Published in final edited form as:

Vaccine. 2009 May 26; 27(25-26): 3349–3358. doi:10.1016/j.vaccine.2009.01.080.

HLA haplotype and supertype associations with cellular immune responses and cytokine production in healthy children after rubella vaccine

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Abstract

Secreted rubella virus-specific cytokines reflect the immunologic mechanisms underlying adoptive immune responses and are significant markers of immunity to rubella. We studied the association between measures of cellular (cytokine and frequency of cytokine-secreting cells) immune responses and HLA haplotypes (with frequencies of $\geq 1\%$) and supertypes among 738 healthy children following two doses of rubella vaccine. Haplotype effects were estimated while accounting for linkage phase ambiguity via an expectation maximization algorithm. Importantly, the majority of HLA class I and class II haplotype associations with different cytokines were consistent between Th1, Th2 and/or innate/proinflammatory cytokine groups. We found few class I supertypes (A1, A2, A3, and B7) with potential associations with IL-10 ELISPOT counts and rubella-specific IL-2, IL-10, TNF- α , and IL-6 cytokine secretion levels. Our data indicate that the presence or absence of certain HLA haplotypes and/or supertypes may influence the cytokine immune response to rubella vaccine, and represents a more advanced analysis compared to individual candidate gene association studies.

Keywords

Rubella vaccine; HLA class I haplotypes; HLA class II haplotypes; HLA supertypes; Cytokines; ELISPOT

1. Introduction

Live attenuated rubella virus vaccine elicits strong humoral immune responses (such as IgM, IgG, and IgA antibody production) as well as cellular immune responses (proliferation and cytotoxic lymphocytes response) [1,2]. Humoral immunity to the rubella vaccine has been shown to persist for up to 15 years [3,4]; however, rubella vaccine-induced cellular immunity is less well understood.

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Financial disclosure: Dr. Poland is the chair of a DMSB for novel non-rubella vaccines undergoing clinical studies by Merck Research Laboratories.

The immune response to rubella and other viruses involves processing of viral peptides and their presentation to both CD8+ cytotoxic T lymphocytes (CTL) restricted by HLA class I alleles and CD4+ T cells restricted by HLA class II alleles. HLA-DR4 restriction of immune recognition of the rubella virus E1 envelope protein was the first major evidence and ‘proof of principle’ that HLA gene polymorphisms influence rubella-induced immunity [5].

Moving from single allele to haplotype association studies represents the “next step” in understanding vaccine immunogenetics. Analysis of inferred HLA haplotypes from unrelated individuals has been extensively used in population-based genetic studies because it provides critical information regarding gene function and allows estimation of haplotype frequencies in a population [6,7]. We demonstrated elsewhere that certain clusters of nonrandomly inherited alleles, called haplotypes, are associated with variations in immune responses to live rubella virus vaccine [8]. In particular, specific class II HLA haplotypes (i.e. DRB1*04-DQB1*03-DPB1*03 and DRB1*13/14-DQB1*06-DPB1*03) were associated with higher lymphoproliferative responses to rubella virus in healthy children after two doses of rubella vaccine and the haplotype DRB1*07-DQB1*02-DPB1*11 was an unfavorable HLA marker due to its association with lower lymphoproliferation to rubella [8].

The notion of grouping HLA class I molecules into broad supertypes or clusters based on a shared sequence motif in peptide-binding pockets of HLA molecules is important in considering population coverage by antigenic epitopes [9]. We reported elsewhere that there are limited associations between HLA supertypes among 346 healthy children and rubella IgG antibody or cellular (lymphoproliferation) immune status [10]. We sought to validate whether these same HLA haplotypes and supertypes are associated with rubella vaccine immune responses in a larger group of 738 healthy children following two doses of rubella vaccine. The study was extended to test the hypotheses that HLA polymorphic haplotypes and supertypes influence T cell (IFN- γ and IL-10 ELISPOT) responses and cytokine (Th1, Th2 and innate/proinflammatory) secretion levels following two doses of rubella vaccine. To test these hypotheses, we recruited 396 new subjects and performed genotyping on the 342 subjects recruited to the previous rubella study.

