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Human Leukocyte Antigen and Interleukin 2, 10 and 12p40 Cytokine Responses to Measles: Is There Evidence of the HLA Effect?

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Abstract

HLA class I and class II associations were examined in relation to measles virus-specific cytokine responses in 339 healthy children who had received two doses of live attenuated measles vaccine. Multivariate linear regression modeling analysis revealed suggestions of associations between the expression of DPA1*0201 ($p=0.03$) and DPA1*0202 ($p=0.09$) alleles and interleukin-2 (IL-2) cytokine production (global p -value 0.06). Importantly, cytokine production and DQB1 allele associations (global p -value 0.04) revealed that the alleles with the strongest association with IL-10 secretion were DQB1*0302 ($p=0.02$), DQB1*0303 ($p=0.07$) and DQB1*0502 ($p=0.06$). Measles-specific IL-10 secretion associations approached significance with DRB1 and DQA1 loci (both global p -values 0.08). Specifically, suggestive associations were found between DRB1*0701 ($p=0.07$), DRB1*1103 ($p=0.06$), DRB1*1302 ($p=0.08$), DRB1*1303 ($p=0.06$), DQA1*0101 ($p=0.08$), and DQA1*0201 ($p=0.04$) alleles and measles-induced IL-10 secretion. Further, suggestive association was observed between specific DQA1*0505 ($p=0.002$) alleles and measles-specific IL-12p40 secretion (global p -value 0.09) indicating that cytokine responses to measles antigens are predominantly influenced by HLA class II genes. We found no associations between any of the alleles of HLA A, B, and Cw loci and cytokine secretion. These novel findings suggest that HLA class II genes may influence the level of cytokine production in the adaptive immune responses to measles vaccine.

Keywords

Measles Vaccine; HLA; Interleukin-2; Interleukin-10; Interleukin-12p40; ELISA

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1. Introduction

Measles is the most transmissible virus known in humans and requires 95–98% vaccine uptake to prevent persisting endemicity [1,2]. A single measles vaccine fails to induce antibody in 2–10% of people [3–6], and only 80.8% of 1,490 children developed seropositivity following a single dose of measles containing vaccine given at 12 months or later in life [7]. Further, only 81.5% of those who were seronegative responded after a second dose [7]. Measles outbreaks still occur in highly-vaccinated populations because of cases introduced from outside the country and primary and possibly secondary vaccine failure [2,8,9]. The importance of measles vaccine failure has become more apparent, and an understanding of this failure may provide a basis for improved vaccines. Thus, examining variability in measles vaccine-induced immunity is critical for its continued use as a tool for measles eradication.

Both CD4 and CD8 T cells are activated by measles virus infection, and cytokine production by these two T cell subsets is essential for the development and regulation of humoral and cellular immunity [10–12]. Two main T helper subsets have been recognized: Th1 cells producing cytokines favoring cell-mediated immunity (IFN- γ , IL-2, TNF- α and IL-12), and Th2 cells producing cytokines necessary for humoral immune responses (IL-4, IL-5, IL-6, IL-10, and IL-13) [13,14]. Measles virus infection induces a Th2 type response and it is likely that cytokine imbalances play a role in immunosuppression [15–18]. In contrast, vaccination with measles virus preferentially induces Th1 type immune responses [19–21]. Therefore, alterations in cytokine levels during vaccination can have dramatic effects on vaccine efficacy.

Our previous work has suggested that humoral and cellular immune responses to measles vaccine are genetically restricted by HLA genes [22–24]. Further, we demonstrated that the HLA genes may also play a role in measles-specific cytokine responses and demonstrated significant associations with measles-induced IFN- γ and IL-4 secretion and HLA alleles [25, 26]. We hypothesized that variations in IL-2, IL-10, and IL-12p40 cytokine responses to measles vaccine are associated in part with genetic polymorphisms of the HLA genes. The aim of this hypothesis-generating study was to investigate IL-2, IL-10, and IL-12p40 cytokine immune responses to measles in healthy children and to determine whether associations exist between these cytokines and HLA class I and class II alleles following two doses of measles vaccine.

2. Materials and methods

2.1 Study subjects

As previously described, between December 2001 and August 2002 we enrolled 346 healthy children (age 12 to 18 years) identified through the Minnesota Independent School District 535 registration rolls [27]. For this sub-study, HLA and cytokine data were available on 339 subjects. Measles vaccination was part of a routine program and all enrolled participants had documentation in their medical records of having received two doses of live measles-mumps-rubella (MMR) vaccine (Merck Research, West Point, PA) containing the attenuated Edmonston B strain of measles virus. Mayo Clinic's Institutional Review Board granted approval for the study, and blood samples were drawn after informed consent, permission, and assent were obtained as appropriate from the subjects and their parents.

