Virus Research 237 (2017) 47–57

Contents lists available at ScienceDirect

Virus Research

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

Interferon-β response is impaired by hepatitis B virus infection in *Tupaia belangeri*

Mohammad Enamul Hoque Kayesh[a,b], Sayeh Ezzikouri[c,d], Haiying Chi[b,c], Takahiro Sanada[e], Naoki Yamamoto[e], Bouchra Kitab[b,c], Takumi Haraguchi[b,c], Rika Matsuyama[b,c], Chimène Nze Nkogue[a,b], Hitoshi Hatai[f], Noriaki Miyoshi[f], Shuko Murakami[g], Yasuhito Tanaka[g], Jun-ichiro Takano[h], Yumiko Shiogama[h], Yasuhiro Yasutomi[h], Michinori Kohara[e], Kyoko Tsukiyama-Kohara[a,b,c,*]

[a] Department of Pathological and Preventive Veterinary Science, The United Graduate School of Veterinary Science, Yamaguchi University, Yamaguchi, Japan
[b] Laboratory of Animal Hygiene, Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima, Japan
[c] Transboundary Animal Diseases Centre, Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima, Japan
[d] Virology Unit, Viral Hepatitis Laboratory, Institut Pasteur du Maroc, Casablanca, Morocco
[e] Department of Microbiology and Cell Biology, Tokyo Metropolitan Institute of Medical Science, Japan
[f] Department of Animal Pathology, Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima, Japan
[g] Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan
[h] Laboratory of Immunoregulation and Vaccine Research, Tsukuba Primate Research Center, National Institute of Biomedical Innovation, Health and Nutrition, 1-1 Hachimandai, Tsukuba, Ibaraki, 305-0843, Japan

ARTICLE INFO

Article history:
Received 27 December 2016
Received in revised form 25 April 2017
Accepted 18 May 2017
Available online 25 May 2017

Keywords:
Tupaia
Hepatitis B virus
Toll-like receptors
Interferon
Chronic infection

ABSTRACT

To date, the chimpanzee has been used as the natural infection model for hepatitis B virus (HBV). However, as this model is very costly and difficult to use because of ethical and animal welfare issues, we aimed to establish the tupaia (*Tupaia belangeri*) as a new model for HBV infection and characterized its intrahepatic innate immune response upon HBV infection. First, we compared the propagation of HBV genotypes A2 and C *in vivo* in tupaia hepatocytes. At 8–10 days post infection (dpi), the level of HBV-A2 propagation in the tupaia liver was found to be higher than that of HBV-C. Abnormal architecture of liver cell cords and mitotic figures were also observed at 8 dpi with HBV-A2. Moreover, we found that HBV-A2 established chronic infection in some tupaias. We then aimed to characterize the intrahepatic innate immune response in this model. First, we infected six tupaias with HBV-A2 (strains JP1 and JP4). At 28 dpi, intrahepatic HBV-DNA and serum hepatitis B surface antigens (HBsAg) were detected in all tupaias. The levels of interferon (IFN)-β were found to be significantly suppressed in the three tupaias infected with HBV A2_JP4, while no significant change was observed in the three infected with HBV A2_JP1. Expression of toll-like receptor (TLR) 1 was suppressed, while that of TLR3 and TLR9 were induced, in HBV A2_JP1-infected tupaias. Expression of TLR8 was induced in all tupaias. Next, we infected nine tupaias with HBV-A2 (JP1, JP2, and JP4), and characterized the infected animals after 31 weeks. Serum HBsAg levels were detected at 31 weeks post-infection (wpi) and IFN-β was found to be significantly suppressed in all tupaias. TLR3 was not induced, except in tupaia #93 and #96. Suppression of TLR9 was observed in all tupaias, except tupaia #93. Also, we investigated the expression levels of cyclic GMP-AMP synthase, which was found to be induced in all tupaias at 28 dpi and in four tupaias at 31 wpi. Additionally, we evaluated the expression levels of sodium-taurocholate cotransporting polypeptide, which was found to be suppressed during chronic HBV infection. Thus, the tupaia infection model of HBV clearly indicated the suppression of IFN-β at 31 wpi, which might have contributed to the establishment of chronic HBV infection.

