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ORIGINAL ARTICLE

Basic Study

Potential role of killer immunoglobulin receptor genes among individuals vaccinated against hepatitis B virus in Lebanon

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Abstract

AIM

To explore the role of killer immunoglobulin receptor (*KIR*) genes in responsiveness or non-responsiveness to vaccination against hepatitis B virus.

METHODS

We recruited 101 voluntary participants between March 2010 and December 2011. Sera samples from vaccinated and non-vaccinated participants were tested for the presence of anti-HBs antibodies as a measure of protection against hepatitis B, hepatitis B surface antigen and hepatitis B core antibody as indicators of

infection by enzyme-linked immunosorbent assay. *KIR* gene frequencies were determined by polymerase chain reaction.

RESULTS

Sera samples from 99 participants were tested for the levels of anti-HBs as an indicator of protection (≥ 10 mIU/mL) following vaccination as defined by the World Health Organization international reference standard. Among the vaccinated participants, 47% (35/74) had anti-HBs titers above 100 mIU/mL, 22% (16/74) had anti-HBs ranging between 10-100 mIU/mL, and 20% (15/74) had values of less than 10 mIU/mL. We report the lack of significant association between the number of vaccine dosages and the titer of antibodies among our vaccinated participants. The inhibitory KIR2DL1, KIR2DL4, KIR3DL1, KIR3DL2, and KIR3DL were detected in more than 95%, whereas KIR2DL2, KIR2DL3, KIR2DL5 (KR2DL5A and KIR2DL5B) were expressed in 56%, 84% and 42% (25% and 29%) of participants, respectively. The observed frequency of the activating *KIR* genes ranged between 35% and 55% except for KIR2DS4, detected in 95% of the study participants (40.6% 2DS4*001/002; 82.2% 2DS4*003/007). KIR2DP1 pseudogene was detected in 99% of our participants, whereas KIR3DP*001/02/04 and KIR3DP1*003 had frequencies of 17% and 100%, respectively. No association between the frequency of *KIR* genes and anti-HBs antibodies was detected. When we compared the frequency of *KIR* genes between vaccinated individuals with protective antibodies titers and those who lost their protective antibody levels, we did not detect a significant difference. KIR2DL5B was significantly different among different groups of vaccinated participants (group Ⅰ > 100 mIU/mL, group Ⅱ 10-100 mIU/mL, group Ⅲ < 10 mIU/mL and group Ⅳ with undetectable levels of protective antibodies).

CONCLUSION

To our knowledge, this is the first study screening for the possible role of *KIR* genes among individuals vaccinated against hepatitis B virus (HBV). Our results can be used to design larger studies to better understand the role of *KIR* genes in protection against or susceptibility to HBV post vaccination.

Key words: Hepatitis B virus; Killer immunoglobulin receptors; Hepatitis B vaccine; Lebanon; Natural killer cells

Core tip: Currently, there are no data supporting the use of booster doses of hepatitis B vaccine among immuno-competent individuals responding to a complete primary vaccination regimen. Importantly, 5%-10% of healthy adults do not generate protective levels of antibodies and are hence considered non-responders. This study aims to explore the role of killer immunoglobulin receptor genes in responsiveness or non-responsiveness to vaccination against hepatitis B virus.

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INTRODUCTION

Infection with hepatitis B virus (HBV) results in a spectrum of clinical outcomes ranging from acute hepatitis to end-stage liver disease and hepatocellular carcinoma[1] with an estimated lifetime risk of 25%-40%[2]. Booster studies suggest that memory begins to decline 15 years following vaccination among adolescents vaccinated in infancy[3-6]. Other studies suggest the persistence of immune memory for 20 years or longer[7-9]. Currently, there are no data supporting the use of booster doses of hepatitis B vaccine among immuno-competent individuals responding to a complete primary vaccination regimen (3 doses). Importantly, 5%-10% of healthy adults do not generate protective levels of antibodies and are hence considered non-responders[10]. Consequently, long-term protection is still debatable[11] and not linked to genetic factors.

Natural killer (NK) cells are known to induce antiviral and antitumor immunity *via* production of pro-inflammatory cytokines and lysis of infected or transformed cells[12]. Killer immunoglobulin receptor (*KIR*) genes encode receptors expressed on NK cells. Based on the gene content, two groups of *KIR* haplotypes are known in humans: A and B. Haplotype A encodes inhibitory receptors and consists of nine genes (3DL3, 2DL3, 2DP1, 2DL1, 3DP1, 2DL4, 3DL1, one activating (2DS4), 3DL2, and 2DL5) whereas haplotype B carries a variety of gene combinations and encodes more activating receptors as compared to haplotype A (3DL3, 2DS2, 2DL2, 2DL5B (inhibitory) 2DS3, 2DP1, 2DL1, 3DP1, 2DL4, 3DS1, 2DL5A (inhibitory), 2DS5, 2DS1, and 3DL2)[13].

