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# HLA-DRB1, DQA1 and DQB1 Alleles and Haplotypes Frequencies in Iranian Healthy Adult Responders and Non-Responders to Recombinant Hepatitis B Vaccine

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## Abstract

**Background:** Different studies have demonstrated that a small proportion of healthy individuals receiving the hepatitis B (HB) vaccine do not produce protective levels of anti-HB antibody, a phenomenon which could be linked to certain human leukocyte antigen (HLA) class-II alleles or haplotypes. **Objectives:** The present study was undertaken to determine the frequency of HLA class-II alleles in Iranian healthy adult responders and non-responders to HB vaccine. **Methods:** Twelve non-responders (anti-HBs antibody < 10 IU/L) and 46 responders (anti-HBs antibody > 100 IU/L) were tissue typed for HLA class-II. HLA-DRB1, DQB1 and DQA1 alleles were determined using polymerase chain reaction based on sequence specific primers (PCR-SSP) technique. Accessibility to excess amount of genomic DNA was possible using Epstein-Barr virus (EBV)-transformed B-cells established from all vaccinees. **Results:** Our results demonstrated increased frequencies of HLA- DRB1\*07, DRB1\*03, DRB1\*04, DQB1\*0201, DQA1\*0201 alleles and HLA- DRB1\*07/DQB1\*0201/DQA1\*0201 and DRB1\*04/DQB1\*0302/DQA1\*03011 haplotypes in the non-responder group. Comparison between responders and non-responders revealed only a significant difference for DQB1\*0201 allele ( $p < 0.05$ ). **Conclusion:** These findings confirm the association of certain HLA alleles and haplotypes with the lack of antibody response to HB vaccine in an Iranian population.

**Keywords:** Human leukocyte antigen, hepatitis B virus, Vaccination, Allele, Haplotype, Iranian

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## INTRODUCTION

Worldwide, more than 350 million people are chronically infected with hepatitis B virus (HBV) (1). Persistence of HBV infection can lead to chronic hepatitis, cirrhosis and hepatocellular carcinoma with an estimated annual death rate of 1-2 million (2). Vaccination with the major surface protein of hepatitis B virus (HBsAg) has been demonstrated to induce a protective antibody response in the majority of healthy adults as well as neonates and children. Despite its proven immunogenicity, lack of protective immune response has been reported in 1-10% of normal population (3-5).

Among a variety of genetic and environmental factors implicated in immune responsiveness to a nominal antigen, the human leukocyte antigen (HLA) complex deserves special consideration. HLA may regulate the immune response to HBsAg by at least two different mechanisms. Lack of immune response could either be a consequence of failure of a given HLA class II molecule to bind an epitope of HBsAg (defect in antigen presentation), or be due to paucity of antigen-specific T-cells recognizing a particular HLA/peptide complex (defect in T-cell repertoire). Evidence for both propositions has been reported to explain unresponsiveness to HBsAg vaccination in humans. Defective HBsAg presentation may result in: a) imbalanced or diminished Th1/Th2 response (6, 7); b) induction of suppressor or regulatory T-cells inhibiting anti-HBs Ab production (8); or c) development of HBsAg-specific cytotoxic T-lymphocytes (CTL) targeting and killing HBsAg-specific B-cells (9).

Several studies have reported the association between certain HLA alleles and haplotypes with the anti-HBs Ab response in different ethnic populations (8, 10-16). Analysis of the association between HLA alleles and immune response to a vaccine formulated antigen, such as HBsAg, in different ethnic populations is of clinical relevance and could shed light on the mechanisms underlying vaccination failure. The present study was undertaken to address this issue in Iranian healthy adult responder and non-responder subjects.

## MATERIALS AND METHODS

**Subjects and Vaccination Schedule.** A total of 252 healthy adult volunteer individuals (HBsAg and anti-HBc/ anti-HBs antibody negative) were vaccinated with triple 20 µg doses of recombinant HBsAg vaccine (Heberbiotec, S.A. Havana, Cuba) as previously described (17). Two to four weeks after completion of vaccination, serum levels of anti-HBsAg antibody was determined by sandwich enzyme-linked immunosorbent assay (ELISA), using a commercial kit (OWRT, Behring, Germany). Based on anti-HBsAg antibody titer, the vaccinees were arbitrarily classified into responders (n=240) (anti-HBsAg antibody >100 IU/L) and non-responders (n=12) (anti-HBsAg antibody <10 IU/L). Of responders, 46 individuals (38 male, 8 female, mean age 27 years) whose B-cells were successfully transformed were randomly selected and from non-responders all 12 subjects (10 male, 2 female, mean age 31 years) were included in this study.

