

# Unresponsiveness to Recombinant Hepatitis B Vaccine in Healthy Iranian Neonates: Association with HLA Antigens

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## Abstract

**Background:** Hepatitis B is an important infectious disease. Since several years ago, mass vaccination against this viral infection has become as part of routine vaccination schedule of Iran. However, some healthy neonates, children and adults fail to generate a protective antibody response after vaccination.

**Objectives:** To investigate distribution of HLA class-I and class-II antigens in healthy Iranian neonates vaccinated with recombinant hepatitis B vaccine.

**Methods:** HLA-typing was performed on peripheral blood lymphocytes isolated from 25 responder and 23 nonresponder (anti-HBs < 10 IU/L) healthy neonates, using the standard microlymphocytotoxicity method. Anti-HBs antibody was quantitated by sandwich ELISA.

**Results:** The frequency of HLA-DR7 ( $p<0.01$ ), DQ2 ( $p<0.02$ ) and DR13 ( $p<0.05$ ) was significantly higher in the nonresponder neonates compared to the responder group. The DR1 and DQ3 antigens were over-represented ( $p<0.05$ ) in the responder vaccinees, implying positive association with the anti-HBs antibody response. Statistical analysis revealed increased frequencies of B7-DR7-DR53-DQ2 and DR13-DR52-DQ2 haplotypes in the nonresponder neonates ( $p<0.05$ ).

**Conclusions:** We found a significant association between lack of antibody response to recombinant hepatitis B vaccine and expression of certain HLA class- II antigens in healthy Iranian neonates.

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**Keywords** • Neonates • hepatitis B vaccine • HLA antigens • anti-HBs antibody

## Introduction

**H**epatitis B virus (HBV) infection and its sequelae which include cirrhosis and hepatocellular carcinoma is still a major public health problem throughout the world. It is estimated that 350 millions of the world population are chronically infected with the virus and two million deaths annually are attributed to HBV

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infection.<sup>1,2</sup> The WHO strategy for effective control of HBV infection and its complications is mass vaccination of neonates and children within the framework of Expanded Programme on Immunization (EPI).<sup>3</sup> Vaccination with the major surface antigen of the virus (HBs Ag) induces protective antibody response in the majority of vaccine recipients. However, 1-10% of healthy neonates, children and adults fail to generate a protective antibody response.<sup>4-6</sup> This unresponsiveness has been attributed to several mechanisms, including defect in antigen presentation,<sup>7</sup> defects in specific T and/or B cell repertoires.<sup>8,9</sup> T-cell suppression,<sup>10</sup> lack of T-cell help necessary for production of anti-HBs by B cells,<sup>11</sup> defect in TH<sub>1</sub> and/or TH<sub>2</sub> cytokine production,<sup>12,13</sup> defect in TCR BV gene repertoire,<sup>14</sup> and over-representation of certain HLA genes and antigens.<sup>13-17</sup> The role of HLA is more important, because most of the mechanisms contributing to lack of immune response to a defined antigen are HLA-linked. Current data in adults suggest considerable ethnic differences in the HLA antigens associated with anti-HBs antibody response.<sup>13-17</sup> A variety of HLA class-I and class-II antigens and extended haplotypes of HLA have been reported to be either increased or decreased in non-responder vaccinees from different ethnic backgrounds.<sup>13-17</sup> A few studies were conducted in neonates which suggested that the mechanism(s) of unresponsiveness to HBs Ag may be different in neonates and adults. In this study, the association between HLA antigens and responsiveness to HBs Ag was investigated in healthy Iranian neonates.

**Patients and Methods**

• *Subjects and vaccination scheme*

Triple doses of 10 µg of a recombinant hepatitis B (HB) vaccine (Heberbiotec, S>A> Havana, Cuba) were administered i.m. to a large group of healthy

Iranian neonates at 0, 1.5 and 9 months intervals. Two to four weeks after completion of vaccination, peripheral blood was collected and anti-HBs antibody was quantitated in serum by sandwich ELISA. Collectively, 23 nonresponders (anti-HBs<10 IU/L) and 25 responder neonates (anti-HBs>1000 IU/L) were randomly selected and included in this study.

• *Cell preparation and HLA typing*

Peripheral blood mononuclear cells (PBMC) were separated from heparinized peripheral blood by Ficoll-paque (Sigma, USA) gradient centrifugation. After washing, PBMC were resuspended in culture medium consisting of RPMI-1640 (Sigma, USA) and antibiotics. HLA class I and II antigens were determined by the standard microlymphocytotoxicity technique, and B-lymphocyte enrichment was achieved by nylon wool method.<sup>18</sup> HLA typing was performed using commercially available plates which contained antisera representing multiple backgrounds (Blood Transfusion Center of Iran, Tehran, Iran).