KIR3DS1- and KIR3DL1-expressing NK cells were reported to expand in acute and chronic human immunodeficiency virus (HIV)-1 infection, respectively[14]. Similarly, reports suggest that KIR2DL2 and/or KIR2DL3 along with their ligand human leukocyte antigen (HLA)-C1 are associated with severe influenza infection; in addition, the frequency of KIR3DS1, KIR2DS5 and KIR2DL5 was also related to the severity of the disease[15]. KIR2DS2 and KIR2DS3 were found to be associated with susceptibility to chronic hepatitis B infection, whereas KIR2DS1, KIR3DS1 and KIR2DL5 may act as protective genes leading to viral clearance among the Chinese Han population[16]. A difference between the frequency of

different *KIR* haplotypes among chronically infected individuals and those spontaneously recovering from HBV infection was also demonstrated in this population[17]. Recently, the rates of KIR2DL3 and 3DS1 were reported to be higher in healthy Turkish individuals as compared to patients with chronic HBV and those with spontaneous remission[18]; authors suggested the possible role of these genes in protection against HBV infection. In addition, genetic factors have been reported to play a role in the regulation of post-vaccine immune responses[19]. This was observed with antibody responses to a number of vaccine antigens including hepatitis B.

It is clear that the interaction between KIRs and their corresponding HLA ligands is implicated in differential responses to HIV, hepatitis C virus (HCV) and HBV as well as other disease conditions[20-23]. Immune responses, like many biological responses, are characterized by a wide range of variation between individuals. This has been described following natural infection or in response to vaccination[24]. HLA, cytokines, toll-like receptors and related gene variants have been associated with a variety of immune responses following vaccinations. Recently, single-nucleotide polymorphism associations were described to be involved in innate and adaptive immune response regulation following measles and rubella vaccinations[25,26]. Similarly, a difference in gene expression was also reported between high and low responders to smallpox vaccine[27]. The fact that antibody response to hepatitis B vaccine is non-protective in up to 10% of individuals[10], and that a genetic basis to non-responsiveness is reported[19,24], prompted us to explore the role of *KIR* genes in response to hepatitis B vaccine in a cohort of healthy vaccinated Lebanese adults.

MATERIALS AND METHODS

Study participants and samples

Human subject approval was obtained for this study from the institutional review board of the American University of Beirut and all the methods were carried out in accordance with the approved ethical guidelines. A written informed consent was signed by the study subjects before participation in the study. A data collection form was administered to the study participants (≥ 18 years old) to collect demographic information, data related to exposure and risk behavior information. One hundred and one subjects were recruited during the time period March 2010-December 2011. Subjects were excluded if they had a prior or current history of HCV, HIV-1, renal disease or cancer. Children or adolescents of HBV carrier mothers and vaccinated in infancy were not included in the study. Blood was drawn from the study participants and peripheral blood mononuclear cells[28] and sera were collected and stored in liquid nitrogen and at -80 ℃, respectively. DNA was extracted from whole blood of the study participants using the QIAamp DNA Blood Midikit (Qiagen, Germany), as per manufacturer's instructions. The integrity of the purified DNA was checked by gel electrophoresis and storage was at -20 ℃.

Enzyme-linked immunosorbent assay

While we recruited 101 voluntary participants, sera samples of 99 HBV vaccinated and non-vaccinated study participants were tested in duplicate for the presence of anti-HBs antibodies as a measure of protection against hepatitis B, hepatitis B surface antigen (HBsAg) and hepatitis B core antibody (anti-HBc) as indicators of infection. We did not have enough sera to use in the analysis for two of our study participants. The Monolisa HBsAg ULTRA, anti-HBs PLUS and anti-HBc PLUS assays (BIO-RAD, France) were used as per manufacturer's instructions, respectively. Anti-HBs antibodies were measured in mIU/mL and levels ≥ 10 mIU/mL were indicative of post-vaccination protection[29,30].

