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Human Leukocyte Antigen Associations with Humoral and Cellular Immunity Following a Second Dose of Measles-Containing Vaccine: Persistence, Dampening, and Extinction of Associations Found After a First Dose

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Abstract

Previously we found Human Leukocyte Antigen (HLA) associations with humoral immunity following a single dose of measles-containing vaccine. In this study, we sought to determine if HLA associations exist with humoral and cellular immunity following a second dose of measles-containing vaccine and if the associations we found with humoral immunity after the first dose persist following a second dose.

We recruited a population-based sample of 346 schoolchildren, all who previously received two doses of a measles-containing vaccine. Molecular HLA class I and II typing as well as humoral and cellular immune assays (measles-specific IgG antibody levels and lymphoproliferative response) were performed in these subjects.

We found significant associations with class I HLA-B ($p=0.05$) as well as class II HLA-DPB1 ($p=0.01$) and -DPA1 ($p=0.03$) genes for measles vaccine-induced antibody levels after the second dose. Similarly, we found significant associations with class II HLA-DQB1 ($p=0.05$) and -DRB1 ($p=0.01$) genes for measles-specific lymphoproliferation after the second dose.

While we found HLA associations after the second dose that we previously found after the first dose of measles containing vaccine, fewer alleles had statistically significant associations, suggesting that the second dose had a dampening or extinguishing effect on the HLA associations. It appears that the second dose overcomes HLA restriction through an as yet unknown mechanism.

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Disclosures

Dr. Poland is the chair of a safety evaluation committee for novel non-measles vaccines undergoing clinical studies by Merck & Co, Inc. Dr. Jacobson served on a Safety Review Committee for a post-licensure study of Gardasil® for Kaiser-Permanente.

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Future studies of HLA associations should consider both the effect of dose and the role that subsequent doses might play on genetic associations found with the response to a first dose.

Keywords

Antibody Formation; Histocompatibility Antigens Class I; Histocompatibility Antigens Class II; Immunogenetics; Lymphocyte Activation; Measles Antibody; Measles Vaccine; Measles-Mumps-Rubella Vaccine

Introduction

The literature and biologic plausibility point to the importance of Human Leukocyte Antigen (HLA) genes in host immune response to measles vaccine [1–10]. Our previous studies found associations with both class I and II HLA alleles and antibody levels following a single dose of measles-containing vaccine [2–5]. Specifically, significant associations with low measles vaccine-induced antibody levels were found with HLA-B*8, B*13, B*44, DRB1*03 and DQA1*0201 alleles, while B*7, DQA1*0104 and DPA1*0202 alleles were found to be associated with high antibody levels [2–4]. The currently licensed measles-mumps-rubella-II (MMR-II) vaccine, consisting of three live attenuated viruses, is highly effective and induces strong long-lasting protective immunity. The current approach to measles prevention utilizes two doses of measles-containing vaccine, which stimulates both measles virus-specific humoral and cellular immune responses [11]. Importantly, St. Sauver *et al* demonstrated the extinction of the HLA homozygosity effect following two doses of the measles-containing vaccine [10]. In this regard, validation of HLA genotype and vaccine-induced immune response associations is important for confidence that such associations are real.

We sought to determine HLA associations with both humoral and cellular immunity following a second dose of measles-containing vaccine. We also sought to determine if the HLA associations that we found with humoral immunity following the first dose persisted after a second dose of measles-containing vaccine.

Material and Methods

A. Subjects

Details of the subject's recruitment have been previously described [6–9]. Briefly, between 2001 and 2002, we conducted a large population-based, stratified random sample of healthy children and young adults (12 to 18 years old) in Olmsted County, Minnesota. School rosters in Minnesota Independent School District 535 were used as the sampling frame from which an age-stratified random sample was obtained. Out of 1,212 contacted individuals, 17 individuals did not meet our eligibility criteria. These criteria included receipt of 2 measles vaccinations, the first at or after 12 months, the second at least a month later with no history of measles including exposure, generally good health, correct age, and county residency. Of the 1,195 remaining individuals, 694 did not respond to the recruitment letter, 119 refused to participate in the study, and 36 did not appear for a blood draw. Thus, we recruited a total of 346 children.

The Institutional Review Board of the Mayo Clinic granted approval for the study, and peripheral venous blood samples were obtained after we obtained written, informed consent from those subjects who were 18 years old. From those younger than 18 years old, we obtained informed consent from parents or guardians of all the subjects at the time of enrollment in the study as well as assent from the subjects.

B. Measles IgG Antibody Assay

We determined measles-specific IgG antibody titers by a whole measles virus-specific enzyme immunoassay (Anti-Measles Virus/IgG EIA, Dade Behring, Marburg, Germany) according to the manufacturer's instructions [12;13]. We have previously found the coefficient of variation for this assay in our laboratory to be 3.8%. The manufacturer reports the limit of detection of the test to be 150 IU/mL.

C. Lymphoproliferative Assay

Using an in vitro lymphoproliferation assay based on [³H]-thymidine incorporation, we assessed cellular immune status to measles-containing vaccine [14]. We measured measles-specific cellular responses by proliferation of fresh peripheral blood leukocytes (2×10^5 cells/well) incubated in RPMI-1640 medium, supplemented with 5% autologous sera, with live attenuated measles vaccine virus (75 PFU/well; Attenuvax, Merck, West Point, PA). Cells were pulsed with 1 μ Ci of [³H]-thymidine (Perkin-Elmer, Wellesley, MA) for 18 hours before harvest. We measured lymphoproliferation after 4 days by [³H]-thymidine uptake, subtracting the background counts. We obtained three replicates of cpm values for unstimulated and measles virus-stimulated cells and expressed the results as antigen-specific stimulation indices (SI), defined as the ratio of the median counts per minute (cpm) of measles vaccine-stimulated wells to the median cpm of unstimulated wells.

