Contribution of HLA class I and class II alleles to the regulation of antibody production to hepatitis B surface antigen in humans

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Abstract

The HLA multigene family consists of HLA class I (HLA-A, B and C) and class II (HLA-DR, DQ and DP) genes, and plays a central role in the regulation of immune response. To investigate how each HLA gene and each HLA allele contribute to the human immune response, we immunized 339 healthy Japanese medical students with recombinant hepatitis B surface antigen (rHBsAg) and determined the HLA types of all vaccinated subjects at the DNA level. The anti-HBs antibody titers showed a log-normal distribution, implying that the immune response to HBsAg in humans is a multifactorial and continuous trait. A stepwise multiple regression analysis demonstrated the alleles at the HLA-class I (HLA-A and B) and class II (HLA-DRB1, DQA1, DQB1, DPA1 and DPB1) loci significantly contributed to antibody production to HBsAg. The predicting equation of anti-HBs antibody levels for individuals with any HLA phenotype was proposed based on a multiple regression analysis. The multiple correlation coefficient of antibody production to HBsAg with the HLA-DRB1 locus was highest (0.34) among all of the HLA loci, whereas those with whole HLA class I or class II loci were 0.36 or 0.44 respectively. The incorporated correlation coefficient of the presence of all HLA gene families with antibody production became 0.50, suggesting that HLA class I and class II loci within the HLA multigene family are dynamically involved in regulation of the immune response to HBsAg.

Introduction

The HLA multigene family consists of HLA class I and class II genes with extremely high polymorphism, and plays a critical role in the regulation of immune response to natural antigens. However, the relative contribution of each gene of the HLA in the regulation of immune responses is unclear. It has been reported that there are low and high antibody responders defined with arbitrary cut-off points to hepatitis B surface antigen (HBsAg) in both mice and humans (1–17). Mechanisms controlling the difference in responsiveness to HBsAg are controversial; however, at least two hypotheses have been proposed. (i) HLA-linked immune response genes (Ir genes) might control high responsiveness, whereas low responsiveness might be due to the absence of the Ir genes. Milich et al. (1–6) reported that MHC-linked Ir genes control the response to HBsAg in mice and that a lack of HBs antibody response is caused by a defect in T cell help. In addition, Alper et al. (9–14) also speculated that MHC-linked Ir genes control the human immune response to HBsAg and that low responsiveness is caused by the absence of these genes. (ii) Alternatively, low responsiveness to HBsAg might be attributable to antigen-specific suppression and be controlled by HLA-linked immune suppression genes (Is genes).
(15–21). We reported that the presence of HLA-linked Is genes in humans, which control antigen-specific low responsiveness to natural antigens, such as HBsAg (15–17), streptococcal cell-wall antigen (18), Cryptomeria japonica pollen antigen (19) and Schistosoma japonica antigen (20), is a dominant genetic trait, presumably functioning via induction of CD8⁺ suppressor T cells.

In these previous reports, HLA was typed using a serologic method. However, recent advances in molecular techniques have allowed more detailed analysis of the polymorphism of HLA at the DNA level. At present, >100 alleles at the HLA-DRB1 locus can be identified by DNA typing, using the PCR-sequence-specific oligonucleotide probe (PCR-SSOP) method, in contrast to serological typing by which only 19 HLA-DR types can be distinguished (22). Serologically determined HLA-DR4, for example, can be subtyped into 22 alleles at the DNA level. Similarly, in the case of the HLA-A locus, serologically determined HLA-A2 can now be subdivided into 17 alleles by the DNA typing (23). Furthermore, the analysis of peptide binding and subsequent T cell activation revealed that even a single amino acid difference in the peptide binding groove of HLA molecules affected the peptide binding and, accordingly, T cell responses to the peptides (24–26).

Furthermore, previous studies were done using a rather crude HBsAg extracted from plasma of HBsAg-positive blood donors as an immunogen. Recently, rHBsAg obtained by recombinant technology has been introduced to vaccination because it has the advantage of purity as compared with the plasma-derived HBsAg.

