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Effects of cytokine and cytokine receptor gene variation on high anti-HB titers: Following up on Taiwan's neonatal hepatitis B immunization program☆

Ying-Ju Lin [a,b,1], Yu-Ching Lan [c,1], Yu-Chuen Huang [a,b], Ting-Hsu Lin [a], Shao-Mei Huang [a], Chien-Chen Lai [d], Chiu-Shong Liu [e], Cheng-Wen Lin [f], Shih-Yin Chen [a,b], Fuu-Jen Tsai [a,b,*]

[a] Department of Medical Research, China Medical University Hospital, Taichung, Taiwan
[b] School of Chinese Medicine, China Medical University, Taichung, Taiwan
[c] Department of Health Risk Management, China Medical University, Taichung, Taiwan
[d] Institute of Molecular Biology, National Chung Hsing University, Taichung, Taiwan
[e] Department of Family Medicine, China Medical University Hospital, Taichung, Taiwan
[f] Department of Medical Laboratory Science and Biotechnology, China Medical University, Taichung, Taiwan

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ABSTRACT

Background: A significant percentage of Taiwanese neonatal HB immunization recipients have subsequently exhibited low anti-HB titers at non-protective or undetectable levels. Several mechanisms have been proposed to explain this phenomenon, including low vaccination responsiveness, deficient lymphocyte function, inappropriate antigen processing and presentation, and abnormal cytokine secretion.
Methods: To determine genetic influences resulting in high anti-HB titers, we divided a study cohort of 183 individuals into an anti-HBs ≥ 1000 mIU/mL group and a 10–1000 mIU/mL anti-HBs titer group. Chi-square tests were used to compare genotype and allelic frequencies between the two groups.
Results: Data from univariate and multivariate regression analyses of cytokine and cytokine receptor gene variants indicate (a) increased potential of high anti-HB titers in the presence of the TT genotype of the *IL-4* rs2243250 SNP (OR = 3.19; $p = 0.012$) and the AA genotype of the *IL-4R* rs1805010 SNP (OR = 2.25; $p = 0.048$), and (b) individuals carrying the TT genotype of the *IL-4* rs2243250 SNP had anti-HB titers at levels that were almost twice as high as those in individuals carrying the CC genotype (478.8 mIU/mL and 290.3 mIU/mL, respectively; $p = 0.033$).
Conclusion: Genetic determinants, especially *IL-4* and *IL-4R*, may contribute to high anti-HB titers in immune responses to HB vaccinations.

1. Introduction

The national hepatitis B (HB) vaccination program in Taiwan, which began in 1984, has resulted in a significant reduction in the carrier rate [1]. However, between 1% and 10% of all vaccinated individuals failed to produce sufficient levels of protective antibodies [2,3], and levels in another segment of the vaccinated population have shown declines to low or virtually undetectable titers over time, resulting in increased risk of HB infection [4–6]. Possible reasons for this phenomenon include low vaccination responsiveness, deficient lymphocyte function, inappropriate antigen processing and presentation, and abnormal cytokine secretion profiles [6–8].

Researchers of inter-individual differences in vaccine responses have focused on immune-related genes, including human leukocyte antigen alleles that modulate antigen processing and presentation [9,10]. Others have addressed genetic variation in immune-related genes such as cytokines, cytokine receptors, and toll-like receptors [6,8,11–13]. Cytokines and cytokine receptors play important regulatory roles in Th1/Th2 balance in immune responses to virus infection and vaccination [14–17]. The *IL-1 beta* (+3953) minor allelic variant is associated with both anti-HB titers and T-cell lymphoproliferative response to HBsAg [17]. Immunoregulatory cytokine gene polymorphisms in *IL-2*, *IL-4*, *IL-10* and *IL-12 B* genes are also correlated with variable immune response to recombinant HBV vaccines [6,16]. These findings have implications for vaccine efficacy—for example, cytokine adjuvant may help maintain Th1/Th2 balance in recipients with diverse genetic backgrounds.