D. HLA Typing

We extracted genomic DNA from blood samples using the Puregene® extraction kit (Gentra Systems Inc., Minneapolis, MN). HLA class I A locus typing was performed with the Pel-Freez SeCore HLA-A locus sequencing kit (Pel-Freez Clinical Systems, LLC, Brown Deer, WI), followed by Pel-Freez SSP (sequence-specific primer) UniTray typing and AmbiSolv, which consisted of specific primer mixes. Mixes were chosen so as to resolve common ambiguities and used AmbiSolv as needed. We performed HLA class I B locus typing with the Pel-Freez reference strand conformation analysis (RSCA) Multi-Dye B locus kit, [15] followed by the ABI B locus sequencing kit, and the Pel-Freez SSP UniTray and AmbiSolv when needed. HLA class I Cw locus typing used the Pel-Freez Cw high resolution SSP Unitray. Ambiguities were resolved using the Forensic Analytical Cw locus sequencing kit and AmbiSolv.

HLA class II DRB1 locus typing was performed using the Pel-Freez DRB1 high resolution RSCA and SSP Unitray typing kits (Pel-Freez Clinical Systems). The HLA-DPA1 locus typing primarily utilized the Dynal AllSet DPA1 Unitray. HLA-DQA1, HLA-DQB1 and HLA-DPB1 loci typing primarily utilized the Pel-Freez DQA1 SSP, DQB1 SSP and DPB1 SSP high resolution Unitray typing kits with the entire locus on a single tray. Ambiguities were resolved, resulting from molecular techniques, including SSP and RSCA, with Pel-Freez AmbiSolv primer mixes. RSCA was performed on an ABI 377 and analyzed using the RSCA Typer software. PCR amplicates were resolved on an ABI 377 and these were analyzed using Match Tools software. All reactions used negative controls. For quality assurance, every 50th PCR reaction was repeated.

E. Statistical Analysis

The analytical methods described herein are similar to those reported in previous manuscripts [7–9]. Plots of immune response by assay date identified an upward trend of cellular proliferation values over time. We fit polynomial linear regression models to evaluate this association and used the resulting models to recalibrate measures of cellular immune response. We needed no recalibration for the antibody determinations. With regard

to the HLA results, we tested for any deviation from the Hardy-Weinberg equilibrium using an exact test [16].

Using analysis of variance methods, we assessed associations of immune response with demographic and clinical variables. Due to skewness seen with the data, we calculated all p-values based on log-transformed values.

We obtained descriptive associations between immune response and HLA loci on an allelic level. Each study participant contributed two observations to these summaries—one for each allele. We grouped alleles for each locus by subtype and summarized the results using medians and interquartile ranges (IQR).

Following these descriptive evaluations, we examined the associations more formally using linear regression analyses. In contrast to the descriptive comparisons, each study participant contributed one observation to these analyses based on an observed genotype. We created regression variables for each allele and coded as 0, 1, or 2 according to the number of copies of the allele that a subject carried. We pooled rare alleles, defined as those with fewer than five occurrences overall, into a category labeled “other.” Again, we replaced original response values with corresponding logarithmic values.

All tests for association were performed after adjusting the humoral and cellular results for potential confounding variables including age, race, gender, age at first measles vaccination, and age at second measles vaccination.

By simultaneously including all but one of the allele variables in a linear regression model, we examined global differences in immune response among all alleles within a given locus. Following these global tests, we examined individual allele effects. We performed the latter series of tests in the spirit of Fisher’s protected least significant difference test. We only considered individual associations statistically significant if we found global significance. We included each allele variable in a separate linear regression analysis, effectively comparing immune response for the allele of interest against all other alleles combined.

Results

We enrolled 346 children. Of these, 47% were female, and 7% were non-white. Since we sampled in blocks by age, the ages were evenly distributed from 12 to 18 years of age. The median age at the time of the first measles-containing vaccine was 15.6 months (IQR 15.1 to 17.4). The median age at the time of the second dose was 12.1 years (IQR 11.5 to 12.6). The median measles-specific IgG antibody was 1,556 IU/mL and the IQR was 746 IU/mL to 2,677 IU/mL. The median lymphoproliferative assay SI was 3.63 and the IQR was 2.13 to 6.17.

As shown in Table 1, we found no statistically significant differences across the age-of-enrollment for either the measles antibody or lymphoproliferation assay. Similarly, we found no differences in either immune measure with regard to gender or race. We then examined the variables age-at-first-MMR and age-at-second-MMR by dividing the subjects into four quartiles for each of those age-variables. We found statistical significance with both variables for the antibody levels but not for lymphoproliferation. The apparent patterns across the four quartiles were non-linear in both cases; however, in each, the fourth quartile, representing the quartile with the oldest age at vaccination, had the highest mean level of antibody. We examined in the same fashion, by quartiles, the time-from-second-MMR-to-blood-draw and the time-from-first-to-second-MMR. We found no statistically significant associations with either immune measure.

We found no violations of Hardy-Weinberg equilibrium for class I HLA A or B loci as well as class II DPB1, DRB1, DPA, and DQA loci. However, a comparison of allele distributions for the HLA class II DQB1 and Cw loci revealed possible departures from equilibrium ($p < 0.001$ and $p=0.03$, respectively). As a result, statistical comparisons involving these loci should be viewed with a certain level of caution.