2.2 In vitro IL-2, IL-10 and IL-12p40 cytokine responses to measles

Peripheral blood mononuclear cells (PBMC) were immediately separated from heparinized venous blood by Ficoll-Hypaque (Sigma, St. Louis, MO) density gradient centrifugation as described elsewhere [25,26]. Cells were resuspended in RPMI 1640 culture media (Celox Laboratories Inc., St. Paul, MN) containing 10% dimethyl sulfoxide (Sigma) and 20% fetal

calf serum (FCS; Hyclone, Logan, UT), frozen at -80°C , and stored in liquid nitrogen. After cryopreservation, PBMC were thawed, resuspended in RPMI 1640 culture media supplemented with 10% normal human serum (NHS; Irvine Scientific, Santa Ana, CA), 100 U/ml penicillin (Sigma), and 100 g/ml streptomycin (Sigma) as previously described [25,26]. For determination of secreted IL-2 and IL-10, PBMC (4×10^5 cells/well) were cultured in duplicate with or without the Edmonston B vaccine strain of measles virus (multiplicity of infection [MOI] of 0.1) diluted in RPMI 1640 culture media supplemented with 1% NHS. Additionally, 2×10^5 PBMC were incubated in triplicate in the same media at a MOI of 0.5 for determination of IL-12p40. Cell-free supernatants were removed after 6 days and measles virus-specific IL-2, IL-10 and IL-12p40 responses were quantitatively determined by ELISA following the manufacturer's protocol (BD Pharmingen, San Diego, CA). The sensitivity of each ELISA assay was 4 pg/ml. The intra-assay coefficient of variation (CV) for IL-2, IL-10 and IL-12p40 assays in our laboratory were 42%, 48%, and 44%, respectively. Median background levels of IL-2, IL-10 and IL-12p40 cytokine production in cultures not stimulated with measles virus were subtracted from the median measles-induced responses to produce corrected secretion values. Negative corrected values indicate that the unstimulated secretion levels were, on average, higher than the stimulated secretion levels.

2.3 Molecular HLA typing

Genomic DNA was extracted from blood samples using the Puregene extraction kit (Gentra Systems Inc., Minneapolis, MN). HLA class I A locus typing was performed using the SeCore HLA-A locus sequencing kit (DynaL Biotech, Brown Deer, WI), followed by sequence-specific primer (SSP) UniTray typing and AmbiSolv, which consisted of specific primer mixes selected to resolve common ambiguities, when needed. HLA class I B locus typing was performed using the reference strand conformation analysis (RSCA) Multi-Dye B locus kit, followed by the ABI B locus sequencing kit, and the SSP UniTray and AmbiSolv (DynaL Biotech). HLA class I Cw locus typing was performed using the Cw high resolution SSP Unitray (DynaL Biotech). Any ambiguities were resolved using the Forensic Analytical Cw locus sequencing kit and AmbiSolv when needed.

HLA class II DRB1 locus typing was performed using the DRB1 high resolution RSCA and SSP Unitray typing kits (DynaL Biotech). HLA-DPA1 locus typing was performed primarily using the Dynal AllSet DPA1 Unitray. HLA-DQA1, HLA-DQB1 and HLA-DPB1 loci typing were performed primarily using the appropriate SSP high resolution Unitray typing kits with the entire locus on a single tray (DynaL Biotech). Any ambiguities resulting from molecular techniques, including SSP and RSCA, were resolved by AmbiSolv primer mixes. RSCA samples were performed on an ABI 377 and analyzed using RSCA Typer software. PCR amplicates were resolved on an ABI 377 and analyzed using Match Tools software. Every 50th PCR reaction was repeated for quality control.

2.4 Statistical Analysis

Three outcomes were of primary interest: measles virus specific-IL-2, IL-10 and IL-12p40 responses. Data were descriptively summarized using frequencies and percentages for all categorical variables, and medians and interquartile ranges (IQRs) for all continuous variables. Associations of cytokine response with demographic and clinical variables of interest were assessed using analysis of variance methods. Due to data skewness, all p-values were calculated using rank-transformed values.

