Associations between Human Leukocyte Antigen Homozygosity and Antibody Levels to Measles Vaccine

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The association between human leukocyte antigen (HLA) homozygosity and measles antibody levels was assessed in a volunteer group of 242 children. Serum samples were tested for measles IgG antibodies, class I and class II HLA alleles were typed, and associations were examined between HLA homozygosity and antibody levels. Children who were homozygous for at least 1 locus were twice as likely to be seronegative (odds ratio, 1.97; 95% confidence interval, 1.08–3.61). Children who were homozygous at ≥1 loci were increasingly likely to be seronegative (χ² test for trend; P = .02). When serum antibody levels were examined as continuous variables, children who were homozygous at certain loci tended to have lower mean antibody levels. These results suggest that lack of HLA diversity may limit the range of peptides that can be presented to antibody-producing cells, potentially resulting in a decreased immune response to viral infections.

HLA genes play an integral role in the immune response to foreign antigen. HLA class I gene products present antigenic peptides to cytotoxic T lymphocytes, and the class II gene products present antigenic peptides to T helper lymphocytes, both of which result in stimulation of an immune response. The HLA genes are highly polymorphic, and the observed diversity at these loci has been suggested to be the result of pathogen-driven natural selection [1–5]. Lack of HLA diversity may limit the range of peptides that can be presented to T helper or T cytotoxic lymphocytes, resulting in a decreased immune response to viral infections. In support of this so-called heterozygote advantage, lack of diversity at specific HLA loci has been associated with failure to mount an effective immune response against a variety of different viral pathogens [6–12].

We previously used the measles vaccine virus as a model for understanding the association between HLA genes and vaccine-induced immune responsiveness [13–15]. In this study, we expanded on our earlier work by testing the hypothesis that both overall and specific homozygosity at the HLA class I and II loci are associated with decreased circulating measles antibody levels.

Methods

Subjects. Many of the details of subject identification and recruitment have been published elsewhere [14–17]. In brief, we collected serum samples from a volunteer group of 876 children aged 5–13 years who attended elementary schools in Olmsted County, Minnesota, and tested their serum for measles antibodies. Olmsted County has a highly vaccinated population that lacks circulating wild measles viruses, as indicated by the lack of resident measles cases in the lifetimes of the study participants (Minnesota Department of Health communication). Therefore, in these children, measured antibody levels reflect receipt of a measles vaccine and not exposure to wild-type virus, assuming that children were not exposed to non-indigenous cases of measles. We attempted to recruit all seronegative and serohyperpositive children for this study. In addition, we attempted to recruit an approximately equal number of seropositive children from the remaining children in our study.

We enrolled 242 children from the initial group, including 72 (87%) of the seronegative children and 77 (86%) of the serohyperpositive children (those whose circulating antibody levels were in the top 10% of the antibody range). We then randomly sampled a subset of the remaining 660 seropositive children (n = 93) to participate in this study. We collected medical history, history of measles exposure or disease, measles vaccination status, and demographic information from a self-administered parental survey and from the children’s community medical records.

HLA typing. Genomic DNA was extracted from fresh or frozen blood clots by using proteinase K digestion followed by phenol/chloroform extraction and ethanol precipitation [18]. DNA was typed for HLA class II alleles DRB1, DQA1, DQB1, and DPA1 by polymerase chain reaction (PCR), using sequence-specific published primers [19–26]. Primer sets and cycle profiles were adapted for use in our laboratory. Amplifications were performed in a GeneAmp PCR system (model 9600; Perkin Elmer–Cetus Instruments). PCR products were separated on 1.5% agarose gels stained with ethidium bromide. DPB1 typing was done with a DNA sequencing kit (Applied
logistic models, odds ratios (ORs) compare homozygosity in the sero-
seronegativity by using Mantel-Haenszel trend tests. In all reported
possible dose-response relationship between homozygosity and
by using a count of 0 as the referent group. Second, we assessed the
count as a set of indicator variables in a logistic regression analysis
was used in 2 separate analyses. First, we represented the
subject. Values of this count could range from 0 to 7, depending on

seronegative, and values

Homozygosity was confirmed either by exon sequencing or by typing
and sequencing the birth parents of our subjects.