2. Materials and methods

2.1. Subject’s recruitment

As previously described, we enrolled 346 healthy children (age: 12–18 years) identified through the Minnesota Independent School District 535 registration rolls in Rochester, MN (cohort 1) [11]. Three hundred forty-two parents agreed to allow their children to take part in the current rubella vaccine study, and from these 342 children we obtained a blood sample. We supplemented this cohort with an additional 396 healthy children and young adults (age: 11–19 years) enrolled between 2006 and 2007 in Rochester, Minnesota (cohort 2). All 738 participants had medical record documentation of having received two doses of measles–mumps–rubella (MMR) vaccine containing the attenuated RA27/3 Wistar strain of rubella virus (≥ 1000 TCID₅₀) (Merck) at or after the age of 12 months. The Institutional Review Board (IRB) of the Mayo Clinic approved the study, and written informed consent was obtained from the parents of all children who participated in the study, as well as written assent from age-appropriate children.

2.2. Cellular assays

ELISPOT assays were performed for the detection of rubella-specific IFN- γ and IL-10 secreting T cells using commercially available kits (Human IFN- γ ELISPOT kit, R&D Systems, Minneapolis, MN, USA and human IL-10 ELISPOT kit, BD Biosciences, San Diego, CA, USA) in samples obtained from 719 and 725 of the 738 subjects, respectively. The assays

were performed in peripheral blood mononuclear cell (PBMC) cultures as previously described [12], following the manufacturer's protocol. The cells were stimulated with live W-Therien strain of rubella virus at a multiplicity of infection (MOI) of 2.5 or 5 µg/ml of phytohemagglutinin (PHA, Sigma) as a positive control. PBMC cultured in triplicate in the absence of live attenuated rubella virus were used as negative controls in each assay. Spot-forming cells (SFC), i.e. rubella-specific T cells were detected 24 h later by scanning and analyzing plates on an ImmunoSpot® S4 Pro Analyzer (Cellular Technology Ltd., Cleveland, OH, USA) using ImmunoSpot® version 4.0 software (Cellular Technology Ltd.).

Cytokine IL-2 ($n = 713$), IL-4 ($n = 691$), IL-5 ($n = 691$), IL-6 ($n = 713$), IL-10 ($n = 713$), IL-12p40 ($n = 711$), IFN- γ ($n = 713$), TNF- α ($n = 713$), and GM-CSF ($n = 711$) secretion levels in response to rubella virus stimulation (W-Therien strain) were determined in PBMC culture supernatants by ELISA. We used pre-optimized conditions for time of incubation and MOI for each cytokine. Rubella-specific cytokine responses were quantitatively determined in cell-free supernatants by ELISA following the manufacturer's protocol (BD Biosciences Pharmingen, San Diego, CA, USA). For all cytokine outcomes, we obtained three rubella virus stimulated measures and three unstimulated measures. Median background levels from unstimulated control cell cultures were subtracted from the median rubella-induced responses to calculate corrected secretion values. Negative corrected values indicate that the unstimulated secretion levels were, on average, higher than the rubella virus stimulated secretion levels.

2.3. HLA genotyping

DNA was extracted from blood using the Puregene® extraction kit (Gentra Systems). HLA class I and class II loci were genotyped at a four-digit specificity level of molecular resolution (Invitrogen) as described previously [11]. Specifics for HLA class I (A and B) supertypes classification, based on a shared sequence motif in peptide-binding pockets of HLA molecules, have been described elsewhere [9,13,14].

