30 ng/ml; Sigma-Aldrich, St. Louis, MO, USA) and ionomycin (1 μg/ml;
Sigma-Aldrich) and monensin (5 μg/ml; Sigma-Aldrich). After 4 h of culture at 37°C and 5% CO₂, the cells were harvested and labelled by PE/CY7-anti-NK1.1 and PerCP/CY5.5-anti-CD3. Then the cells were fixed, permeabilized and stained with PE-anti-INF-γ. The stained cells were analysed using a flow cytometer.

Cell depletion and in vivo blocking

For NK cell depletion, mice were intravenously (i.v.) injected with 200 μg of protein-G-purified monoclonal anti-NK1.1 (PK136) per mouse 24 h before challenge. To chronically deplete NK cells, mice were treated with PK136 every 72 h for 5 weeks. The elimination of NK cells was confirmed by flow cytometry. Functional grade purified anti-mouse IFN-NAR1 and corresponding amount of Mouse IgG1 isotype control were purchased from ebioscience (San Diego, CA).

Quantitative PCR

Total liver RNA was extracted by using Trizol Reagent (Invitrogen, Carlsbad, CA, USA). Two microgram RNA was reverse transcribed. Quantitative PCR was performed with Rotor-Gene 6000 (Corbett Research, Sidney, Australia) and a SYBR Premix Ex Taq (Takara, Dalian, China), according to the manufacturer’s instructions. The primer sequences used were as follows: β-actin, sense, 5′-TGGAATCTGGCATCATTGAAA-3′; antisense, 5′-CCGACTTACTAGCTGCGTTTA-3′; iNOS, sense, 5′-CACCTTGAGTTCAACCA GT-3′; anti-sense, 5′-ACCACCTGATCTTGATGATGC-3′; IFN-α, sense, 5′-TCTCTCCTGCTGGAAGGAC-3′; anti-sense, 5′-ACACAGTGTACCTGTTGGA-3′; IFN-β, sense, 5′-CGCTCTCAAGAAAAGGACAA-3′; anti-sense, 5′-TGGCTGTGTTTGACTCATGAG-3′; IFN-γ, sense, 5′-TAGCCAAGACTTGATGTGGCG-3′; anti-sense, 5′-AGACATCTCCTCCATGACAGC-3′; TNF-α, sense, 5′-AA GCCCTTAGCCACGCT GTA-3′; anti-sense, 5′-CCAGCCATGGGTTGACCT-3′. Results were analysed by ΔΔCt method as described (23). Values were expressed as fold change compared with control.

Statistical analysis

Results were analysed by using Student’s t-test or ANOVA where appropriate. All data are expressed as the mean and standard error of the mean (SEM). P values < 0.05 were considered significant.

Results

Establishment of a mouse model for HBV tolerance

Recent reports showed that hydrodynamic delivery of a HBV genome-containing plasmid pAAV/HBV1.2 into mouse liver allowed persistent HBV viraemia and anti-genemia in C57BL/6 mice (17). In our study, C57BL/6 mice were hydrodynamically injected with 6 μg of pAAV/HBV1.2 plasmid; the levels of HBsAg and HBeAg in the serum were determined. Serum HBsAg (Fig. 1A) and HBeAg (Fig. 1B) can be detected 1 day post-injection and expression of the two proteins declined during the first 2 weeks but remained relatively constant thereafter. Secretion of HBsAg and HBeAg into the serum remained high 6 weeks post-pAAV/HBV1.2 administration. Moreover, both cytoplasmic and nuclear HBCAg were also detected in the livers of HBV-carrier mice at 6 weeks after injection (Fig. 1C). There were no detectable anti-HBs antibodies in the sera of these animals (data not shown). Serum alanine aminotransaminase activity (ALT) was high on day 1 because of the hydrodynamic procedure and then returned to baseline levels thereafter (Fig. 1D), suggesting that there was no hepatitis flare following pAAV/HBV1.2 injection. Overall, hydrodynamic injection of pAAV/HBV1.2 leads to a persistent HBV gene expression in mice, which might mimic human chronic HBV infections in the immune tolerant stage.

Liver over-expression of IL-15 breaks HBV-induced immunotolerance

Hydrodynamic-based gene transfer is a relatively simple, yet very effective technique to express biologically active proteins in vivo (24). To evaluate the immunological activities of IL-15 in vivo, an expression plasmid encoding mouse IL-15 (pLIVE-IL-15) was constructed to overexpress this cytokine in mice. To determine the time course and duration of IL-15 secretion, we hydrodynamically injected mice with 10 μg of either pLIVE-IL-15 or pLIVE-EGFP plasmid and measured serum IL-15 levels at indicated time points post-injection (Fig. 2A). IL-15 was undetectable or very low before treatment. Administration of pLIVE-IL-15 resulted in a persistent expression of IL-15 whose concentration peaked in serum at 1 week post-injection and remained at a fairly constant level through the 8-week period of study. By contrast, administration of pLIVE-EGFP did not produce elevations in serum IL-15 levels.