The association between class I HLA alleles and antibody level was examined (Table 2). For class I, the global p values were 0.19, 0.05, and 0.28 for HLA-A, -B, and -Cw, respectively. Accepting that the global test for HLA-B was statistically significant, we found that the individual allele B*3503 ($p=0.012$) was associated with a higher than average antibody level.

Of note, other alleles had p values between 0.05 and 0.10 and thus are suggestive of an association. HLA class I alleles B*1302 and B*3501 had median measles antibody values that were higher overall than average. HLA class I alleles B*4403, B*5501, and B*5701 were associated with lower antibody levels. See Table 2 for individual p values.

For the HLA class II genes and measles antibody, the global p values for HLA-DPB1 and HLA-DPA1 loci were significant with p values at 0.01 and 0.03, respectively. Table 3 demonstrates, for HLA-DPB1, three alleles with statistically significant p values. Allele DPB1*0201 was associated with a lower median antibody level ($p=0.030$) as was allele DPB1*1301 ($p=0.008$). HLA Class II allele DPB1*1501 was associated with an increased antibody level ($p=0.005$). In addition, the DPA1*0104 allele was associated with increased antibody response among measles-vaccinated individuals ($p=0.02$). For the other three loci, HLA-DQB1, HLA-DRB1 and HLA-DQA1, the global p values were 0.39, 0.21 and 0.23, respectively.

For class I genes and the measles-specific lymphoproliferation responses, the global p values for the three genes HLA-A, HLA-B, and HLA-Cw were 0.69, 0.48, and 0.21 respectively. As shown in Table 4, no locus demonstrated statistically important associations among study subjects, nor did any association with individual sets of alleles.

For HLA class II genes and lymphoproliferation, the global p values for HLA-DPB1 and HLA-DPA1 loci were not significant ($p=0.94$ and 0.47 , respectively). For two other genes, HLA-DQB1 and HLA-DRB1, the global p values were statistically significant ($p=0.05$ and 0.01 respectively). As shown in Table 5, the class II allele DQB1*0202 has a $p=0.012$ associated with a lower median SI value.

Discussion

In this study we found HLA associations with both humoral and cellular immune status following a second dose of measles-containing vaccine. For humoral immunity, we found a statistically equivocal class IB HLA association with one allele (*3503). We also found a statistically significant class II HLA association with three DPB1 alleles (*0201, *1301 and *1501) and one DPA1 allele (*0104). For cellular immunity, we found no class I HLA associations, but we did find a statistically equivocal class II association with one DQB1 allele (*0202) and statistically significant associations with four DRB1 alleles (*0102, *0701, *1104 and *1201). Of note, our previous work with rubella vaccine using the same cohort of children demonstrated significant associations between two DPB1 (*1301 and *1501) and one DRB1 (*1104) alleles and rubella-specific antibody levels. [15;17]. We also found associations between class IB (*3503), DQB1 (*0202) and DRB1 (*0701 and *1104) alleles and rubella vaccine cellular immunity [15;17]. Moreover, alleles DQB1 (*0201) and DRB1 (*1201) were found to be associated with mumps vaccine-specific cellular immunity in our previous study [18], suggesting that measles, rubella and mumps virus antigens may

be presented in association with certain common HLA alleles. Additional research is required to verify these findings and to study the mechanisms for these associations.

Our results for B*3503 and DPB1*0201 alleles are consistent with those for A*02-Cw*04-B*35 and DRB1*07-DQB1*02-DPB1*02 haplotypes in our previous study demonstrating associations with measles vaccine-induced higher and lower antibody levels, respectively [6]. In addition, the DQB1*0202 allele and a haplotype DRB1*03-DQB1*02-DPB1*04 are found to be associated with higher measles virus-specific lymphoproliferation in our current and a previous studies, respectively. However, due to high polymorphisms of DRB-DQB and DPA-DBP genes and their linkage disequilibrium (LD) it is not feasible to distinguish which allele could have the functional or mechanistic consequences.

We recognize our approach enjoys a number of strengths but also suffers from some limitations. Strengths of our approach include the fact that our subjects resided in an area where for their entire lifetime there had never been circulating measles. Thus, the immune measures reflected exposure to the vaccine rather than wild virus. In addition, we worked from medical records with documentation of two appropriately timed doses of MMR-II vaccine. Limitations include our primarily non-Hispanic, Caucasian population, which limits the inferences we can make with regard to race and ethnicity. In addition, our sample size of 346 subjects provides ample statistical power to detect large biological differences, but may limit our ability to detect very small effect sizes.

This investigation expands upon our previous work with this cohort. We have already reported HLA associations following a single dose for both HLA class I and II alleles [2;3]. We also examined the phenomenon of very high levels of measles antibody following a single dose with respect to HLA class I and class II alleles [4]. In addition to examining HLA associations with the first dose of measles-containing vaccine, we also examined relationships between HLA—both class I and II—and a variety of important cytokines following two doses of measles-containing vaccine [7–9].

In the studies where we identified associations of specific HLA class I and class II alleles with measles-containing vaccine-induced antibody levels following a single dose of the measles-mumps-rubella (MMR-II) vaccine, we did not test for cellular immune status and no longer can with the samples we have remaining [2;3]. See Table 6 for those results. Furthermore, for HLA class I alleles, our original assay gave us only two-digit typing resolution. In examining those results, we note that five of the associations we found after the first dose disappeared after the second dose and two more became equivocal at best.

