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Short Communication

Genetic Factors of Low-responsiveness to Hepatitis B Virus Vaccine Confirms the Importance of Human Leukocyte Antigen Class II Types in a Japanese Young Adult Population

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We investigated the genetic mechanisms underlying the association between human leukocyte antigen (HLA) types and the immune response to hepatitis B virus (HBV) vaccination in 84 healthy Japanese adults, and found that the HLA-DRB1*04 and HLA-DQB1*03 frequencies were higher in the low responders (<10 mIU/ml; n=9, 10.7%) compared to the responders (≥10 mIU/ml, n=75, 89.3%). The combination of DRB1*04 and DQB1*03 was associated with a low response to vaccination. The DRB1*04 and DQB1*03 haplotypes' frequencies were significantly higher in the low responders compared to responders. Novel candidate HLA types may be important in Japanese individuals.

Key words: HBV vaccine, antibody response, low-responder, HLA class II, Japanese

Hepatitis B virus (HBV) infection affects more than 350 million people worldwide and is the leading cause of cirrhosis and hepatocellular carcinoma [1]. HBV surface antigen (HBsAg) vaccination programs have been carried out in many countries since the 1980s and have been highly effective in decreasing the number of new HBV infections [2]. The protective effect of such a vaccination is dependent mainly on the induction of a specific antibody response. However, approx. 5-10% of healthy adults fail to develop anti-HBs antibodies after a standard course of vaccination [3].

A twin vaccination study revealed that >60% of the observed variability in immune responses can be explained by genetic factors [4]. That study's authors also suggested that approx. 40% of the genetic contribution is determined by human leukocyte antigen (HLA) genes, with the DRB1* locus estimated to account for 0.25 and the remaining heritability of 0.36 being assigned to other gene loci [4]. Several case control studies have compared the frequencies of HLA class II antigens among HBsAg responders and non-responders, revealing both alleles strongly associated with a high response [DRB1*01 (DR1), DRB1*11 (DR5), DRB1*15 (DR2), DQB1*0501, and DPB1*0401] and alleles strongly associated with a poor response/non-responsiveness [DRB1*03, DRB1*07, DQB1*02, and DPB1*1101] [5-7]. However, these alleles tend to have a low frequency in the Japanese population.

HLA class II molecules mediate the activation of helper T (Th) cells by antigen binding. Several groups have suggested that antibody non-responders to vaccination fail to show HBsAg-specific Th cell activation *in*

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vitro [8]. Most of the genes involved in the regulation of responsiveness to HBsAg are still unknown, particularly with regard to the influence of ethnicity and geographic location. Little is known about the HLA types related to the induction of immunity by HBsAg vaccination in the Japanese population, although an association has been demonstrated between HLA haplotypes and response/non-response to HBsAg [9,10]. It has also been suggested that age-associated changes of humoral immunity may result in decreased effectiveness of vaccinations [11].

We designed the present study to examine two polymorphisms of HLA class II alleles (HLA-DRB1* and HLA-DQB1*) in a genetically homogenous population of Japanese university students, in order to avoid the influence of host-related factors (such as age, gender, obesity, chronic disease and immunodeficiency), by comparing good responders and poor responders participating in the same HBsAg vaccination program.

Subjects and Methods

The subjects were selected in order to minimize the influence of age and to allow the elucidation of genetic factors influencing the response to HBsAg vaccine in a Japanese population. The subjects were 123 volunteers who were healthy, non-obese, non-smoking, fourth-year students from the Laboratory of Science, Saitama Prefectural University School of Health and the Department of Medical Technology, Kagawa Prefectural University of Health Sciences (26 men and 97 women aged 21-24 years; body mass index <25.0 kg/m^2). This study was approved by the Ethics Committees of Saitama Prefectural University and Kagawa Prefectural University of Health Sciences. The objectives of the study were explained to all of the subjects, and written consent was obtained in accord with the Declaration of Helsinki.

The primary objective of the HBsAg vaccination program was to prepare the subjects for clinical practice. They received the first, second, and third doses of a yeast-derived recombinant HBsAg vaccine (Bimmugen; The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan) in May, June, and November of their third year in university. Blood samples for the evaluation of the anti-HBs antibody titer were collected in May of the subjects' fourth year in university, approx. 6 months after the third dose of the vaccine.