• *Statistical analysis*

The statistical significance of the observed association between responder and nonresponder groups and HLA phenotype was analyzed by 2x2 contingency tables using the chi-square test with Yate's correction. Woolf's relative risk (RR), etiologic fraction (EF) and preventive fraction (PF) were calculated as described elsewhere.<sup>18</sup> The association was considered positive if the calculated EFs were higher than 0.15 and negative if the calculated PFs were more than 0.15. P values <0.05 were considered significant.

**Results**

The observed frequencies of the HLA class I and class II antigens in the nonresponder vaccinees

**Table 1:** Frequencies of HLA class-I antigens associated with unresponsiveness to hepatitis B vaccine

HLA	Responders (n=25)	Non-responders (n=23)	RR	EF	PF	Association	p value
A2	7(28)*	10 (43.4)*	1.97	0.21	-	PA	NS
A9	6(24)	2 (8.6)	0.3	-	0.17	NA	NS
A25	1(4)	5 (21.7)	6.66	0.18	-	PA	NS
A28	9(36)	4 (17.3)	0.37	-	0.22	NA	NS
A29	2(8)	0	-	-	-	NA	NS
B8	6(24)	1 (4.3)	0.14	-	0.21	NA	NS
B14	2(8)	0	-	-	-	NA	NS
B21	0	3 (13)	-	-	-	PA	NS
B53	7(28)	3 (13)	0.38	-	0.772	NA	NS
CW3	9(36)	13 (56.5)	2.31	0.32	-	PA	NS

RR: Relative risk; EF: Etiologic fraction; PF: Preventive fraction; PA: Positive association (EF>0.15); NA: Negative association (PF>0.15); NS: Not significant. \*Number (%) of subjects.

## Unresponsiveness to hepatitis B vaccine and HLA antigens

**Table 2: Frequencies of HLA class-II antigens associated with unresponsiveness to hepatitis B vaccine**

HLA	Responders (n=25)	Non-responders (n=23)	RR	EF	PF	Association	p value
DR1	6 (24)*	1 (4.3)*	0.18	-	0.165	NA	<0.05
DR2	8 (32)	4 (17.3)	0.44	-	0.18	NA	NS
DR3	7 (28)	9 (39.1)	1.65	0.154	-	PA	NS
DR7	3 (12)	11 (47.8)	8	0.456	-	PA	<0.01
DR13	3 (12)	9 (39.1)	4.71	0.3	-	PA	<0.05
DR52	17 (68)	18 (78)	1.37	0.21	-	PA	NS
DR53	10 (40)	14 (60.8)	2.33	0.34	-	PA	NS
DQ2	10 (40)	17 (73.9)	4.25	0.56	-	PA	<0.02
DQ3	15 (60)	7 (30.4)	0.34	-	0.37	NA	<0.05

RR: Relative risk; EF: Etiologic fraction; PF: Preventive fraction; PA: Positive association (EF>0.15); NA: Negative association (PF>0.15); NS: Not significant. \*Number (%) of subjects.

were statistically analyzed and compared with those of the responder group. No significant association was observed between HBs Ag responsiveness and HLA class I antigens. The results showing positive or negative associations are illustrated in Tables 1 to 3. Our results demonstrated that the frequencies of HLA-DR7 ( $p<0.01$ ), DQ2 ( $p<0.02$ ) and DR13 ( $P<0.05$ ) antigens and B7-DR7-DR53-DQ2 and DR13-DR52-DQ2 ( $p<0.05$ ) haplotypes were significantly higher in non-responder neonates. On the other hand, the frequencies of DR1 and DQ3 ( $p<0.05$ ) were higher in responder neonates.

### Discussion

Production of anti-HBs antibody is helper T cell dependent.<sup>14</sup> CD4<sup>+</sup> lymphocytes recognize different epitopes within the HBs Ag molecule that can be presented by certain HLA class II antigens.<sup>19</sup> Defective antigen presentation, therefore, could result in inefficient anti-HBs antibody production. Unresponsiveness to HBs Ag vaccination has been associated with HLA (MHC) antigens in human and murine.<sup>13-17,20-24</sup> Different mechanisms have been proposed to be involved in non-responsiveness to HBs Ag. Taking into account the influence of HLA antigens, including: absence of dominant immune response genes located in the HLA complex,<sup>25</sup> ac-