While we recognize the possibility that our first round of results were false positives, we hypothesize that the second dose overcame the HLA restriction and dampened the genetic effect. The two-vaccination schedule results in higher levels of sero-positivity and antibody levels and has clearly reduced the occurrence of measles in the United States [19–21]. Our hypothesis would also be consistent with our previous observation that two doses of MMR-II vaccine appear to overcome HLA homozygosity associations with low measles antibody levels observed after one dose of vaccine [10]. We have previously identified and replicated a number of HLA genotypes associated with variations in rubella-vaccine immune responses. Other viral vaccines demonstrate a similar extinction of the first-dose genetic restriction. For example, a second series of hepatitis B vaccine can result in seroconversion in HLA-defined “non-responders” [22;23]. McDermott *et al.* have demonstrated that, in non-responders to three doses of hepatitis B vaccine, a single revaccination with hepatitis B vaccine produced a dose-dependent hepatitis B surface antigen antibody (anti-HBs) response [24]. A similar dampening effect has been found with tetanus toxoid vaccine, where the HLA region was implicated in high response after one dose but not after booster

immunization [25]. Unfortunately, similar studies have not been performed with the rubella or mumps component of the MMR vaccine analyzed here. Further investigation into the effect of multiple doses of live, inactivated, and subunit vaccines on genetic control of immunity will not only confirm the generalizability of our findings, but may also provide insights into the mechanisms of genetic control of vaccine-induced immune responses.

These results imply that we may find evidence of the ability to overcome genetic (HLA) restriction by repeat viral vaccine dosing. It is possible that subsequent immunizations boost the weaker immune responses restricted by HLA alleles associated with lower response that were elicited during the primary vaccination, thereby overcoming any HLA restrictions. Alternatively, repeated immunization may provide the necessary antigenic stimulation to elicit T cell responses restricted by alternate HLA alleles, which, in turn, compensate for the initial HLA restriction and promote robust humoral immunity. The mechanisms behind genetic restrictions of vaccine response require further investigation. Future investigations should pursue whether persons carrying the HLA alleles associated with measles-containing vaccine failure who failed to respond to the first dose do respond to a second dose, and pursue mechanistic studies to ascertain the underlying functional defects. This would pave the way toward practical solutions to inter-individual immunogenetic variation in vaccine response beyond measles-containing vaccines [5].

We did, of course, find genetic associations that appeared after the second dose with humoral immunity that we did not detect with the first dose. Of note, however, these appeared with four-digit HLA typing and with a much lower rate of seronegativity. Thus, the genetic associations that remain after two doses still point toward explication of variation in response but do not negate the impact of the second dose. Unfortunately, we lack samples following the first dose that would permit more precise HLA typing and linkage with the sampling after the second dose. Future studies should consider this hypothesis and test it directly.

Conclusions

In summary, our study provides evidence that HLA genes have important immunogenetic associations with circulating antibody levels and measles-specific lymphoproliferative responses in children following the second dose of MMR-II. Our results demonstrate that class I and class II HLA molecules may act as restriction elements for the recognition of measles virus peptides by human T cells [26]. Further, our results suggest an extinction effect after a second dose of vaccine involving the interplay of class I and class II HLA molecules and the humoral response to measles. Our study of HLA allele associations with immune responses to measles should provide the experimental framework for the selection of immunodominant epitopes that trigger immune responses in the human population. Such data may inform the development of next-generation peptide-based vaccines and are consistent with the personalized vaccinology and vaccinomics approach to vaccine-induced immunity that have been described elsewhere [27–32].

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Abbreviations

³H	Tritiated Hydrogen
anti-HBs	Hepatitis B Surface Antigen Antibody
Ci	Curie
CPM	Counts Per Minute
EIA	Enzyme ImmunoAssay
HLA	Human Leukocyte Antigen
IgG	Immunoglobulin G
IQR	InterQuartile Range
IU	International Units
mL	Milliliters
MMR-II	Measles-Mumps-Rubella-II vaccine
PFU	Plaque Forming Unit
RPMI	Roswell Park Memorial Institute medium
RSCA	Reference Strand Conformation Analysis
SI	Stimulation Index
SSP	Sequence-Specific Primer

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Highlights

- Significant associations were found with class I HLA-B ($p=0.05$) as well as class II HLA-DPB1 ($p=0.01$) and -DPA1 ($p=0.03$) genes for measles vaccine-induced antibody levels after the second dose
- Second dose of measles vaccine overcomes HLA restriction through an as yet unknown mechanism
- Class I and class II HLA molecules may act as restriction elements for the recognition of measles virus peptides by human T cells

Table 1
Comparison of selected demographic and clinic variables with lymphocyte proliferation and antibody levels to measles-containing vaccine

Variable	N	Lymphoproliferation (SI)		IgG antibody level (IU/mL)	
		Median (IQR ^a)	P value ^b	Median (IQR ^a)	P value ^b
Age at enrollment			0.25		0.09
12	53	3.01 (2.02–5.31)		1380 (823–2335)	
13	47	3.40 (1.75–5.74)		1143 (432–2127)	
14	53	3.87 (2.49–5.96)		1572 (868–2335)	
15	48	3.79 (1.84–6.77)		1962 (597–3787)	
16	48	3.88 (2.61–7.86)		1881 (859–3805)	
17	49	3.16 (2.03–4.90)		1425 (584–2389)	
18	48	4.73 (2.95–6.49)		1762 (1021–2835)	
Gender			0.24		0.88
Male	185	3.81 (2.34–6.60)		1480 (682–2651)	
Female	161	3.45 (2.04–5.76)		1572 (784–2700)	
Race			0.31		0.85
White, non-Hispanic	323	3.67 (2.13–6.32)		1563 (737–2677)	
Other	23	3.24 (1.53–4.86)		1380 (763–2919)	
Age at first MMR (in quartiles)			0.30		0.05
≤15.12 months	84	4.02 (2.53–6.92)		1452 (593–2326)	
15.13, 15.61 months	90	3.44 (1.92–5.69)		1381 (684–2384)	
15.62, 17.45 months	85	3.35 (1.81–6.60)		1563 (874–2702)	
> 17.45 months	87	3.79 (2.43–5.40)		1848 (1019–3265)	
Age at second MMR (in quartiles)			0.30		0.03
≤ 11.50 years	86	3.37 (1.92–6.76)		1564 (906–2577)	
11.51, 12.07 years	87	3.26 (1.97–5.43)		1623 (700–2962)	
12.08, 12.56 years	86	3.70 (2.03–5.96)		1193 (532–2073)	
> 12.56 years	87	4.06 (2.95–6.60)		1878 (1060–2924)	
Time from second MMR to blood draw (in quartiles)			0.46		0.20
≤ 2.79 years	87	3.12 (1.80–5.25)		1380 (680–2127)	