2.4. Statistical methods

The focus of the statistical analyses was to assess the association between HLA haplotypes, and HLA supertypes with nine *in vitro* measures of rubella virus-specific cytokine secretion (measured in units of pg/ml), and two measures of cell-mediated immunity (CMI) measured as the counts of rubella vaccine-induced memory T cells positive for IFN- γ and IL-10. Each of these 11 immune response outcomes resulted in six measures per individual: three prior to stimulation with rubella virus and three post-stimulation. For descriptive purposes, a single value per individual was obtained for each outcome by subtracting the median of the three unstimulated values from the median of the three stimulated values. Data were descriptively summarized across individuals using frequencies and percentages for categorical variables, and medians and inter-quartile ranges for continuous variables.

2.4.1. HLA haplotypes—Separate analyses were carried out for each of the 11 outcomes. Two sets of haplotypes were considered: one for the three class I loci (A, C and B), and one for the five class II loci (DRB1, DQA1, DQB1, DPA1, and DPB1). Because genotyping is performed for each locus individually, the phase of the alleles on a specific chromosome for any individual is unknown and there may be multiple pairs of haplotypes which are consistent with an individual's observed HLA alleles. Therefore, the posterior probabilities of all possible haplotypes for an individual, conditional on the observed genotypes, were estimated using the expectation maximization (EM) algorithm implemented in the Haplo.Stats package [15]. This provided an expected design matrix that contained as variables the expected number of copies of each haplotype that an individual carries, estimated while accounting for phase ambiguity. Because of the imprecision involved in estimating the effects of low-frequency haplotypes,

estimates of specific haplotype effects were only estimated if the haplotypes occurred in our entire cohort with an estimated frequency of at least 1%.

As with supertype design variables, the resultant haplotype design variables range from 0 to 2, allowing for a similar analysis approach. We first examined global class I and class II associations with each of the 11 outcomes, and followed with assessments of individual haplotypes. The same methodology described in the HLA supertype section below was used, with one exception. Due to phase ambiguity, haplotype-specific descriptive summaries using medians and inter-quartile ranges could not be obtained. Instead *t*-statistics reflecting the direction, and relative magnitude, of the estimated haplotypic effect on the immune response measure; negative values correspond to haplotype-specific decreases in immune response and positive values correspond to increases in response, and larger values of the *t*-statistics are indicative of larger haplotype effects.

2.4.2. HLA supertypes—Separate analyses were carried out for each outcome and each HLA locus. Alleles for HLA loci were grouped into their respective supertypes, and summaries for the measures of rubella immune response were obtained using medians and inter-quartile ranges. Individuals contributed two observations to these descriptive summaries: one for each of their two alleles. Associations between HLA supertypes and immune response measures were then formally evaluated using linear regression models. In these models, regression variables were created for each HLA allele that reflected the number of copies of the allele that a subject carried (0, 1, or 2). Alleles occurring fewer than five times among all subjects, collected into a variable labeled “other”. Linear mixed models were employed for the cytokine secretion and CMI variables, simultaneously modeling all six observed measurements. These models essentially average the observed within-individual differences between stimulated and unstimulated states among those with the same HLA variant, and compare differences in these averages among those with different variants. In these models, we allowed for within-subject correlations without imposing any constraints on the within-person variance–covariance matrix.

Differences in immune response among all supertype alleles of each HLA locus were first assessed globally and simultaneously tested for statistical significance. Following these global tests, individual allele associations with immune response were examined by modeling each supertype allele in separate linear mixed models. These tests were performed in the spirit of Fisher’s protected least significant difference test; individual allele associations were not considered statistically significant in the absence of locus-specific global significance.

All analyses described above were adjusted for covariates potentially associated with immune response. These included age at enrollment, race, gender, age at first rubella vaccination, age at second rubella vaccination, and cohort status (old versus new). Data transformations were used to correct for data skewness in all linear regression models. An inverse cumulative normal (probit) transformation was used to correct for the skewed distributions of all cytokine secretion and ELISPOT outcome variables while performing the linear mixed models analyses. All statistical tests were two-sided.