© 2017 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The innate immune response is considered the first line of immune defense that halts many viral infections (Zuniga et al., 2015). Viral nucleic acids and proteins are recognized by differ-

* Corresponding author at: Transboundary Animal Diseases Center & Department of Animal Hygiene, Joint Faculty of Veterinary Medicine, Kagoshima University 1-21-24, Korimoto, Kagoshima-city, 890-0065, Japan.
E-mail address: kkohara@vet.kagoshima-u.ac.jp (K. Tsukiyama-Kohara).

http://dx.doi.org/10.1016/j.virusres.2017.05.013
0168-1702/© 2017 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

ent pattern recognition receptors (PRRs), such as toll-like receptors (TLRs), RIG-I-like receptors, and NOD-like receptors (Kawai and Akira, 2011). TLRs, one of the important components of innate immunity, play a crucial role in sensing invaders and initiating innate immune response, thus limiting the spread of infections and modulating efficient adaptive immune responses (Takeuchi and Akira, 2010). Recently discovered Cyclic GMP-AMP (cGAMP) synthase (cGAS), a cytosolic DNA sensor is an important element in the induction of innate immune response (Civril et al., 2013; Gao et al., 2013). NTCP has been found to be involved in Hepatitis B virus (HBV) infection (Yan et al., 2012).

HBV is major causative agent of chronic hepatitis, liver cirrhosis, and/or hepatocellular carcinoma. HBV, a member of the *Hepadnaviridae* family, is an enveloped, circular, and partially double-stranded DNA virus (Lee, 1997). More than 248 million people worldwide, i.e., approximately 5% of the world's population, are chronically infected with HBV (Ganem and Prince, 2004; Schweitzer et al., 2015). Based on phylogenetic studies and sequence analyses, HBV strains have been classified into 10 genotypes (A–J) (Lin and Kao, 2015). These different genotypes have not all been characterized, and HBV-induced immunopathogenesis is poorly understood because of the lack of an appropriate animal infection model. Animal infection models are also essential for the development of new drugs and vaccines (Keating and Noble, 2003). Although the role of adaptive immunity in the control of HBV infection is well documented, the effects of innate immunity in this regard are yet to be explored.

So far, chimpanzees have been used as infection models for HBV; however, owing to economic and ethical reasons, it is now difficult to use chimpanzees for experimental infection. Recently, humanized chimeric mice were developed (Mercer et al., 2001), and HBV have been reported to efficiently infect these mice (Nakagawa et al., 2013). However, the use of mice also possess some disadvantages, including high cost, immunocompromised animal status, and inability to examine chronic infections.

Tupaia belangeri belongs to the Tupaiidae family, which comprises four genera and 19 extant species (Tsukiyama-Kohara and Kohara, 2014). The evolutionary characterization of 7S RNA-derived short interspersed elements (SINEs) showed that tupaias possess specific chimeric Tu-type II SINEs, and can be grouped with primates (Kriegs et al., 2007). In addition, genomic analysis has suggested that the *Tupaia* genus is more closely related to humans than to rodents (Fan et al., 2013; Kriegs et al., 2007). *T. belangeri* has been previously reported to be susceptible to HBV (Sanada et al., 2016; Walter et al., 1996) and so, can be developed as an immunocompetent animal infection model. However, the molecular basis of HBV pathogenesis has not been fully characterized in the tupaia model because of the lack of characterization tools (such as specific antibodies, quantitative polymerase chain reaction [qPCR] assays, and cDNAs).

In this study, we investigated the susceptibility of tupaias to several strains of HBV and established a qPCR assay for TLRs, cGAS, and cytokines to characterize the innate immune response against HBV infection in tupaia.