Our goal for this study was to determine the likelihood of cytokine and cytokine receptor gene variants to regulate anti-HB titer variation in immune responses to HB neonatal vaccinations. In addition to the pro-inflammatory cytokines *IL-1* and *TNF-alpha*, we also examined genetic variants of Th1 (*IL-2RA*, *IL-12B*, *IL-12RB1* and *IL-12RB2*) and

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* Corresponding author at: Department of Medical Research, China Medical University Hospital, Taichung, Taiwan. Tel.: +886 4 22062121; fax: +886 4 22033295.
E-mail address: d0704@mail.cmuh.org.tw (F.-J. Tsai).
[1] Contributed equally to this work.

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Table 1
Clinical characteristics of students presenting moderate and high titers to the HBV vaccine.

	Total (n = 176)	Anti-HBs titer group		*p* value
		I: anti-HBs ≥ 1000 mIU/mL	II: 1000 > anti-HBs ≥ 10 mIU/mL	
		(n = 42)	(n = 141)	
Age(years)	18.75(±0.733)	18.83(±0.775)	18.72(±0.723)	0.430
BMI (kg/m^2, mean (±SD))	21.51 (±3.183)	21.51 (±3.240)	21.50 (±3.009)	0.681
Systolic BP (mm Hg, mean (±SD))	116.5 (±13.07)	116.4(±12.59)	116.5 (±13.24)	0.890
Diastolic BP (mmHg, mean (±SD))	71.11 (±9.632)	71.61 (±8.891)	70.97 (±9.849)	0.840
Hb (gm/dL, mean (±SD))	14.45 (±1.290)	14.49 (±1.097)	14.43 (±1.341)	0.9 12
AST(IU/L, mean(±SD))	25.40 (±25.04)	22.58 (±8.265)	26.16 (±27.88)	0.064
ALT(IU/L, mean(±SD))	19.60 (±18.05)	20.24(±23.96)	19.42 (±16.18)	0.864
Creatinine (mg/dL, mean (±SD))	0.9640 (±0.9942)	1.247 (±2. 128)	0.8871 (±0.1591)	0.448
Uric acid(mg/dL, mean(±SD))	5.713 (±1.440)	5.605 (±1.719)	5.743 (±1.360)	0.874
Cholesterol (mg/dL, mean(±SD))	163.0 (±29.59)	165.2 (±39.78)	162.4(±26.31)	0.457
Triglyceride (mg/dL, mean (±SD))	66.60 (±42.17)	82.39 (±73.71)	62.31 (±27.04)	0.191
Glucose AC (mg/dL, mean (±SD))	92.57 (±15.25)	89.47 (±10.24)	93.41 (±16.28)	0.872
Anti-HB titers (mIU/mL, mean (±SD))	373.5 (±398.0)	1000 (±0.0)	203.4 (±255.5)	<**0.0001**

BMI, body mass index; BP, blood pressure; Hb, hemoglobin; ALT, alanine aminotransferase; AST, aspartate aminotransferase.
Bold font emphasizes statistical significance ($p < 0.05$).

Th2 (*IL-4*, *IL-4R*, *IL-10* and *IL-13*). All selected SNPs have known effects on biological functions.

2. Participants and methods

The research design was approved by the Human Subjects Committee of the Institutional Review Board of CMU Hospital.

2.1. Hepatitis B (HB) vaccination program

The start date of Taiwan's national HB vaccination program was July 1, 1984 [18]. During the first twelve months it only covered the newborns of HBsAg-carrier mothers, but starting in July 1986 the program covered all newborns, and one year later it was extended to all preschool and elementary school students, teenagers, and adults. Starting in July 1991, the vaccination records of all children entering the first grade had to include HB, with vaccinations or boosters given to those with no or partial records. Today, all pregnant Taiwanese women are screened for HBsAg, and those identified as HBsAg-positive are tested for the hepatitis B e antigen (HBeAg). All newborns of high-titer HBsAg carriers or HBeAg-positive mothers are given 0.5 ml (100 IU) of hepatitis B immunoglobulin within 24 h of birth.

2.2. Study population and design

We conducted a cross-sectional seroprevalence survey in September 2005. Study data were collected for new Taiwanese graduate and freshman students of China Medical University, who are given health screenings prior to admission. Voluntary participants signed informed consent documents. The final sample of 183 students did not include those with histories of HCV infection, chronic disease, cancer, pregnancy, lactation, excessive alcohol consumption, drug abuse, or immunosuppressant treatment. Participants were divided into two groups: group I, anti-HBs ≥ 1000 mIU/mL, and group II, 1000 > anti-HBs ≥ 10 mIU/mL. All participants were born in 1986 or later, and therefore had received neonatal HB immunizations at birth and at 1, 2 and 12 months of age.

