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HLA Genotypes and Rubella Vaccine Immune Response: Additional Evidence

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Abstract

Recent population-based studies have demonstrated the genetic heritability of rubella vaccine response and assessed that the HLA system may explain about 20% of the inter-individual variance in humoral immune response to this vaccine. Our earlier studies compared HLA allelic associations with rubella vaccine-specific antibodies between two smaller cohorts of healthy Rochester, MN, children (346 and 396 subjects) after two doses of rubella-containing vaccine. This study found that specific HLA alleles were consistently associated with rubella-specific antibody titers (B*27:05, DPA1*02:01, and DPB1*04:01 alleles). The current study examined HLA associations in an independent larger cohort of 1,012 healthy San Diego, CA, subjects (age 19-40 years) after rubella vaccine in order to replicate our previous findings in the Rochester subjects. Two HLA associations of comparable magnitudes were consistently observed between B*27:05 (median NT_{50} Rochester cohort 48.9, p=0.067; San Diego cohort 54.8, p=0.047) and DPB1*04:01 (median NT_{50} Rochester cohort 61.6, p<0.001; San Diego cohort 70.8, p=0.084) alleles and rubella virus-neutralizing antibody titers. Additional HLA alleles resulted in consistent effects on IL-6 production in both cohorts, but did not meet criteria for statistical significance. Our data suggest these HLA alleles play a role in rubella vaccine-induced immunity and provide the

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Conflict of interest

Dr. Poland is the chair of a Safety Evaluation Committee for novel non-rubella investigational vaccine trials being conducted by Merck Research Laboratories. Dr. Poland offers consultative advice on vaccine development to Merck & Co. Inc., CSL Biotherapies, Avianax, Sanofi Pasteur, Dynavax, Novartis Vaccines and Therapeutics, PAXVAX Inc, and Emergent Biosolutions. Drs. Poland and Ovsyannikova hold two patents related to measles and vaccinia peptide research. These activities have been reviewed by the Mayo Clinic Conflict of Interest Review Board and are conducted in compliance with Mayo Clinic Conflict of Interest policies. This research has been reviewed by the Mayo Clinic Conflict of Interest Review Board and was conducted in compliance with Mayo Clinic Conflict of Interest policies.

basis for future studies that may explain the mechanism(s) by which these HLA polymorphisms affect immune responses to rubella vaccine.

MeSH Keywords

HLA antigens; alleles; rubella vaccine; vaccination; rubella; antibodies; neutralizing; cytokines

1. Introduction

Rubella RA27/3 vaccine, developed in 1969, induces a protective response in the majority of healthy recipients, as indicated by production of rubella-specific neutralizing antibodies [1]. Yet, we have no explanation for why the vaccine fails to induce protective titers of antibody in up to 10% of healthy individuals [2, 3], causing failure to protect against disease and outbreaks [2, 3]. New concerns about waning of rubella vaccine-induced immunity have also recently been published [2].

Recent studies have demonstrated that the heritability of rubella vaccine response is approximately 46% [4]. It is important to note that variation in the human leukocyte antigen (HLA) genes account for up to 20% of the overall genetic variation in rubella vaccine-induced antibodies [5]. The immune response to rubella vaccine, which is influenced by HLA-specific genotypes, other genes, immune response pathways, and single-nucleotide polymorphism (SNP)-defined alleles that tag HLA alleles, are being studied and validated [6-8], providing approaches for functional studies and the design of new candidate rubella vaccines [9]. Replication of genetic study findings is essential to diminish the possibility of false associations and to direct efforts in identifying the most promising variants for functional studies.

In our previous work, we compared HLA allelic associations with rubella vaccine-specific antibodies between two cohorts comprised of healthy school children, ages 11-22 years, enrolled in Rochester, MN, (346 and 396 subjects, respectively) after two doses of rubella vaccine [5]. We found that HLA alleles consistently associated with rubella-specific antibody titers in these two cohorts were B*27:05, DPA1*02:01, and DPB1*04:01 alleles. Specifically, the B*27:05 and DPA1*02:01 alleles were significantly associated with differential (lower) antibody responses to rubella vaccine, and the DPB1*04:01 allele was associated with higher antibody titers in both cohorts [5].

The objective of the current study was to assess HLA associations in a larger (San Diego, CA) independent cohort of healthy subjects after rubella vaccine in order to replicate and validate our previous findings. Validated HLA genetic variants are valuable for understanding mechanisms influencing immune response, and for identifying biomarkers of rubella vaccine-induced immunity that might help in optimizing the development of new vaccine candidates and therapeutics.