Variable	N	Lymphoproliferation (SI)		IgG antibody level (IU/mL)	
		Median (IQR) ^a	P value ^b	Median (IQR) ^a	P value ^b
2.80, 4.68 years	86	3.79 (2.35–6.83)		1759 (1024–3109)	
4.69, 6.07 years	86	3.74 (2.40–5.69)		1713 (588–3041)	
> 6.07 years	87	3.87 (2.03–6.53)		1527 (763–2555)	
Time from first to second MMR (in quartiles)			0.30		0.33
≤ 9.9 years	86	3.46 (2.02–6.74)		1596 (907–2651)	
10.0, 10.7 years	87	3.45 (1.97–5.76)		1583 (701–2962)	
10.8, 11.2 years	87	3.09 (2.03–5.40)		1314 (538–2399)	
> 11.2 years	86	4.29 (2.83–6.83)		1762 (819–2700)	

^aInter-quartile range (1st quartile – 3rd quartile)

^bAnalysis of variance. Due to data skewness, p-values were calculated based on log-transformed data. Statistically significant findings ($p \leq 0.05$) are bolded.

TABLE 2

Class I HLA allelic associations with measles antibody levels after two doses

Locus	Allele	Allele counts	Median, IU/mL	Lower Quartile	Upper Quartile	P value ^a
Class I HLA-A	Overall	692	1556	746	2677	0.190
	*0101	111	1647	820	2637	0.529
	*0201	199	1697	906	2482	0.493
	*0301	108	1808	1088	3205	0.019
	*1101	30	1575	657	2677	0.601
	*2301	12	1015	612	4491	0.514
	*2402	60	1322	590	2360	0.182
	*2501	16	920	590	1474	0.381
	*2601	16	1811	1052	3906	0.721
	*2901	5	2421	533	3720	0.645
	*2902	18	695	377	2335	0.111
	*3001	6	1939	1196	2420	0.419
	*3101	19	1683	1019	4352	0.273
	*3201	22	1058	557	1430	0.167
	*6801	43	1127	470	2637	0.151
	Other ^b	27	1023	699	2719	0.312
Class I HLA-B						0.050
	*0702	83	1860	779	3145	0.302
	*0801	79	1590	765	2908	0.901
	*1302	17	2237	1196	3436	0.075
	*1402	7	1395	660	2719	0.675
	*1501	52	1366	706	2613	0.578
	*1801	22	1231	642	4570	0.813
	*2705	28	1847	1161	2989	0.253
	*3501	38	1912	1255	2987	0.055

Locus	Allele	Allele counts	Median, IU/mL	Lower Quartile	Upper Quartile	P value ^d
	*3503	18	2703	1585	3781	0.012
	*3701	5	2633	379	7001	0.445
	*3801	16	1646	496	2691	0.239
	*3901	8	1377	1135	2201	0.829
	*4001	44	1230	819	2421	0.412
	*4002	9	1467	1063	4468	0.448
	*4402	73	1426	737	2076	0.238
	*4403	27	820	404	2335	0.054
	*4501	7	2127	393	4231	0.527
	*4901	15	1641	765	2719	0.976
	*5001	7	929	533	1885	0.495
	*5101	31	1587	1025	2708	0.478
	*5201	6	984	460	5304	0.929
	*5501	15	1528	435	2677	0.069
	*5701	20	1062	509	2128	0.060
	Other ^b	65	1426	657	2309	0.506
Class I HLA-Cw						0.280
	*0102	25	1670	835	2309	0.765
	*0202	38	1634	1023	2652	0.450
	*0303	43	929	454	1689	0.005
	*0304	67	1476	785	2637	0.925
	*0401	79	1899	1196	2925	0.018
	*0501	59	1573	1015	2399	0.874
	*0602	54	1331	646	2370	0.365
	*0701	101	1647	820	2908	0.462
	*0702	96	1575	790	3127	0.254
	*0704	16	1174	662	2166	0.795
	*0802	10	1066	701	1413	0.464
	*1203	34	1312	574	2180	0.149

Locus	Allele	Allele counts	Median, IU/mL	Lower Quartile	Upper Quartile	P value ^a
	*1402	5	1380	1059	1380	0.814
	*1502	16	1717	682	3028	0.574
	*1601	21	875	405	2758	0.278
	Other ^b	28	1422	702	4996	0.793

^aLinear regression analysis. Due to data skewness, p-values were based on log-transformed data. Analyses adjust for age at blood draw, gender, race, age at first MMR, and age at second MMR. Statistically significant findings ($p \leq 0.05$) are bolded.