KIR genotyping

The polymerase chain reaction (PCR)-based *KIR* genotyping SSP Kit (Invitrogen, Brown Deer, WI, United States) was used to detect the presence and absence of *KIR* genes, as per manufacturer's instructions. Briefly, 25 μL of DNA was used along with the primer sets to amplify the alleles described by the World Health Organization (WHO) international nomenclature committee (http://www.ebi.ac.uk/ipd/kir/). All amplifications were performed using PX2 thermocycler (ThermoHybrid, United Kingdom) programmed with a 1-min denaturation step at 95 ℃, followed by 30 cycles of 94 ℃ for 20 s, 63 ℃ for 20 s, and 72 ℃ for 90 s and finally 4 ℃ in the thermal cycler. PCR products were gel-purified and visualized under UV transillumination (Sigma, California, United States)[31]. The presence and absence of the following gene loci and variants were tested: 2DL1, 2DL2, 2DL3, 2DL4, 2DL5A, 2DL5B, 2DS1, 2DS2, 2DS3, 2DS5, 3DL1, 3DL2, 3DL3, 3DS1, 2DP1, and 3DP1. The variants of the KIR3DP1 pseudogene, KIR3DP*001/002/004 and KIR3DP1*003 were also detected in addition to KIR2DS4 variants: 2DS4*001/002 and 2DS4*003/007. The frequency of *KIR* was calculated by direct count of the observed phenotype and referred to as observed frequency (OF). In addition, the estimated *KIR* gene frequency (KF) for the putative loci was calculated using the following formula: KF = 1 - $\sqrt{}$ (1-OF) based on the assumption of Hardy-Weinberg equilibrium[32]. The frequencies of haplotype *A* and *B* were calculated using the following formula: haplotype $A = (2n_{AA} + n_{AB})/2n$ and haplotype $B = (2n_{BB} + n_{AB})/2n$, where n_{AA}, n_{AB} and n_{BB} are the numbers of individuals with haplotype group AA, AB and BB, respectively and n is the total number of individuals[33].

Statistical analysis

SPSS 19 was used for statistical analyses. We compared vaccinated and non-vaccinated subjects for each of the *KIR* polymorphisms using χ^2 and Fisher-exact test (FET) and reported the odds ratio and 95%CI. Similar analyses

Table 1 Demographics and characteristics of participants

	n	%
Gender (*n* = 101)		
Male	39	38.6
Female	62	61.4
Age (yr) (*n* = 95)		
19-29	38	40.0
30-39	19	20.0
40-49	17	17.9
50-59	11	11.6
60-69	6	6.3
70-79	2	2.1
80-89	2	2.1
Education (*n* = 99)		
Illiterate	3	3.0
Primary School Education	3	3.0
Secondary School Graduate	9	9.1
High School Education	10	10.1
University Undergraduate Level	50	50.5
Others	24	24.2
Occupation (*n* = 99)		
Student	15	15.2
Employed	74	74.7
Unemployed	7	7.1
Retired	3	3

were conducted for the comparison of protected and non-protected subjects within the vaccinated group. We also examined the relationship between genotypes and the presence or absence of *KIR* genes and the levels of anti-HBs and *KIR* genes among the vaccinated subjects using χ^2 and FET; for these comparisons, post-hoc tests were conducted only if the omnibus test was significant. We corrected for multiple comparisons for post-hoc tests using Bonferroni correction.

RESULTS

Characteristics of study participants

One hundred and one subjects were recruited between March 2010 and December 2011; 39% of the study participants were males and 61% were females. The majority of our study participants were 19-29 years old (40%). Table 1 summarizes the characteristics of the study participants. The majority (75%) held a university undergraduate degree or higher and was employed. During recruitment and when participants were asked about their vaccine status, 50% of our voluntary participants self-reported that they were vaccinated against HBV whereas 25% thought they were not vaccinated and 26% did not know their vaccine status.