2. Materials and methods

2.1. Study cohorts

Recruitment of subjects described herein is similar or identical to those published for our previous HLA association studies [7, 10-13]. The study participants whose data were used in this research comprised 1,718 healthy children, older adolescents, and healthy adults (age 11 to 40 years), consisting of study cohorts enrolled from two distinct locations: Rochester, MN, and San Diego, CA (706 and 1,012 subjects, respectively). Clinical and demographic characteristics were previously reported [7, 10, 11]. The cohort from Rochester, MN, comprised a large sample from two independent age-stratified random samples of healthy schoolchildren and young adults from all socio-economic strata. Specifically, between December 2001 and August 2002, we enrolled 346 healthy children, age 12 to 18 years. A detailed description of this study cohort has been previously published [12, 13]. Between December 2006-August 2007, we enrolled 396 healthy children, age 11 to 22 years, as previously published [7, 14]. Of these 742 subjects, 706 parents permitted their children to join the current rubella vaccine study. A blood sample was obtained from each of these children. All 706 participants had records of receiving two doses of measles-mumps-rubella (MMR, Merck) vaccine and had phenotype (IL-6 and IFN-γ) data available. No circulating rubella virus was witnessed since the earliest year of birth for any subject in Rochester, MN. We enrolled an additional 1,076 healthy older adolescents and healthy adults (San Diego cohort) during July 2005-September 2006. Their ages ranged from 18 to 40 years. Of these 1,076 subjects, 1,012 provided a blood sample and met our inclusion criteria. Subject enrollment for this study has been fully described in our previous publications [10, 11]. These subjects represent a cross section of the U.S. population with confirmed vaccine-induced immunity to MMR, and documented receipt of MMR vaccine. The Institutional Review Boards of the Mayo Clinic and the U.S. Naval Health Research Center (NHRC, San Diego, CA) approved the study, and written informed consent was obtained from each subject, or from parents of children who participated in the study, as well as written assent from age-appropriate participants.

2.2. Antibody measurement

Rubella virus-specific neutralizing antibody titers were assessed using a serum-based soluble immunocolorimetric neutralization assay (sICNA) [15]. Rochester and San Diego cohort antibody measurements were performed at the same time. Serial dilutions of subject sera were incubated with the rubella virus vaccine strain HPV77. After incubation, the virus/sera mixture was added to Vero cells cultured in a flat bottom 96-well plate and incubated for 72 hrs at 37°C, 5% CO_2. Cells were fixed in cold methanol for 10 minutes, blocked with PBS supplemented with 5% skim milk (BD Difco, NJ) and 0.1% Tween-20 for 30 minutes. Fixed cells were washed and incubated with anti-E1 glycoprotein (CDC, GA) for 30 minutes, and washed three times with PBS-T. Secondary goat anti-mouse horseradish peroxidase (HRP)-conjugated antibody (Invitrogen, CA) was added for 30 minutes. Plates were washed again and antibody conjugate was visualized by adding NeA-Blue 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate (Clinical Science Products, MA) for 10 minutes. The optical density (OD) values were read at 450 nm/630 nm on an Eon® microplate spectrophotometer (BioTek, VT). The Loess method of statistical interpolation was used to

estimate 50% neutralization titers (NT50) from observed values [16]. Measurements of neutralizing antibody titer at the 95% confidence interval for the intra-class correlation coefficients (ICC) were 0.69-0.82.

2.3. Rubella-specific cytokine secretion

Rubella virus-specific cytokine secretion assay methods described herein are similar or identical to those published for our previous studies [17, 18]. Concentrations of secreted cytokines following stimulation of PBMCs with live rubella virus were assessed, as previously described [17, 18]. Cytokine secretion assays for the Rochester cohort were carried out a few years prior to those of the San Diego cohort, but cytokine measurements used an identical protocol and assay conditions. Briefly, 2×10^5/ml PBMCs were stimulated with the W-Therien strain of rubella virus (a gift from Dr. Teryl Frey, Georgia State University, Atlanta, GA) with optimized multiplicity of infection (MOI) and incubation times depending on the specific cytokine measured. For the measurement of IL-6 (Rochester cohort, n=685; San Diego cohort, n=969) and IFN-γ (Rochester cohort, n=685; San Diego cohort, n=948), PBMCs were stimulated with an MOI of 5. The supernatants were removed post-stimulation at 24 hrs for IL-6 and 48 hrs for IFN-γ. Cytokine levels were quantified using BD OptEIA™ Human ELISA kits. Absorbance units were measured using a Molecular Devices SpectraMax 340PC (Sunnyvale, CA). Negative IFN-γ secretion values suggest that the unstimulated secretion concentrations were, on average, higher than the rubella virus-stimulated secretion concentrations.

2.4. Genotyping

Our HLA genotyping procedures are similar or identical to what we have previously published [5, 11]. HLA class I A, B, and C typing was implemented using high-resolution SSP (sequence-specific primer) A, B, and C Unitray typing assays (Invitrogen), respectively. HLA class II typing was done with high-resolution DRB1-SSP, DQA1-SSP, DQB1-SSP, DPA1-SSP, and DPB1-SSP Unitray typing assays (Invitrogen). All PCR amplifications were completed on an ABI-377 (Applied Biosystems) and examined using MatchTools (Applied Biosystems) software. The genome-wide SNP typing for the Rochester cohort (n=706) was performed using the Infinium Omni 1M-Quad SNP array (Illumina, San Diego, CA). The genome-wide SNP typing for the San Diego cohort (n=1,012) was performed using the Infinium HumanHap550 BeadChip array (Illumina, San Diego, CA) for the self-declared Caucasian subjects, and the 650K Infinium HumanHap650Y SNP BeadChip array for the subjects indicating their race as either African-American or unknown [19].