Antibody measurement. We tested the subjects’ serum for mea-
les-specific IgG antibody levels by using whole virus ELISA (Mea-
sleELISA; BioWhittaker). Tests were performed in duplicate, and the
mean value of the replicates was used for the analyses. The coeffi-
cient of variation of this assay in our laboratory was 6.6%. Optical
density (OD) values were indexed according to the manufacturer’s
instructions. Index values ≤ 0.80 index units were considered to be
seronegative, and values ≥ 1.00 were considered to be seropositive.
We classified the top 10% of index values as “serohiperpositive.”
Index values were obtained by dividing the measles ELISA OD val-
dues by the minimum value of the low-positive range

Statistical methods. Prior to analysis, by comparing alleles of
probands, parents, and siblings, we examined whether the alleles
at each locus were inherited according to Mendelian transmission.
Subjects who exhibited inconsistent inheritance for a given locus,
given the alleles of parents and siblings, were coded as missing for
that locus. When analyses involved multiple loci, subjects found
have inconsistent allele values for any of the loci involved were
excluded (table 1).

We compared demographic and clinical characteristics across sero-
negativity status by using t tests for continuous variables and \( \chi^2 \) tests
for categorical variables or, if any of the expected cell counts were
< 5, by Fisher exact tests. We compared frequencies of homozy-
gosity in seronegative subjects with those of seropositive and serohy-
perpositive subjects by use of unconditional logistic regression. An
indicator variable of homozygosity for any allele was computed for
each of the 7 loci. Children who were homozygous for
any of the 7 HLA loci. Children who were homozygous for
≥ 1 locus were no more likely to be seronegative than children who were
not homozygous (OR, 1.78; 95% confidence interval [CI], 0.80–
3.98). However, the DPA allele was not very polymorphic in our
population (DPA*0103 accounted for 80% of the DPA alleles), and
163 (68%) of the children in the study were homozygous at
this locus. Therefore, to maximize our ability to detect associ-
ations between homozygosity and low-level circulating anti-
bodies, we excluded DPA and reran the analysis considering any
homozygosity among the 6 remaining loci. After this exclusion,
children who were homozygous for ≥ 1 locus were almost twice
as likely to be seronegative than children who were not homo-
ygous (OR, 1.97; 95% CI, 1.08–3.61).

Children who were homozygous at multiple loci were also at an
increased risk for seronegativity, compared with children who
were not homozygous. As the number of loci at which subjects
were homozygous increased, the odds of seronegativity also in-
creased. Children who were homozygous at ≥ 4 loci were 4–5.5

Results

In total, 242 children participated in our study. Population charac-
teristics did not differ between the children who were sero-
negative and those who were seropositive for measles antibodies
(table 2). Adjusting for age at immunization, sex, and time since
immunization did not significantly affect any of our analyses;
therefore, we report unadjusted results.

Overall, 171 (71%) of the children were homozygous for ≥ 1
of the 7 HLA loci. Children who were homozygous for ≥ 1 locus
were no more likely to be seronegative than children who were
not homozygous (OR, 1.78; 95% confidence interval [CI], 0.80–
3.98). However, the DPA allele was not very polymorphic in our
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were not homozygous. As the number of loci at which subjects
were homozygous increased, the odds of seronegativity also in-
creased. Children who were homozygous at ≥ 4 loci were 4–5.5

Table 1. No. of Olmsted County, Minnesota, children found to
have missing or inconsistent allele values for any of the HLA loci
and no. of children eligible for analysis.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Missing allele</th>
<th>Mendelian inheritance errors</th>
<th>Eligible for analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPB</td>
<td>19</td>
<td>0</td>
<td>223</td>
</tr>
<tr>
<td>DPA</td>
<td>2</td>
<td>0</td>
<td>240</td>
</tr>
<tr>
<td>DQB</td>
<td>1</td>
<td>0</td>
<td>241</td>
</tr>
<tr>
<td>DQA</td>
<td>4</td>
<td>1</td>
<td>237</td>
</tr>
<tr>
<td>DRB</td>
<td>0</td>
<td>1</td>
<td>241</td>
</tr>
<tr>
<td>Class IB</td>
<td>3</td>
<td>1</td>
<td>238</td>
</tr>
<tr>
<td>Class IA</td>
<td>3</td>
<td>2</td>
<td>237</td>
</tr>
</tbody>
</table>

NOTE. Data are no. of children.

 Biosystems). HLA class I A and B alleles were typed by microlym-
phocytotoxicity assays in the Mayo Tissue Typing Laboratory [13].

Table 2. Distribution of demographic characteristics among Olm-
sted County, Minnesota, children who were seronegative or seros-
positive for measles antibody response.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Seronegative</th>
<th>Seropositive</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%) girls</td>
<td>37 (51.4)</td>
<td>82 (48.2)</td>
<td>.65*</td>
</tr>
<tr>
<td>No. (%) white</td>
<td>69 (95.8)</td>
<td>162 (95.3)</td>
<td>.76*</td>
</tr>
<tr>
<td>Mean (SD) age at immunization, months</td>
<td>15.8 (2.0)</td>
<td>16.9 (7.8)</td>
<td>.22*</td>
</tr>
<tr>
<td>Mean (SD) time since immunization, years</td>
<td>8.3 (2.2)</td>
<td>8.5 (2.2)</td>
<td>.47*</td>
</tr>
</tbody>
</table>

* \( \chi^2 \) test.
* Fisher’s exact test.
* Fisher’s exact test.
times more likely to be seronegative than children who were not homozygous (table 3).

