Vaccine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

A mouse model with age-dependent immune response and immune-tolerance for HBV infection

Xuerui Yi *, Youcheng Yuan, Na Li [1], Lu Yi, Cuiling Wang, Ying Qi, Liang Gong, Guangze Liu, Xiangping Kong

Liver Diseases Research Center, The 458th Hospital of Guangzhou, Guangzhou 510600, China

ARTICLE INFO

Article history:
Received 12 April 2017
Received in revised form 10 December 2017
Accepted 27 December 2017
Available online xxxx

Keywords:
Hepatitis B virus
Mouse model
Age-dependent
Immune tolerance
GS-9620
Functional cure

ABSTRACT

Background: Viral clearance of human HBV infection largely depends on the age of exposure. Thus, a mouse model with age-dependent immune response and immune-tolerance for HBV infection was established.

Methods: HBVRag1 mice were generated by crossing Rag1$^{-/-}$ mice with HBV-Tg mice. Following adoptive transfer of splenocytes adult (8–9 weeks old) and young (3 weeks old) HBVRag1 mice were named as HBVRag-ReA and HBVRag-ReY mice respectively. The biochemical parameters that were associated with viral load and immune function, as well as the histological evaluation of the liver tissues between the two mouse models were detected. The immune tolerance of HBVRag-ReY mice that were reconstituted at the early stages of life was evaluated by quantitative hepatitis B core antibody assay, adoptive transfer, and modulation of gut microbiota with the addition of antibiotics.

Results: HBVRag-ReA mice indicated apparent hepatocytes damage, clearance of HBsAg and production of HBsAb and HBcAb. HBVRag-ReY mice did not develop ALT elevation, and produced HBcAb and HBsAg. A higher number of hepatic CD8$^+$ T and B cells promoted clearance of HBsAg in HBVRag-ReA mice following 30 days of lymphocyte transfer. In contrast to HBVRag-ReA mice, HBVRag-ReY mice exhibited higher levels of Th1/Th2 cytokines. HBVRag-ReY mice exhibited significantly higher ($P < .01$, approximately 10-fold) serum quantitative anti-HBc levels than HBV-Tg mice, which might be similar to the phase of immune clearance and immune tolerance in human HBV infection. Furthermore, the age-related tolerance in HBVRag-ReY mice that were sensitive to antibiotic treatment was different from that noted in HBV-Tg mice. GS-9620 could inhibit the production of HBsAg, whereas HBV vaccination could induce sustained seroconversion in HBVRag-ReY mice with low levels of HBsAg.

Conclusions: The present study described a mouse model with age-dependent immunity and immune-tolerance for HBV infection *in vivo*, which may mimic chronic HBV infection in humans.

1. Introduction

Approximately 400 million people are chronically infected with hepatitis B virus. The viral infection results in one million deaths annually worldwide [1,2]. The unique feature of viral clearance of HBV infection is age-dependent in humans. Following HBV infection, 95% of adults will achieve spontaneous clearance, while 90% of neonates will develop chronic infection [2]. "Immune system immaturity", "neonatal tolerance" and/or "liver tolerance" may result in chronic HBV infection notably in young subjects [2,3]. A diverse and strong adaptive immune response is necessary for viral clearance in the adult [4]. Nevertheless, the nature of the disease and the change in the outcome according to the time period of the infection remain unclear. Early events of immune response, which determine HBV clearance and/or persistence, should be considered [5–7].