2.5. Statistical methods

Statistical methods described herein are similar to those published for our previous genetic association studies [5, 11]. Demographic and immune outcome variables were summarized within cohorts. Counts and percentages were used for categorical variables, and medians and 25^{th} and 75^{th} percentiles were used for quantitative variables. For immune outcomes measured from rubella virus-stimulated and unstimulated cells (IL-6 and IFN-γ cytokine secretion), the difference between the median values from the stimulated and unstimulated replicate values was calculated prior to summarizing the immune response outcome. Chi-square tests for categorical variables, and Kruskal-Wallis tests for quantitative variables,

were conducted to investigate differences between cohorts. Immune outcome variables were transformed prior to statistical analyses to satisfy modeling assumptions. Specifically, the neutralizing antibody NT_{50} was log transformed while IL-6 and IFN-γ underwent Van der Waerden inverse-normal transformations. The log of the NT_{50} was used as the response in linear regression models, which tested for HLA associations while adjusting for assay batch, gender, age at enrollment, age at vaccination, time from vaccination to enrollment. We also took advantage of genome-wide genotyping data to quantify population genetic differences among individuals. As reported in our prior publications [19, 20], we identified SNPs spanning the genome that were not in linkage disequilibrium from the available genotyping panels. From these SNPs, we extracted the first three axes of genetic variation using the approach implemented in the EIGENSTRAT software package [21], and used them as covariates in our regression analyses in order to adjust for population genetic differences among individuals. The age and time variables were categorized into quartiles prior to analysis. Cytokine IL-6 and IFN-γ Van der Waerden scores were used as responses in linear mixed models that tested for HLA associations while adjusting for the same features as the NT_{50} analyses. In these linear mixed effects models, all of the repeated stimulated and unstimulated results were included in a single model and the repeated nature of the data was accounted for by estimating the covariances among measurements within an individual using an unstructured covariance matrix. Global models were first performed to assess the significance of the association between each HLA locus and each of the three immune outcomes in the Rochester cohort. These global models were followed by additional models that individually tested the association between the dosage of each HLA four-digit allele and the immune outcomes. A parallel series of analyses were conducted in the San Diego cohort to test for associations within HLA loci following global locus-level tests of association. Our primary assessment of statistical significance focused on HLA alleles that were individually associated with immune outcomes for each HLA locus whose global test was significant in the Rochester cohort. Because of the high heterozygosity in the HLA loci, and the relatively small size of this cohort, we opted to set our level of significance at $p<0.10$. Those alleles that met our threshold for significance in our discovery work in the Rochester cohort were subsequently assessed for association with the same immune outcome in the San Diego cohort on a per-allele basis using the analysis strategies employed for the per-allele tests in the Rochester cohort. As there were significant differences between the two cohorts, we also report results from alleles that did not reach our criteria for statistical significance in the Rochester cohort, but which showed consistent results between cohorts.

3. Results

3.1. Demographic characteristics

Table 1 shows the demographic characteristics and rubella vaccine-specific immune outcomes for subjects in the Rochester (n=706) and San Diego (n=1,012) study cohorts. These study cohorts tend to be heterogeneous with respect to age, race, ethnicity, and phenotype assessments. The median age in the Rochester cohort was significantly lower than in the San Diego cohort (15 vs. 24 years, $p< 0.0001$), and there was a significantly lower proportion of males in the Rochester (54.0%) than in the San Diego (73.2%) cohort ($p<0.0001$). The San Diego cohort was also more racially diverse than the Rochester cohort

(p<0.0001), which comprised 53.2% and 91.1% Caucasian participants, respectively. In addition to the demographic differences, there were also significant differences between the Rochester and San Diego cohorts with regard to median NT_{50} neutralizing antibody titers (57.4 and 66.9, respectively, p<0.0001) and median IL-6 secretion levels (3,680 vs. 4,117 pg/ml, respectively, p<0.0001). Median IFN-γ secretion levels in the Rochester cohort were low (8.5 pg/ml) and when measured in the San Diego cohort, they were hardly detectable (p<0.0001). No deviations from Hardy-Weinberg Equilibrium (HWE) were detected for any of the HLA loci in either Rochester or San Diego cohorts.

3.2. Associations between HLA alleles and rubella vaccine-specific immune responses

Neutralizing antibody titers against rubella virus were measured in 699 Rochester subjects and 1,008 San Diego study subjects (Table 1). The findings for the associations identified in the Rochester cohort (p<0.10) between HLA alleles and three measures of rubella virus-specific immune response are shown in Table 2. Also shown are the results from the tests for association for these alleles in the San Diego cohort. Of the 11 alleles from the loci whose global tests of significance were significant at the p<0.10 level, we identified three alleles that met the same level of significance in the San Diego cohort. Two HLA associations of comparable magnitudes were consistently observed between HLA-B*27:05 (median NT_{50} Rochester cohort 48.9, p=0.067; San Diego cohort 54.8, p=0.047) and HLA-DPB1*04:01 (median NT_{50} Rochester cohort 61.6, p<0.001; San Diego cohort 70.8, p=0.084) alleles and rubella neutralizing antibody titers. Specifically, the B*27:05 allele was associated with lower titers and the DPB1*04:01 allele was associated with higher antibody titers in both study cohorts. Alternatively, the association observed between the HLA-B*50:01 allele (median NT_{50} Rochester cohort 111.3, p<0.001; San Diego cohort 49.7, p=0.099) and neutralizing antibody titers, while found in both Rochester and San Diego cohorts, was in the opposite direction.