When the loci were examined individually, children who were homozygous for classes IB and DQA were more likely to be seronegative than children who were heterozygous at these loci (table 4). When we examined serum antibody levels as continuous variables, we found that, in general, children who were homozygous at each of the loci tended to have mean antibody levels lower than those of children who were heterozygous at the same locus (table 5 and figure 1), although these results were statistically significant only for the class IA and DQA loci. In addition, children who were homozygous for any locus (excluding DPA) had antibody levels significantly lower than those of children who were heterozygous at all loci (mean [SD] antibody level, 1.68 [1.44] for homozygotes vs. 2.05 [1.52] for heterozygotes; \( P = .001 \)).

Discussion

Our results suggest that both overall and specific HLA homozygosity is associated with reduced measles-specific antibody levels after measles vaccination. Taken together, our results suggest a cumulative effect of increasing HLA homozygosity, in which homozygosity at increasing numbers of loci results in progressively lower measles-specific antibody levels, supporting the hypothesis of the heterozygote advantage [4].

In addition, we found that homozygosity at specific loci tended to be associated with an overall decrease in antibody level, even if homozygosity was not associated with the standard measure of seronegativity (ELISA value \( < 0.80 \)). For example, although class IA homozygosity was not significantly associated with seronegativity, class IA homozygosity was associated with an overall decrease in antibody level, compared with class IA heterozygosity. We did not observe a statistically significant difference between continuous antibody levels among homozygotes and heterozygotes at every locus we examined. However, the distribution of the antibody levels among the homozygotes tended to be shifted to the left of the distribution of heterozygous antibody levels at nearly all loci. This suggests that homozygosity at HLA loci generally results in at least slightly lower measles antibody levels.

Our results are in agreement with those of studies of other viruses, which indicated that HLA heterozygotes are better able to mount an immune response to viral pathogens than persons who are homozygous at specific HLA loci. A heterozygote advantage has been observed among subjects infected with hepatitis B virus [7, 9, 11, 27], human immunodeficiency virus [8, 10], and human T cell lymphotropic virus type 1 [12]. Taken together, these studies lend support to the hypothesis that lack of HLA genetic diversity may limit the range of peptides that can be presented to antibody-producing or cytotoxic cells, resulting in a decreased immune response to viral infections.

Our study results were limited by our use of circulating antibody levels several years after vaccination as a proxy for initial vaccine response. However, time since vaccination did not affect any observed relationships between HLA homozygosity and seronegativity. In addition, there were no reported wild measles virus cases in Olmsted County during the lifetime of our study participants. Therefore, even though antibody levels may have waned over time, we believe that our measurement of antibody levels is a reasonable measure of vaccine response.

Our study also focused on antibody levels as a measure of measles immunity. However, a strong cellular immune response is important in clearing a measles infection [28, 29], and it is currently unclear how well humoral and cellular immune measures are correlated. Our current work is focused on determining whether HLA homozygosity is also associated with measures of decreased cellular immunity.

The results of this study did not confirm our preliminary report, which indicated that homozygosity at the DRB and DPB loci was associated with seronegative measles antibody levels [14, 30]. Our study population was larger in this report than in the previous one \(( n = 237 \text{ vs. } n = 149)\), and we confirmed all of our typing by specifically sequencing alleles. In addition, previous studies included only seronegative and serohyperpositive subjects. These differences in methods presumably account for the lack of association between the DRB and DPB loci and seronegativity we observed in this study. However, though not statisti-

### Table 3. Odds ratios and 95% confidence intervals predicting measles antibody seronegativity in Olmsted County, Minnesota, children homozygous at \( \geq 1 \) of 7 HLA loci.

<table>
<thead>
<tr>
<th>Homozygosity locus count</th>
<th>Including DPA(^{a})</th>
<th>Excluding DPA(^{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio</td>
<td>95% Confidence interval</td>
</tr>
<tr>
<td>0 of 7</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>1 of 7</td>
<td>1.89</td>
<td>0.78–4.58</td>
</tr>
<tr>
<td>2 of 7</td>
<td>1.46</td>
<td>0.58–3.65</td>
</tr>
<tr>
<td>3 of 7</td>
<td>1.33</td>
<td>0.40–4.40</td>
</tr>
<tr>
<td>4 of 7</td>
<td>3.56</td>
<td>0.74–17.11</td>
</tr>
<tr>
<td>( \geq 5 ) of 7</td>
<td>4.74</td>
<td>0.89–25.18</td>
</tr>
</tbody>
</table>

\(^{a}\) \( P = .10 \), \( \chi^2 \) test for trend.

