Relationship of HLA-DQA1 Alleles and Humoral Antibody following Measles Vaccination

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ABSTRACT

Background: The human leukocyte antigen (HLA)-DQA1 locus is only moderately polymorphic compared to other HLA class II loci; however, we hypothesized that these polymorphisms could be important in determining the humoral antibody response to measles vaccine virus.

Methods: The seroprevalence of measles antibody was determined in 881 school children who had been immunized with MMR-II at age approximately 15 months. All subjects resided in a geographic area with no circulating measles virus. The IgG antibody levels were determined by a measles-specific whole virus enzyme immunoassay (EIA) (BioWhittaker, Walkersville, MD). Subjects who were nonresponders (IgG seronegative or equivocal) (n = 46) and hyperresponders (upper 10th percentile of IgG levels of all subjects) (n = 64) were HLA-DQA1 typed using polymerase chain reaction with sequence-specific primers (PCR-SSP). The HLA-DQA1 allele frequencies, as well as homozygosity rates, were compared between the nonresponders and hyperresponders.

Results: The overall allele frequency distribution of alleles between the nonresponders and hyperresponders was significantly different (P = 0.05), with nonresponders having an excess of HLA-DQA1*05 alleles (P = 0.017) and hyperresponders having an excess of HLA-DQA1*01 alleles (P = 0.016). The homozygosity rate among nonresponders was significantly higher than among hyperresponders (23.9% vs. 9.4%, P = 0.037).

Conclusion: HLA-DQA1 alleles have important associations with the antibody response to measles vaccine. Specifically, the carriage of the HLA-DQA1*05 alleles is associated with nonresponse and that of HLA-DQA1*01 alleles with hyperresponse. In addition, HLA-DQA1 homozygosity is significantly associated with poor antibody response to measles vaccine.

Key Words: HLA alleles, humoral antibody response, measles, vaccine


The human leukocyte antigen (HLA) loci exhibit a high degree of polymorphism. Numerous investigations have suggested an association between HLA alleles and susceptibility to both autoimmune disorders and infectious diseases. Although the HLA-DQA1 locus is only moderately polymorphic in comparison to other HLA loci,¹ the presence of specific DQA1 alleles has been associated with several diseases in several different ethnic populations.²⁻⁶ Sollid and colleagues have suggested that specific residues (69 and 75) may play a critical role in antigen binding and, therefore, may have a role in the specificity of antigens presented to T cells.² If indeed certain alleles preferentially present specific antigens, the presence of these alleles may restrict the immunologic response to an infectious agent, such as live measles vaccine virus.

We have demonstrated previously that other HLA class I and II loci have strong associations with the circulating humoral antibody to measles vaccine virus.⁷⁻⁹ Homozygosity at the HLA-DRB1, TAP2 position 665, and HLA-B genes are associated with nonresponse to measles vaccine. In an effort to define the association between measles vaccine antibody levels and other class II HLA genes further, we examined the distribution of HLA-DQA1 alleles and homozygosity rates between nonresponders and hyperresponders to measles vaccine.
METHODS

The seroprevalence of measles antibody was determined in 881 healthy school children, ages 5 to 13 years, who had been immunized with MMR-II at approximately age 15 months. All subjects resided in a geographic area with no circulating measles virus; therefore, the presence of measurable measles antibody could reasonably be attributed to immunization. Levels of IgG antibody were determined in duplicate, and the mean used, by a measles-specific whole virus enzyme immunoassay (EL4) (BioWhittaker, Walkersville, MD). Subjects who were nonresponders (n = 46) (from the group who were IgG seronegative or equivocal) and hyperresponders (n = 64) (from the upper 10th percentile of IgG levels of all subjects) were HLA-DQAI-typed by polymerase chain reaction (PCR) with sequence-specific primers (PCR-SSP) using published primers10,11 and methods adapted for use in our laboratory. Briefly, genomic DNA was extracted from blood clots using a standard proteinase K digestion, followed by phenol/chloroform extraction and ethanol precipitation.12 Genomic DNA was amplified in 10 μL reactions containing 4 μM primers, 800 μM dNTPs, 0.4 units Taq polymerase (Perkin Elmer Cetus, Norwalk, CT), 5% glycerol (v/v), and 0.1 mg/mL cresol red. The PCR amplifications were carried out in a GeneAmp System 9600 (Perkin Elmer Cetus Instruments, Norwalk, CT), and the PCR products were separated on a 1.5% agarose gel stained with ethidium bromide.

When performing HLA-typing by PCR, homozygosity is assigned when only one allele is found. Subjects with only one allele could therefore be homozygous or carry an allele that our typing system is unable to detect, such as a new unreported allele. To overcome this limitation, we employed an internal positive amplification control. The absence of a sequence-specific product in the presence of a positive amplification-control product demonstrates that the PCR did work and the reaction can be considered negative. The absence of the internal positive amplification product avoids mistakenly labeling a reaction negative when it was due to failure of the reaction. Secondly, we enrolled the birth parents of several of these subjects and HLA-DQAI typed them. From the alleles carried by the parents, we are able to infer accurately whether the subjects are true homozygotes.

The HLA-DQAI allele frequencies, as well as homozygosity rates, were compared between the nonresponders and hyperresponders using Pearson's chi-square, and Fisher's exact test when expected counts were small. A paired t-test was used for analysis of antibody levels.