Since HBV infection is restricted to humans, the development of animal models has been the greatest challenge in the examination of the mechanism of action of chronic HBV infection. In a suitable model, persistent HBV replication and expression require to be established, while HBV-specific adaptive immune responses must be induced [8]. Baron and colleagues set up model systems with C57 background mouse, which could mimic human HBV clearance and persistence in order to overcome this difficulty and investigate the immunological mechanisms of age-dependent viral clearance during HBV infection [6,9]. IL-21 and CXCL13 that were expressed in the liver by T follicular helper cells and macrophages respec-

* Corresponding author at: Liver Diseases Research Center, The 458th Hospital of Guangzhou, No. 801 Dongfeng East Road, Guangzhou 510600, China.
E-mail address: yixuerui65@163.com (X. Yi).
[1] Present address: The Health Dept. of People's Hospital of Qinghai Province, Xining 810007, China.

https://doi.org/10.1016/j.vaccine.2017.12.071
0264-410X/

tively, promoted hepatic lymphoid organization and successfully induced immunity against HBV infection.

Accumulated evidence demonstrates that host genetic factors can determine chronic HBV infection [3]. Although age-dependent viral clearance of HBV infection in the immune reconstitution mouse model was not affected by genetic background [5,6], it varied significantly in the HDI (hydrodynamic injection) mouse model [3]. For example, age-dependent viral clearance was not found in the BALB/c background HDI mouse model [3]. Therefore, a BALB/c reconstituted mouse model is essential in order to investigate age-dependent HBV clearance.

In the present study, we obtained BALB/c background HBVRag1 mice by crossing HBV-Tg mice with Rag1$^{-/-}$mice. The HBVRag1 mice expressed HBsAg and HBcAg in the absence of an adaptive immune system. Adoptive transfer of splenocytes from BALB/c mice, which were naive to HBV, mimicked the primary HBV infection, and revealed age-dependent viral clearance and immune responses. The immune tolerance in young mice was similar to that noted during chronic HBV infection in humans, and could mimic host anti-HBV immunity during the changes of quantitative anti-HBc (qAnti-HBc) levels. In addition, the immunotherapy that was based on the agonist GS-9620 and the HBV vaccine was examined in the mouse model.

2. Material and methods

2.1. Mice and experimental system

BALB/c background HBV-Tg mice were produced in our center by 1.3 copies of the HBV (ayw subtype) genome, which has detectable HBV DNA and expresses high levels of HBsAg [10–12]. BALB/c background Rag1$^{-/-}$ mice (Strain Name: C.129S7 (B6)-Rag-1^{tm1Mom}/J) provided by Dr. Shengdian Wang of the Institute of Biophysics, CAS, were purchased from the Jackson Laboratory. BALB/c mice were from the Southern Medical University (Guangzhou, China). BALB/c background HBVRag1 mice were produced using HBV-Tg mice crossed with Rag-1$^{-/-}$. Young (3 weeks old) and/or adult (8–9 weeks old) HBVRag1 mice were reconstituted via tail vein injection with 5×10^7 syngeneic splenocytes. The injection speed was 50 μL/s and the injection volume was 200–500 μL with 1 × phosphate buffer saline (PBS), at pH 7.2. The ALT detection Kit was from Jiancheng Co., (Nanjing, China). All mice were kept under the specific pathogen-free conditions at No. 458 Hospital (Guangzhou, China) as described in the institutional guidelines.

2.2. PCR analysis integrated transgenes

Genomic DNA was isolated from the 2-week-old mouse-tail samples (2–3 mm) as described in the protocol of E.Z.N.A. Tissue DNA kit (OMEGA bio-tek). DNA was amplified using HBV primers. The sequences were as follows: P1: 5-ACGCAGGATAACCACATT-3, P2: 5-ACAACCTTCCACCAAACT-3, P3: 5-TGGATGTGGAATGTGTGCG AG-3, P4: 5-GAGGTTCCGCTACGACTCTG-3, P5: 5-CCGGACAAG TTTTTCATCGT-3 [13,14]. The last 3 primer sets were used for Rag-1$^{-/-}$ mouse genomic DNA analysis.