In an attempt to confirm the vaccination status of our study participants, sera samples from 99 participants were tested for the levels of anti-HBs as an indicator of protection ($\geqslant$ 10 mIU/mL) as defined by the WHO international reference standard[30,34]. This is especially due to the lack of documented dosages of hepatitis B vaccine for many of the study participants as well as lack of knowledge of the vaccination status of many of the study participants. In the subsequent analyses, data on these 99 voluntary subjects are reported; participants with anti-HBs antibodies $\geqslant$ 10 mIU/mL will be considered vaccinated against hepatitis B. We also tested the sera samples for anti-HBc as a marker of previous infection and for HBsAg, a marker associated with recent exposure to HBV. All our participants were negative for HBsAg. Among the voluntary participants, 74/99 (75%) were vaccinated against hepatitis B as judged by the detection of anti-HBs titers; whereas 25% (25/99) were classified as non-vaccinated against hepatitis B. Among the vaccinated participants, 47% (35/74) had anti-HBs titers above 100 mIU/mL, 22% (16/74) had anti-HBs ranging between 10-100 mIU/mL, and 20% (15/74) had values of less than 10 mIU/mL. The time of vaccination (when available) ranged between the years 1999 and 2011, with some participants receiving 2 doses and others receiving 3 or more doses. When we tested for an association between the age groups of our study participants (19-29, 30-39, 40-49, 50-59, 60-69, 70-79 and 80-89) and the concentration of anti-HBs antibodies among vaccinated subjects, a *P* value of 0.047 was detected (FET). Nine percent (7/74) of the vaccinated participants had undetectable levels of protective antibodies. Five out of 7 (71%) of the former group were health-care workers; the latter are expected to be continuously monitored for protection against HBV due to the nature of their work. Importantly, anti-HBc was positive in 3% (3/99) of our study participants presenting with anti-HBs levels ranging between 110-1000 mIU/mL. This is associated with protection as a result of natural infection. One of these participants is a vaccinated female nurse, whereas the other 2 are non-vaccinated and are not in the health care profession. None of the anti-HBc positive participants were HBsAg positive.

KIR genotypes and genes frequencies

We next determined the *KIR* genotypes among the study participants: 44%, 40% and 16% were carriers of AA, AB and BB genotypes, respectively, with a 1.77 A to B ratio. The genotype was classified as B if any of the following genes was detected: 2DL2, 2DL5, 3DS1, 2DS1, 2DS2, 2DS3 and 2DS5. If none of these was detected, the genotype was considered as AA. Similarly, if none of the A haplotypes was detected, the genotype was classified as BB; 77%, 75% and 69% of the AA, AB and BB carriers were vaccinated, respectively. There was no significant difference between the expression of AA, AB and BB among vaccinated and non-vaccinated participants (FET, *P* = 0.784). Similarly, we did not detect any significant difference when we compared the frequencies of *KIR* genotypes among the vaccinated with anti-HBs levels less than 10, 10-100 and above 100 mIU/mL. We did not detect any difference between the frequencies of *KIR* genotypes among vaccinated participants with protective and non-protective levels of anti-HBs (FET, *P* = 0.865).

Table 2 The observed and estimated killer immunoglobulin receptor gene frequencies in the study participants

	Inhibitory *KIR*								Non-inhibitory *KIR*						Pseudogene		
	2DL1	2DL2	2DL3	2DL4	2DL5	3DL1	3DL2	3DL3	2DS1	2DS2	2DS3	2DS4	2DS5	3DS1	2DP1	3DP*001/002/004	3DP1*003
OF	99	56	84	100	42	96	100	100	40.6	55.4	46.5	95	34.7	41.6	99	17	100
KLF	0.9	0.34	0.6	1	0.24	0.8	1	1	0.23	0.33	0.27	0.78	0.19	0.24	0.9	1	1

2DL5A, 24.8%; 2DL5B, 28.7%; 2DS4*001/*002, 40.6%; 2DS4*003/007, 82.2%. KIR: Killer immunoglobulin receptor; OF: Observed frequency calculated by direct counting; KLF: Gene frequency calculated using the formula 1 - √ (1 - OF).

Table 3 Killer immunoglobulin receptor gene frequencies among study participants vaccinated against hepatitis B virus (protected and non-protected by hepatitis B vaccine) *n* (%)

KIR genes	Anti-HBsAg < 10 mIU/mL (*n* = 22)	Anti-HBsAg ⩾ 10 mIU/mL (*n* = 52)	Test[1]	OR (95%CI)	*P* value
2DL2	14 (63.60)	27 (51.90)	0.858	0.62 (0.22-1.72)	0.446
2DL3	18 (81.80)	45 (86.40)	FET	1.43 (0.37-5.48)	0.723
2DL5A	8 (36.40)	13 (25.00)	0.982	0.58 (0.20-1.70)	0.4
2DL5B	5 (22.70)	19 (36.50)	1.346	1.97 (0.62-6.16)	0.289
2DS1	11 (50.00)	19 (36.50)	1.162	0.58 (0.21-1.58)	0.31
2DS2	14 (63.60)	26 (50.00)	1.157	0.57 (0.21-1.59)	0.318
2DS3	9 (40.90)	23 (44.20)	0.069	1.15 (0.42-3.15)	0.804
2DS4*001/002	8 (36.40)	20 (38.40)	0.029	1.09 (0.39-3.072)	1.00
2DS4*003/007	19 (86.40)	41 (78.85)	FET	0.59 (0.15-2.36)	0.534
2DS5	10 (45.50)	17 (32.60)	1.087	0.58 (0.21-1.62)	0.428
3DS1	11 (50.00)	21 (40.40)	0.582	0.68 (0.25-1.85)	0.608
3DP*001/002/004	6 (27.30)	9 (17.30)	FET	0.56 (0.17-1.82)	0.355