**Establishment of B-Lymphoblastoid Cell Lines.** Considering the small volume of blood taken from each subject and the need for large amount of genomic DNA for this and other studies being conducted on these samples, isolated B-cells were immortalized with Epstein-Barr virus (EBV) as previously described (18). Briefly, peripheral blood mononuclear cells (PBMC) were isolated from heparinized peripheral venous blood by

Ficoll-Paque (Sigma, USA) centrifugation. The cells were resuspended in filtered supernatant produced by EBV-infected B95.8 marmoset cells (NCBI C-110, National Cell Bank of Iran, Pasteur Institute of Iran). After 1 hour incubation at 37°C in a CO<sub>2</sub> incubator with periodic agitation, the cells were washed with RPMI-1640 medium (Sigma, USA) and resuspended in culture medium supplemented with 10% heat-inactivated fetal calf serum (FCS) (Seromed, Germany) and antibiotics, including penicillin (100 U/ml) and streptomycin (100 µg/ml) (Gibco, Scotland). Memory cytotoxic T-cells in individuals seropositive for EBV, which can attack the EBV-infected B-cells, were inhibited by addition of 1 µg/ml cyclosporine-A (Sandoz, Switzerland) in the culture medium. Proliferating foci of transformed lymphocytes were visible 2-3 weeks after infection by EBV. Immortalized lymphoblastoid B-cells were then expanded and cryopreserved in 10% dimethylsulfoxide (Sigma, USA) and 90% FCS in liquid nitrogen.

**DNA Extraction and HLA Typing.** Genomic DNA was extracted from 10 ml peripheral blood in 5% EDTA by a modified salting out method (19). HLA typing was performed by polymerase chain reaction based on sequence specific primers (PCR-SSP), following Olerup and Zetterquist method (20).

The PCR reactions were carried out in 10 µl volumes. The haplotypes were calculated according to linkage disequilibrium among Iranian DRB1, DQA1, and DQB1 alleles.

**Statistical Analysis.** The differences in HLA allele and haplotype frequencies among the studied groups were analyzed using Chi-Square test after Yates correction. Each allele frequency in non-responders was compared with the same allele in responders. The odds ratio (OR) with 95% confidence intervals (CI) was calculated and a p-value of less than 0.05 was considered to be significant.

## RESULTS

The results of HLA DRB1, DQB1 and DQA1 alleles typing in responders and non-responders to recombinant HBsAg vaccine have been shown in tables 1-3, respectively. Comparison of alleles frequencies between the two groups has demonstrated higher expression of DRB1\*0301, DRB1\*04, DRB1\*07, DRB1\*1303, DQB1\*0201 and DQA1\*0201 alleles in non-responders, of which only DQB1\*0201 allele was found to be significant ( $p < 0.05$ ). The most frequent alleles in responders compared to non-responders were HLA-DRB1\*15, DRB1\*11, DRB1\*1001, DRB1\*0101, DRB1\*1301, DQB1\*05031, DQB1\*0602, DQA1\*0103 and DQA1\*0105 alleles. Analyzing haplotype frequencies showed that the most frequent haplotypes in non-responders were HLA-DRB1\*07/DQB1\*0201/DQA1\*0201, DRB1\*04/DQB1\*0302/DQA1\*03011 and DRB1\*1303/ DQB1\*03011/ DQA1\*0505, whereas in responders HLA-DRB1\*07/DQB1\*0201/DQA1\*0201, DRB1\*11/DQB1\*0301/DQA1\*0505 and DRB1\*0101/DQB1\*0501/DQA1\*0101 were the most frequent haplotypes (Table 4). Significant differences were not observed for any of these haplotypes between responder and non-responder vaccinees.