Descriptive summaries of cytokine response with HLA loci were obtained on an allelic level. Each person contributed two observations to these summaries--one for each allele. Alleles were grouped for each locus by allele subtype, and summarized using medians and interquartile ranges. In addition to obtaining these simple summaries of the outcomes of interest, we also

assessed whether the genotype proportions fit Hardy-Weinberg proportions as a measure of genotyping quality using the software HWE [28].

Following the descriptive comparisons, associations were more formally evaluated using linear regression analyses. In contrast to the descriptive comparisons, each subject contributed one observation to the regression analysis, based on his or her genotype. Regression variables were created for each allele and were coded as 0, 1, or 2, according to the number of copies of the allele that a subject carried. Rare alleles, defined as those with fewer than five occurrences among all individuals, were pooled into a category labeled “other.” Again, due to data skewness, the original response values for each regression model were replaced with corresponding ranked values. Global differences in cytokine response among all alleles within a given HLA locus were carried out by simultaneously including all but one of the allele variables in a multivariate linear regression model.

Following these global tests, we examined individual allele effects on cytokine response. This latter series of tests was performed in the spirit of Fisher’s Protected Least Significant Difference test: individual allele associations were not considered statistically significant in the absence of statistically or marginally significant global tests. Each allele variable was included in a separate linear regression analysis, effectively comparing cytokine response for the allele of interest against all other alleles combined.

All global and allelic analyses described above were adjusted for potential confounding variables associated with cytokine response. The following set of variables was included in all models: age at enrollment, race, gender, age at 1st MMR, and age at 2nd MMR. P-values less than 0.05 were considered significant; however, p-values between 0.05 and 0.10 were considered as being worthy of further investigation. All statistical tests were two-sided, and all analyses were carried out using the SAS software system (SAS Institute Inc., Cary, NC).

3. Results

3.1 Study population and in vitro cytokine responses

We genotyped 339 children enrolled in the study ranging in age from 12 to 18 years. The majority of the children were Caucasian (93%) and the median age of participants at the first and second immunization was 15.6 months and 12.1 years, respectively. The median time since second immunization was 4.7 years. There were a total of 180 (53%) males and 159 (47%) females in the study. IL-2 and IL-10 cytokine responses to measles virus within the total population were similar between genders (median IL-2 secretion of -2.80 (IQR -15.60, 14.10 pg/ml for males versus -1.90 (IQR -14.30, 13.70 pg/ml for females [p=0.51], and median IL-10 secretion of 24.50 (IQR 10.00, 71.50 pg/ml for males versus 32.00 (IQR 10.00, 75.00) pg/ml for females, respectively [p=0.19]). Examination of measles-specific IL-12p40 cytokine responses revealed that male subjects had marginally lower IL-12p40 cytokine responses than females (median 6.50 [IQR 2.00, 15.90 pg/ml for males versus 9.00 [IQR 2.80, 20.00] pg/ml for females, respectively [p=0.07]). We found no violations of Hardy-Weinberg equilibrium for HLA-A (p=0.38), -B (p=0.91), -DRB1 (p=0.11), -DQA1 (p=0.09), -DPA1 (p=0.12) or -DPB1 (p=0.61) loci. However, a comparison of allele distributions for the HLA-Cw and HLA-DQB1 loci revealed possible departures from equilibrium (p=0.03 and p<0.001, respectively). As a result, statistical comparisons involving the Cw and DQB1 loci should be viewed with a certain level of caution.

3.2 HLA associations with measles-specific IL-2 production

Medians and IQRs for each cytokine tested, overall and by HLA polymorphisms, are shown in Tables 1–3. At the 4-digit allele specificity level, we observed 116 unique alleles across the