\(^{b}\) No children were homozygous at \( \geq 5 \) loci. \( P = .02 \), \( \chi^2 \) test for trend.

### Table 4. Odds of seronegativity in Olmsted County, Minnesota, children homozygous at specific HLA loci.

<table>
<thead>
<tr>
<th>HLA locus with homozygosity</th>
<th>Odds ratio</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class IA</td>
<td>1.33</td>
<td>0.60–2.93</td>
</tr>
<tr>
<td>Class IB</td>
<td>3.39</td>
<td>1.21–9.51</td>
</tr>
<tr>
<td>DPA</td>
<td>0.71</td>
<td>0.40–1.26</td>
</tr>
<tr>
<td>DPB</td>
<td>1.01</td>
<td>0.51–1.98</td>
</tr>
<tr>
<td>DQA</td>
<td>2.11</td>
<td>1.06–4.22</td>
</tr>
<tr>
<td>DQB</td>
<td>1.00</td>
<td>0.50–2.00</td>
</tr>
<tr>
<td>DRB</td>
<td>1.30</td>
<td>0.61–2.78</td>
</tr>
</tbody>
</table>
cally significant, mean antibody titers were lower in both DRB and DPB homozygotes than in heterozygotes.

Because we tested 7 loci and did not adjust for multiple comparisons, it is possible that our results are due to type I error. However, we found that 2 of these loci (classes IA and DQA) were significant at the \( \alpha = .05 \) level. The possibility that this could have happened by chance is < 5\%, which, together with the consistent direction of our other results, suggests that type I error probably does not account for our findings.

Finally, because we did not choose a random sample of the population, but rather a volunteer sample, to participate in our study, it is possible that our study sample is biased and does not accurately reflect the general population. However, since antibody testing and HLA typing are not routinely performed, it is highly unlikely that subjects would have elected to participate or not participate in this study on the basis of either their antibody status or their HLA type. It is possible that persons might have preferentially enrolled in the original sample because parents were unsure of their child’s vaccine status and wanted to find out whether their children were immune. However, we did not rely on parental recall of vaccine receipt; instead, we checked vaccine status by medical and school records. Therefore, we believe that we did not introduce significant selection bias into our study design.

In conclusion, our results suggest that both overall and specific homozygosity at HLA loci are associated with lowered levels of measles antibodies. These results lend support to studies of other

Table 5. Differences in distribution of continuous antibody ELISA values (measured as mean [SD] optical density index units) in Olmsted County, Minnesota, schoolchildren homozygous or heterozygous at specific HLA loci, by Wilcoxon rank-sum test.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Heterozygotes</th>
<th>Homozygotes</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class IA</td>
<td>1.86 (1.50)</td>
<td>1.49 (1.42)</td>
<td>.04</td>
</tr>
<tr>
<td>Class IB</td>
<td>1.82 (1.51)</td>
<td>1.59 (1.23)</td>
<td>.48</td>
</tr>
<tr>
<td>DPA</td>
<td>1.83 (1.53)</td>
<td>1.79 (1.48)</td>
<td>.89</td>
</tr>
<tr>
<td>DPB</td>
<td>1.87 (1.51)</td>
<td>1.76 (1.49)</td>
<td>.44</td>
</tr>
<tr>
<td>DQA</td>
<td>1.85 (1.50)</td>
<td>1.53 (1.41)</td>
<td>.03</td>
</tr>
<tr>
<td>DQB</td>
<td>1.81 (1.52)</td>
<td>1.79 (1.41)</td>
<td>.82</td>
</tr>
<tr>
<td>DRB</td>
<td>1.85 (1.51)</td>
<td>1.63 (1.33)</td>
<td>.12</td>
</tr>
<tr>
<td>Any locus*</td>
<td>2.05 (1.52)</td>
<td>1.68 (1.44)</td>
<td>.001</td>
</tr>
</tbody>
</table>

NOTE. Distribution estimates are weighted to account for the sampling scheme.

*Analysis does not include DPA.
viruses that indicated that HLA restriction results in decreased immune response to viral pathogens. These results also fit well with evolutionary theories suggesting that infectious pathogens contribute to the natural selection of heterozygosity. Our results may offer insight into why some persons respond well to measles and possibly other viral vaccination, whereas others fail to respond with protective antibody titers. These findings may also have implications for peptide-based vaccine development, taking into account the genetic diversity, or lack of diversity, among populations.

Acknowledgments

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