^b“Other” includes the following HLA-A alleles: *0102, *0202, *0205, *0206, *0207, *0302, *2403, *2608, *3002, *3206, *3301, *6601, *6802, *6803, *6901; HLA-B alleles: *0704, *0705, *0706, *0714, *1301, *1401, *1502, *1503, *1506, *1510, *1516, *1517, *1518, *1522, *1525, *1803, *2702, *3502, *3504, *3508, *3523, *3902, *3906, *4101, *4201, *4404, *4405, *4601, *4701, *4801, *4803, *5301, *5601, *5702, *5801, *5802, *7301; and HLA-Cw alleles: 0302, *0403, *0604, *0706, *0710, *0712, *0801, *1202, *1505, *1602, *1604, *1701, *1703, *1802, respectively.

Table 3

Class II HLA allelic associations with measles antibody levels after two doses

Locus	Allele	Allele counts	Median antibody level (IU/mL)	Lower Quartile	Upper Quartile	P value ^a
HLA-DPB1	Overall	692	1556	746	2677	
	*0101	52	1589	492	2452	0.010
	*0201	82	1129	501	2498	0.030
	*0202	5	642	516	835	0.274
	*0301	70	1709	6657	3436	0.390
	*0401	269	1553	785	2708	0.170
	*0402	84	1556	836	2402	0.715
	*0501	18	1487	824	2309	0.974
	*0601	15	1131	997	2498	0.867
	*0901	6	2441	1584	2946	0.308
	*1001	6	1310	683	3456	0.760
	*1101	13	1395	533	2758	0.416
	*1301	15	967	646	1543	0.008
	*1401	12	1923	1463	4317	0.233
HLA-DQB1	*1501	8	2900	2522	5760	0.005
	*1701	13	2237	1152	3265	0.173
	Other ^a	24	1554	732	1844	0.478
						0.390
	*0201	104	1585	691	2702	0.750
	*0202	57	1152	557	2920	0.417
	*0301	131	1745	765	2652	0.697
	*0302	66	1617	675	3090	0.722
	*0303	24	1129	848	1742	0.100
	*0402	18	1194	779	2074	0.557
	*0501	79	1803	997	2963	0.215

Locus	Allele	Allele counts	Median antibody level (IU/mL)	Lower Quartile	Upper Quartile	P value ^d
	*0502	13	1380	683	1495	0.867
	*0503	17	1480	555	2309	0.409
	*0602	91	1885	765	3634	0.068
	*0603	45	1430	1063	2127	0.955
	Other ^a	47	1380	685	2133	0.101
HLA-DRB1						0.210
	*0101	55	1770	1015	2701	0.210
	*0102	5	2719	1508	2762	0.890
	*0103	7	1255	460	2987	0.465
	*0301	94	1532	642	2555	0.513
	*0401	62	1665	785	2983	0.557
	*0402	6	1488	249	2180	0.471
	*0403	5	1899	1467	3720	0.678
	*0404	28	1666	1117	2731	0.514
	*0405	5	3090	584	3865	0.560
	*0407	6	1156	393	2762	0.382
	*0701	73	1060	533	2335	0.026
	*0801	22	1421	907	3041	0.621
	*0901	11	1619	834	2133	0.641
	*1001	6	4584	642	5030	0.145
	*1101	28	1929	895	3602	0.160
	*1103	5	1131	1019	1131	0.509
	*1104	18	2110	1099	3977	0.075
	*1201	20	1611	616	2737	0.896
	*1301	48	1370	920	2002	0.478
	*1302	36	1397	824	2049	0.365
	*1303	10	1346	368	2237	0.203
	*1401	18	1341	397	1689	0.187
	*1501	88	1977	853	3656	0.013

Locus	Allele	Allele counts	Median antibody level (IU/mL)	Lower Quartile	Upper Quartile	P value ^a
HLA-DPA1	Other ^a	36	1154	710	2190	0.043
						0.030
	*0103	515	1495	699	2578	0.620
	*0104	7	2970	2367	7245	0.020
	*0201	114	1633	701	2918	0.610
	*0202	29	1196	433	2309	0.190
	Other ^a	5	4231	2677	4815	0.010
						0.230
	*0101	65	1670	1015	2719	0.430
	*0102	130	1662	824	3145	0.150
HLA-DQA1	*0103	48	1348	819	2033	0.390
	*0104	20	1341	476	2709	0.290
	*0105	6	4584	642	5031	0.140
	*0201	73	1060	533	2335	0.030
	*0301	66	1575	675	2983	0.800
	*0302	10	1639	834	2133	0.560
	*0303	42	1706	801	2420	0.710
	*0401	24	1379	843	2152	0.950
	*0501	94	1420	516	2494	0.340
	*0505	82	1745	765	2946	0.800
	Other ^b	12	1278	750	2394	0.520

^aLinear regression analysis. Due to data skewness, p-values were based in log-transformed data. Analyses adjust for age at blood draw, gender, race, age at 1st MMR, and age at 2nd MMR. Statistically significant findings ($p \leq 0.05$) are bolded.

^b“Other” includes the following DPB1 alleles: *1601, *1901, *2001, *2101, *2301, *2401, *2601, *3001, *5001, *5101, *6301, *6601, *0304, *0306, *0601, *0604, *0608, *0609, *0614; DRB1 alleles: *0302, *0408, *0802, *0803, *0804, *1102, *1106, *1111, *1119, *1202, *1208, *1304, *1305, *1315, *1405, *1406, *1410, *1424, *1502, *1503, *1601, *1602; DPA1 alleles: *0101, *0105, *0301; DQA1 alleles: *0203, *0402, *0503, *0601.

