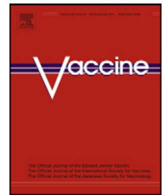
Vaccine 30 (2012) 5708–5713

Contents lists available at SciVerse ScienceDirect

Vaccine

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



Association of polymorphisms of cytokine and TLR-2 genes with long-term immunity to hepatitis B in children vaccinated early in life

Yangyang Wang[a,e,1], Peizhen Xu[b,1], Dongmei Zhu[c], Shu Zhang[d], Yongchun Bi[a], Yali Hu[d,e,**], Yi-Hua Zhou[a,c,e,*]

[a] Department of Laboratory Medicine, Nanjing Drum Tower Hospital, Nanjing University Medical School, Jiangsu, China
[b] Changzhou Women's and Children's Hospital, Jiangsu, China
[c] Department of Infectious Diseases, Nanjing Drum Tower Hospital, Nanjing University Medical School, Jiangsu, China
[d] Department of Obstetrics and Gynecology, Nanjing Drum Tower Hospital, Nanjing University Medical School, Jiangsu, China
[e] Jiangsu Key Laboratory for Molecular Medicine, Nanjing University Medical School, Jiangsu, China

ARTICLE INFO

Article history:
Received 26 February 2012
Received in revised form 29 June 2012
Accepted 8 July 2012
Available online 20 July 2012

Keywords:
Hepatitis B vaccine
Long-term immune response
Cytokine genes
Polymorphism

ABSTRACT

Hepatitis B vaccine is effective in preventing hepatitis B virus (HBV) infection. However, 5–10% of vaccinees fail to produce sufficient antibody against hepatitis B surface antigen (anti-HBs). In this study, we investigated the association of genetic polymorphisms with long-term response to hepatitis B vaccine in 301 children who received the vaccine 5–7 years ago. Of them, 86 (28.6%) had anti-HBs <10 mIU/ml (group A) and 215 (71.4%) had anti-HBs ≥10 mIU/ml (group B). While the frequencies of T allele and TT genotype in single nucleotide polymorphisms (SNP) rs2243250 and rs2070874 of interleukin (*IL*)-*4* in group A were higher than those in group B (all $P < 0.05$ and $q < 0.2$), the frequency of C allele in SNP rs2243250, rs2070874 and rs2227284 of *IL-4* in group B was higher than that in group A (all $P < 0.05$ and $q < 0.2$). None of 11 other SNP in IL-2, IL-10, IL-1β, IL-13, IL-12B, tumor necrosis factor-α, and toll-like receptor-2 genes was found to associate with anti-HBs response. SNP rs2070874 was associated with humoral response to hepatitis B vaccine after analyzed by multivariable logistic regression analysis ($P = 0.015$). The haplotype TT defined by SNP rs2243250 and rs2070874 in *IL-4* was associated with the poor humoral response (adjusted $P = 0.037$). Our findings demonstrate that *IL-4* gene polymorphisms may affect the long-term immune response to hepatitis B vaccine.

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1. Introduction

Hepatitis B virus (HBV) infection is a worldwide health problem. Hepatitis B vaccine, composed of hepatitis B surface antigen (HBsAg), is effective in preventing HBV infection. Vaccination with three-dose series on a 0-, 1-, 6-month schedule induces long-term protection in most vaccinees for more than two decades [1,2]. Since the introduction of universal vaccination in infants, chronic HBV infections have been substantially reduced in children [3,4]. However, the vaccine-induced immunity protection is inadequate in 5–10% vaccinees [5,6]. Deficiency of enough protective immunity poses the risk of infection with HBV.