**Table 1. HLA-DRB1 alleles frequency in healthy adult responders and non-responders to recombinant HBsAg vaccine**

DRB1 alleles	High-responders (n=46)	Non-responders (n=12)	Odds Ratio (95% CI)	P value
DRB1*0101	9(9.78%)	1(4.16%)	2.49(0.29-54.24)	0.68
DRB1*0301	8(8.69%)	4(16.66%)	0.48(0.11-2.11)	0.26
DRB1*04	8(8.69%)	4(16.66%)	0.48(0.11-2.11)	0.26
DRB1*07	17(18.47%)	6(25%)	0.68(0.21-2.25)	0.56
DRB1*1001	6(6.52%)	0	Undefined	0.34
DRB1*11	11(11.95%)	1(4.16%)	3.12(0.38-68.05)	0.45
DRB1*12	1(1.08%)	0	Undefined	1.00
DRB1*1301	8(8.69%)	1(4.16%)	2.19(0.25-49.06)	0.68
DRB1*1302	3(3.26%)	1(4.16%)	0.78(0.07-20.29)	1.00
DRB1*1303	2(2.17%)	2(8.33%)	0.24(0.02-2.61)	0.18
DRB1*1401	4(4.34%)	1(4.16%)	1.05(0.1-25.78)	1.00
DRB1*15	12(13.04%)	1(4.16%)	3.45(0.42-74.70)	0.29
DRB1*16	3(3.26%)	2(8.33%)	0.37(0.05-3.41)	0.27

**Table 2. HLA-DQB1 alleles frequency in healthy adult responders and non-responders to recombinant HBsAg vaccine**

DQB1 alleles	Responders (n=46)	Non-responders (n=12)	Odds Ratio (95% CI)	P value
DQB1*0201	20(22.22%)	11(45.83%)	0.34(0.12-0.96)	0.04
DQB1*03011	13(14.44%)	3(12.5%)	1.18(0.28-5.98)	1.00
DQB1*03012	3(3.33%)	0	Undefined	1.00
DQB1*0302	5(5.55%)	3(12.5%)	0.41(0.08-2.39)	0.36
DQB1*03032	3(3.33%)	0	Undefined	1.00
DQB1*0305	3(3.33%)	0	Undefined	1.00
DQB1*0501	17(18.88%)	3(12.5%)	1.63(0.39-7.76)	0.56
DQB1*05031	7(7.77%)	0	Undefined	0.34
DQB1*0505	0	1(4.16%)	0.00(0.00-4.64)	0.21
DQB1*06011	4(4.44%)	1(4.16%)	1.07(0.1-26.39)	1.00
DQB1*0602	11(12.22%)	1(4.16%)	3.2(0.39-69.79)	0.45
DQB1*0604	4(4.44%)	1(4.16%)	1.07(0.1-26.39)	1.00

**Table 3. HLA-DQA1 alleles frequency in healthy adult responders and non-responders to recombinant HBsAg vaccine**

DQA1 alleles	Responders (n=46)	Non-responders (n=12)	Odds Ratio (95% CI)	P value
DQA1*0101	10(10.86%)	2(8.33%)	1.34(0.25-9.59)	1.00
DQA1*01021	12(13.04%)	2(8.33%)	1.65(0.31-11.56)	0.73
DQA1*0103	12(13.04%)	1(4.16%)	3.45(0.42-74.70)	0.29
DQA1*0105	10(10.86%)	1(4.16%)	2.80(0.34-61.56)	0.45
DQA1*0201	17(18.47%)	8(33.33%)	0.45(0.15-1.38)	0.19
DQA1*03011	8(8.69%)	3(12.5%)	0.67(0.14-3.5)	0.69
DQA1*05011	7(7.6%)	3(12.5%)	0.58(0.12-3.1)	0.42
DQA1*0505	15(16.3%)	4(16.66%)	0.97(0.26-3.92)	1.00
DQA1*06011	1(1.08%)	0	Undefined	1.00

**Table 4. HLA class-II haplotypes frequency in healthy adult responders and non-responders to recombinant HBsAg vaccine**