[1]The statistical test performed to compare the frequencies of *KIR* gene expression among vaccinated and non-vaccinated study participants where by FET refers to Fisher's exact test and the rest of the values represent the Pearson χ^2 value. We compared the frequencies of *KIR* genes with enough variability between study participants that were vaccinated against hepatitis B virus. The vaccinated participants were divided for this analysis into 2 groups depending on the level of anti-HBsAg. Anti-HBsAg < 10 mIU/mL, not protected against hepatitis B virus infection; anti-HBsAg ⩾ 10 mIU/mL, protected against hepatitis B virus infection as a result of vaccination. KIR: Killer immunoglobulin receptor; FET: Fisher-exact test; HBsAg: Hepatitis B surface antigen. OR: Odds ratio.

We divided the *KIR* genes expressed in our study participants into inhibitory, non-inhibitory (or activating) and those encoding inhibitory and activating signals, as previously described[13]. The inhibitory KIR2DL1, KIR2DL4, KIR3DL1, KIR3DL2, and KIR3DL were detected in more than 95%, whereas KIR2DL2, KIR2DL3, KIR2DL5 (KR2DL5A and KIR2DL5B) were expressed in 56%, 84% and 42% (25% and 29%) of participants, respectively (Table 2). The OF of the activating *KIR* genes ranged between 35% and 55% except for KIR2DS4, detected in 95% of the study participants (40.6% 2DS4*001/002; 82.2% 2DS4*003/007). KIR2DP1 pseudogene was detected in 99% of our participants, whereas KIR3DP*001/02/04 and KIR3DP1*003 had frequencies of 17% and 100%, respectively. The corresponding estimated frequencies of each *KIR* gene followed the same trend or order as the OF data.

For this analysis and thereafter, we studied the genes with enough variability, particularly 2DL2, 2DL3, 2DL5 (2DL5A, 2DL5B), 2DS1, 2DS2, 2DS3, 2DS4 (and its variants), 2DS5, 3DS1 and the pseudogene 3DP1*001/002/004 (Table 2). We report the lack of significant difference in the frequency of *KIR* genes among vaccinated and non-vaccinated participants (Table 3). Moreover, there was no significant difference in the frequency of these genes among participants protected against HBV and those that are not protected against HBV, as judged by their level of anti-HBs antibodies (Table 4).

We performed similar analyses to determine the relationship between AA, AB, BB genotypes and the expression of *KIR* genes showing enough variability (Table 5). 2DL2 (χ^2, P = 0.00), 2DL3 (χ^2, P = 0.00), 2DS2 (χ^2, P = 0.00), 2DS3 (χ^2, P = 0.00), 2DL5B FET (P = 0.00), and 3DP1001/002/004 (FET, P = 0.006) were found to be significantly different between genotypes but not 2DS1 (χ^2, P = 0.749), 2DL5A (FET, P = 0.782), 2DS4*001/002 (χ^2, P = 0.621), 2DS4*003/007 (FET, P = 0.392) and 3DS1 (χ^2, P = 0.948). For those genes showing significant differences among the AA, AB and BB genotypes, we computed post-hoc comparisons. These differences still hold significance between subgroups except for 2DL5B, 2DS2, 2DS3 and 3DP1*001/002/004 between genotypes AB and BB.

KIR gene frequencies among vaccinated and non-vaccinated participants

To evaluate the role of *KIR* genes in protection against or susceptibility to HBV, we next compared the frequency of *KIR* genes among HBV-vaccinated and non-vaccinated participants (as judged by the level of anti-HBs antibodies). Depending on their anti-HBs levels that we tested for in this study, participants vaccinated against hepatitis B were divided into 4 groups: Group Ⅰ >

Table 4 Killer immunoglobulin receptor gene frequencies among study participants vaccinated against hepatitis B virus as compared to non-vaccinated subjects *n* (%)