These findings have facilitated studies on the contribution of HLA alleles to the immune response to HBsAg more precisely. We immunized 339 healthy Japanese medical students with rHBsAg, determined the HLA types of all vaccinated subjects at the DNA level, measured the levels of antibody response specific for HBsAg and analyzed all the data using multiple regression analysis. We report here that all HLA genes are dynamically involved in the regulation of the immune response to rHBsAg.

**Methods**

**Immunization**

After obtaining informed consent from each subject, 339 healthy Japanese medical students (aged 21–25 years, 288 men and 51 women, all negative for the presence of HBsAg, anti-HBs antibody and anti-HBc antibody) were immunized s.c. with 20 µg rHBsAg (Derived Yeast, Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan) three times at 0, 4 and 24 weeks respectively.

**HLA typing**

DNA was extracted from peripheral granulocytes. All subjects were genotyped for HLA class I (HLA-A) and class II (HLA-DRB1, DQA1, DQB1, DPA1 and DPB1) by the PCR-SSOP method, as described previously (27–31). Briefly, 1 µg of genomic DNA was subjected to PCR for the HLA genes with primers, designed to specifically amplify each HLA gene. PCR products were hybridized with ³²P-labeled SSOP by dot-blot hybridization. The HLA-B locus of HLA class I was serotyped using the microcytotoxicity test because precise DNA typing was not yet available (32).
We applied a stepwise multiple linear regression model of individuals positive for the allele and the individuals negative for the allele was below five, consequently a total of 84 HBs antibody production on HLA alleles 

\[ Y = a + bX_i \]

To estimate the contribution of each allele of each HLA locus to HBs antibody production, we conducted a stepwise multiple regression analysis (details as described in Methods). The alleles at each HLA locus that either positively or negatively contribute to the anti-HBs antibody production with statistical significance and the partial correlation coefficients of these alleles are listed in Table 1. In the HLA-A and HLA-B loci, HLA-A*2602, A*1101, B70 and B35 contributed negatively to the anti-HBs antibody response. HLA-B46 and B7 correlated with the enhanced anti-HBs antibody production, indicating that these two alleles contributed positively to the anti-HBs antibody response. In the HLA-DRB1 locus, HLA-DRB1*08032, 0101 and 1403 positively contributed to the response, whereas HLA-DRB1*0405, 0406, 0802, 0401 and 1101 negatively contributed to the response. Alleles identified in the HLA-DOA1 and HLA-DOB1 loci associated with reduced anti-HBs antibody responses were HLA-DOA1*0302, 0301, 0104 and 0601, and HLA-DOB1*0401, 0303 and 0302 respectively. In the HLA-DPA1 locus, HLA-DPA1*0103 positively contributed to the response. In the HLA-DRB1 locus, HLA-DRB1*0402, 0202 and 1301 positively contributed to the response, whereas HLA-DRB1*1401 negatively contributed to the response. It is difficult to identify the genes primarily responsible for HBs antibody production in the HLA-A-B-DR-DQ-DP haplotypes, because of the linkage disequilibria among certain alleles in the HLA-A, -B, -DR, -DQ and -DP loci.

**Results**

**Distribution of anti-HBs antibody titers among vaccinated subjects**

The distribution of anti-HBs antibody titers among 339 vaccinated subjects ranged from <1 to >8000 mlU/ml and showed a log normal distribution, as analyzed by a test of goodness of fit (df = 7, \( \chi^2 = 8.3 \)) (Fig. 1). This suggests that the immune response to rHBsAg in human is a multifactorial and continuous trait.

**Contribution of each allele of the HLA loci to anti-HBs antibody production**

To estimate the contribution of each allele of each HLA locus to the HBs antibody production, we used a commercial test system (AUSAB, Abbott, IL). The assay has a sensitivity of 1 mlU/ml. Serum samples were obtained from vaccinated subjects 4 weeks after the third vaccination. The levels of antibody responses specific for HBsAg were assayed by radioimmunoassay, using a commercial test system (AUSAB, Abbott, IL). The assay has a sensitivity of 1 mlU/ml.