Fewer associations between HLA loci and the other two immune measures were observed in the Rochester cohort. Rubella virus-specific IL-6 and IFN-γ secretion levels were measured in 685 Rochester subjects and 969 San Diego study subjects, respectively (Table 1). No loci were significantly associated with IL-6 secretion, even at the liberal p<0.10 threshold, and only the HLA-DQB1 locus had a significant global test (p=0.045) when examined for an association with IFN-γ secretion. However, the direction of association for the DQB1*51:01 allele was in the opposite direction between the two cohorts (Table 2). A parallel series of sensitivity analyses were conducted in Caucasian subjects only. The findings for the associations identified in the Caucasian Rochester and Caucasian San Diego cohorts (p<0.10) between HLA alleles and measures of rubella virus-specific immunity are shown in Supplemental Table 1.

3.3. Exploratory comparisons of HLA associations between two cohorts

Because of the significant differences between the two cohorts, not only in demographic characteristics but in immune phenotypes, we were interested to further examine in an exploratory fashion the degree to which individual allelic associations agreed between the two cohorts. Likewise, because of the high heterozygosity in the HLA loci, and the relatively small size of the cohorts, we decided to set our level of significance at p<0.10.

Tables 3 through 5 show the findings for all of the alleles where a potential association ($p<0.10$) was observed in either of the two cohorts for neutralizing antibody titer, and for IL-6 and IFN-γ secretion.

The data in Table 3 illustrate suggestive associations ($p<0.10$) between HLA alleles and rubella virus-specific neutralizing antibody titers across the Rochester and San Diego cohorts. Several (n=18) potential associations between HLA type and neutralizing antibody titers were observed in each study cohort. Among these are the two alleles that met our criteria for replication. Of note, several loci are suggestive of associations that are consistent between cohorts, but the allele counts are low in one or both of them, which makes it difficult to conclude that the data indeed reflect a consistent association between cohorts. Table 4 shows the results for associations between HLA alleles and rubella-specific IL-6 secretion in the Rochester (n=13 associations) and San Diego (n=17 associations) cohorts. The HLA-A*31:01 allele was consistently associated with lower rubella vaccine-specific IL-6 secretion in both Rochester (median 3,483 pg/ml, p=0.084) and San Diego (median 3,811 pg/ml, p=0.023) cohorts, although global tests were not significant, and this may represent an issue of statistical power. An additional finding of interest was observed, with the HLA-DQA1*04:01 allele (median Rochester cohort 3,725, p=0.061; San Diego cohort 3,995, p=0.180) being potentially associated with variations in IL-6 production, although statistical significance thresholds were not met. The HLA-B*51:01 allele was associated with very low rubella vaccine-specific IFN-γ secretion in both Rochester (median 5.47 pg/ml, p=0.039) and San Diego (median -0,48 pg/ml, p=0.037) cohorts; however, global tests were not significant. The results for the association between HLA alleles and IFN-γ secretion for both cohorts are presented in Table 5.

4. Discussion

Identification of the host genetic factors related to the generation of protective immune responses to rubella vaccine is fundamental for understanding mechanisms underlying rubella-induced immunity and rational vaccine development using the vaccinomics paradigm [22-24]. HLA genotypes play a critical part in adaptive immunity to rubella vaccine, accounting for up to 20% of the overall genetic variance in rubella antibodies [5]. Several studies have linked HLA gene polymorphisms to differences in the immune response to rubella vaccine [6, 25-28]. However, discordant associations between HLA genotypes and other genes and vaccine-induced immune response outcomes were frequently reported for many population-based vaccine studies, including ours. Replication studies in different populations are needed to identify an association that is not a chance finding.

In our study, we successfully replicated two HLA allelic associations found in the Rochester and San Diego cohorts. In fact, associations of comparable magnitudes between HLA class IB (B*27:05) and class II DPB1 (DPB1*04:01) alleles were similar between the two study cohorts. The associations between B*27:05 and DPB1*04:01 alleles with rubella virus-specific enzyme immunoassay (EIA) IgG antibody levels were also observed in our previous study that utilized two smaller cohorts of schoolchildren following two doses of rubella vaccine [5]. Of note, the direction of the relationship between these two alleles and IgG antibody outcome was in the same direction that was found in this replication study. The

main role of the class I B27 and class II DPB1*04 molecules is to present self and viral epitopes to activated CD8+ and CD4+ T cells, respectively, which stimulates an adaptive immune response. In this regard, HLA-B27 is one of the most “fascinating protective” molecules that efficiently bind processed peptides of diverse length and affinity [29, 30]. Infected individuals who carry B27 (B*27:05) alleles exhibit a slower disease progression to hepatitis C virus (HCV), influenza, Epstein-Barr virus (EBV), and human immunodeficiency virus (HIV) through the recognition of immunodominant HLA-B27-restricted CD8+T cell epitopes [31-34]. The exact immunological mechanisms behind this phenomenon are still not well understood, but the greater capacities of HLA-B27-restricted CD8+ T cells in protective anti-viral immunity are likely associated with polyfunctional CD8+ T cells that produce IL-2 and proliferate in an antigen-specific manner [29].

