Available online at www.sciencedirect.com
SCIENCE DIRECT
Hepatology Research 35 (2006) 118–126
HEPATOLOGY RESEARCH
http://www.elsevier.com/locate/hepres

Alteration of the influences of HLA classes I and II alleles on the perinatal hepatitis B virus infection after immunoprophylaxis in Korean children

Jong-Hyun Kim [a], Chul-Woo Pyo [b], Dae Kyun Koh [a], Jae Kyun Hur [a], Jin Han Kang [a], Tai-Gyu Kim [b,c,*]

[a] *Department of Pediatrics, St. Vincent's Hospital, The Catholic University of Korea, 93 Ji-dong, Paldal-gu, Suwon, Kyonggi-do 442-060, Republic of Korea*
[b] *Catholic Hemopoietic Stem Cell Bank, College of Medicine, The Catholic University of Korea, 505 Banpo-dong, Seocho-gu, Seoul 137-701, Republic of Korea*
[c] *Department of Microbiology, College of Medicine, The Catholic University of Korea, 505 Banpo-dong, Seocho-gu, Seoul 137-701, Republic of Korea*

Received 14 December 2005; received in revised form 2 March 2006; accepted 12 March 2006

Abstract

Some HLA alleles are known to be associated with hepatitis B virus (HBV) persistence. In order to find out the relationship between HLA and perinatal HBV infection after prophylaxis, we typed HLA classes I and II in 38 HBeAg-positive mothers, their children (19 succeeded and 19 failed in prophylaxis) and 198 HBsAg-negative healthy controls. HLA-B35 (RR = 2.8, $p < 0.03$), Cw*07 (RR = 2.7, $p < 0.02$), DRB1*07 (RR = 3.6, $p < 0.006$), DQA1*02 (RR = 3.6, $p < 0.02$) and DQB1*02 (RR = 2.4, $p < 0.05$) alleles were higher and DRB1*13 (RR = 0.3, $p < 0.03$) and DPB1*0401 (RR = 0.1, $p < 0.01$) alleles were lower frequencies in HBeAg-positive mothers than in the control. In failed children to the perinatal HBV prophylaxis, HLA-Cw*0303 allele was significantly higher ($p < 0.05$) and DPB1*0202 allele was lower ($p < 0.03$) than in succeeded children. These results suggest the influences of certain HLA alleles on naturally acquired chronic HBV infection may be changed by perinatal HBV prophylaxis.

Keywords: Hepatitis B vaccine; Hepatitis B immunoglobulin; Prophylaxis failure; Vertical transmission

1. Introduction

Hepatitis B virus (HBV) infection is one of the major global human health problems. It results in a broad spectrum of liver diseases, ranging from subclinical infection to acute, self-limited hepatitis and fatal fulminant hepatitis. Furthermore, they can progress to chronic liver diseases, like cirrhosis, and eventually hepatocellular carcinoma [1]. Depending on the onset age of infection, their outcomes vary widely. Only about 5% of adults remain chronically infected, however, without specific preventive perinatal prophylaxis, approximately 90% of infected neonates become chronic HBV carriers [2,3].

Since the vaccination of HBV began in early 1980s, the rate of infection has decreased worldwide. However, persistent infection still occurs in about 5–10% of babies born to HBeAg-positive mothers [4–6]. Many studies have tried, but failed to fully discover the mechanism of prophylaxis failure on perinatal HBV infection [4,7–13]. Prophylaxis failure of perinatal HBV infection is likely due to the interplay of the virus and host immune response. On the viral side, several

Abbreviations: ALT, alanine aminotransferase; Anti-HBc IgG, immunoglobulin G to hepatitis B virus core antigen; ARMS, amplification refractory modification system; HBeAg, hepatitis B virus e antigen; HBIG, hepatitis B virus immunoglobulin; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; HLA, human leukocyte antigen; PCR, polymerase chain reaction

* Corresponding author. Tel.: +82 2 590 1216; fax: +82 2 594 7355.
E-mail addresses: jh00mn@catholic.ac.kr (J.-H. Kim), kimtg@cmc.cuk.ac.kr (T.-G. Kim).

1386-6346/$ – see front matter © 2006 Published by Elsevier Ireland Ltd.
doi:10.1016/j.hepres.2006.03.004

hypotheses have been suggested. First, babies from mothers with high viral load during delivery can be subjected to a higher prophylaxis failure rate [4,7,8]. Second, HBV surface gene mutant viruses unneutralizable with anti-HBs can be emerged because of the immune pressure of HBV vaccine or immunoglobulin in perinatal prophylaxis [9–12]. Third, HBV carrier mothers may already have the unneutralizable surface gene mutant viruses among their viral population which can transfer to their babies [13,14].