KIR genes	Vaccinated (*n* = 74)	Non-vaccinated (*n* = 25)	Test[1]	OR (95%CI)	*P* value
2DL2	41 (55.40)	15 (60.00)	0.161	0.83 (0.33-2.082)	0.436
2DL3	63 (85.10)	20 (80.00)	0.364	1.43 (0.44-4.62)	0.374
2DL5A	21 (28.40)	4 (16.00)	1.517	2.08 (0.64-6.79)	0.168
2DL5B	24 (32.40)	5 (20.00)	1.395	1.92 (0.64-5.73)	0.178
2DS1	30 (40.50)	10 (40.00)	0.002	1.02 (0.41-2.58)	0.577
2DS2	40 (54.10)	15 (60.00)	0.268	0.78 (0.31-1.97)	0.39
2DS3	32 (43.20)	14 (56.00)	1.223	0.60 (0.24-1.49)	0.191
2DS4*001/002	28 (37.80)	11 (44.00)	0.297	0.77 (0.31-1.94)	0.376
2DS4*003/007	60 (81.10)	22 (88.00)	FET	0.58 (0.15-2.23)	0.324
2DS5	27 (36.50)	7 (28.00)	0.597	1.47 (0.55-3.99)	0.302
3DS1	32 (43.20)	9 (36.00)	0.246	1.35 (0.53-3.46)	0.401
3DP*001/002/004	15 (20.30)	2 (8.00)	FET	2.92 (1.62-13.80)	0.134

We compared the frequencies of *KIR* genes with enough variability between study participants that were vaccinated against hepatitis B virus and those that were not. [1]The statistical test performed to compare the frequencies of *KIR* gene expression among vaccinated and non-vaccinated study participants whereby FET refers to Fisher's exact test and the rest of the values represent the Pearson χ^2 value. KIR: Killer immunoglobulin receptor; FET: Fisher-exact test; OR: Odds ratio.

Table 5 The relationship between AA, AB and BB genotypes and the expression of killer immunoglobulin receptor genes among the study participants *n* (%)

KIR genes	AA (*n* = 44)	AB (*n* = 41)	BB (*n* = 16)	Test[1]	*P* value
2DL2	0 (0.00)	41 (100.00)	16 (100.00)	101	0.00[a]
2DL3	44 (100.00)	41 (100.00)	0 (0.00)	101	0.00[a]
2DL5A	10 (22.70)	10 (22.70)	5 (31.25)	FET	0.782
2DL5B	1 (2.27)	20 (24.39)	8 (50.00)	FET	0.00[a]
2DS1	16 (36.30)	18 (43.90)	7 (43.75)	0.579	0.749
2DS2	0 (0.00)	40 (97.50)	16 (100.00)	97.051	0.00[a]
2DS3	3 (6.80)	30 (73.17)	14 (87.50)	50.38	0.00[a]
2DS4*001/002	16 (36.40)	19 (37.50)	6 (37.50)	0.952	0.621
2DS4*003/007	38 (86.40)	31 (75.60)	14 (87.50)	FET	0.392
2DS5	14 (31.80)	16 (39.00)	5 (31.30)	0.584	0.747
3DS1	18 (40.90)	18 (43.90)	6 (40.00)	0.107	0.948
3DP*001/002/004	2 (4.50)	10 (24.40)	5 (31.30)	FET	0.0006[a]

[1]The statistical test performed to determine the relationship between AA, AB and BB genotypes and the expression of *KIR* genes among vaccinated and non-vaccinated study participants. FET refers to the Fisher's exact test and the rest of the values represent the Pearson χ^2 value. [a]$P < 0.05$, significant. KIR: Killer immunoglobulin receptor; FET: Fisher-exact test.

100 mIU/mL, group Ⅱ 10-100 mIU/mL, group Ⅲ < 10 mIU/mL and group Ⅳ with undetectable levels of protective antibodies. The frequency of only KIR2DL5B was significantly different among these categories (FET, $P = 0.0263$) (Table 6). When we performed post-hoc comparisons between these groups, we detected no significant difference in the expression of KIR2DL5B. These groups of vaccinated participants were also similar in relation to the expression of AA, AB and BB genotypes (FET, $P = 0.669$).

We identified 3 participants testing positive for anti-HBc with anti-HBs levels higher than 100 mIU/mL. Two out of three of these participants were non-vaccinated and thus we believe they are protected as a result of natural infection. The third participant was vaccinated against HBV. Two out of three (66.7%) of these participants carry the AB genotype and one participant is AA positive. The three participants expressed 2DL1, 2DL3, 3DL1, 3DL2, 3DL3 (inhibitory), activating genes (2DS3, 2DS4*001/002 variant) and both inhibitory and activating genes (2DL4, 2DP1 and 3DP1*003). These subjects did not express 2DL5A or 3DP1*001/002/004. We did not test for the presence of HBV DNA among these participants. We did not find any significant relationship between the genotype of these participants and the expression of *KIR* genes, or between the *KIR* genes and the susceptibility to natural or breakthrough infection.