eight loci with sufficient cell sizes to permit statistical analyses; however, only alleles significantly or suggestively associated with cytokine secretion ($p < 0.10$) are presented. Little IL-2 release was detected after stimulation of PBMC with measles virus and overall median measles-specific IL-2 secretion level was -2.35 (IQR -14.98, 13.85) pg/ml. The associations between HLA class I (A, B and Cw) and class II (DRB1, DQA1, DQB1, DPA1 and DPB1) alleles and measles virus induced IL-2 levels were examined and statistically significant or suggestive associations are summarized in Table 1. The global p-values for HLA-A, HLA-B, HLA-Cw, HLA-DRB1, HLA-DQA1, HLA-DQB1, HLA-DPA1 and HLA-DPB1 loci were 0.69, 0.49, 0.53, 0.73, 0.77, 0.54, 0.06 and 0.89, respectively. For the HLA-DPA1 locus and IL-2 secretion, the global p-value was marginally significant ($p = 0.06$). Allele DPA1*0201 was associated with a higher median IL-2 level (median 2.81 pg/ml, $p = 0.03$). In addition, a marginally significant increase in the frequency of DPA1*0202 (median -5.26 pg/ml, $p = 0.09$) was found among subjects who failed to produce measles-specific IL-2 responses. However, these potential associations should be interpreted with caution, due to the absence of a significant global test as well as cytokine secretion detected below the sensitivity of the immunoassay. The entire associations between HLA class I (A, B and Cw) and class II (DRB1, DQA1, DQB1, DPA1 and DPB1) alleles and measles virus-specific IL-2 cytokine responses in the population cohort analyzed in this study are available as on-line supplementary materials.

3.3 HLA associations with measles-specific IL-10 production

Stimulation of PBMC from previously vaccinated subjects with measles virus induced recall measles-specific IL-10 cytokine responses. The overall median measles-specific IL-10 secretion level was 29.00 (IQR 10.00, 72.50) pg/ml. HLA alleles significantly or suggestively associated with cytokine secretion of IL-10 production ($p < 0.10$) by PBMC stimulated with measles virus antigen are listed in Table 2. The global p-values for HLA-A, HLA-B, HLA-Cw, HLA-DRB1, HLA-DQA1, HLA-DQB1, HLA-DPA1 and HLA-DPB1 loci and measles-specific IL-10 production were 0.58, 0.80, 0.93, 0.08, 0.08, 0.04, 0.10 and 0.85, respectively. The global tests suggested associations between IL-10 secretion and DRB1 and DQA1 loci that approached significance (global p-value 0.08). Alleles DRB1*1103 (median 113.50 pg/ml, $p = 0.06$) and DQA1*0101 (median 39.75 pg/ml, $p = 0.08$) had marginally significant associations with increased IL-10 production. In contrast, alleles DRB1*0701 (median 18.50 pg/ml, $p = 0.07$), DRB1*1302 (median 15.75 pg/ml, $p = 0.08$), DRB1*1303 (median 14.50 pg/ml, $p = 0.06$) and DQA1*0201 (median 18.50 pg/ml, $p = 0.04$) had marginally significant associations with decreased IL-10 production. Importantly, for the HLA-DQB1 locus and IL-10 secretion, the global p-value was significant ($p = 0.04$). Allele DQB1*0302 (median 46.00 pg/ml, $p = 0.02$) was associated with a higher median IL-10 level. In addition, alleles DQB1*0303 (median 17.25 pg/ml, $p = 0.07$) and DQB1*0502 (median 4.00 pg/ml, $p = 0.06$) had marginally significant associations with decreased IL-10 production. The entire associations between HLA class I (A, B and Cw) and class II (DRB1, DQA1, DQB1, DPA1 and DPB1) alleles and measles virus-specific IL-10 cytokine responses in the population cohort analyzed in this study are available as on-line supplementary materials.

3.4 HLA associations with measles-specific IL-12p40 production

Measles virus was also able to induce recall measles-specific IL-12p40 cytokine secretion from PBMC of previously vaccinated subjects (overall median 8.00, [IQR 2.00, 17.00]) pg/ml. HLA alleles significantly or suggestively associated with cytokine secretion levels ($p < 0.10$) of IL-12p40 production by PBMC stimulated with measles virus are listed in Table 3. The global p-values for HLA-A, HLA-B, HLA-Cw, HLA-DRB1, HLA-DQA1, HLA-DQB1, HLA-DPA1 and HLA-DPB1 loci and measles-specific IL-12p40 production were 0.32, 0.57, 0.26, 0.73, 0.09, 0.25, 0.96 and 0.62, respectively. For the HLA-DQA1 locus and IL-12p40 secretion, the global p-value was marginally significant ($p = 0.09$). Allele DQA1*0505 was significantly associated with a higher median IL-12p40 level (median 12.67 pg/ml, $p = 0.002$). Again, this

potential association should be interpreted with caution due to the absence of a significant global test. The entire associations between HLA class I (A, B and Cw) and class II (DRB1, DQA1, DQB1, DPA1 and DPB1) alleles and measles virus-specific IL-12p40 cytokine responses in the population cohort analyzed in this study are available as on-line supplementary materials.