tive suppression of immune response,<sup>10</sup> selective killing of HBs Ag-specific B-cells by HLA-restricted cytotoxic T lymphocytes,<sup>26</sup> defective APC/T cell interaction,<sup>27</sup> selective deletion of specific T-cells<sup>14,16</sup> and induction of TH<sub>1</sub>/TH<sub>2</sub> imbalances.<sup>12,13,28</sup> In the present study we observed significantly increased frequencies of the DR7, DQ2 and DR13 antigens in our non-responder neonate vaccinees (Table 2). Similar studies have mostly been performed on adults and limited data are available from neonates.<sup>29</sup> Our finding of the increased expression of DR7 and DQ2 molecules has already been reported in healthy non-responder adults<sup>14,16,21-23</sup> and neonates<sup>29</sup> from different countries using both serological and molecular techniques. Like others, we found an over-representation of DR1 and DQ3 gene products in responder vaccinees, suggesting a positive association between these antigens and anti-HBs antibody production in human.<sup>16,17</sup> Higher representation of the DR13 antigen in our non-responder subjects, however, has not already been reported and seems to be restricted to the Iranian neonate population. Most of the studies published to date that have examined the relationship between the immune response to HBsAg and HLA type have used serological methods. Recent advances in molecular techniques have allowed more detailed analysis of the polymorphism of HLA at the DNA level. Due to some limitations, particularly inability to obtain

**Table 3: Frequencies of HLA haplotypes associated with lack of response to hepatitis B vaccine**

HLA	Responders (n=25)	Non-responders (n=23)	RR	EF	PF	Association	p value
DR7-DR53-DQ2	2 (8)*	7 (30.4)*	5.03	0.24	-	PA	NS
B7-DR7-DR53-DQ2	0	5 (21.7)	-	-	-	PA	<0.05
A2-DR7-DR53-DQ2	1 (4)	6 (26)	8.47	0.23	-	PA	NS
DR13-DR52-DQ2	0	5 (21.7)	-	-	-	PA	<0.05

RR: Relative risk; EF: Etiologic fraction; PF: Preventive fraction; PA: Positive association (EF>0.15) NA: Negative association (PF>0.15); NS: Not significant; \*Number (%) of subjects.

larger volumes of peripheral blood samples from neonates and ethical restrictions, we were unable to perform DNA typing in our study. Molecular analyses have demonstrated that the lack of antibody response to HBs Ag is significantly associated with the HLA haplotype DRB1\* 0701-DQB1\* 0202. A second HLA haplotype, DRB1\* 0701-DQB1\* 0303 was not associated with the lack of antibody response to HBs Ag vaccination, indicating a crucial role for the DQB1\* 0202 allele.<sup>21</sup> Furthermore, DRB1\* 1301 and DRB1\* 1302 alleles have been associated with responsiveness and unresponsiveness to HBs Ag, respectively. There is only one amino-acid differences between the alleles at position 86 in the peptide binding groove. At this position DRB1\* 1301 has a valine while DRB1\* 1302 has glycine.<sup>17</sup> It seems that loss of glycine or existence of valine at position of 86 in DRB1 molecules is a determining factor for a positive influence on anti-HBs production. Interestingly, DRB1\* 1501 and DRB1\* 0401 alleles that were also found to be associated with response to HBsAg have a valine at position of 86.<sup>17</sup> Based on some reports<sup>14,16</sup> it is likely that nonresponsiveness to HBs Ag is linked to a defect in the T-cell antigen recognition repertoire. It is conceivable that T cells recognizing the dominant T-cell epitope(s) of HBs Ag that can associate with certain class II molecules (e.g., DR7) are absent, because these HBs Ag-derived peptide-MHC class II complexes so closely resemble self peptide-MHC class II (molecular mimicry) that they have been eliminated in the thymus or in the periphery.<sup>16</sup> However, our recent findings of the overexpression of certain TCR VB genes in CD4<sup>+</sup> T-cells from adult non-responders do not support this proposition. HLA antigens may induce insufficient or imbalanced TH<sub>1</sub>/TH<sub>2</sub> response, resulting in inappropriate cellular or humoral immune response to HBs Ag, due to the profile of secreted cytokines.<sup>12,13,28</sup> We have recently demonstrated inhibition of both TH<sub>1</sub> and TH<sub>2</sub> responses in adult and neonate nonresponders.<sup>30</sup> This inhibitory effect was influenced by those antigens and haplotypes of HLA that were positively associated with unresponsiveness (unpublished observation). Secretion of TH<sub>1</sub> and TH<sub>2</sub> cytokines is tightly regulated by APC function. Thus, defective TH<sub>1</sub> and TH<sub>2</sub> responses are most likely attributed to APC function. Since HLA antigens play a pivotal role in presentation of antigenic peptides to T helper cells and thereby induction of their activation, therefore, the quality of T helper response is linked to the composition of HLA antigens which act as restrictive elements. Our recent observation of the decreased production of IL-12 by PBMC from non-responder neonates, suggests APC dysfunction in non-responder neonates. Since the TH<sub>1</sub> and TH<sub>2</sub> re-

sponses<sup>33</sup> and HLA antigens,<sup>31</sup> are similarly represented in healthy adult non-responders, similar mechanisms seem to be involved in lack of response to HBs Ag in both adults and neonates.

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