2.3. HB seromarkers and genotyping

Serological measurements focused on HBsAg and anti-HB titers, which were determined by enzyme immunoassays (Abbott Laboratories, North Chicago, IL). Protective anti-HB titer was defined as > 10 mIU/mL, and non-protective as < 10 mIU/mL. HBsAg-positive individuals were assumed to be HB carriers. Standard protocols were used to extract Genomic DNA from participants identified as HBsAg-negative (Roche Genomic DNA kit). Primers and probes were established using the Applied Biosystems Assay-by-Design™ service. Cytokine and cytokine receptor gene polymorphisms were detected by TaqMan® Genotyping Assays (Applied Biosystems) (Table 2).

2.4. Statistical analyses

Unless otherwise indicated, data are expressed as mean ± SD for the continuous variables. SPSS 12.0 for Windows was used to analyze all data. Specifically, $\chi 2$ tests were used to determine differences in categorical variables, and odds ratios (OR) and 95% confidence intervals (CI) were calculated for the factors under consideration. We also performed forward stepwise multivariate regression analyses to identify factors contributing independently to anti-HB titers. Genotypes were obtained by direct count, followed by allele frequency calculations. In addition to χ^2 tests, p values were calculated using the Minitab program; p values < 0.05 were considered statistically significant.

3. Results

3.1. Demographic characteristics and anti-HB titers

The only statistically significant difference found between the two groups was for mean anti-HB titers ($p < 0.0001$) (Table 1), indicating that all other demographic characteristics were similar between individuals with high and moderate titers to the HB vaccine. No significant differences were found in overall demographic characteristics between groups I and II.

Table 2
Genes and SNPs analyzed for associations with vaccine-induced immune response.

Gene	Chromosome	Position	Position	Nucleotide change	SNP ID
TNF alpha	6	31,651,010	−308	A/G	rs1800629
IL-1 beta	2	113,311,338	−511	G/A	rs16944
IL-2 RA	10	6,126,391	−17,245	C/T	rs706781
IL-4	5	132,037,053	−590	C/T	rs2243250
IL-4 R	16	27,263,704	I75V	A/G	rs1805010
IL-4 R	16	27,281,901	Q576R	A/G	rs1801275
IL-10	1	206,946,634	−819	A/G	rs1800871
IL-12 B	5	158,759,900	+8275	A/G	rs2546890
IL-12 RB1	19	18,034,603	−517	C/T	rs372889
IL-12 RB2	1	67,794,818	−593	A/G	rs1495964
IL-13	5	131,995,964	Q144R	A/G	rs20541

3.2. Allele and genotype frequencies of cytokine gene polymorphisms

We genotyped cytokine and cytokine receptor gene polymorphisms to determine associations between genetic variants and variation in anti-HB titers. Genotype, genotype frequency, and allele data for eleven genes are shown in Tables 2 and 3. As shown, statistically significant differences were observed between groups I and II for the *IL-4* (rs2243250) and *IL-4R* (rs1805010) genetic variants (allelic frequencies: $p = 0.010$ and $p = 0.026$, respectively). Frequencies of individuals carrying the T allele of *IL-4* (rs2243250) were 89.2% for group I and 75.2% for group II ($p = 0.010$). Frequencies of individuals carrying the A allele of *IL-4R* (rs1805010) were 61.1% for group I and 46.4% for group II ($p = 0.026$).

We also investigated associations between cytokine gene SNPs and anti-HB titers in terms of immune responses to HB vaccination; results are shown in Table 4. The only significant association was observed for the *IL-4* (rs2243250) SNP. Individuals carrying the TT genotype of that SNP had almost twice as much anti-HB titer as individuals carrying the CC genotype (mean 478.8 mIU/mL, 95% CI = 398.8–558.8 mIU/mL versus mean 290.3 mIU/mL, 95% CI = 56.88–523.8 mIU/mL; $p = 0.0033$).

3.3. Genetic factors for high anti-HB titers

According to results from a univariate regression analysis, statistically significant ORs were noted for the *IL-4* (rs2243250) and

Table 3
Allelic and genotype frequencies of the regulatory genes for the cytokines in vaccinated students presenting moderate and high titers to the HBV vaccine.