TABLE 4

Class I HLA allelic associations with measles-specific lymphoproliferative responses after two doses

Locus	Allele	Allele counts	Median, SI value	Lower Quartile	Upper Quartile	P value ^a
	Overall	692	3.63	2.13	6.17	
Class I HLA-A						0.690
	*0101	111	3.09	1.76	5.69	0.109
	*0201	199	3.75	2.27	6.32	0.609
	*0301	108	3.86	2.03	6.03	0.591
	*1101	30	3.13	1.81	5.96	0.721
	*2301	12	4.92	3.46	7.61	0.239
	*2402	60	3.8	2.53	6.2	0.646
	*2501	16	3.74	1.84	6.84	0.319
	*2601	16	5.33	2.79	7.96	0.038
	*2901	5	4.21	0.89	4.97	0.424
	*2902	18	3.8	3.35	6.6	0.464
	*3001	6	3.98	2.35	5.04	0.748
	*3101	19	3.78	1.87	8.12	0.754
	*3201	22	3.01	1.66	5.23	0.386
	*6801	43	3.4	2.52	5.31	0.899
	Other ^b	27	3.59	2.03	4.86	0.560
Class I HLA-B						0.480
	*0702	83	3.73	1.98	6.17	0.737
	*0801	79	3.09	1.76	5.43	0.256
	*1302	17	4.71	2.35	6.74	0.872
	*1402	7	4.37	1.8	6.2	0.983
	*1501	52	3.88	2.08	5.92	0.740
	*1801	22	3.3	2.13	6.83	0.744
	*2705	28	3.16	2.47	7.28	0.965
	*3501	38	3.71	2.44	5.39	0.375

Locus	Allele	Allele counts	Median, SI value	Lower Quartile	Upper Quartile	P value ^d
	*3503	18	4.36	3.22	5.69	0.534
	*3701	5	8.73	4.21	8.77	0.228
	*3801	16	4.96	3	6.88	0.081
	*3901	8	2.26	1.4	2.72	0.021
	*4001	44	3.35	2.43	6.07	0.589
	*4002	9	3.26	3.07	4.37	0.572
	*4402	73	3.26	1.92	4.86	0.070
	*4403	27	3.48	2.45	6.43	0.927
	*4501	7	3.81	3.36	4.89	0.740
	*4901	15	4.86	2.07	6.69	0.305
	*5001	7	1.9	0.89	3.59	0.022
	*5101	31	3.86	2.52	8.49	0.096
	*5201	6	5.78	2.03	9.15	0.649
	*5501	15	4.85	2.55	6.53	0.881
	*5701	20	2.93	2.49	6.17	0.700
	Other ^b	65	3.86	2.4	7.82	0.254
Class I HLA-Cw						0.210
	*0102	25	3.28	1.81	8.55	0.881
	*0202	38	3.29	2.55	6.78	0.500
	*0303	43	3.84	2.17	6.45	0.747
	*0304	67	3.67	2.52	6.17	0.894
	*0401	79	4.38	2.73	6.94	0.064
	*0501	59	3.18	1.87	4.9	0.042
	*0602	54	3.52	2.35	6.03	0.613
	*0701	101	3.24	1.76	5.68	0.337
	*0702	96	3.5	1.94	5.83	0.519
	*0704	16	3.62	1.85	4.85	0.349
	*0802	10	4.12	1.8	6.2	0.846
	*1203	34	4.33	2.53	7	0.289

Locus	Allele	Allele counts	Median, SI value	Lower Quartile	Upper Quartile	P value ^a
	*1402	5	2.52	2.43	4.86	0.749
	*1502	16	5.85	3.35	9.76	0.006
	*1601	21	3.48	1.77	6.11	0.401
	Other ^b	25	3.7	1.93	7.45	0.953

^aLinear regression analysis. Due to data skewness, p-values were based on log-transformed data. Analyses adjust for age at blood draw, gender, race, age at 1st MMR, and age at 2nd MMR.

^b“Other” includes the following HLA-A alleles: *0102, *0202, *0205, *0206, *0207, *0302, *2403, *2608, *3002, *3206, *3301, *6601, *6802, *6803, *6901; HLA-B alleles: *0704, *0705, *0706, *0714, *1301, *1401, *1502, *1503, *1506, *1510, *1516, *1517, *1518, *1522, *1525, *1803, *2702, *3502, *3504, *3508, *3523, *3902, *3906, *4101, *4201, *4404, *4405, *4601, *4701, *4801, *4803, *5301, *5601, *5702, *5801, *5802, *7301; and HLA-Cw alleles: 0302, *0403, *0406, *0604, *0706, *0710, *0712, *0801, *1202, *1505, *1602, *1604, *1701, *1703, *1802, respectively.

Abbreviations: SI = stimulation indices.

Table 5

Class II HLA allelic associations with measles-specific lymphoproliferative responses after two doses

Locus	Allele	Allele counts	Median SI value	Lower Quartile	Upper Quartile	P value ^a
HLA-DPB1	Overall	692	3.63	2.13	6.17	
						0.940
	*0101	52	3.56	2.3	6.04	0.591
	*0201	82	3.59	2.03	6.32	0.824
	*0202	5	3.56	1.59	6.85	0.896
	*0301	70	3.85	2.35	5.9	0.987
	*0401	269	3.68	2.07	6.32	0.937
	*0402	84	3.7	2.42	6.44	0.476
	*0501	18	4.41	2.82	5.96	0.436
	*0601	15	3.57	1.8	8.53	0.912
	*0901	6	5.02	3.07	5.39	0.762
	*1001	6	3.17	2.39	5.58	0.724
	*1101	13	3.36	0.91	6.11	0.134
	*1301	15	3	2.43	4.64	0.507
	*1401	12	3.84	3.08	8.54	0.359
	*1501	8	3.25	1.78	5.31	0.670
HLA-DQB1	*1701	13	2.35	1.59	5.04	0.141
	Other ^a	24	3.8	2.28	5.6	0.863
						0.050
	*0201	104	3.26	1.79	5.24	0.070
	*0202	57	3.25	1.53	4.94	0.012
	*0301	131	3.78	2.52	6.67	0.261
	*0302	66	4.5	2.27	7	0.418
	*0303	24	3.4	2.7	6.24	0.971
	*0402	18	3.26	1.87	10.2	0.857
	*0501	79	4.17	2.54	8.02	0.070