For assessment of anti-HBV activity of IL-15 produced upon hydrodynamic injection, we treated HBV-carrier mice with 10 μg of either pLIVE-EGFP or pLIVE-IL-15. Liver IL-15 over-expression resulted in great suppression of HBsAg and HBeAg expression early after injection and eliminated the expression of the two proteins at 5 weeks post-injection (Fig. 2B and C). HBV replication intermediates in the livers of IL-15-treated and mock-treated mice were also assayed. IL-15 treatment dramatically reduced the level of HBV replication 3 days post-pAAV/HBV1.2 injection (Fig. 2D). Furthermore, input DNA that served as a template for replication and HBV replication intermediates in the livers of IL-15-treated mice could not be detected 5 weeks...
Fig. 1. Establishment of a mouse model for HBV tolerance by hydrodynamic injection of pAAV/HBV1.2 plasmid. C57BL/6 mice were hydrodynamically injected with 6 μg of pAAV/HBV1.2. (A, B) Titers of serum HBsAg (A), HBeAg (B) were determined at indicated time points using radioimmunoassay (RIA). (C) At 6 weeks after injection, Livers were processed for HBcAg expression (original magnification, ×100). (D) Serum ALT levels were measured at the indicated time points after injection. Data are shown as mean ± SEM. These results are taken from three independent experiments.

Fig. 2. Liver over-expression of IL-15 breaks HBV-induced immunotolerance. (A) C57BL/6 mice were hydrodynamically injected with 10 μg of either pLIVE-EGFP or pLIVE-IL-15 plasmid. Serum samples were collected at various time points after injection and IL-15 was determined by enzyme-linked immunosorbent assay (ELISA). (B, C, D, E) C57BL/6 mice were hydrodynamically injected with 6 μg of HBV plasmid plus 10 μg of either pLIVE-EGFP or pLIVE-IL-15. Titers of serum HBsAg (B), HBeAg (C) were determined at indicated time points. The level of HBV DNA replicative intermediates in the liver (D) was determined by southern blot analysis. RC, Relaxed circular; SS, single-strand. Serum ALT levels (E) were measured at the indicated time points after injection. Data are shown as mean ± SEM. *P < 0.05 vs. pLIVE – EGFP + pAAV/ HBV1.2 group.
Suppression of HBV replication by IL-15

Rag1<sup>−/−</sup> mice (mice deficient in T and B cells) were treated with pAAV/HBV1.2 along with either pLIVE-EGFP or pLIVE-IL-15. As shown in Figure 3A and B, over-expression of IL-15 similarly decreased HBsAg and HBeAg levels in HBV-carrier mice during the time course examined, suggesting that IL-15 exerted anti-HBV activity in a T-cell and B-cell independent manner.

NK cells are not involved in IL-15-mediated anti-HBV activity

Previous study has demonstrated that NK cell activation could inhibit HBV replication in HBV transgenic mice (25), we then examined whether the anti-HBV activity of IL-15 was mediated by NK cells in our study. As shown in Figure S1A, the percentage and number of NK cells in both spleen and liver of IL-15-gene delivered mice were increased significantly compared with control mice. However, expression of the early activation marker CD69 on NK cells (Fig. S1B) was only slightly upregulated and intracellular expression of IFN-γ by NK cells (Fig. S1C) was also only slightly enhanced in IL-15 treated mice, indicating NK cell function was not improved. To further elucidate the role of NK cells, PK136 mAb was injected before IL-15 treatment to deplete NK and also NKT cells (Fig. 4A). As shown in Figure 4B and C, to our surprise, NK and NKT cell depletion did not abrogate the anti-HBV activity of IL-15. Furthermore, treatment of pLIVE-IL-15 also resulted in great suppression of HBV expression in IL-2Rγc<sup>−/−</sup> mice (mice deficient in NK cells) (Fig. 4D). These findings suggest that

IL-15 exerts anti-HBV function in T cell- and B cell-deficient Rag1<sup>−/−</sup> mice

It has been clearly shown that the adaptive immune response is needed for an efficient clearance of HBV (14), so we want to know whether inhibition of HBV expression by IL-15 is dependent on T cells and B cells.

Post-injection (Fig. 2D), probably because plasmid-bearing hepatocytes were cleared by immune response. These data demonstrate that IL-15 expression can enhance the immune response to HBV. Additionally, pLIVE-IL-15 treatment did not increase serum ALT levels (Fig. 2E), indicating that IL-15 inhibited HBV expression in a noncytolytic process.