On the aspect of host immune system, individual differences in immune systems like human leukocyte antigen (HLA) can cause different responses to virus and vaccination. HLA classes I and II molecules control viral antigen recognition by T-lymphocytes, and hence, their vaccinations can cause immune responses different in nature or magnitude [15]. Many studies have associated HLA with chronic HBV infection [16–27]. Definite relationships between the two factors have not been found because of inconsistency in results. However, several studies have demonstrated that DRB1*07 [20] and DRB1*13 [21–23,25,27] are associated with the infection. Almost all of these studies compared patients with a chronic natural HBV infection against either those on the recovery from an acute infection [16,19–25,27] or general population [17,18,26,27]. Few studies have shown the influence of HLA on perinatal HBV infection after prophylaxis [28]. Unlike natural infection cases, HBV vaccine or immunoglobulin given to prevent perinatal infection in this case can influence the immune system of babies born to HBV carrier mothers, and cause different results of infection.

We investigated the HLA classes I and II alleles in mothers with chronic HBV infection and their babies whom were succeeded or failed to perinatal prophylaxis, and compared the frequencies with those of healthy control subjects. Moreover, in order to strengthen the results, we extended a study to sequence the HBV surface gene in failed babies for eradicating the influence of the mutation in the failure of perinatal HBV prophylaxis.

2. Subjects and methods

2.1. Study subjects

During the period from 1996 to 2000, we screened all pregnant women for HBsAg in St. Vincent's Hospital, The Catholic University of Korea, and conducted a follow-up study on babies born to the HBsAg-positive mothers in order to keep track of the outcome of perinatal HBV prophylaxis administered by the pediatric hepatitis clinic in the same hospital. On that period, the rate of HBsAg-positive mothers was 4.8% (340/7038), and that of HBeAg positive was 37.8% (124/328). All of the babies born to HBsAg-positive mothers were vaccinated with plasma-derived vaccine (0.3 μg HBsAg, Hepaccine-B; CJ Co., Seoul, Korea) according to a three-dose vaccination schedule. Despite HBeAg status of their mothers, they also received 0.5 ml HBIG (100 IU anti-HBs, Hepabig; Green Cross Co., Yongin, Korea) within 12 h of birth.

We conducted the follow-up study on 202 babies (81 and 121 from HBeAg-positive and negative mothers, respectively) for more than 12 months. Eleven babies born to the HBeAg-positive mothers became HBV carriers despite perinatal prophylaxis, but no baby born to the HBeAg-negative mothers became a HBV carrier.

Between January 1999 and June 2001, we sampled subjects to get the HLA typing on HBeAg-positive pairs of mother and baby both attending the same clinic. There were 19 failed and 19 succeeded perinatal prophylaxis pairs. Among these sample subjects, we conducted follow-up on 11 failed pairs and all succeeded pairs in our hospital from birth. In addition, we kept track of eight failed pairs who received perinatal prophylaxis at another clinics after confirming their vaccination history with certifications. They received the same HBIG and vaccine at our hospital, or received another plasma-derived vaccine (0.3 μg HBsAg, Hepavax-B; Green Cross, Yongin, Korea), as a prophylaxis.

All of the mothers with HBeAg positive could not received perinatal prophylaxis for HBV infections because the HBV vaccine was introduced to Korea in 1983. We considered those mothers as a natural chronic HBV infection group. And, a control group of HLA typing was made up of 198 Koreans drawn from HBsAg-negative healthy individuals.

Informed consents were obtained from parents, and human experimentation guidelines of St. Vincent's Hospital, The Catholic University of Korea, were followed in the conduct of this research

2.2. Serologic test, ALT and DNA titers for HBV

We tested all of the maternal serum specimens for HBsAg, anti-HBs (AUSZYME and AUSAB; Abbott Laboratories, Tokyo, Japan), HBeAg and anti-HBe (Abbott Laboratories, Abbott Park, IL) by enzyme immunoassay according to the manufacturer's specification. We tested all babies' specimen for HBsAg, anti-HBs, and anti-HBc IgG (CORZYME; Abbott Laboratories, Abbott Park, IL) at first. Then, we tested HBsAg-positive specimens for HBeAg and anti-HBe. Alanine aminotransferase (ALT) as a liver function test, were checked by Sequential Multiple Autoanalyzer 747–200 (Hittachi, Tokyo, Japan).

The titers of HBV-DNA in the babies with failed perinatal prophylaxis were checked by LightCycler-FastStart DNA Master SYBR Green I kit (Roche Applied Science, Mannheim, Germany) using the LightCycler instrument (Roche Applied Science) according to the manufacturer's specification.

2.3. PCR amplification and nucleic acid direct sequencing of HBV-DNA

HBV-DNA was extracted from 50 μL heparinized blood samples with the MasterPure™ DNA Purification Kit (Epi-