2.3. HBV protein and antibody detections

Initially, HBsAg and HBeAg were detected with an ELISA kit (Kehua, Shanghai, China) in the serum derived from mice. HBsAb was assayed with the ETI-AB-AUK PLUS and ABAU set kits (DiaSorin, Italy, sensitivity5 mIU/ml). Subsequently, serum from reconstituted HBVRag1 mice was used for HBcAb detection by the ETI-AB-COREK PLUS (DiaSorin, Italy) kit, whereas the double sandwich immunoassay (dynamic range 0.08–2.5 IU/ml; Wantai, Beijing, China) method was used for the quantitative HBcAb detection. The samples were tested at dilutions of 1:10 to 1:40 if the Anti-HBc level was higher than 2.5 IU/mL (>2.5 IU/mL). All assays were recorded on Multiskan FC (Thermo SCIENTIFIC).

2.4. Liver lymphocyte preparations

Briefly, mice were perfused with 20 mL cold PBS (pH7.2), until swelling of the liver tissue was achieved. The liver tissues were removed from the abdomen and forced through filters with nylon film (75 μm). Following a centrifugation for 60 s at 35*g*, the supernatant was collected and centrifuged further at 550*g* for 8 min. The pellet was resuspended in PBS and liver-derived lymphocytes were isolated with 70/40 percoll [6,15].

2.5. ELISpot assay

Splenocytes were inserted in 96-well plates at a density of 2×10^5 liver splenocytes/well and the concentration of IL-4 and IFN-γ was estimated by ELISpot assays (Dakewe, Shenzhen, China). The cells were unstimulated for 21 h at 37 °C, while the positive control samples were stimulated with 2 g/ml PMA/Ionomycin. Following washing and staining, the spot forming cells in each well (SFC) were counted by the ELISpot Reader Champ SpotⅢ.

2.6. Flow cytometry

Lymphocytes were stained using the following antibodies and fluorochromes (the antibodies were from BD Pharmingen, USA): APC-conjugated anti-IgM (Ⅱ/41), PE–conjugated anti-CD45R/B220 (clone RA3-6B2), PE-conjugated anti-CD4 (GK1.5), FITC–conjugated anti-CD8a (clone 53-6.7), and PE–Cy^{TM} conjugated anti-CD3e (clone 145-2C11). The cell parameters were measured with a FACS Calibur flow cytometer (BD) and analyzed using the CellQuest software (BD).

2.7. Liver histology

Tissues were fixed with 10% formalin and embedded in paraffin. The sections with a thickness of 5-μm and/or higher were stained with hematoxylin and eosin (H&E) according to the protocol provided by the manufacturer. Immunohistochemistry was conducted using goat anti-HBsAg (Abcam ab17183) and/or rabbit anti-HBcAg (GB058602). The slides were visualized using microscopy (OLYMPUS BX43F).

2.8. Antiviral effect of the GS-9620 agonist and the HBV vaccine in the mouse model

The young HBVRag1 mice (3 weeks old) were reconstituted with syngeneic splenocytes and were denoted as HBVRag1-reconstituted and/or HBVRag-ReY. The mice that corresponded to the HBVRag1 strain that was reconstituted during adulthood (8–9 weeks old), were denoted as HBVRag-ReA mice.

Following 6 weeks of reconstitution, the HBVRag-ReY mice (9 weeks old) were treated with the agonist GS-9620 and the HBV vaccine. (1) The GS-9620 treatment was conducted as follows: GS-9620 was purchased from MedChem Express with purity > 98 %. The mice were treated by gavage with GS-9620 at a dose of 5 mg/kg body weight 3 times a week for 4 weeks. (2) The HBV vaccine treatment was conducted as follows: The recombinant yeast HBsAg vaccine (subtype: adw) was provided by the BioKangtai Company of Shenzhen. The mice were vaccinated with 5 μg of HBV vaccine and were boosted on day 14. HBsAg and anti-HBsAg were analyzed in the serum of mice that was derived from blood samples collected from the tail.

Please cite this article in press as: Yi X et al. A mouse model with age-dependent immune response and immune-tolerance for HBV infection. Vaccine (2018), https://doi.org/10.1016/j.vaccine.2017.12.071