Variation in immune response to vaccine is influenced by several factors such as age, gender, smoking and immunologic tolerance [7]. In addition, genetic factor is also considered to be important in the production of vaccine induced immunity [8,9]. A number of studies about correlations between immune response to hepatitis B vaccine and polymorphisms of human leukocyte antigen [10–17], immunoregulatory cytokine genes [18–22], cytokine receptor genes [22,23], or toll-like receptors (TLR) genes [22] have been reported. These studies mostly grouped the participants based on the peak antibody against hepatitis B surface antigen (anti-HBs) levels, which usually occur 1–2 months after the third vaccine dose. The peak anti-HBs level may indicate the vaccine efficacy, while the duration of hepatitis B vaccine-induced immunity could reflect long-term antibody protection. Recently, the influence of 3 single nucleotide polymorphisms (SNP) in interleukin (*IL*)-*10* and 6 *IL-10*

Abbreviations: HBV, hepatitis B virus; anti-HBs, antibody against hepatitis B surface antigen; SNP, single nucleotide polymorphisms; HBsAg, hepatitis B surface antigen; TLR, toll-like receptors; anti-HBc, antibody against hepatitis B core antigen; IL, interleukin; TNF, tumor necrosis factor; RFLP, restriction fragment length polymorphism; HWE, Hardy–Weinberg Equilibrium; Ors, odds ratios; 95% CI, 95% confidence intervals.

* Corresponding author at: Departments of Laboratory Medicine and Infectious Diseases, Nanjing Drum Tower Hospital, Nanjing 210008, China.
Tel.: +86 25 8330 4616x10373; fax: +86 25 8310 5987.
** Corresponding author at: Department of Obstetrics and Gynecology, Nanjing Drum Tower Hospital, 321 Zhongshan Road, Nanjing 210008, China.
Tel.: +86 25 8330 4616x66808; fax: +86 25 8310 5998.
E-mail addresses: dtylhu@126.com (Y. Hu), yzh20061111@yahoo.com (Y.-H. Zhou).
[1] These authors contributed equally to this work.

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http://dx.doi.org/10.1016/j.vaccine.2012.07.010

Table 1
Primers, restriction enzymes and restriction fragments length of investigated single nucleotide polymorphisms (SNP).

SNP	Gene	Primers	Restriction enzymes	Restriction fragments (bp)
rs1143627	*IL-1β*	F: TCTTTTCCCCTTTCCT TTAACT R: GAGAGACTCCCTTAGCAC	Alu I	T: 150, 84 C: 234
rs16944	*IL-1β*	F: TGGCATTGATCTGGTTCATC R: GTTTAGGAATCTTCCCACTT	Bsu36 I	C: 305 T: 191, 114
rs1143634	*IL-1β*	F: CTCAGGTGTCCTCGAAGAAATCAAA R: GCTTTTTTGCTGTGAGTCCCG	Taq I	C: 110, 85 T: 195
rs2243250	*IL-4*	F: ACTAGGCCTCACCTGATACG R: GTTGTAATGCAGTCCTCCTG	BsmF I	C: 209, 45 T: 254
rs2070874	*IL-4*	F: CTCATTTTCCGTCGGTTTCAGC R: GAAGCAGTTGGGACGTGAGA	Mnl I	C: 93, 47 T: 150
rs2227284	*IL-4*	F: TAGGTCCTGGGCTTCACAG R: TTAGCTCTCTTTGGTAAATAGGGAA	Hinf I	G:133, 27 T: 160
rs2069762	*IL-2*	F: GTGATAGCTCTAATTCATGC R: ATTCACATGTTCAGTGTAGTTCT	Mae I	C: 110, 21 T: 131
rs2069763	*IL-2*	F:ATGTACAGGATGCAACTCCT R:TGGTGAGTTTGGGATTCTTG	Mwo I	G: 151+111 T: 262
rs1800872	*IL-10*	F: ATCCAAGACAACACTACTAA R: TAAATATCCTCAAAGTTCC	Rsa I	A: 240, 233, 65, 42, 8 C: 305, 233, 42, 8
rs1800896	*IL-10*	F: CTCGCCGCAACCCAACTGGC R: TCTTACCTATCCCTACTTCC	Mnl I	G: 111, 31 A: 142
rs3212227	*IL-12B*	F: GGCATGAAATCCCTGAAACC R: TACATCCTGGCAGACAAACG	Taq I	C: 269, 152 A: 421
rs1295686	*IL-13*	F: GGCTGAATATCCATGGTGTGTGTCC R: GGCTGAGGTCGGCTAGGCTGAAGAC	BsaA I	C: 310, 249 T: 559
rs1800629	*TNF-α*	F: AGGCAATAGGTTTTGAGGGCCAT R: ACACTCCCCATCCTCCCGGCT	Nco I	G: 97, 20 A: 117
rs3804100	*TLR-2*	F: AGCCTGTGAGGATGCCTG R: AACATGGGTAAGAGGGAGGC	Mwo I	C: 210, 152 T: 362