IL-15 exerts anti-HBV function in T cell- and B cell-deficient Rag1<sup>−/−</sup> mice
although NK cell numbers were upregulated in IL-15 treated mice, NK cells were not involved in IL-15-mediated anti-HBV activity.

Anti-HBV activity of IL-15 is independent of IFN-γ

IFN-γ is known to inhibit HBV gene expression noncytopathically (26) and IL-15 can enhance IFN-γ production by several types of lymphocytes (27). Here, we investigated whether IFN-γ was required for the anti-HBV effect of IL-15. To examine the role of IFN-γ in the IL-15-induced antiviral activity, we monitored the ability of IL-15 to inhibit HBV gene expression in IFN-γ−/− mice (mice deficient in IFN-γ). As shown in Figure 5, serum HBsAg levels were much lower in IL-15 treated mice than in control mice and HBsAg expression became undetectable at 4–5 weeks following IL-15 treatment, indicating that IFN-γ did not play a critical role in IL-15-mediated anti-HBV activity.

Anti-HBV activity of IL-15 is dependent on IFN-β

Type I IFNs (IFN-α/β) play a vital role in host antiviral response and have been widely used for treatment of chronic HBV infection (28). As shown in Figure 6A and B, IL-15 treatment increased intrahepatic IFN-β mRNA expression as well as circulating serum IFN-β levels,
whereas IFN-α mRNA and protein levels were unchanged. To further evaluate the role of increased IFN-β in the anti-HBV activity of IL-15, mice were injected with an IFNAR1 blocking antibody before IL-15 treatment. As shown in Figure 6C and D, blockade of IFN-β function led to a significant increase in the expression of HBsAg and HBeAg and the level of HBV replication in IL-15 treated mice, indicating the anti-HBV activity of IL-15 was probably mediated by IFN-β.

Discussion

In this study, a mouse model for HBV tolerance was established by hydrodynamical injection of pAAV/HBV1.2 plasmid into C57BL/6 mice, which was simultaneously hydrodynamically injected with either an IL-15-expression plasmid pLIVE-IL-15 or a mock control vector pLIVE-EGFP. Simultaneously hydrodynamic injection of the plasmid pLIVE-IL-15 resulted in sustained high serum level of IL-15, which then markedly decreased the titers of serum HBsAg and HBeAg and the level of liver HBV DNA replicative intermediates. Interestingly, IL-15-mediated anti-HBV activity was not dependent on adaptive immunity, NK cells, IL-2Rγc-related immune cells and even IFN-γ but absolutely required presence of IFN-β. Taken together, liver over-expression of IL-15 may prevent HBV-induced immune tolerance in an IFN-β-dependent manner, which is an undescribed anti-HBV function of IL-15.

IL-15 mRNA has been found to be constitutively expressed by a variety of cell types and tissues and IL-15 expression is tightly regulated (7, 29), probably because of its pleiotropic activity. Some papers reported the construction of several optimized vectors for high expression of bioactive IL-15 upon hydrodynamic injection in vivo (18, 20). However, these plasmids did not result in persistent expression of IL-15, not suitable for studying the long-term effects of IL-15 on immune response to chronic infections such as HBV. In our study, the pLIVE-IL-15 plasmid was constructed for sustained in vivo expression of IL-15 (Fig. 2A). We show that a single hydrodynamic injection of the plasmid pLIVE-IL-15 resulted in a sustained high level of IL-15 in the serum over 2 months after injection.

To understand the role of IL-15 in defense against HBV infection, pLIVE-IL-15 or pLIVE-EGFP (Ctrl) and pAAV/HBV1.2 vectors were simultaneously hydrodynamically injected into C57BL/6 mice. It was noted that IL-15 treatment resulted in low expression of HBsAg and HBeAg in serum and low levels of HBV DNA replicative intermediates in liver and clearance of HBV virus at 5 weeks post-injection (Fig. 2B, C and D). Because of the short serum half-life and rapid clearance of most cytokines, it is expensive and unpractical to repeat injection. Thus, hydrodynamic delivery of pLIVE-vectors containing cytokine genes, as in our study, may provide a more efficient method to observe the effects of cytokines on immune response to chronic infections than the systemic administration of the proteins in mice. This approach is also a valuable implication for future liver-specific deliver of cytokine genes.

Acquired immune systems (T cells and B cells) have been recognized as the key effectors mediating HBV clearance (14). IL-15 plays a pivotal role in T cell homeostasis and effector functions, including T cell proliferation, IFN-γ production, effector T cell survival and generation of memory T cells (7, 8). IL-15 also has the ability to stimulate the proliferation and differentiation of activated B cells (30). IL-15 treatment resulted in great suppression of serum HBsAg and HBeAg levels in Rag1−/− mice (Fig. 3A and B), indicating that T cells and B cells were not involved in IL-15-mediated anti-HBV activity.