DRB1/DQB1/DQA1 haplotypes	Responders (n=45)	Non-responders (n=12)	Odds Ratio (95% CI)	P value
0101/0501/0101	7(7.77%)	1(4.16)	1.94(0.22-44.08)	1.00
07/0201/0201	12(13.33%)	5(20.83)	0.58(0.16-2.18)	0.34
1303/03011/0505	1(1.11%)	2(8.33)	0.12(0.00-1.86)	0.11
1301/0602/0103	6(6.66%)	1(4.16)	1.64(0.18-38.04)	1.00
11/0301/0505	10(11.11%)	1(4.16)	2.88(0.34-63.12)	0.45
04/0302/03011	5(5.55%)	3(12.5)	0.41(0.08-2.39)	0.36
15/06011/0103	4(4.44%)	0	Undefined	0.57
15/0602/01021	3(3.33%)	0	Undefined	1.00

## DISCUSSION

Generally, several parameters affect vaccine efficacy which could broadly be classified into environmental and genetic factors. Although the type and dose of administered vaccine could significantly increase the seroprotection rate, a sizable proportion of vaccinees remain non-responders (3-5). Recent studies conducted on mono- and dizygotic twins have indicated that genetic factors may account for nearly 60% of immune responsiveness to HBsAg vaccination, of which HLA genes are the major contributors (21). In congenic mice HBsAg-specific immune response shows major histocompatibility complexes (MHC)-associated hierarchy with certain haplotypes being associated with high anti-HBs response, while others with absolute failure of response. Thus, the most frequent MHC alleles in high, intermediate and low or non-responder mice were found to be H-2<sup>d,q</sup>, H-2<sup>a</sup> and H-2<sup>s,f</sup> (22). In humans current data suggests considerable ethnic differences. A variety of HLA class I and class II alleles and extended haplotypes of HLA have been reported to be either increased or decreased in non-responder individuals from different ethnic backgrounds (8, 10-16, 23; Table 5).

**Table 5. HLA alleles association with response to recombinant HBsAg vaccine in different ethnic populations**

Country	HLA alleles and haplotypes	Response to HBsAg vaccine	Reference
Belgium	DRB1*01	Increase	12
	DRB1*07	Decrease	
	DQB1*0201	Decrease	
Germany	DRB1*03	Decrease	13
	DRB1*07	Decrease	
	DRB1*13	Increase	
England	DRB1*07	Decrease	14
	DQB1*02	Decrease	
	DRB1*0701/DQB1*0201	Decrease	
Italy	DRB1*07	Decrease	30
	DQB1*0201	Decrease	
	DRB1*04,08,1101	Increase	
Japan	DQB1*03,04	Increase	16
	DQA1*03,0104,0601	Increase	
	DRB1*08032,0101,1403	Decrease	
France	DRB1*01	Increase	15
	DRB1*03	Decrease	
	DRB1*14	Decrease	
USA	DRB1*07	Decrease	10
	DRB1*0404	Decrease	
	DRB1*0401	Increase	
Spain	DRB1*07	Decrease	22
	DQ2	Decrease	
	DRB1*03	Decrease	
Iran	DRB1*11,15	Increase	Present study
	DQB1*0602/DQA1*0103	Increase	
	DRB1*0301,04,07	Decrease	
	DQB1*0201/DQA1*0201	Decrease	

In the present study, we have investigated the association between HLA class II alleles and haplotypes with antibody response to recombinant HBsAg vaccination in Iranian healthy adult individuals. A number of DRB1, DQB1 and DQA1 alleles including DRB1\*15, DRB1\*1001, DRB1\*11, DRB1\*0101, DRB1\*1301, DQB1\*05031, DQB1\*0602, DQA1\*0103 and DQA1\*0105 were over-expressed in responders

compared to non-responders. The most frequent alleles in our non-responders were DRB1\*1303, DRB1\*0301, DRB1\*04, DRB1\*16, DRB1\*07, DQB1\*0201, DQB1\*0505, DQB1\*0302, DQA1\*0201 and DQA1\*05011 (Tables 1-3). Our results, in parallel with other reports (Table 5), confirm the association of certain HLA class-II alleles with lack of antibody response to HBsAg vaccine. However, due to the small sample size, particularly limited number of the non-responder subjects, significant association was observed only for HLA-DQB1\*0201 ( $p < 0.05$ ) with vaccination failure. Sample collection from non-responders is practically difficult, because a small proportion of the vaccinated subjects are categorized as non-responders.