RESULTS

The allele frequency distribution (each subject carries two alleles) between the measles vaccine nonresponders and hyperresponders was significantly different (p = 0.05, chi-square) (Figure 1). We then compared individual allele frequencies between nonresponders and hyperresponders. Nonresponders had an excess of HLA-DQA1*05 alleles (33.7%, n = 31 vs. 19.5%, n = 25, P = 0.017) and hyperresponders had an excess of HLA-DQA1*01 alleles (50.0%, n = 64, vs. 33.7%, n = 31; P = 0.016). We also compared the homozygosity rates between the two groups. The homozygosity rate among nonresponders was significantly higher than among hyperresponders (23.9% [n = 11] vs. 9.4% [n = 6], P = 0.037, chi-square), and among the 17 homozygotes, the frequency of the different homozygote genotypes differed significantly between the nonresponders and hyperresponders (P = 0.01, Figure 2). As the allele frequency data suggest, homozygous nonresponders were more likely to be HLA-DQA1*05 (54.5%, n = 6) than any other homozygous genotype. In fact, no hyperresponders in this cohort were found to be HLA-DQA1*01 homozygous. We were able to confirm homozygosity in all subjects (n = 8) for whom we had family data available.

The demographics of the measles vaccine nonresponders was similar to those of the measles vaccine hyperresponders. There was an equal gender distribution between the groups (nonresponders 48% female and hyperresponders 56% female, P = 0.46). Since all subjects were immunized at approximately 15 months of age, the age of the subject at enrollment can be used as an accurate proxy for time since immunization. To confirm that waning measles antibody levels over time were not responsible for the difference in antibody levels between the nonresponders and hyperresponders, the age range

Figure 1. The distribution of HLA-DQA1 alleles between measles vaccine nonresponders and hyperresponders. The distribution of alleles between the groups differed significantly (P = 0.05). Measles vaccine nonresponders (#) were more likely to carry HLA-DQA1*05 alleles, whereas hyperresponders (*) had more HLA-DQA1*01 alleles.
of the nonresponders was 5 to 13 years (median, 9 years) and the age range of the hyperresponders was 5 to 12 years (median, 8 years). Furthermore, we examined measles antibody levels from six hyperresponders. None of these subjects experienced a statistically or clinically significant decline in measles antibody levels over the approximately 3-year time frame. Similar data are not available for nonresponders, because these subjects have been re-immunized.

DISCUSSION

In this study, HLA-DQA1 alleles were found to be associated with circulating humoral antibody to measles vaccine virus. Furthermore, the presence of specific alleles, HLA-DQA1*01, correlates with a vigorous antibody response to measles vaccine virus, whereas the presence of HLA-DQA1*05 alleles correlates with a poor antibody response. Other work has demonstrated similar associations between specific class II HLA alleles and antibody response to vaccines. We previously reported that HLA-DRB1*13 alleles are associated with strong antibody response to measles vaccine. An extended HLA haplotype has been identified that is associated with failure to develop protective antibody levels after multiple doses of hepatitis B vaccine.

Potentially more important is our finding that HLA-DQA1 homozygosity is significantly associated with nonresponse to measles vaccine and is consistent with our findings for three other HLA loci. We previously reported a significant association between seronegative humoral antibody levels after measles vaccination and HLA-DRB1 homozygosity. Similarly, homozygotes of TAP2 amino acid position 665 and HLA-B homozygotes were more likely to be measles-vaccine nonresponders than were hyperresponders. These findings, together with this report of HLA-DQA1 homozygosity, lend further support to the theory that allelic diversity and heterozygosity within the HLA system is advantageous for an optimal range of immune responses.

Although there is a 1-year age difference between the hyperresponders and nonresponders, we do not believe this difference to be of importance. Evidence for this is that we previously reported that the average decline of measles antibody over time in this cohort was 0.06 ELA units per year. Although this decline is measurable, it is not clinically significant and is not large enough to allow subjects to move from the hyperresponder group to the nonresponder group over the time frame represented by this study.

Strong linkage disequilibrium exists among the genes of the HLA system. We used logistic regression to assess the independent effects of HLA-DRB1, TAP2 position 665 and HLA-DQA1 homozygosity on measles vaccine nonresponse and hyperresponse. The association of antibody levels with HLA-DQA1 homozygosity was not significant (P = 0.16), with an odds ratio of 4.6 (95% confidence interval, CI: 0.55–38.81) adjusted for the effects of TAP2 and DRB1 homozygosity. Not surprisingly, the significance of the contribution of individual genes may diminish, but our power to rule out the effects of individual genes is very small; however, homozygosity at DRB1 remains statistically significant after adjusting for DQA and TAP2 (odds ratio OR = 12.3, CI: 1.89–80.14, P = 0.009). With the data available thus far, we have inadequate power to address the impact of linkage disequilibrium on these findings.

As stated in our hypothesis, we believe that there is likely a multigene influence over antibody response and we plan to examine extended HLA haplotypes. However, we recognize that this study design decreases our power to detect associations in this preliminary communication, particularly for the purposes of addressing linkage disequilibrium.

CONCLUSION

HLA-DQA1 alleles are associated with humoral antibody levels to measles vaccine virus. The HLA-DQA1*01 alleles are associated with hyperresponse, whereas HLA-DQA1*05 alleles are associated with nonresponse to measles vaccine. Overall, and consistent with previous findings, HLA-DQA1 homozygosity is significantly associated with negative or poor antibody levels to measles vaccine virus.
REFERENCES