It is well known that IL-15 is an absolutely essential cytokine for the development, proliferation and function of NK cells (7). Previous reports suggested that the antiviral activity of IL-15 was primarily mediated through the activation of NK cells (31, 32). In this study, not surprisingly, increased levels of IL-15 resulted in expansion of NK cells in both spleen and liver (Fig. S1A), but we found that NK cells were only slightly activated (Fig. S1B and C) and depletion of NK cells did not abrogate anti-HBV activity of IL-15 (Fig. 4A, B and C). To further confirm the anti-HBV activity of IL-15 is independent of NK cells, pLIVE-IL-15 and pAAV/HBV1.2 were delivered into IL-2Rγc−/− mice. Over-expression of IL-15 in IL-2Rγc−/− mice suppressed HBV expression if compared with pLIVE-EGFP group (Fig. 4D), once again suggesting that NK cells were not involved in anti-HBV activity of IL-15. This result also suggests that unlike NK cells, IL-15 can activate some kinds of cells independent of IL-2Rγc, indicating other activated cells may play an important role in IL-15-mediated anti-HBV activity, similarly reported by Davies et al. (33) that IL-15 had innate anti-tumor activity independent of NK cells and other IL-2Rγc-related immune cells.

Our results suggest that IFN-β, but not IFN-γ, plays an important role in IL-15 induced anti-HBV activity. In our model, intrahepatic IFN-β mRNA expression and serum IFN-β levels were increased after pLIVE-IL-15 treatment (Fig. 6A and B). This is consistent with previous data that IL-15-transgenic mice had higher levels of type I interferon production (33). We further found that blockade of IFN-β activity abrogated the anti-HBV effect of IL-15 (Fig. 6C and D). In an in vitro study, treatment of macrophage cell line RAW264.7 cells with IL-15 induced the production of IFN-β and protected them against virus infection (34, 35). However, depletion of macrophages with clodronate liposomes did not block the ability of IL-15 to inhibit HBV expression in our study (data not shown), indicating that IFN-β was not produced by macrophages. In addition to being an important factor for regulating the activity of immune cells, IL-15 is known to act on many differ-
ent types of non-immune cells such as fibroblasts, hepatocyte, epithelial cells and endothelial cells (27,36). Further experiments are needed to determine which type of cell secretes IFN-β.

The outcome of HBV infection in humans ranges from transient, self-resolving acute infections to lifelong viral persistence. Viral clearance or viral persistence is determined by complicated, as yet not fully understood, viral-host interactions. Many recent papers have found that HBV proteins like HB×Ag protein (37,38), polymerase (39) can interfere with IFN-β production, thereby attenuating the antiviral response of the innate immune system and ultimately contributing to the establishment of persistent infections. Our results demonstrate that IL-15 over-expression in liver enhanced production of IFN-β and eventually promoted viral clearance. Thus, boosting of the innate immune response by IL-15 during primary HBV infection may prove valuable to help reduce viral spread and break the immune tolerance to HBV. Our results also suggest that outcomes of HBV infection might be influenced by the initial activation and strength of the immune response during the early phase of HBV infection. Following infection with HBV, approximately 95% of adults clear the virus spontaneously, whereas the remaining 5% fail to respond to viral infection and evolve to persistent infection. The liver is continuously exposed to pathogens from the gastrointestinal tract which might induce the expression of IL-15 (40). Indeed, IL-15 protein is constitutively expressed in human liver and significantly increased upon stimulation (41). Thus, it is conceivable that IL-15 may be involved in HBV clearance in human beings during infection. So, this study on a novel function of IL-15 in anti-HBV immunity might provide an implication in clinical practice.

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Conflict of interest

No conflict of interest.

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References

Suppression of HBV replication by IL-15

Additional Supporting Information may be found in the online version of this article:

Fig. S1. NK cells after liver over-expression of IL-15. C57BL/6 mice were hydrodynamically injected with 6 μg of HBV plasmid plus 10 μg of either pLIVE-EGFP or pLIVE-IL-15. After 2 weeks of injection, splenocytes and hepatic mononuclear cells (MNCs) were isolated and analyzed by flow cytometry with anti-NK1.1 and anti-CD3 antibodies. (A) The percentage and number of NK cells in spleen and liver was analyzed. Data are shown as mean ± SEM; *P < 0.05. (B) The surface expression of CD69 on CD3-NK1.1+ cells in spleen and liver was also analyzed (bold lines). The thin lines represent negative control stained with isotype control IgG. (C) Intracellular IFN-γ production by spleen and liver NK cells (CD3-NK1.1+) was examined by flow cytometry. These results are taken from three independent experiments.

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