Both candidate gene and genome-wide association studies (GWAS) have offered a novel view on the mechanistic role of the HLA-DPB1 genes in host immune response to vaccines and susceptibility to infection [13, 35-37]. A recent GWAS confirmed the role of the HLA-DPB1 protein and gene expression in protection against hepatitis B virus (HBV) infection [38, 39]. In our study, the DPB1*0401 allele was significantly associated with higher rubella vaccine antibody titers in both cohorts. The HLA-DPB1*04:01 allele has also been linked with variations in antibody titers to other viral vaccines, including measles and hepatitis B [35, 40]. It is possible that the DPB1*0401 molecule may be particularly effective at processing and presenting self- and viral-derived epitopes to CD4+ T cells [41], providing the mechanism by which class II DPB1 molecules participate in the induction and regulation of viral-specific adaptive immune responses. To clarify the molecular mechanisms underlying the effects of DPB1*04:01 on higher antibody response, further functional studies of the DPB1 molecule are necessary.

Contrary to HLA molecules with well described function, the causal relationship between HLA-A*31:01 and HLA-DQA1*04:01 alleles and rubella vaccine-specific IL-6 secretion is not well understood [28]. In our secondary analyses, we found that the HLA-A*31:01 and DQA1*04:01 alleles were associated with variations in rubella-specific IL-6 production in both cohorts, although global tests were not significant. Of note, in a study of schoolchildren vaccinated with two doses of MMR vaccine, the A*31:01 allele was found to be associated with higher measles-induced IFN-γ response [42]. Another study reported a role for HLA-A*31:01-restricted CTL response in chronic HIV-1 infections [43]. The DQA1 locus in our study subjects was represented by four genotypes (DQA1*01:01, *01:05, *03:01, and *04:01), and only the DQA1*04:01 allele demonstrated a suggestive association with IL-6 responses in both cohorts.

The strengths of our study include a large sample size (total 1,718 subjects), a candidate gene approach, and a comprehensive examination of rubella vaccine-specific genotype-phenotype associations. Because rubella virus-neutralizing antibody titers are considered to be the best predictable marker of vaccine-induced protective immunity, rubella virus-specific neutralizing antibody titers in serum in our study were tested using a sensitive immunocolorimetric neutralization assay. Our study was also designed to follow the National Cancer Institute and the National Human Genome Research Institute (NCI-NHGRI) Working Group principles for validating genotype-phenotype associations [44].

Among these principles is the use of a sufficient sample size and independent data sets, analysis of phenotypes comparable to those reported in the discovery study, the use of comparable populations, and conducting several rounds of replication studies [44]. To our knowledge, this is the largest study that replicates candidate HLA genes and their potential effect on rubella vaccine-induced immunity in independent cohorts.

In our studies of HLA associations with rubella vaccine-specific immune measures, we enrolled participants from two distinct study cohorts. These cohorts differed significantly in regard to demographic and vaccination history, and represent a study limitation. These cohorts were also studied several years after their second dose of rubella vaccine. They also differed significantly in their measured immune responses. In fact, IFN-γ secretion levels in the Rochester cohort were very low; when assessed in the San Diego cohort, IFN-γ secretion was hardly detectable. Further, HLA allele frequencies may be different among these two cohorts. This issue is further compounded by the extreme heterozygosity present in the various HLA loci. The resulting numbers of alleles observed in small numbers of participants limits the statistical power to detect significant associations. The fact that we observed a number of consistent HLA associations between the cohorts confirms that HLA loci are involved in response to rubella vaccination, and suggests several specific alleles that play a role. However, further work in different cohorts is needed in order to more completely elucidate the full complexity of the role that HLA loci/alleles and other genes play in response to immunization against rubella.

In summary, our previous rubella vaccine replication [5] and current validation studies have demonstrated repeated associations of HLA genes on antibody (and cytokine) responses to rubella vaccine in healthy individuals. Our data suggest that rubella vaccine-induced adaptive immune responses are significantly influenced by polymorphisms of HLA class I and class II alleles, particularly A*31:01, B*27:05, and DPB1*04:01. The lack of confirmatory data from other rubella vaccine immunogenetic studies warrants further evaluation of these HLA associations in additional populations. This work has also provided the foundation for future studies that may explain the mechanism(s) through which such HLA polymorphisms affect immune responses to rubella.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

Certain HLA alleles were associated with rubella-specific antibody titers.

Two HLA associations were observed between B*27:05 and DPB1*04:01 alleles.

HLA polymorphisms affect immune responses to rubella vaccine.

Table 1

Demographic characteristics and immune outcomes in the Rochester and San Diego study cohorts.