3. Results

3.1. Study subjects and CMI outcomes

We genotyped 738 children aged 11–19 years (396 males and 342 females) who previously received two doses of rubella vaccine. The majority of subjects were Caucasians (91%) and the median age at the first and second vaccination was 15 months and 11 years, respectively. Rubella virus-specific ELISPOT counts were generally low: median values (25th, 75th percentiles) for IFN- γ and IL-10 spot-forming cells (SFC per 1×10^6 cells) were –20 (–60, 0)

and 5 (−35, 45), respectively. Six of the nine cytokines (IL-2, IL-6, IL-10, IFN- γ , TNF- α , and GM-CSF) were detected by ELISA in our study subjects, whereas three cytokines (IL-4, IL-5, and IL-12p40) had extremely low levels. Median values (25th, 75th percentiles) for rubella-specific IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p40, IFN- γ , TNF- α and GM-CSF cytokine secretion levels were 17.59 (7.73, 30.48) pg/ml, 0.30 (−0.31, 0.96) pg/ml, 0.47 (0.00, 1.09) pg/ml, 3680.99 (3159.97, 4051.96) pg/ml, 4.20 (2.29, 6.69) pg/ml, 0.00 (−7.15, 7.17) pg/ml, 8.53 (2.97, 23.41) pg/ml, 29.74 (−7.00, 89.23) pg/ml and 28.04 (23.56, 32.55) pg/ml, respectively.

3.2. Associations between HLA haplotypes and rubella virus vaccine immune responses

A total of 37 haplotypes (15 class I and 22 class II) with frequencies of $\geq 1\%$ were identified. Separate analyses were performed for every measure of CMI. Due to space limitations, Tables 1–4 only report haplotypes with significant ($p < 0.05$) or suggestive ($p \leq 0.10$) associations with measures (ELISPOT and secreted cytokines) of rubella-specific CMI.

The four global tests failed to show a statistically significant association between class I and class II haplotypes and rubella-specific T cell responses measured by IFN- γ and IL-10 ELISPOT (Table 1). When examining haplotypes individually, the class II haplotypes DRB1*13-DQA1*01-DQB1*06-DPA1*01-DPB1*03 (t -statistic of -2.30 , $p = 0.022$) and DRB1*03-DQA1*05-DQB1*02-DPA1*01-DPB1*04 (t -statistic of -2.78 , $p = 0.006$) were suggestive of lower rubella IFN- γ and IL-10 ELISPOT T cell responses, respectively.

Associations between class I and class II haplotypes and Th1 cytokines (IFN- γ , IL-2, and IL-12p40) secretion were examined and specific haplotypic associations with p -values less than 0.10 are summarized in Table 2. The global tests failed to show a statistically significant association between rubella-specific IFN- γ secretion levels and HLA class I and II haplotypes (p -value of 0.305 and 0.895, respectively). However, the individual haplotypes with the strongest indication for association with higher rubella-specific IFN- γ responses were A*02-C*07-B*07 (t -statistic of 2.10, $p = 0.036$), A*03-C*04-B*35 (t -statistic of 2.31, $p = 0.021$) and DRB1*03-DQA1*05-DQB1*02-DPA1*01-DPB1*03 (t -statistic of 2.26, $p = 0.024$).

For the class I HLA haplotypes, the global tests did not reach statistical significance for IL-2 response ($p = 0.356$), although for the class II haplotypes and IL-2 secretion to rubella virus the global p -value was suggestive ($p = 0.091$) (Table 2). In particular, DRB1*04-DQA1*03-DQB1*03-DPA1*01-DPB1*02 (t -statistic of 2.86, $p = 0.004$) and DRB1*04-DQA1*03-DQB1*03-DPA1*01-DPB1*04 (t -statistic of 3.06, $p = 0.002$) were observed to be associated with increased rubella-specific IL-2 secretion levels. In contrast, the DRB1*13-DQA1*01-DQB1*06-DPA1*01-DPB1*03 (t -statistic of -1.98 , $p = 0.049$) haplotype was observed to be associated with decreased IL-2 secretion levels.