DISCUSSION

The administration of hepatitis B vaccine in infancy is 95% effective and correlates with long-term protection[8,35,36]. However, vaccine failure has been reported in 5% of hepatitis B-vaccinated persons; moreover, breakthrough infection has also been reported following vaccination with hepatitis B vaccine[37]. The increase

Table 6 Killer immunoglobulin receptor gene expression and levels of anti-HBs among the vaccinated study participants *n* (%)

KIR genes	Group Ⅰ[1] (*n* = 36)	Group Ⅱ[2] (*n* = 16)	Group Ⅲ[3] (*n* = 15)	Group Ⅳ[4] (*n* = 7)	Test[5]	*P* value
2DL2	17 (47.20)	10 (62.50)	11 (73.30)	3 (42.90)	FET	0.362
2DL3	31 (86.10)	14 (87.50)	12 (80.00)	6 (85.70)	FET	0.824
2DL5A	9 (25.00)	4 (25.00)	4 (26.70)	4 (57.10)	FET	0.987
2DL5B	9 (25.00)	10 (62.50)	4 (26.70)	1 (14.30)	FET	0.023[6]
2DS1	11 (30.60)	8 (50.00)	7 (46.70)	4 (57.10)	FET	0.530
2DS2	16 (44.40)	10 (62.50)	11 (73.30)	3 (42.90)	FET	0.270
2DS3	12 (33.30)	11 (68.80)	8 (53.30)	1 (14.30)	5.01	0.060
2DS4*001/002	15 (41.70)	5 (31.30)	6 (40.00)	2 (28.60)	0.568	0.920
2DS4*003/007	28 (77.80)	13 (81.30)	13 (86.70)	6 (85.70)	FET	0.860
2DS5	11 (30.60)	6 (37.50)	6 (40.00)	3 (42.90)	0.846	0.850
3DS1	14 (38.90)	7 (43.80)	7 (46.70)	6 (85.70)	FET	0.960
2DL4	36 (100.00)	16 (100.00)	15 (100.00)	4 (57.10)	0.258	0.970
3DP*001/002/004	8 (22.20)	1 (6.30)	4 (26.70)	1 (14.30)	FET	0.420

[1]Group Ⅰ: Vaccinated participants and anti-HBs titers > 100 mIU/mL; [2]Group Ⅱ: Vaccinated participants and anti-HBs titers 10-100 mIU/mL; [3]Group Ⅲ: Vaccinated participants and anti-HBs titers 0.1-9.99 mIU/mL; [4]Group Ⅳ: Vaccinated participants and anti-HBs titers = 0 mIU/mL; [5]The statistical test performed to compare the frequencies of *KIR* gene expression among the vaccinated study participants whereby FET refers to Fisher's exact test and the rest of the values represent the Pearson χ^2 value; [6]Significant difference of KIR2DL5B expression among vaccinated study participants ($P < 0.05$). KIR: Killer immunoglobulin receptor; FET: Fisher-exact test.

in circulating NK cells, major players in the innate immune system and regulators of the virus-specific T cell responses through their cross-talk with dendritic cells and T cells[38-40], was suggested to contribute to HBV viral control[41]. The impact of genetic regulation on immune responses following vaccinations has been previously reported[19,24]. This evidence prompted us to explore the potential role of KIR following hepatitis B vaccination. In Lebanon, hepatitis B vaccine is offered as part of the immunization program early in childhood as per the WHO guidelines. In this study, 69% of the vaccinated participants retained more than 10 mIU/mL of anti-HBs antibodies and hence are immune to HBV infection; whereas 30% are susceptible to the latter due to either undetectable levels of antibodies or levels below 10 mIU/mL. We do not have data on the time of vaccination of these participants to reflect on the duration of the retention or the loss of the immune response post-vaccination. We report the lack of significant association between the number of vaccine dosages (when vaccine dosage is available) and the titer of antibodies among vaccinated participants. Recent reports show that multiple immunizations against hepatitis B are inefficient at mounting antibody responses[42], while others suggest that immunization against hepatitis in infancy is associated with a seroprotective response to a challenge dose of vaccine with extended duration of protection through adolescent years[43]. We cannot suggest similar trends from our results due to the lack of data on the time of vaccination, the age at vaccination, as well as the number of dosages administered for many participants.