	Rochester cohort (N=706)	San Diego cohort (N=1,012)	p-value
Gender			< 0.0001
Male	381 (54.0%)	741 (73.2%)	
Female	325 (46.0%)	271 (26.8%)	
Race (self-declared)			< 0.0001
American Indian, Alaska Native	4 (0.6%)	19 (1.9%)	
Asian, Hawaiian, Pacific Islander	22 (3.1%)	49 (4.8%)	
Black or African American	8 (1.1%)	172 (17.0%)	
White	643 (91.1%)	538 (53.2%)	
Multiple	20 (2.8%)	86 (8.5%)	
Other	3 (0.4%)	127 (12.5%)	
Unknown	6 (0.8%)	21 (2.1%)	
Ethnicity			< 0.0001
Not Hispanic or Latino	688 (97.5%)	755 (74.6%)	
Hispanic or Latino	11 (1.6%)	215 (21.2%)	
Don't Know	7 (1.0%)	42 (4.2%)	
Age (years)			< 0.0001
N	706	1012	
Median (25^{th} – 75^{th} Percentile)	15 (13 – 17)	24 (22 – 27)	
Age at most recent vaccination (years)			< 0.0001
N	706	720	
Median (25^{th} – 75^{th} Percentile)	11 (5 – 12)	19 (18 – 22)	
Years from second vaccination to enrollment			< 0.0001
N	706	720	
Median (25^{th} – 75^{th} Percentile)	5.8 (3.9 – 7.4)	3.0 (2.2 – 4.0)	
Rubella-specific antibody titer (NT50/ml)			< 0.0001
N	699	1,008	
Median (25^{th} – 75^{th} Percentile)	57.4 (35.4 – 95.7)	66.9 (43.9 – 113.9)	
IL-6 concentration (pg/ml)			< 0.0001
N	685	969	
Median (25^{th} – 75^{th} Percentile)	3680.5 (3134.6 – 4051.2)	4117.3 (3524.7 – 4787.3)	
IFN-concentration (pg/ml)			< 0.0001
N	685	948	
Median (25^{th} – 75^{th} Percentile)	8.5 (3.0 – 23.4)	-1.4 (-6.4 – 3.1)	

N: number of subjects/assays.

Table 2

HLA allelic associations with measures of rubella vaccine-specific immune response responses in the Rochester and San Diego study cohorts.

HLA locus	Allele	Neutralizing antibody titer (NT_{50}/ml)										
		Rochester cohort						San Diego cohort				
		Allele counts	**Median**	**25th Percentile**	**75th Percentile**	**Allelic p-value**	**Global p-value**	**Allele counts**	**Median**	**25th Percentile**	**75th Percentile**	**Allelic p-value**
	Overall	1398	57.4	35.4	95.7			2016	66.9	43.9	113.9	
HLA-B	*27:05	58	48.9	34.2	88.4	0.067	0.056	55	54.8	39.4	93.1	0.047
	*50:01	12	111.3	31.4	277.3	<0.001		12	49.7	45.0	64.5	0.099
	*57:01	35	48.7	34.2	69.9	0.022		48	59.1	43.4	93.8	0.697
	*58:01	7	109.8	95.1	136.8	0.025		34	100.4	64.4	133.7	0.289
HLA-DPA1	*01:04	7	93.4	66.1	178.7	0.053	0.010	-	-	-	-	-
	*02:01	213	54.0	34.6	87.3	0.015		367	66.8	43.8	113.3	0.561
	*01:09	13	4.6	1.7	18.8	0.092		171	65.4	44.8	111.0	0.960
HLA-DPB1	*03:01	140	45.5	32.1	77.4	0.004	<0.001	136	54.5	36.6	92.0	0.267
	*04:01	567	61.6	38.2	104.6	<0.001		608	70.8	44.6	113.3	0.084
	*06:01	30	34.1	27.0	64.2	<0.001		26	57.2	39.9	112.9	0.396
	*15:01	9	121.4	67.5	178.7	0.005		8	78.0	43.0	163.5	0.677
		IFN-γ secretion (pg/ml)										
		Rochester cohort						San Diego cohort				
		Allele counts	Median	25th Percentile	75th Percentile	Allelic p-value	Global p- value	Allele counts	Median	25th Percentile	75th Percentile	Allelic p-value
	Overall	1370	8.5	3.0	23.4			1896	-1.4	-6.4	3.1	
HLA-DQB1	*06:03	93	5.0	1.9	11.5	0.0003	0.045	90	-0.7	-5.9	4.9	0.576
	*51:01	64	5.5	1.4	17.0	0.039		94	-0.5	-6.6	4.5	0.037

Associations tested using linear models (linear regression for neutralizing antibody and linear mixed models for IFN-γ) using log-transformed antibody levels and inverse-normal transformations for IFN-γ. Analyses adjusted for age at blood draw quartile, quartiles for age at vaccination, time from most recent vaccination to enrollment, gender, race, and rubella vaccination assay run. Negative IFN-γ values indicate that the unstimulated secretion values were, on average, higher than the rubella virus-stimulated secretion values.

Table 3

Suggestive HLA allelic associations with rubella vaccine-specific neutralizing antibody responses in the Rochester and San Diego study cohorts.