4. Discussion

Widespread use of the live measles virus vaccine, which has been licensed since 1963, has significantly reduced both the morbidity and mortality attributable to the disease; however, outbreaks still occur in highly vaccinated populations. The immune responses generated following measles immunization vary from individual to individual. The outcome of an immune response to measles virus vaccine is regulated by a number of different factors, including the class I and class II genes of the HLA complex. In fact, the HLA complex controls the outcome of the immune response (including vaccine failure) at the level of cytokine production since the HLA-peptide complex generated after measles antigen stimulation determines which type of cytokine production is preferentially induced [29]. A study of twins demonstrated that variations in antibody levels to measles vaccine showed a high degree of heritability (88.5%) [30]. Prior population-based studies found associations with both class I and class II alleles and measles vaccine immune response [22–24]. Subsequent studies suggest the significance of HLA genes in measles virus-specific IFN- γ and IL-4 cytokine immune responses [25,26]. The class I HLA-A (*0101 and *3101) and HLA-Cw (*0303 and *0501) alleles have been significantly associated with measles virus-induced IFN- γ secretion [25]. Likewise, class II HLA-DRB1 (*0301, *0901 and *1501) and DRB1 (*0103, *0701 and *1101) alleles were associated with IFN- γ and IL-4 secretion, respectively. In addition, the DQB1 alleles with an association with IFN- γ secretion were *0201, *0303, *0402 and *0602 [26]. These previous studies could not rule out the role of other cytokines, including IL-2, IL-10 and IL-12, associated with HLA complex since they were not studied.

Public health remains concerned with measles vaccine failure and hence the need to study immunogenetic regulation and mechanism for this failure. Vaccine failure has historically been defined in terms of antibody response. Primary vaccine failure is mainly accounted by the lack of antibody response and appears to occur in 2–10% of vaccinated individuals [31–33]. The secondary vaccine failure rate (development of the disease upon exposure despite vaccination) was estimated to be less than 0.2% [6,34]. In previous studies, we demonstrated associations between HLA genes and low antibody levels after measles immunization [22,23]. However, it is not clear whether HLA gene polymorphisms influence the variability in measles-induced cytokine responses following measles virus vaccine. Cytokine production is an important component in the shaping of the immune response to measles vaccine and natural measles infection [35]. These measles-induced cytokine responses are characterized by early secretion of IFN- γ and IL-2 followed by a change to Th2 type IL-4 and IL-10 spontaneous secretion [18,36]. IL-10, an immunoregulatory and immunosuppressive cytokine, is known to be induced upon both live attenuated measles vaccination and natural measles infection [37,38]. Increased levels of IL-10 suppress T cell proliferation, macrophage activation and down regulates the synthesis of IL-4 and IL-5 [17,39,40]. Earlier studies have shown that IL-12 is important to the development of cell-mediated immunity and is a necessary cytokine for acquisition of a Th1 type immune response [17,35]. It was demonstrated that measles virus binding to the CD46 receptor leads to down-regulation of IL-12 secretion in measles-infected monocytes after *in vitro* measles stimulation [16,21]. The decrease in IL-12 production by monocytes was shown to be greater when the p40 monomer was measured rather than the bioactive p70 heterodimer [16,41]. While cytokines are believed to be important in developing and controlling measles immunity, the underlying immunogenetic regulation responsible for variations in cytokine responses after two doses of measles vaccine have not been studied.

In this study, multivariate linear regression modeling of associations between HLA class I and class II antigen groups and cytokine secretion suggested that HLA class II alleles may significantly influence cytokine responses to the measles virus. We found that the level of IL-2 secretion was associated with the DPA1 (*0201, *0202) alleles. We also found associations between specific DRB1 (*0701, *1103, *1302, *1303), DQA1 (*0101, *0201) and DQB1 (*0302, *0303, *0502) alleles and measles-specific IL-10 secretion. In addition, suggestive associations were observed between DQA1 (*0505) alleles and IL-12p40 secretion. Thus, it may be hypothesized that cytokine responses to measles antigens may be influenced by HLA class II genes. However, results presented here should be viewed as hypothesis-generating and used in prospective, more directed, investigations.