Gene	Position	SNP ID	Allele/ Genotype	Group 1: anti-HBs ≥ 1000 mIU/mL (n = 42)	Group II: 1000 > anti-HBs ≥ 10 mIU/mL (n = 141)	*p* value	Odds ratio (95% CI)
TNF alpha	−308	rs1800629	AA	0 (0.0)	0 (0.0)	0.745	–
			AG	8 (21.1)	26 (18.7)		1.16 (0.48–2.82)
			GG	30 (78.9)	113 (81.3)		1
			A	8 (10.5)	15 (9.0)	0.758	1.14 (0.49–2.63)
			G	68 (89.5)	153 (91.0)		1
IL-1 beta	−511	rs16944	AA	5 (13.2)	29 (20.7)	0.463	0.49 (0.16–1.53)
			AG	20 (52.6)	74 (52.9)		0.77 (0.34–1.72)
			GG	13 (34.2)	37 (26.4)		1
			A	30 (39.5)	132 (47.1)	0.234	0.73 (0.44–1.23)
			G	46 (60.5)	148 (52.9)		1
IL-2 RA	−17,245	rs706781	CC	6 (15.8)	9 **(6.5)**	0.125	2.38 (0.77–7.37)
			CT	9 (23.7)	48 (34.5)		0.67 (0.29–1.56)
			TT	23 (60.5)	82 (59.0)		1
			C	21 (27.6)	66 (23.7)	0.485	1.23 (0.69–2.18)
			T	55 (72.4)	212 (76.3)		1
IL-4	−590	rs2243250	CC	1 (2.7)	10 (7.2)	***0.031***	**0.27 (0.03–2.17)**
			CT	6 (16.2)	49 (35.3)		**0.33 (0.13–0.84)**
			TT	30(81.1)	80(57.6)		**1**
			C	8(10.8)	69(24.8)	***0.010***	**0.37(0.17–0.80)**
			T	66 (89.2)	209 (75.2)		**1**
IL-4R	175 V	rs1805010	AA	14 (38.9)	33 (23.6)	0.102	3.04 (1.05–8.76)
			AG	16(44.4)	64(45.7)		1.79 (0.65–4.94)
			GG	6 (16.7)	43 (30.7)		1
			A	44 (61.1)	130(46.4)	***0.026***	**1.81(1.07–3.08)**
			G	28 (38.9)	150 (53.6)		**1**
IL-4R	Q576R	rs1801275	AA	26 (68.4)	106 (75.7)	0.241	–
			AG	12(31.6)	29(20.7)		–
			GG	0 (0.0)	5 (3.6)		1
			A	64 (84.2)	241 (86.1)	0.681	0.86 (0.43–1.74)
			G	12 (15.8)	39 (13.9)		1
IL-10	−819	rs1800871	AA	16 (41.0)	69 (50.7)	0.563	0.70 (0.24–2.03)
			AG	17 (43.6)	49 (36.0)		1.04 (0.35–3.05)
			GG	6 (15.4)	18 (13.2)		1
			A	49 (62.8)	187 (68.8)	0.325	0.77 (0.45–1.30)
			G	29 (37.2)	85 (31.2)		1
IL-12 B	+8275	rs2546890	AA	5 (13.2)	24 (17.1)	0.484	0.59 (0.194.79)
			AG	16 (42.1)	68 (48.6)		0.66 (0.31–1.44)
			GG	17(44.7)	48(34.3)		1
			A	26 (34.2)	116 (41.4)	0.254	0.74 (0.43–1.25)
			G	50(65.8)	164(58.6)		1
1L-12 RB1	−517	rs372889	CC	4 (10.5)	25 (18.0)	0.545	0.53 (0.16–1.78)
			CT	19 (50.0)	64 (46.0)		0.99 (0.46–2.14)
			TT	15 (39.5)	50 (36.0)		1
			C	27 (35.5)	114 (41.0)	0.387	0.79 (0.47–1.34)
			T	49(64.5)	164(59.0)		1
IL-12 RB2	−593	rs1495964	AA	2 (5.3)	22 (16.1)	0.167	0.35 (0.07–1.68)
			AG	23 (60.5)	65 (47.4)		1.36 (0.63–2.95)
			GG	13 (34.2)	50 (36.5)		1
			A	27 (35.5)	109 (39.8)	0.501	0.83 (0.49–1.41)
			G	49 (64.4)	165 (60.2)		1
IL-13	Q144R	rs20541	AA	6 (16.2)	15 (12.6)	0.743	1.49 (0.49–4.51)
			AG	16 (43.2)	48 (40.3)		1.24 (0.56–2.78)
			GG	15 (40.5)	56 (47.1)		1
			A	28 (37.8)	78 (32.8)	0.422	1.25 (0.73–2.15)
			G	46 (62.2)	160 (67.2)		1