2. Materials and methods

2.1. Animals

A total of 21 adult and 10 newborn tupaia were used in this study. Adult tupaia were obtained from the Laboratory Animal Center at the Kunming Institute of Zoology, Chinese Academy of Sciences (Kunming, China). This study was carried out in accordance with both the Guidelines for Animal Experimentation of the Japanese Association for Laboratory Animal Science and the Guide for the Care and Use of Laboratory Animals of the National Institute of Health. All experimental protocols used in this study were approved by the institutional review boards of the regional ethics committees of the Kagoshima University (VM15051, VM13044).

Animals were housed in separate cages and fed a daily regimen of eggs, fruit, water, and dry mouse food; they were handled as humanely as possible in accordance with the guidelines of the Institutional Animal Care and Use Committee for Laboratory Animals.

2.2. Viruses

In this study, we used the virus preparation of HBV genotypes A (A2_JP1, A2_JP2, A2_JP4) and C (C_JPNAT; accession number: AB246345.1) for infecting tupaias, which was used in a previous study (Sanada et al., 2016). Originally, the virus HBV A2_JP1 was isolated from chronic patient, and HBV A2_JP2 and A2_JP4 virus from acute patients and propagated in chimeric mice with humanized liver. The virus preparations used for infecting tupaias were ensured negative for HCV-RNA and HDV-RNA by PCR.

2.3. Infection

Before viral infection, all animals used in this study were confirmed negative for HBV by PCR. Six newborn tupaias were infected with HBV genotype A (A2_JP4) (#336, #337, #338, #339, #N48, and #N93), while four newborn tupaias were infected with HBV genotype C (#59, #60, #340, and #341). The infection was administered subcutaneously and the dose was adjusted to 10^6 copies/tupaia. Tupaia #336, #337, #338, and #339 were sacrificed for sampling at 12, 11, 10, and 8 days post infection (dpi), respectively. Tupaia #340 and #341 were sacrificed at 10 dpi, whereas tupaia #59 and #60 were sacrified at 12 dpi. For histological analysis, liver tissues were extracted, fixed with paraformaldehyde, and stained with hematoxylin and eosin.

Fifteen adult (1 year-old) tupaia were inoculated with HBV genotype A (A2_JP1, A2_JP2, and A2_JP4). The infection was administered intraperitoneally and the dose was adjusted to 10^{6-7} copies/tupaia. Nine adults, including three uninfected controls (#N1, #N2, and #N3) and six HBV (A2_JP1 and A2_JP4)-infected ones (#17, #32, #36, #22, #43, and #48) were sacrificed at 28 dpi. Twelve adults, including three uninfected controls (#N4, #N5, and #N6) and nine HBV (A2_JP1, A2_JP2, and A2_JP4)-infected ones (#84, #85, #92, #93, #86, #94, #88, #96, and #97) were sacrificed at 31 weeks post infection (wpi). Liver tissues were extracted from all sacrificed animals. RNA from liver tissues was isolated using the acid guanidium-phenol chloroform extraction method and purified using an RNeasy Mini Kit (Qiagen, Valencia, CA, USA). Before inoculation as well as at determined time points, blood samples were collected by venipuncture; the levels of serum alanine aminotransferase (ALT), hepatitis B surface antigen (HBsAg), and serum HBV-DNA were estimated.

2.4. Measurement of ALT and viral load

The serum ALT level was determined using a Transnase Nissui kit (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) and standardized; it was represented in IU/liter. HBV-DNA was isolated from the sera samples by using the SMITEST EX-R&D Kits according to the manufacturer's instructions (Medical & Biological Laboratories Co. LTD., Nagano, Japan). The total DNA was extracted from the liver tissues with a homogenizer, treated with proteinase K in a lysis buffer (0.1 M Tris [pH 7.5], 12.5 mM EDTA, 0.15 M NaCl, 1% SDS, and 10 mg/mL proteinase K), purified using the Qiagen QIAamp DNA extraction kit, and finally eluted in 400 µL of deionized water. HBV-DNA quantification was performed using qPCR, based

Download English Version:

https://daneshyari.com/en/article/5675532

Download Persian Version:

https://daneshyari.com/article/5675532

Daneshyari.com