Anti-HBc antibodies, indicators of HBV infection, were detected in 3 participants (3%) in the absence of HBsAg, with one being a nurse suspected of being exposed to HBV at the work place. This might suggest a "breakthrough" infection occurring following vaccination against hepatitis B; this is suggested since health care workers are regularly monitored for protective levels of anti-HBs antibodies. However, due to the lack of data on the timing of vaccination and/or infection of this participant, we cannot confirm whether exposure to HBV has occurred before or after vaccination. The other 2 participants are non-vaccinated and are protected with high levels of anti-HBs antibodies as a result of natural infection. We do not have data pertaining to the time of infection following vaccination; moreover, we did not perform HBV DNA testing. Health care workers with undetectable anti-HBsAg levels detected in our study are clearly susceptible to HBV infection and consequently in need of booster vaccination to induce an anamnestic response in order to prevent acute disease and carrier state.

While hepatitis B vaccine booster doses are not currently recommended following vaccination, a better understanding of the correlates of long-term immunity is needed. This is critical especially since several studies show that vaccines with anti-HBs levels of 10-99 mIU/mL achieved following primary vaccination are less likely to produce an anamnestic response following a booster HBV vaccine as compared to those with anti-HBs $\geqslant$ 100 mIU/mL[35,44]. NK cells play a major role in the innate immune system as first line of defense and in the regulation of the virus-specific T cell responses through their cross-talk with dendritic cells and T cells[38-40]. Moreover, NK cells are suggested to contribute to HBV control[41]. Our data show that genotypes with 11 *KIR* genes were most prevalent, with AA genotype being more frequent among the study participants. The inhibitory *KIR* genes were more frequent among our study participants than the activating genes, which is in agreement with a finding associated with A haplotype being present in higher numbers in inhibitory *KIR* genes[39].

KIR2DL4, KIR3DL2, KIR3DL3 and KIR3DP1*003 were present in every participant. This is expected since these

are framework genes. The frequency of KIR2DL5B is the only significantly different gene among the vaccinated participants with different anti-HBs antibodies titer. The role of KIR2DL5, expressed at frequencies ranging between 26% and 86% in all human populations, is not completely understood[45]. The ligand of KIR2DL5 is also still unknown.

A number of limitations exist, and these include the lack of data on the time of vaccination and corresponding age at time of vaccination of the study participants, and more importantly, the small sample size. Our sample size is powered to detect medium to large effect sizes when some of the effect sizes for group differences are small. However, medium to large effect sizes are those where group differences have more clinical significance, which we are powered to detect. Consequently, the clear impact that *KIR* genes have on susceptibility to acquiring hepatitis B or protection against the infection cannot be addressed in these small groups.

To our knowledge, this is the first study screening for the possible role of *KIR* genes among individuals vaccinated against HBV. While studies have shown the association between gene variants and immune responses to a variety of vaccines, little is known about the strength and the sustainability of antibody responses following vaccination against HBV in relation to expression of *KIR* genes. Our results are useful to design larger studies to better elucidate the role of KIR in susceptibility or long-term protection against HBV as well as other diseases.

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COMMENTS

Background

Killer immunoglobulin receptor (*KIR*) genes encode receptors expressed on the surface of natural killer cells. The literature has described the relationship between KIRs and differential responses in many disease conditions, specifically human immunodeficiency virus and hepatitis C virus. The authors thought to explore the role of *KIR* genes in response to hepatitis B vaccine in a cohort of Lebanese adults.

Research frontiers

This study aims at elucidating the possible role of genetic factors such as KIRs in the regulation of post-vaccine immune responses specifically following hepatitis B vaccine.

Applications

While the sample size is powered to detect medium to large effect sizes, the impact that KIR and HLA have on the susceptibility to acquiring hepatitis B virus (HBV) or protection against the infection cannot be addressed in our sample. Nevertheless, our results are useful to design larger studies to better elucidate the role of KIR in susceptibility or long-term protection against HBV and other diseases.

Terminology

Two groups of *KIR* haplotypes are known in humans: A and B. Haplotype A encodes inhibitory receptors and consists of nine genes [3DL3, 2DL3, 2DP1, 2DL1, 3DP1, 2DL4, 3DL1, one activating (2DS4), 3DL2 and 2DL5]. Haplotype B carries a variety of gene combinations and encodes more activating receptors as compared to haplotype A [3DL3, 2DS2, 2DL2, 2DL5B (inhibitory), 2DS3, 2DP1, 2DL1, 3DP1, 2DL4, 3DS1, 2DL5A (inhibitory), 2DS5, 2DS1, and 3DL2].

Peer-review

This is an interesting study aiming to screen for a possible role of *KIR* gene expression and antibody response following hepatitis B vaccination. The major point of this manuscript is that there is no significant association between the frequency of *KIR* genes and anti-HBs antibodies detected. Although it is a negative result, it could be an indicant for understanding the role of *KIR* loci in response to HB vaccine.

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