Blood samples were drawn into blood collection tubes with EDTA-2Na, plasma was separated by centrifugation, and the plasma level of HBs antibody was quantified by a chemiluminescence enzyme immunoassay (CLEIA, Lumipulse presto II; Fuji Rebio, Tokyo, Japan). When the antibody level reached 1,000 mIU/ml or higher, the sample was determined by using an enzyme-linked immunosorbent assay (ELISA; Siemens Healthcare Japan, Tokyo, Japan). Genomic DNA was extracted from buffy coat white blood cells with a DNA extraction kit (QIAamp DNA Blood mini kit; Qiagen, Hilden, Germany) and was subjected to a sequence-specific primers-polymerase chain reaction (SSP-PCR) to amplify the HLA-DRB1 or HLA-DQB1 loci for "low-resolution to their two-to four-digit specificities" typing using synthetic oligonucleotide primers, as described [12,13]. With reference to the known frequencies in the Japanese population (jshi. umin. ac. Jp/databank/index. html), the target HLA-DRB1 alleles were *0101, *0401-0410, *0801, *0901, *1101, and *1501-1502, and the HLA-DQB1 alleles were *0301, *0302-0303, *0401, *0402, and *0601.

Based on the anti-HBs antibody level, we divided the subjects into low-responder (<10 mIU/ml), middle-responder (10-100 mIU/ml), and high-responder (>100 mIU/ml) groups, and we compared the HLA types of these groups. The independence of the frequencies of HLA types and each anti-HBs antibody group was analyzed by Fisher's exact test. We performed a multiple regression analysis to confirm the influence of the individual factors on anti-HBs antibody production. StatFlex ver. 6 software (ArTec, Osaka, Japan) was used for all statistical analyses and p-values <0.05 were considered significant.

Results

In the primary analysis, to estimate the frequency of low responders to HBsAg vaccine in the Japanese population, we analyzed the anti-HBs antibody level in 84 subjects given three separate programed recombinant HBsAg vaccines at Saitama Prefectural University. These were divided into the nine low responders (<10 mIU/ml, 10.7%) and the 75 responders (≥ 10 mIU/ml, 89.3%). Several case control studies have compared the frequencies of HLA class II antigens among HBsAg responders and low responders, revealing various strong associations [5-8].

To identify HLA class II alleles associated with the response to anti-HBs vaccine, we performed a DNA analysis of HLA-DRB1 and HLA-DQB1 types in another 39 subjects (17 low responders and 22 responders) for a total of 123 subjects. Table 1 shows the frequency of HLA class II types in the 3 groups (low-responder, <10 mIU/ml; middle-responder, 10-100 mIU/ml; and high-responder; >100 mIU/ml). At the HLA-DRB1 locus, HLA-DRB1*0401-0410 was positively associated with low mean anti-HBs antibody levels (n=123, mean ± standard error [SE] 252.3±82.4 vs. 674.4±151.7 mIU/ml, positive vs. negative, $p<0.05$). At the HLA-DQB1 locus, HLA-DQB1*0301 was positively associated with low mean anti-HBs antibody levels (n=117, mean±SE; 340.2±125.7 vs. 540.4±118.1 mIU/ml, positive vs. negative, $p>0.05$). There were significant differences in the frequency of DRB1*0401-0410 and DQB1*0301 by Fisher's exact test ($p<0.05$ and $p<0.01$ respectively), but there were no significant differences in the other HLA class II types (HLA-DRB1*0301, *0901, *1501-1502, HLA-DQB1*0302; 0303, *0401; 0402, *0601, *0602).

The multiple regression analysis confirmed the influence of HLA-DRB1*0401-0410 and HLA-DQB1 *0301 on the anti-HBs antibody level ($p<0.05$). Fig. 1 shows the anti-HBs antibody levels in relation to the HLA-DR and HLA-DQ types. In particular, the high-responder group dominated the combination of being HLA-DRB1*0401-0410-negative and DQB1 *0301-positive ($p<0.05$). In addition, there was a significant difference between the low-responder and high-responder groups according to a multiple cluster analysis by Dunn's method ($p<0.01$).

Table 1 HLA class II frequencies of the vaccinated subjects in each anti-HBs titer group

HBsAb (mIU/mL)		Low-responder <10.0	Middle-responder 10-100	High-responder >100	*P* value
HLA-DRB1*0101	Neg.	21	29	44	0.756
	Pos.	5	8	16	
HLA-DRB1*0401-0410	Neg.	9	19	38	**0.046**
	Pos.	17	18	22	
HLA-DRB1*0801	Neg.	12	26	45	0.737
	Pos.	4	5	10	
HLA-DRB1*0901	Neg.	10	26	43	0.422
	Pos.	5	5	12	
HLA-DRB1*1101	Neg.	16	30	48	0.788
	Pos.	1	4	8	
HLA-DRB1*1501–1502	Neg.	16	25	41	0.189
	Pos.	1	9	15	
HLA-DQB1*0301	Neg.	16	20	50	**0.005**
	Pos.	10	17	10	
HLA-DQB1*0302–0303	Neg.	17	27	40	0.506
	Pos.	7	7	19	
HLA-DQB1*0401	Neg.	12	26	52	0.093
	Pos.	3	5	3	
HLA-DQB1*0402	Neg.	9	26	41	0.227
	Pos.	6	5	14	
HLA-DQB1*0601	Neg.	10	16	33	0.597
	Pos.	6	15	20	

Numerical values are the numbers of people. Neg., negative; Pos., positive. Differences in the frequency of each DRB1 and DQB1 type were determined by Fisher's exact test. Significant associations are shown in **bold**.