Locus	Allele	Allele counts	Median SI value	Lower Quartile	Upper Quartile	P value ^d
	*0502	13	2.43	1.36	6.78	0.616
	*0503	17	3.87	3	5.37	0.969
	*0602	91	4.38	2.72	7.43	0.157
	*0603	45	3.81	2.53	5.39	0.536
	Other ^a	47	2.82	1.59	5.69	0.064
HLA-DRB1						0.010
	*0101	55	4.38	2.49	6.76	0.144
	*0102	5	13.54	3.87	16.4	0.045
	*0103	7	2.8	1.77	8.55	0.623
	*0301	94	3.2	1.77	5.23	0.070
	*0401	62	3.67	2.47	5.37	0.927
	*0402	6	5.8	1.8	8.38	0.466
	*0403	5	4.89	4.27	4.97	0.673
	*0404	28	4.87	3.2	7.27	0.277
	*0405	5	3.74	2.54	4.78	0.811
	*0407	6	4.75	3.84	5.76	0.217
	*0701	73	3.34	1.77	5.24	0.028
	*0801	22	2.77	1.65	6.43	0.381
	*0901	11	5.2	3.4	7.68	0.273
	*1001	6	4.64	3.84	8.48	0.232
	*1101	28	3.74	1.98	6.05	0.536
	*1103	5	5.87	3.73	6.03	0.526
	*1104	18	6.88	3.66	10.4	0.005
	*1201	20	2.51	1.67	3.43	0.030
	*1301	48	3.8	2.53	5.48	0.535
	*1302	36	2.82	1.62	5.54	0.061
	*1303	10	3.29	1.83	8.73	0.926
	*1401	18	3.8	2.43	5.37	0.689
	*1501	88	3.77	2.43	7.19	0.358

Locus	Allele	Allele counts	Median SI value	Lower Quartile	Upper Quartile	P value ^a
	Other ^a	36	4.1	2.61	8.56	0.168
HLA-DPA1						0.470
	*0103	515	3.67	2.13	6.17	0.730
	*0104	7	3.41	1.49	4.63	0.590
	*0201	114	3.38	1.92	6.11	0.640
	*0202	29	4.44	3.24	8.02	0.060
	Other ^a	5	4.86	3.09	5.69	0.440
HLA-DQA1						0.080
	*0101	65	4.00	2.54	6.76	0.110
	*0102	130	3.35	1.87	6.78	0.790
	*0103	48	4.13	2.59	5.86	0.120
	*0104	20	3.80	2.72	5.91	0.990
	*0105	6	4.64	3.84	8.48	0.230
	*0201	73	3.34	1.77	5.23	0.020
	*0301	66	4.07	2.27	6.15	0.600
	*0302	10	5.83	4.71	7.68	0.190
	*0303	42	3.81	2.55	5.76	0.290
	*0401	24	2.77	1.44	5.97	0.080
	*0501	94	3.20	1.81	5.20	0.060
	*0505	82	3.73	2.40	6.74	0.630
	Other ^b	12	3.90	2.65	4.73	0.680

^aLinear regression analysis. Due to data skewness, p-values were based on log-transformed data. Analyses adjusted for age at blood draw gender, race, age at 1st MMR, and age at 2nd MMR. Statistically significant findings ($p \leq 0.05$) are bolded.

^b“Other” includes the following DPB1 alleles: *1601, *1901, *2001, *2101, *2301, *2401, *2601, *3001, *5001, *5101, *6301, *6601; DQB1 alleles: *0203, *0304, *0306, *0601, *0604, *0608, *0609, *0614; DRB1 alleles: *0302, *0408, *0802, *0803, *0804, *1102, *1106, *1111, *1119, *1202, *1208, *1304, *1305, *1310, *1315, *1405, *1406, *1410, *1424, *1502, *1503, *1601, *1602; DPA1 alleles: *0101, *0105, *0301; DQA1 alleles: *0203, *0402, *0503, *0601.

Abbreviations: SI=stimulation indices.

Table 6

HLA associations found after the first dose and after the second dose (Reporting only those with statistically significant global P values)

Allele	After the First Dose	After the Second Dose
HLA-B7	Seropositivity	None
HLA-B8	Seronegativity	None
HLA-B13	Seronegativity	HLA-B*1302 equivocal seropositivity
HLA-B35	None	HLA-B*3503 seropositivity HLA-B*3501 equivocal seropositivity
HLA-B44	Seronegativity	HLA-B*4403 equivocal seronegativity
HLA-B51	Seropositivity	None
HLA-B55	None	HLA-B*5501 equivocal seronegativity
HLA-B57	None	HLA-B*5701 equivocal seronegativity
HLA-DRB1*03	Seronegativity	None
HLA-DPA1*0104	None	Seropositivity
HLA-DPA1*0201	Equivocal seronegativity	None
HLA-DPB1*0201	None	Seronegativity
HLA-DPB1*1301	None	Seronegativity
HLA-DPB1*1501	None	Seropositivity