genetic haplotypes on immune response to hepatitis B vaccine has been analyzed in 454 students born 20 years ago in Taiwan. This study found that ATA/ACC genotype defined by SNP (rs1800896, rs1800871 and rs1800872) in *IL-10* was associated with poor antibody response to hepatitis B vaccine, while ACC/ACC genotype was associated with good response [24]. In the present study, we investigated the association of nucleotide variants of the cytokine genes and TLR-2 gene with the duration of hepatitis B vaccine-induced immunity in 301 healthy children who received three doses vaccination 5–7 years ago.

2. Materials and methods

2.1. Subjects

Since 2002, vaccination against hepatitis B has been integrated into the China's Planned Vaccination Program; all newborns in China may receive 3 doses (5 μg HBsAg/dose) charge-free recombinant (yeast) hepatitis B vaccine [3]. The first dose is used in the hospital within 24 h after birth and the second and third doses are administered in the local Children's Immunization Clinic at age of 1 and 6 months respectively. The vaccine, licensed by Merck & Dohme Co. through the technology transfer, is manufactured by Kangtai Biological Company, Shenzhen, China.

In a long-term follow-up of the provincial prevalence of birth defects conducted October 2009 through March 2010, blood samples (~3 ml from each subject) were collected from 374 HBsAg-negative Han Chinese children, who were born in 2003 and 2004. Blood cells and serum were separated and were kept at −20 °C. Of them, 70 subjects who received booster vaccination and 3 subjects who were antibody against hepatitis B core antigen (anti-HBc) positive, indicating the occurrence of resolved infection, were excluded. Thus, a total of 301 subjects were included in the further analysis. All 301 participants received 5 μg hepatitis B vaccine according to a 0-, 1-, 6- month vaccination schedule after birth. This study was approved by the ethics committees of Nanjing Drum Tower Hospital, Nanjing University Medical School. Written or witnessed verbal informed consent was obtained from all the children's parents.

HBsAg, anti-HBs, and anti-HBc were quantitatively tested using microparticle enzyme immunoassay (AxSYM AUSAB kit; Abbott Laboratories, North Chicago, USA). Subjects with anti-HBs titers <10 mIU/ml and ≥ 10 mIU/ml were divided into group A and group B, representing the long-term low responders and long-term responders, respectively.

2.2. DNA extraction and genotyping

Genomic DNA was extracted from white blood cells by rapid isolation. Briefly, the cells were digested by proteinase K, followed by precipitating protein with acetate solution, and precipitating DNA by isopropanol respectively. After rinsed by 70% ethanol, the DNA was dissolved in 100 μl Tris-EDTA buffer. Fourteen SNP in *IL-4* (3 SNP), *IL-2* (2), *IL-10* (2), *IL-1β* (3), *IL-13*, *IL-12B*, tumor necrosis factor (*TNF*)-*α* and *TLR-2* were detected using polymerase chain reaction-restriction fragment length polymorphism (RFLP) technique. Details of primers that amplify DNA fragments containing the polymorphism sites are listed in Table 1. The restriction fragments were separated on 2–3.5% ethidium bromide-stained agarose gel, and visualized with ultraviolet light. Furthermore, for each SNP, the PCR products of three to five samples were selected to subject direct DNA sequencing on ABI Prism 3130

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