Molecular analysis performed in a British population has demonstrated that the lack of antibody response to HBsAg is significantly associated with the HLA DRB1\*07/DQB1\*02 haplotype. A second HLA haplotype, DRB1\*07/DQB1\*03 was not associated with the lack of antibody response to HBsAg vaccination, indicating a crucial role for the DQB1\*02 allele (14). We observed a similar association in our subjects, 4 of 46 responders displayed the HLA-DRB1\*07/DQB1\*03 haplotype, but it was absent in our non-responders. In contrast, DRB1\*07/DQB1\*02 haplotype frequency was higher in non-responders (5 of 12; 42%) as compared to our responder subjects (10 of 46; 22%), though the difference did not reach statistical significance.

Looking at the major HLA class II alleles associated to HBsAg vaccination failure reported by different investigators, DRB1\*07 and DQB1\*0201 are the most common alleles observed in the majority of Caucasian populations, but not Japanese people (Table 5). The profile of the Iranian population seems to be very much similar to the Caucasian group. Similarly, alleles associated to HBsAg responsiveness seem to be similar in Iranian and many Caucasian populations with DRB1\*01, DRB1\*11 and DRB1\*1301 being elevated in high responders (Table 5). Contrary to the results observed in many Caucasian populations as well as our own results, in Japanese population HLA DRB1\*04, DRB1\*08021, DQB1\*03, DQB1\*04, DQA1\*03 and DQA1\*0104 alleles were found to be associated with anti-HBs antibody response, whereas DRB1\*0803, DRB1\*0101 and DRB1\*1403 alleles were associated with low or non-responsiveness (16). Association of DRB1\*0301 allele with HBsAg vaccine unresponsiveness has also been suggested by the low affinity binding of overlapping peptides from HBsAg to HLA glycoproteins encoded by DRA1\*0101-DRB1\*0301 (HLA-DR3) molecules (24). Our finding of increased frequency of HLA DRB1\*0301, DRB1\*07, DQB1\*0201 and DQA1\*0201 alleles in Iranian healthy adult non-responder individuals, which has also been reported in many other populations with different ethnic backgrounds, suggests involvement of these alleles in down-regulation of anti-HBs antibody response by induction of either inappropriate Th1 / Th2 cytokine production or immune suppression. These immunoregulatory mechanisms are both regulated at the level of antigen presentation. However, the finding that antigen presenting cells (APC) from non-responders can present HBsAg to DR-matched T-cells of responders indicates that defective HBsAg-specific T-cell repertoire rather than APC dysfunction is likely to be involved in vaccination failure (25, 26). Discordant HLA/peptide binding and cytokine production patterns observed in genetically identical monozygotic twins vaccinated with HBsAg (27) suggest involvement of postgenetic and environmental factors influencing the T-cell repertoire. Lower frequency of circulating HBsAg-specific T helper cells in non-responders ( $< 1/50000$ ) as compared to good responders ( $\sim 1/2000$ ) (28), has been taken to support the latter proposition.

Low frequency of antigen-specific T-cells, however, could be a secondary defect resulting from dysregulated antigen presentation and cytokine production. We have recently demonstrated lack of HBsAg-specific circulating B-cells in non-responders (17) which seems to be a consequence of diminished Th1 and Th2 cytokine secretion in both neonate and adult healthy non-responders (6, 29). Dysfunction of T helper response, in turns, was found to be associated with inhibition of IL-12 production by APCs from non-responders (30), highlighting the importance of the former mechanism, that is defective antigen presentation. Despite suppression of Th1 and Th2 responses, we found clonal expansion of a subset of CD4+ T-cells expressing a particular T-cell receptor (TCR) variable beta (BV) gene family (31). Thus it appears that presentation of HBsAg peptides in context with certain alleles or haplotypes of HLA molecule has resulted in stimulation of a restricted subpopulation of CD4+ T-cells, presumably T regulatory or suppressor cells, down-regulating the anti-HBs Ab response in non-responder vaccinees.

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