95% CI, 95% confidence intervals; ND, not determined.
The significance of data in bold emphases indicated *p* value < 0.05.

Table 4
Influences of the cytokine gene SNPs on anti-HB titers.

Gene	Position	SNP ID	Genotype	N	Anti-HBs titer (mIU/mL, mean [95%CI])	*p* value
TNF alpha	−308	rs1800629	AA	0	ND	
			AG	33	290.3 [56.88–523.8]	
			GG	133	332.8 [233.0–432.6]	0.3893 (AA + AG vs. GG)
IL-1beta	−511	rs16944	AA	34	395.9 [263.5–528.3]	
			AG	86	411.0 [324.7–497.4]	
			GG	47	373.3 [251.5–495.1]	0.5170 (AA + AG vs. GG)
IL-2 RA	−17,245	rs706781	CC	13	569.2 [306.2–832.1]	
			CT	56	357.3 [256.1–458.5]	
			TT	97	401.0 [319.3–482.7]	0.7642 (CC + CT vs. TT)
IL-4	−590	rs2243250	CC	11	290.3 [56.88–523.8]	
			CT	53	306.2 [206.5–405.9]	
			TT	108	478.8 [398.8–558.8]	***0.0033*** (CC + CT vs. TT)
IL-4 R	175 V	rs1805010	AA	44	416.4 [287.2–545.5]	
			AG	75	408.4 [319.3–497.5]	
			GG	46	334.9 [220.2-449.5]	0.7004 (AA + AG vs. GG)
IL-4 R	Q576R	rs1801275	AA	125	373.0 [303.9–442.1]	
			AG	37	521.5 [380.9–662.2]	
			GG	5	86.94 [−102.8-276.6]	0.3282 (AG + GG vs. AA)
IL-10	−819	rs1800871	AA	78	378.5 [291.9–465.2]	
			AG	63	455.1 [349.0–561.3]	
			GG	31	417.2 [−260.7–573.8]	0.8928 (AG + GG vs. AA)
IL-12 B	+8275	rs2546890	AA	28	331.9 [183.1–480.8]	
			AG	80	361.1 [273.5–448.8]	
			GG	59	477.5 [371.1–583.8]	0.0682 (AA + AG vs. GG)
IL-12 RB1	−517	rs372889	CC	28	331.4 [197.0–465.7]	
			CT	77	383.1 [289.7–476.6]	
			TT	61	451.6 [347.4–555.8]	0.2900 (CC + CT vs. TT)
IL-12 RB2	−593	rs1495964	AA	22	354.1 [200.3–507.9]	
			AG	83	436.8 [344.6–529.0]	
			GG	59	371.3 [269.1–473.5]	0.6854 (AA + AG vs. GG)
IL-13	Q144R	rs20541	AA	18	477.9 [262.8–693.1]	
			AG	61	430.8 [322.5–539.1]	
			GG	66	441.1 [347.0–535.1]	0.8071 (AA + AG vs. GG)

95% CI, 95% confidence intervals; ND, not determined.
The significance of data in bold emphases indicated p value<0.05.

IL-4R (rs1805010) genetic variants (allelic frequencies: $p = 0.010$ and $p = 0.026$, respectively) (Table 3). After adjusting for these potential factors, results from a multivariate regression analysis using anti-HB titers as the dependent variable indicate associations between high anti-HB titers and both *IL-4* (rs2243250) and *IL-4R* (rs1805010) SNPs (Table 5). Specifically, significant correlations were found between high anti-HB titers and the presence of both the TT genotype of the *IL-4* rs2243250 SNP (OR = 3.19; $p = 0.012$) and AA genotype of the *IL-4R* rs1805010 SNP (OR = 2.25; $p = 0.048$).