Further, global tests revealed significant associations between class I A-C-B haplotypes and rubella-specific IL-12p40 secretion (p -value of 0.026) (Table 2). We found that although IL-12p40 secretion levels were extremely low, the A*03-C*03-B*40 (t -statistic of 2.94, $p = 0.003$), A*03-C*07-B*07 (t -statistic of 2.14, $p = 0.033$) haplotypes were associated with higher IL-12p40 production. On the contrary, the A*02-C*03-B*40 haplotype (t -statistic of -2.20 , $p = 0.028$) was associated with suppressed IL-12p40 production. We also found potential evidence for higher levels of IL-12p40 associated with the class II haplotype DRB1*07-DQA1*02-DQB1*03-DPA1*01-DPB1*04 (t -statistic of 2.27, $p = 0.024$). These associations must be considered preliminary given the extremely low levels of IL-12p40 secretion in rubella virus-stimulated PBMC cultures.

The associations between HLA haplotypes and rubella-specific Th2 cytokine (IL-4, IL-5, and IL-10) immune response profile following two doses of live rubella vaccination were examined and the results are summarized in Table 3. The global tests failed to provide evidence of

statistically significant associations. The individual class II haplotypes with the strongest evidence for association with higher IL-4 responses were DRB1*15-DQA1*01-DQB1*06-DPA1*01-DPB1*02 (t -statistic of 2.78, $p = 0.006$) and DRB1*07-DQA1*02-DQB1*02-DPA1*02-DPB1*11 (t -statistic of 2.08, $p = 0.038$). However, IL-4 secretion was minimally detectable in rubella virus stimulated cell cultures. Similarly, IL-5 was barely detectable, yet we found potential evidence for augmented levels of IL-5 associated with the class I A*02-C*07-B*08 (t -statistic of 2.68, $p = 0.008$) haplotype and evidence for lesser IL-5 levels with class II DRB1*11-DQA1*05-DQB1*03-DPA1*01-DPB1*02 (t -statistic of -2.99 , $p = 0.003$) haplotype. Lastly, only two haplotypes, A*01-C*07-B*07 (t -statistic of 1.96, $p = 0.050$) and DRB1*03-DQA1*05-DQB1*02-DPA1*01-DPB1*02 (t -statistic of 2.03, $p = 0.043$), were potentially associated with elevated IL-10 secretion levels.

The associations between HLA haplotypes and innate/proinflammatory (TNF- α , GM-CSF, and IL-6) cytokines were also examined and potential associations are shown in Table 4. For both class I and class II haplotypes, the global tests did not reach statistical significance for either of the cytokines responses, although we found suggestive evidence for higher levels of TNF- α with class I A*29-C*16-B*44 (t -statistic of 2.38, $p = 0.017$), and for IL-6 with class I A*24-C*07-B*07 (t -statistic of 2.58, $p = 0.010$) haplotypes. We did find evidence for lower levels of rubella-specific IL-6 secretion associated with the DRB1*03-DQA1*05-DQB1*02-DPA1*01-DPB1*04 (t -statistic of -2.20 , $p = 0.028$) haplotype. While we do not conclude that these differences are statistically significant, due to a lack of global significance, these observations are of potential interest in future studies.

3.3. Associations between HLA supertypes and rubella virus vaccine immune responses

The most common HLA class I A (A1, A2, A3, and A24) and B (B7, B27, B44, B58, and B62) supertypes in our study population were expressed in frequencies comparable to the HLA supertype frequencies published elsewhere [9,14]. The associations between HLA class I supertypes and rubella-specific T cell (ELISPOT) immune responses following two doses of rubella vaccination were examined and the results are summarized in Table 5. IFN- γ producing T cells detected by ELISPOT assay were low; however, the global tests revealed a significant association between IL-10 ELISPOT counts and class I A supertype (p -value of 0.003). In particular, the A2 supertype (median SFC per 1×10^6 T cells of 10, $p = 0.004$) was associated with higher rubella-specific IL-10 ELISPOT counts, whereas the A1 supertype was associated with low spot-forming cell counts (median 0, $p = 0.002$).