**Measurement of serum levels of antibody to HBsAg**

Serum samples were obtained from vaccinated subjects 4 weeks after the third vaccination. The levels of antibody responses specific for HBsAg were assayed by radioimmunoassay, using a commercial test system (AUSAB, Abbott, IL). The assay has a sensitivity of 1 mlU/ml.

**Statistical analysis**

A standardized log anti-HBs titer was defined as: (log anti-HBs antibody titer – average of log anti-HBs antibody titer)/standard deviation of log anti-HBs antibody titers. A test of goodness of fit was conducted to test whether the distribution of anti-HBs antibody titers fitted a log normal distribution or not. A total of 129 alleles at the seven loci within the HLA multigene family was identified in this population (19 alleles at the HLA-A locus, 33 alleles at the HLA-B locus, 28 alleles at the HLA-DRB1 locus, 14 alleles at the HLA-DQA1 locus, 16 alleles at the HLA-DQB1 locus, five alleles at the HLA-DPA1 locus and 14 alleles at the HLA-DPB1 locus). We excluded the alleles in which the number of the individuals with the allele was below five, consequently a total of 84 alleles were analyzed as determinants. We compared the average of standardized log anti-HBs titer between the individuals positive for the allele and the individuals negative for the allele by Student’s t-test, and selected the alleles with significant difference in the titer.

To estimate the contribution of each allele of each HLA locus to HBs antibody production, under the assumption that HLA alleles contribute additively to HBs antibody production, we applied a stepwise multiple linear regression model of HBs antibody production on HLA alleles 

\[ Y = a + bX_i \]

where \( Y \) denotes the observed values and \( y \) the predicted values obtained by the equation for prediction of the antibody level and \( y \) denotes the observed values.

![Fig. 2. Correlation between the observed value and the predicted value of HBs antibody production](https://academic.oup.com/intimm/article-abstract/8/4/525/852181)
Table 1. Multiple regression analysis of anti-HBs antibody titers with HLA types

<table>
<thead>
<tr>
<th>Class</th>
<th>R²</th>
<th>A</th>
<th>B</th>
<th>DRB1</th>
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</tr>
</tbody>
</table>

The broken line denotes linkage disequilibrium

R: multiple correlation coefficient of antibody production with each HLA locus
r: partial correlation coefficient of antibody production with each allele
N: number of individuals in parentheses

The squares represent selected alleles by the stepwise multiple regression analysis

especially between DR and DQ alleles. However, by applying a stepwise multiple regression analysis to the alleles described above, one can discern statistically which allele on each of the haplotypes would mainly contribute to the antibody production. For instance, DRB1*08032 in the B46-DRB1*08032 haplotype, DPA1*0103 and DPB1*0402 in the B7-DRB1*0101-DRB1*0103-DPB1*0402 haplotype, and DQA1*0503 in the DRB1*1403-DQA1*0503 haplotype were identified as primarily and positively contributing to the antibody production. DRB1*0405 in the DRB1*0405-DQA1*0302-DQB1*0401 haplotype, DQB1*0302 in the DRB1*0405-DRB1*0301-DQA1*0302-DQB1*0302 haplotype and B70 in the B70-DRB1*0401 haplotype were defined as primarily and negatively contributing to the antibody production.

On the other hand, HLA alleles which were not necessarily in linkage disequilibrium with any alleles in the other HLA loci were also identified to be independently involved in antibody production. For instance, HLA-A*2602, which negatively contributed to the antibody production, did not form any specific HLA-A-B-DR-DQ-DP haplotypes.

Contribution of each HLA locus to HBs antibody production

The multiple correlation coefficients of anti-HBs antibody production with each HLA locus are shown in Table 1. Those with the HLA-A, B, DRB1, DQA1, DQB1, DPA1 and DPB1 loci were 0.23, 0.27, 0.34, 0.27, 0.29, 0.18 and 0.25 respectively. The contribution of the DRB1 locus was the greatest among all the HLA loci. Those with HLA-class I (HLA-A and B) and class II (HLA-DRB1, DQA1, DQB1, DPA1 and DPB1) genes accounted for 0.36 and 0.44 respectively. Moreover, the incorporated correlation coefficient of the entire HLA gene family with the antibody production was 0.50. All these results led to the conclusion that not only the HLA-DRB1 gene, but also all other polymorphic genes of the HLA multigene family significantly influenced the immune response to HBsAg.