4. Discussion

We previously reported an association between low anti-HB titers in immune responses to HB vaccination and *IL-10* genetic variants [6]. In this study we screened for cytokine and cytokine receptor gene variants, and found (a) increased potential for high anti-HB titers in the presence of the TT genotype of the *IL-4* rs2243250 SNP and the AA genotype of the *IL-4R* rs1805010 SNP; and (b) that individuals carrying the TT genotype of the *IL-4* rs2243250 SNP had anti-HB titers at levels that were almost twice as high as in individuals carrying the CC genotype. Combined, these results suggest that genetic determinants, especially *IL-4* and *IL-4R*, contributed to high anti-HB titers in immune responses to HB vaccination.

Table 5
Results from multivariate regression for genetic factors associated with high anti-HB titers between groups I and II.

Characteristic	Odds ratio	95% Confidence Interval	*p* value
AST	0.99	0.941–1.031	0.510
IL-4 rs2243250 TT/CC + CT	3.19	1.287–7.895	***0.012***
IL-4 R rs1805010 AA/AG + GG	2.25	1.007–5.029	***0.048***

Bold font emphasizes statistical significance (p<0.05).

Cytokines and cytokine receptors play important regulatory roles in Th1/Th2 balance in immune responses to virus infections and vaccinations [14–17]. We found that the frequencies of one SNP in the 5′ near gene of *IL-4* (−590 or rs2243250) and one in the coding region of *IL-4R* (I75V or rs1805010) were significantly different in individuals with high and moderate anti-HB titers. In addition, multivariate regression results indicate that rs2243250 and rs1805010 had significant associations with high anti-HB titers. Further, we found associations between *IL-4* and *IL-4R* SNPs and high antibody responses to HBV vaccination.

IL-4, a T_H2 cytokine, is the primary cytokine in T cell-driven humoral immune responses [19]. IL-4 and IL-4 receptor (IL-4R) play important roles in antibody response regulation by B lymphocytic cells [20]. Recent reports indicate that the IL-4 −590 T allele (rs2243250) increases transcriptional activity, is associated with higher antibody responses to diphtheria and HBV, and is associated with lower antibody responses to pneumococcal serotypes [16,21,22]. Another research team has reported an association between the I75V SNP (rs1805010) in the *IL-4R* gene and increased sensitivity to IL-4 stimulation and measles vaccine-induced immunity [12]. As a T_H2 typical cytokine, IL-4 is an antagonistic cytokine of typical T_H1 cytokine IFN-gamma. The T_H1 cytokines contain IL-2, IFN-gamma, TNF-alpha and TGF-beta and trigger the cell-mediated immune response. There was a reported genetic association but with no significant results between *IFN-gamma* SNPs and measles vaccine immunity [12]. However, there were currently no reported genetic associations between *IFN-gamma* and its receptor and anti-HB titers. Several studies have focused on analysis of in vitro HBsAg-induced cytokine production but with contradictory results [23–25]. Tsutsui et al. reported that there was no correlation between function and

cytokine production of HBsAg-specific human CD4(+)-cloned T cells [25]. Other studies showed a default of IFN-gamma production among non-responders [23,24]. For Jarrosson et al. [26], the IFN-gamma production was detected in both responders and non-responders, and lesser IFN-gamma production was found in non-responders. Our results indicate an association between *IL-4* and *IL-4R* functional SNPs and high anti-HB titers during the 20 years following the initiation of a neonatal HB vaccination program in Taiwan, suggesting that genetic variability in the *IL-4* promoter and coding region may play an important role in regulating immediate responses to HBsAg, as well as maintaining serum anti-HB titers.

In conclusion, our findings support the idea that cytokine and cytokine receptor gene SNPs may contribute to variation in anti-HB titers in response to hepatitis B vaccinations. Our data also suggest the involvement of genetic variation in *IL-4* and *IL-4R* regarding HB vaccine-induced immunity. Research using larger cohorts is required to confirm these results. It is our belief that understanding the genetic influences of hepatitis B immunity will support the development of novel HB vaccines in combination with immune-regulatory cytokines to improve vaccine efficacy in recipients who have a range of genetic backgrounds.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.cca.2012.03.004.

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