Associations between HLA supertypes and measures of Th1 (IFN- γ , IL-2, and IL-12p40) cytokine immune responses to rubella virus are presented in Table 6. The global tests for association failed to show any statistically significant associations. However, when examining class I A supertypes individually, the A3 supertype (median 18.92 pg/ml, $p = 0.017$) approached statistical significance with higher IL-2 secretion levels.

We found no associations with any of the HLA-A or B supertypes and either rubella-specific IL-4 or IL-5 secretion profile (Table 7). Further, the global tests for association also failed to reveal a statistically significant association between IL-10 secretion and the class I A and B supertypes (p -values of 0.140 and 0.575, respectively). The A3 supertype (median 4.01 pg/ml, $p = 0.022$) was marginally associated with slightly lower rubella-specific IL-10 production. These data suggest that HLA class I supertypes may have limited associations with the Th2-type cytokine immune responses to rubella vaccine.

Lastly, the associations between HLA supertypes and levels of innate/proinflammatory (TNF- α , GM-CSF, and IL-6) cytokine responses to rubella virus are presented in Table 8. The global tests revealed a statistically significant association between TNF- α secretion levels and class

I A supertype (p -value of 0.010). In particular, the A3 supertype (median 25.64 pg/ml, $p = 0.015$) was associated with lower rubella-specific TNF- α production.

The global tests for association failed to demonstrate a statistically significant association between GM-CSF secretion and the class I A and B superotypes (p -values of 0.210 and 0.881, respectively). When examining class I A superotypes independently, the A24 supertype (median 28.48 pg/ml, $p = 0.052$) appeared to be marginally associated with higher rubella-specific GM-CSF responses (Table 8).

Again, the global tests for association failed to show a statistically significant association between rubella-induced IL-6 secretion levels and class I superotypes (p -value of 0.272 and 0.143, respectively). When examining all superotypes individually, the A2 (median 3718.26 pg/ml, $p = 0.030$) and B7 (median 3630.54 pg/ml, $p = 0.041$) superotypes appeared to be marginally associated with higher and lower rubella-specific IL-6 responses, respectively, after two doses of rubella vaccine (Table 8).

4. Discussion

Given that the cellular (cytokine) immune responses to rubella virus vaccine are important in protection against disease, this study sought to investigate whether rubella vaccination has an effect on cytokine production based on the HLA haplotypes and superotypes of the host. An important finding of our study was the discovery of consistent associations between HLA haplotypes and rubella-induced cytokine immune responses. Our findings show that among healthy schoolchildren some HLA class I and class II haplotype associations with different cytokines were shared between Th1, Th2 and innate/proinflammatory cytokine groups. When we compared all haplotypes across cytokine immune responses, we observed that the class I haplotype A*02-C*07-B*07 was associated with both higher rubella-specific T cell responses (measured by IFN- γ ELISPOT) and IFN- γ secretion levels (Tables 1 and 2). Similarly, the A*11-C*04-B*35 haplotype was commonly observed in association with higher T cell IFN- γ and secreted IL-2 responses. Further, the class I haplotype A*02-C*03-B*40 was associated with variations in both rubella-induced T cell (measured by IL-10 ELISPOT) and IL-12p40 responses. Likewise, the class I HLA haplotype A*01-C*06-B*57 was associated with variations in IL-5 and IL-6 secretion levels (Tables 3 and 4). In addition, the A*24-C*07-B*07 haplotype was marginally associated with elevated rubella-specific TNF- α and IL-6 production. These preliminary data suggest that cytokine immune responses to rubella virus in previously immunized individuals are, in part, restricted by HLA haplotypes by the mechanism of restricting which pathogen-derived peptides are presented. These observations are important as they point to potential immunologic approaches to indirect influencing cellular immune responses to rubella.