	Rochester cohort						San Diego cohort				
HLA locus/antibody titer	**Allele**	**Allele counts**	**Median, NT_{50}/ml[1]**	**25th Percentile, NT_{50}/ml**	**75th Percentile, NT_{50}/ml**	**p-value**	**Allele counts**	**Median, NT_{50}/ml**	**25th Percentile, NT_{50}/ml**	**75th Percentile, NT_{50}/ml**	**p-value**
Antibody titer (NT_{50}/ml)	Overall	1398	57.4	35.4	95.7		2016	66.9	43.9	113.9	
HLA-A	*24:07	-	-	-	-	-	7	55.4	50.7	101.0	0.063
	*31:01	41	44.0	31.7	82.2	0.280	56	58.0	37.4	114.0	0.072
	*33:03	8	78.8	49.8	119.6	0.137	38	108.3	53.5	145.3	0.039
HLA-B	*44:05	5	59.5	39.7	115.4	0.916	6	87.1	61.9	139.0	0.003
	*49:01	29	57.9	39.3	78.1	0.807	30	92.7	41.6	123.0	0.046
HLA-C	-	-	-	-	-	-	-	-	-	-	-
HLA-DPA1	*03:01	-	-	-	-	-	30	83.7	60.0	128.3	0.057
	*04:01	-	-	-	-	-	7	133.7	67.0	167.6	0.008
HLA-DPB1	*06:02	7	83.2	53.1	92.6	0.563	48	75.2	53.4	122.2	0.041
	*14:01	22	50.9	42.5	68.9	0.316	19	55.4	41.3	80.5	0.055
HLA-DQB1	*04:02	42	44.4	28.8	63.2	0.006	117	62.5	37.1	104.7	0.188
	*05:02	24	56.3	40.7	123.1	0.997	50	79.3	55.4	142.5	0.048
	*05:03	36	67.0	38.0	102.0	0.055	52	81.5	54.7	120.7	0.387
HLA-DRB1	*01:02	15	88.0	68.0	113.0	0.099	39	56.6	41.1	100.5	0.244
	*04:03	10	76.2	31.6	126.3	0.395	23	122.0	62.0	155.3	0.059
	*08:02	-	-	-	-	-	50	45.0	32.7	69.5	0.009
	*08:04	-	-	-	-	-	34	96.7	57.8	150.5	0.065
	*11:01	61	60.6	37.6	104.5	0.863	107	54.7	35.1	106.1	0.052
	*11:03	11	39.7	30.6	64.2	0.096	11	72.5	55.3	103.6	0.841
	*12:01	29	72.4	50.0	115.8	0.030	38	70.5	55.5	130.2	0.485
	*13:01	92	53.3	36.4	80.6	0.075	87	62.4	42.1	100.6	0.350
HLA-DQA1	*01:03	99	53.3	35.2	82.5	0.068	118	62.8	39.7	114.5	0.303
	*01:04	38	70.4	43.8	111.6	0.046	48	81.5	56.3	116.7	0.152

	Rochester cohort						San Diego cohort				
HLA locus/antibody titer	**Allele**	**Allele counts**	**Median, NT_{50}/ml^{l}**	**25th Percentile, NT_{50}/ml**	**75th Percentile, NT_{50}/ml**	**p-value**	**Allele counts**	**Median, NT_{50}/ml**	**25th Percentile, NT_{50}/ml**	**75th Percentile, NT_{50}/ml**	**p-value**
	*06:01	11	53.3	33.7	136.3	0.958	18	99.2	62.8	141.7	0.050

Associations tested using linear regression models using log-transformed antibody levels. Analyses adjusted for age at blood draw quartile, quartiles for age at vaccination, time from most recent vaccination to enrollment, gender, race, and rubella vaccination assay run.

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Table 4

Suggestive HLA allelic associations with rubella vaccine-specific IL-6 immune responses in the Rochester and San Diego study cohorts.

	Rochester cohort						San Diego cohort				
HLA locus/cytokine	**Allele**	**Allele counts**	**Median, pg/ml**	**25th Percentile, pg/ml**	**75th Percentile, pg/ml**	**p-value**	**Allele counts**	**Median, pg/ml**	**25th Percentile, pg/ml**	**75th Percentile, pg/ml**	**p-value**
IL-6 (pg/ml)	Overall	1370	3,681	3,135	4,061		1938	4,117	3,525	4,787	
HLA-A	*02:05	9	3,953	3,015	3,975	0.073	21	3,836	3,246	5,082	0.214
	*31:01	42	3,483	2,916	4,006	0.084	56	3,811	3,455	4,235	0.023
HLA-B	*14:01	11	3,367	3,008	3,936	0.408	15	4,711	3,950	5,021	0.026
	*35:01	70	3,634	2,994	3,945	0.062	106	3,960	3,516	4,867	0.251
	*41:02	6	3,710	3,382	4,185	0.051	7	4,672	3,651	5,333	0.563
	*45:01	8	4,012	3,044	4,186	0.398	35	4,302	3,415	5,071	0.028
	*48:01	-	-	-	-	-	16	4,720	3,581	5,244	0.080
	*49:01	30	3,964	3,668	4,566	0.017	30	3,933	3,448	4,607	0.674
HLA-C	*03:02	5	3,690	3,387	3,783	0.096	14	4,185	3,612	4,919	0.198
	*04:01	139	3,620	2,994	3,970	0.060	257	4,026	3,502	4,789	0.208
	*08:02	31	3,637	3,008	4,041	0.973	74	4,418	3,817	4,942	0.015
	*15:02	25	3,585	3,131	3,989	0.414	47	3,750	3,371	4,349	0.017
HLA-DPA1	*01:03	1060	3,669	3,127	4,030	0.555	1181	4,076	3,501	4,768	0.031
	*02:02	59	3,690	3,144	4,183	0.361	187	4,251	3,667	4,820	0.080
HLA-DPB1	*05:01	41	4,061	3,397	4,369	0.120	73	4,360	3,580	5,111	0.074
HLA-DQB1	*04:02	41	3,650	2,981	4,085	0.221	115	4,132	3,579	4,702	0.104
	*05:01	166	3,732	3,168	4,033	0.014	216	4,109	3,445	4,609	0.973
	*05:02	23	3,746	3,094	4,306	0.526	49	4,410	3,760	4,847	0.064
	*06:02	188	3,629	3,075	3,992	0.463	234	4,209	3,474	4,702	0.083
HLA-DRB1	*01:01	117	3,675	3,090	4,001	0.035	100	4,071	3,433	4,560	0.389
	*01:03	13	3,542	3,024	3,983	0.008	22	3,988	3,410	4,569	0.378
	*10:01	14	3,786	3,666	4,162	0.449	23	4,527	3,386	5,200	0.001
	*11:01	61	3,797	3,345	4,172	0.854	101	4,151	3,559	4,577	0.015
	*15:01	190	3,619	3,058	3,989	0.882	171	4,032	3,407	4,679	0.020
	*15:03	4	3,868	3,411	3,966	0.004	48	4,255	3,614	4,574	0.549