Discussion

HBsAg vaccine is an exogenous antigen. It is well documented that type differences of HLA class II molecules influence the helper T-cell recognition of exogenous antigens, as well as the generation of antibodies by B cells [14]. HLA class II genes have very high levels of polymorphism, especially the beta 1 chain. The previous studies investigated mainly the response to HBsAg vaccine in western subjects, with HLA-DRB1*0301, *0701 and HLA-DQB1*0201 being consistently confirmed to be associated with non-responsiveness to vaccination [5-7]. However, the frequency of these HLA-DR and HLA-DQ types is very low in the Japanese population. In the present study we therefore focused on the relation between HLA-DRB1 and HLA-DQB1 types and the response to an HBsAg vaccination in Japanese subjects. Our analyses revealed that an increased likelihood of a low response to HBsAg vaccination was significantly associated with the HLA-DRB1 *0401-0410 and HLA-DQB1 *0301 alleles in young Japanese adults. On the other hand, a particular HLA class II type was not associated with a high antibody response to vaccination.

Our multiple regression analysis indicated that the HLA-DRB1*0401-0410 and HLA-DQB1*0301 alleles influenced the anti-HBs antibody production after vaccination. This combination of HLA-DR and HLA-DQ types may modify the conformation of the peptide-binding groove and thus influence antigen presentation, resulting in weak responsiveness to the HBsAg vaccine in a segment of

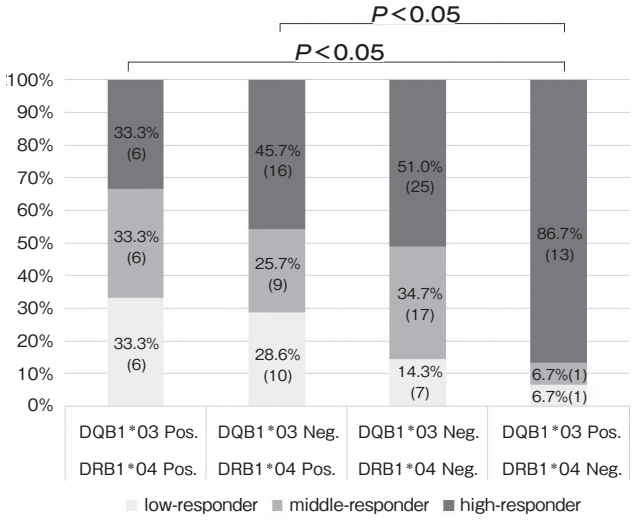

Fig. 1 The multiple regression analysis results. The distribution of the low-, middle- and high-responder groups is shown for each HLA-DRB1*04 and HLA-DQB1*03 type.
The numbers of subjects are shown in parentheses (n=117 total).

the Japanese population. Interestingly, the combination of HLA-DRB1*0401-0410 negativity and HLA-DQB1*0301 positivity was most effective and seems to be an important combination of HLA class II molecules (HLA class II haplotype).

Our results are not in agreement with reports on vaccinated HBsAg antibody non-responders in western countries [5-7]. In the Japanese, there is a reason why the HLA molecule conformation that influences an affinity with HBsAg will be decided by combinations of the different HLA-DR, -DQ, and -DP types with western populations. In an earlier study we investigated the association between cytokine gene polymorphisms and the immune response to HBsAg vaccination, and the results revealed that single nucleotide polymorphisms (SNPs) of the TNF-α and IL-10 genes may lead to ethnic differences in the response of Japanese individuals to HBsAg vaccine [15]. HLA gene polymorphisms are extremely common, and the same observation applies to HLA class II gene polymorphisms in the Japanese population. A recent genome-wide association study (GWAS) showed that SNPs in the HLA-DR region are associated with the response to HBsAg vaccine [16], and thus it may be important to study responsible SNPs rather than assessing the influence of HLA types. It was indicated that the HLA class II region has the key influence on antibody titers after HBsAg vaccination [14]. Although we did not do so in the present study, it appears to be necessary to investigate HLA-DP alleles in the future. Further well-designed analyses of the Japanese population and other ethnicities are required to confirm our findings, toward the development of more effective hepatitis B vaccine. It may also be helpful to design more effective vaccination methods by using a pre-inoculation test to check the influencing factors such as the HLA types and polymorphisms of cytokine genes.

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