For the class II haplotypes, the HLA haplotype sharing phenomenon was also repeatedly observed. When all class II haplotypes were compared across cytokine immune responses, we found that the class II haplotype DRB1*13-DQA1*01-DQB1*06-DPA1*01-DPB1*03 was associated with lesser rubella-specific T cell responses (measured by IFN- γ ELISPOT) and IL-2 secretion levels (Tables 1 and 2). Interestingly, the same DRB1*13-DQA1*01-DQB1*06-DPA1*01-DPB1*03 haplotype was also found to be associated with elevated IL-10 ELISPOT T cell responses and TNF- α secretion levels (Tables 1 and 4). Similarly, the DRB1*03-DQA1*05-DQB1*02-DPA1*01-DPB1*04 haplotype was associated with lower T cell IL-10 ELISPOT responses and IL-6 secretion. Subjects carrying the DRB1*03-DQA1*05-DQB1*02-DPA1*02-DPB1*01 haplotype demonstrated low *in vitro* IL-10 ELISPOT and IL-12p40 cytokine responses to rubella virus. We also found evidence for poorer rubella-specific IFN- γ , IL-5, and TNF- α secretion associated with the DRB1*11-DQA1*05-DQB1*03-DPA1*01-DPB1*04 haplotype in our study subjects (Tables 2–4). Intriguingly, we

found that the same haplotype, DRB1*11-DQA1*05-DQB1*03-DPA1*01-DPB1*04, was also associated with higher IL-12p40 secretion levels. The DRB1*07-DQA1*02-DQB1*02-DPA1*01-DPB1*04 haplotype was a favorable HLA marker for higher IL-2 and IL-5 secretion levels (Tables 2 and 3). These data suggest that genetic variation in HLA may play a dual role in modulating both poorer and higher cytokine immune responses to rubella virus.

We reported elsewhere that the DRB1*04-DQB1*03-DPB1*03 and DRB1*07-DQB1*02-DPB1*04 haplotypes were associated with high lymphocyte proliferation to rubella virus antigens in 346 healthy children previously vaccinated with two doses of rubella vaccine [8]. In the same study we reported that the DRB1*07-DQB1*02-DPB1*11 haplotype was associated with low rubella-induced lymphoproliferation [8]. In the current study, the DRB1*04-DQB1*03-DPB1*03 haplotype, which is a part of the DRB1*04-DQA1*03-DQB1*03-DPA1*01-DPB1*03 larger haplotype, was also found to be associated with increased rubella-specific IFN- γ secretion levels. Furthermore, we found in the current study that the DRB1*07-DQB1*02-DPB1*04 haplotype, which is a part of the DRB1*07-DQA1*02-DQB1*02-DPA1*01-DPB1*04 larger haplotype, was associated with increased levels of rubella-specific IL-2 and IL-5. Additionally, we found that the DRB1*07-DQB1*02-DPB1*11 haplotype, which is a part of the DRB1*07-DQA1*02-DQB1*02-DPA1*02-DPB1*11 larger haplotype, was associated with augmented rubella-specific IL-4 production. Thus, analyses that incorporated information from additional subjects did not change the originally-observed associations, providing an admittedly incomplete, but encouraging, confirmation of these associations in a larger group of 738 subjects, which included 342 subjects from the previous rubella vaccine study.

Certain HLA supertypes may, in part, contribute to or account for variations in vaccine immune responses and susceptibility to disease. For instance, the B27 and B58 supertypes are associated with protection against HIV disease in Caucasian individuals [16]. Immunization with the hepatitis B virus core epitope has been reported to elicit CTL responses in humans expressing different HLA-A2 supertype molecules [17]. Viral epitopes restricted by HLA-A1, A2, A24, A26, and B44 supertypes can induce IFN- γ T cell responses in subjects more than 30 years after smallpox vaccination [18]. This study also sought to determine whether HLA class I supertypes influenced rubella-specific cytokine immune responses. Our findings show that a few class I supertypes were associated with rubella-specific cytokine secretion levels. For example, the most common A2 supertype was associated with higher rubella-specific T cell responses (measured by IL-10 ELISPOT) and increased IL-6 secretion levels. However, the most prevalent HLA-B supertype, B7, was associated with lower IL-6 production.