Prediction

The predicting equation for anti-HBs antibody levels for the individuals with any HLA types was proposed by the multiple regression analysis as follows:

\[ Y = -0.048 + 0.367X_{\text{DRB1*08032}} - 0.922X_{\text{B70}} - 0.459X_{\text{DQA1*0302}} + 0.166X_{\text{DPA1*0301}} - 0.788X_{\text{B70}} - 0.299X_{\text{DPB1*0402}} + 0.462X_{\text{DQB1*0401}} + 0.439X_{\text{DPB1*0103}} + 0.221X_{\text{DPB1*0202}} - 0.359X_{\text{DRB1*1403}} + 0.444X_{\text{DPB1*0401}} \]

where Y is the predictive normalized value of log₂ (anti-HBs
Table 2. The HLA types of lowest responders and low responders

A. The HLA types of 6 lowest responders.

<table>
<thead>
<tr>
<th></th>
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B. The HLA types of 14 low responders among 333 responders.

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*a* the standardized log anti-HBs antibody titer of individuals
*b* Box represent the alleles negatively contributing to anti-HBs antibody production
*c* Underlines represent the alleles positively contributing to anti-HBs antibody production

The alleles either negatively or positively contributing to anti-HBs antibody production are identified by multiple regression analysis and details are shown in Table 1

The anti-HBs titer of 339 HLA-typed vaccinated subjects predicted by using this equation correlated well with the observed anti-HBs titers (r = 0.50) (Fig. 2).

**Discussion**

This report is apparently the first that the anti-HBs antibody titers of a large group of healthy donors vaccinated with rHBsAg showed a log-normal distribution. Since this observation suggested that the human immune response to rHBsAg was a multifactorial and continuous trait, we introduced multiple regression analysis, and revealed the contribution of each HLA allele and each HLA gene to the immune response to rHBsAg. The HLA-DRB1 locus proved to be the most responsible among all other HLA loci involved in antibody production to HBsAg. This is consistent with previous observations that the high immune response to HBsAg was associated with serologically defined HLA-DR1 both in Japanese and Caucasian populations (7,17). In the current study, however, it appeared that the presence of HLA-DPA1*0103-DPB1*0402 in linkage disequilibrium with HLA-DRB1*0101 was more influential than that of HLA-DRB1*0101, with regard to the antibody production to HBsAg. Besides the HLA-DRB1*0101 haplotype, it was also observed that the presence of HLA-DRB1*08032 was associated with elevated antibody production, a finding not reported in previous studies. HLA-DRB1*0405, on the other hand, contributed negatively to antibody production, thereby supporting our previous observation that serologically defined HLA-DR4 was strongly associated with the non-responsiveness to HBsAg (15-17). In this study we also observed that HLA-DRB1*1101 negatively contributed to antibody production to HBsAg. The findings reported here were made possible by the DNA typing of HLA and using a large sample size for study.
In the Caucasian population, a statistical association has been reported between low antibody response to HBsAg and serologically defined HLA-DR3 and DR7 (7,8), as well as with their extended haplotypes HLA-B8, SC01, DR3 and HLA-B44, FC31, DR7, and, in particular, with homozygotes for the latter haplotypes (9,10). Further evidence to support this was obtained in family studies (11). Although these alleles (HLA-DR3 and DR7) were extremely rare in the subjects in our study, the average of standardized anti-HBs antibody titers of the individuals with HLA-DRB1*0301 (n = 2) or DRB1*0701 (n = 2) was low (-0.225σ, -1.175σ), a finding consistent with data on Caucasians.