Of note, we controlled our experiment-wise Type I error rate by focusing only on global tests of significance. Nonetheless, we recognize that multiple testing issues exist, and attempts at replicating these results in prospective studies should be carried out. Our data are hypothesis-generating, but since our sample size is only moderate relative to the HLA allelic diversity in the population, it is important that our findings be confirmed in a larger and more ethnically diverse study population. Taken together, these novel observations suggest that HLA class II genes may significantly influence the level of cytokine production in the adaptive immune responses to measles vaccine. These data emphasize the importance of examining T cell cytokine responses to measles virus and the need to define such immune responses in measles vaccinated individuals from a diverse genetic background.

We recognize that secreted cytokine values cannot be truly negative and that negative values are a reflection of measurement variability. These negative cytokine secretion results may also be related to the HLA class I and class II alleles that vary in their capacity to stimulate cytokine production by CD8 and CD4 T cells, or that other non-HLA genes may play a role during measles virus antigen processing and presentation [42]. It is possible that cryopreserved PBMC did indeed secrete cytokines, but at levels that were below the sensitivity range of the immunoassay (minimum detection of 4 pg/ml). Ultimately, the observed IL-2 and IL-12p40 decreased secretion levels may indicate that measles virus infection suppresses IL-2 and IL-12 *in vitro* production by PBMC.

We conclude that HLA class II polymorphisms may influence cytokine immune responses in healthy individuals after two doses of measles vaccine. We believe that this information can be useful for understanding the variability in measles vaccine-induced immunity and variations in cytokine levels during vaccination. Further, this study provides further data regarding the complexity and inter-related roles of immune response genes, such as HLA genes, and effector messengers, such as cytokines, in generating an immune response.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1
HLA allelic associations with measles virus-specific IL-2 cytokine responses

Locus	Allele	Allele counts	IL-2 median secretion value (pg/ml)	Lowest Quartile (pg/ml)	Uppermost Quartile (pg/ml)	p-value ^a
	Overall	672	-2.35	-14.98	13.85	
HLA-A						0.69
HLA-B						0.49
HLA-Cw						0.53
HLA-DRB1						0.73
HLA-DQA1						0.77
HLA-DQB1						0.54
HLA-DPA1						0.06
	*0201	110	2.81	-12.12	19.28	0.03
	*0202	27	-5.26	-31.45	0.63	0.09
HLA-DPB1						0.89

^aLinear regression analysis. Due to data skewness, p-values were based on rank-transformed data. Analyses adjusted for age at blood draw, gender, race, age at first MMR, and age at second MMR. Suggestive findings (p<0.10) are presented.

Table 2
HLA allelic associations with measles virus-specific IL-10 cytokine responses

Locus	Allele	Allele counts	IL-10 median secretion value (pg/ml)	Lowest Quartile (pg/ml)	Uppermost Quartile (pg/ml)	p-value ^a
	Overall	672	29.00	10.00	72.50	
HLA-A						0.58
HLA-B						0.80
HLA-Cw						0.93
HLA-DRB1						0.08
	*0701	68	18.50	7.25	47.50	0.07
	*1103	5	113.50	71.50	171.50	0.06
	*1302	36	15.75	0.00	71.50	0.08
	*1303	9	14.50	6.00	24.00	0.06
HLA-DQA1						0.08
	*0101	64	39.75	18.50	80.00	0.08
	*0201	68	18.50	5.75	43.50	0.04
HLA-DQB1						0.04
	*0302	65	46.00	19.00	89.00	0.02
	*0303	22	17.25	0.50	37.50	0.07
	*0502	13	4.00	-9.00	22.50	0.06
HLA-DPA1						0.10
HLA-DPB1						0.85

^aLinear regression analysis. Due to data skewness, p-values were based on rank-transformed data. Analyses adjusted for age at blood draw, gender, race, age at first MMR, and age at second MMR. Suggestive findings (p<0.10) are presented.

Table 3
HLA allelic associations with measles virus-specific IL-12p40 cytokine responses

Locus	Allele	Allele counts	IL-12p40 median secretion value (pg/ml)	Lowest Quartile (pg/ml)	Uppermost Quartile (pg/ml)	p-value ^a
	Overall	676	8.00	2.00	17.00	
HLA-A						0.32
HLA-B						0.57
HLA-Cw						0.26
HLA-DRB1						0.73
HLA-DQA1						0.09
	*0505	81	12.67	5.00	28.00	0.002
HLA-DQB1						0.25
HLA-DPA1						0.96
HLA-DPB1						0.62

^aLinear regression analysis. Due to data skewness, p-values were based on rank-transformed data. Analyses adjusted for age at blood draw, gender, race, age at first MMR, and age at second MMR. Suggestive findings (p<0.10) are presented.