	Rochester cohort						San Diego cohort			
HLA locus/cytokine	**Allele**	**Allele counts**	**Median, pg/ml**	**25th Percentile, pg/ml**	**75th Percentile, pg/ml**	**p-value**	**Allele counts**	**Median, pg/ml**	**25th Percentile, pg/ml**	**75th Percentile, pg/ml**
	*16:01	12	3,691	3,137	4,285	0.538	16	4,662	3,888	4,958
HLA-DQA1	*01:01	140	3,712	3,095	4,031	0.021	161	4,138	3,450	4,611
	*01:05	16	3,722	3,520	4,136	0.802	44	4,186	3,602	4,925
	*03:01	142	3,667	3,081	4,047	0.806	167	4,061	3,528	4,830
	*04:01	46	3,725	3,122	4,091	0.061	122	3,995	3,542	4,676

Associations tested using linear mixed models using log-transformed antibody levels and inverse-normal transformations for IL-6. Analyses adjusted for age at blood draw quartile, quaritles for age at vaccination, time from most recent vaccination to enrollment, gender, race, and rubella vaccination assay run.

Table 5

Suggestive HLA allelic associations with rubella vaccine-specific IFN-γ immune responses in the Rochester and San Diego study cohorts.

	Rochester cohort						San Diego cohort				
HLA locus/cytokine	**Allele**	**Allele counts**	**Median, pg/ml**	**25th Percentile, pg/ml**	**75th Percentile, pg/ml**	**p-value**	**Allele counts**	**Median, pg/ml**	**25th Percentile, pg/ml**	**75th Percentile, pg/ml**	**p-value**
IFN-γ (pg/ml)	Overall	1370	8.50	3.00	23.40		1896	-1.40	-6.40	3.10	
HLA-A	*03:01	199	8.29	2.73	24.14	0.743	209	-0.46	-5.93	4.18	0.042
	*36:01	-	-	-	-	-	7	1.41	-12.76	6.39	0.041
	*33:01	9	3.35	-0.97	12.05	0.034	25	-1.29	-5.73	0.93	0.167
	*68:01	64	5.25	2.97	12.64	0.023	77	0.51	-6.87	4.52	0.680
	*68:02	6	5.41	1.72	13.93	0.736	50	-1.60	-5.76	1.40	0.038
HLA-B	*13:02	33	6.61	2.84	21.57	0.197	31	-1.52	-5.74	2.93	0.070
	*18:01	40	4.76	2.06	14.26	0.766	62	-1.05	-5.20	2.42	0.045
	*49:01	30	8.09	2.00	21.30	0.491	30	0.15	-5.44	4.76	0.085
	*50:01	12	9.45	2.52	34.53	0.554	11	-0.06	-2.29	3.44	0.086
	*57:01	33	11.62	2.98	27.09	0.074	45	-2.07	-7.42	2.36	0.435
HLA-C	*07:04	33	5.25	2.97	29.17	0.127	18	2.25	-4.49	6.70	0.086
	*15:05	6	7.58	-0.16	21.8	0.669	10	-4.83	-8.03	1.96	0.042
HLA-DPA1	*01:03	1060	9.23	3.12	23.83	0.094	1159	-1.57	-6.51	3.14	0.848
	*01:09	13	4.6	1.73	18.76	0.092	160	-0.4	-4.24	3.91	0.019
HLA-DPB1	*03:01	138	11.98	3.61	32.52	0.004	119	-0.17	-4.39	4.20	0.704
	*05:02	9	12.03	5.95	21.80	0.772	33	-0.16	-3.93	4.91	0.028
	*17:01	18	6.17	0.16	31.16	0.460	47	-3.64	-10.58	1.15	0.013
HLA-DQB1	*04:01	-	-	-	-	-	7	-4.71	-20.46	-1.25	0.071
HLA-DRB1	*04:03	9	28.76	11.29	42.55	0.090	22	-3.03	-5.91	2.08	0.927
	*04:07	9	13.48	4.06	34.09	0.970	38	-2.00	-8.57	3.88	0.020
	*13:01	91	5.22	2.07	11.91	<0.001	85	-1.99	-7.38	4.2	0.997
	*13:03	12	20.98	9.63	25.25	0.090	36	-1.01	-5.47	3.00	0.775
HLA-DQA1	*01:03	99	5.35	1.93	13.00	0.002	112	-0.99	-6.5	4.55	0.80

Associations tested using linear mixed models using log-transformed antibody levels and inverse-normal transformations for IFN-γ. Analyses adjusted for age at blood draw quartile, quartiles for age at vaccination, time from most recent vaccination to enrollment, gender, race, and rubella vaccination assay run. Negative IFN-γ values indicate that the unstimulated secretion values were, on average, higher than the rubella virus-stimulated secretion values.