It was assumed that the HLA class II molecules, coded for by alleles positively contributing to the antibody production, may bind oligopeptides derived from HBsAg to activate CD4⁺ T cells (T₄2) which help B cells to differentiate to antibody-producing plasma cells. In this study, almost all vaccinated subjects, especially those with higher antibody titers, showed strong T cell responses to HBsAg in vitro (data not shown). On the contrary, it was not easy to explain how the HLA molecules coded for by the alleles negatively contributing to HBs antibody production function in the immune regulation. Simply, the HLA class II molecules, coded for by the negatively contributing alleles, might not be able to bind peptides from HBsAg and failed to activate CD4⁺ helper T cells. However, we also observed that even most of the vaccinated subjects with lower antibody titers showed vigorous proliferative T cell responses in vitro (data not shown), implying that their HLA class I and/or class II molecules apparently bound the peptides from HBsAg to activate CD8⁺ and/or CD4⁺ T cells, but they failed to help or interfere with the antibody production.

It should be noted that not only HLA class II alleles but also HLA class I alleles were seen to be involved in the regulation of antibody production. HLA-A*2602 and 1101, defined at the DNA level, and serologically defined HLA-B35 negatively contributed to the antibody production. It is apparent from the current analysis that the contribution of these HLA class I alleles is primary and not due to strong linkage disequilibria with any HLA class II alleles which contribute negatively to the antibody production. These HLA class I molecules might bind self or non-self peptides to activate CD8⁺ T cells which might contribute to down-regulation of the immune response to HBsAg. Recently, Schirmeck et al. (34) reported that peptides from HBsAg were presented by HLA class I and that they were targets for the CD8⁺ cytotoxic T lymphocytes (CTL). Therefore, it is conceivable that the CD8⁺ CTL recognizing the peptide from HBsAg in the context of HLA class I molecule might down-regulate the antibody production by killing the B cells and/or antigen-presenting cell.

Thus, it is most likely that HLA class I and class II alleles within the HLA multigene family may control the immune response to HBsAg by activating functionally distinct T cell subsets including T₄1, T₄2 and CD8⁺ T cells like CTL. The Ir gene effect or Is gene effect might be operating in this regulation. However, we have to await further investigation at the cellular and molecular levels based on these observations.

In our previous studies using an arbitrary cut-off point to define non-responders and responders, we reported that non-responsiveness was associated with the HLA-B54-DR4-DQ4 haplotype that might generate suppressor T cells (15-17). In the current study, as listed in Table 2(A), the six lowest responders whose HBs antibody productions were below −3σ (undetectable by the current method) had numerous alleles which negatively contributed to the antibody production. Five of them had the HLA-DRB1*0405-DQA1*0302-DQB1*0401 haplotype and these findings were not inconsistent with the results of the previous study. On the other hand, the HLA types of 14 low responders whose HBs antibody productions were over −3σ and below −1.5σ were composed of the negatively contributing alleles (Table 2B), implying that those lowest responders and low responders were continuous in view of the HLA types. This observation corroborated our analysis of all these data based on the hypothesis that the immune response to HBsAg was a continuous trait.

It should be noted that the combined correlation coefficient of the entire HLA gene family with anti-HBs titer was estimated to be 0.50, suggesting the contribution of other factors to the anti-HBs antibody response. In this study, although a linear regression model was applied, the interaction among HLA alleles might be operating. If the dominant or epistatic interaction is demonstrated by using a larger sample or by analysis at the cellular level, the multiple correlation coefficient should be >0.50 by taking into account the interaction among HLA alleles. Actually dominant or epistatic interactions among alleles at several HLA loci were observed even though statistical significance did not reach the 5% level, most likely due to the small sample size. In addition, polymorphic genes in the immune system, such as genes involved in the antigen processing pathway (TAP, etc.) and other non-HLA-linked genes (Ig allotype gene, etc.), ought to be surveyed.

In conclusion, this is the first report to demonstrate how all HLA class I and class II genes contribute quantitatively to regulating the immune response to HBsAg in humans. The predicting equation for anti-HBs antibody production could make it possible to predict the anti-HBs titers of individuals prior to immunization. This basic information will provide clues for future investigations to uncover mechanisms governing immune regulation.

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Abbreviations

CTL: cytotoxic T lymphocyte
HBsAg: hepatitis B surface antigen
PCR-SSOP: PCR-sequence-specific oligonucleotide probe

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