Our findings demonstrate that among our study subjects, the supertype A3 was associated with elevated IL-2 and slightly decreased IL-10 production. These A2 and A3 supertypes were associated with higher levels of cytokine secretion and could be considered as favorable HLA supertypes, although these associations must be considered preliminary given the lack of global significance. In contrast, lower rubella-specific IL-10 ELISPOT counts were significantly associated with the A1 supertype. This information can be useful with respect to personalized vaccine design, since rubella-derived viral peptides may be presented by multiple alleles of the HLA supertype, and because the frequency of these supertypes worldwide are high. Irrespective of ethnicity, the frequency of some class I supertypes, such as A2, A3, B7, and B44, is as high as 35–55% of the general population [19]. Accordingly new vaccines comprised of various immunodominant epitopes restricted by multiple HLA supertypes can be designed to maximize population coverage [20,21].

In conclusion, the results of this study suggest that HLA haplotypes and supertypes may be important in generating of rubella immunity and that further investigation of the roles of both HLA haplotypes and supertypes in rubella vaccine-induced immunity should be pursued.

Acknowledgments

We thank the Mayo Vaccine Research Group and subjects who participated in our studies. We thank Cheri A. Hart for her editorial assistance. This work was supported by NIH grants AI 48793, AI 33144 and 1 UL1 RR024150-01 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health, and the NIH Roadmap for Medical Research. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NCRR or NIH.

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Table 1
Associations between rubella virus-specific T cell (ELISPOT) responses and HLA haplotypes.

ELISPOT/HLA locus	Haplotype	Frequency	Test statistic (haplotype <i>t</i> -statistic)	Allele <i>p</i> -value	Global <i>p</i> -value
<i>IFN-γ</i> ELISPOT					
Class I	A-C-B				0.559
	*02- <i>*07-*</i> 07	0.034	1.82	0.069	
	*11- <i>*04-*</i> 35	0.011	1.85	0.064	
Class II	DRB1-DQA1-DQB1-DPA1-DPB1				0.666
	*13- <i>*01-*</i> 06- <i>*01-*</i> 03	0.013	-2.30	0.022	
<i>IL-10</i> ELISPOT					
Class I	A-C-B				0.248
	*02- <i>*03-*</i> 40	0.033	1.64	0.101	
Class II	DRB1-DQA1-DQB1-DPA1-DPB1				0.722
	*03- <i>*05-*</i> 02- <i>*01-*</i> 04	0.048	-2.78	0.006	
	*03- <i>*05-*</i> 02- <i>*02-*</i> 01	0.037	-1.87	0.063	
	*13- <i>*01-*</i> 06- <i>*01-*</i> 03	0.013	1.87	0.062	

Only haplotypes with estimated frequencies ≥ 0.01 and *p*-values ≤ 0.10 are presented. Haplotype effects estimated using a haplotype *t*-statistic that assesses associations of cytokine ELISPOT levels across haplotypes. Allele *p*-value compares haplotype of interest to all other haplotypes combined. Statistically significant findings (*p* < 0.05) are bolded. Analyses adjust for age at blood draw, gender, race, age at first rubella vaccine, age at second rubella vaccine, and cohort status.

Table 2
Associations between rubella virus-specific Th1 (IFN- γ , IL-2 and IL-12p40) cytokine responses with HLA II haplotypes.

Cytokine/HLA locus	Haplotype	Frequency	Test statistic (haplotype <i>t</i> - statistic)	Allele <i>p</i> -value	Global <i>p</i> -value
<i>IFN-γ</i> Class I	A-C-B				0.305
	*02-*07-*07	0.034	2.10	0.036	
	*03-*04-*35	0.016	2.31	0.021	
	DRB1-DQA1-DQB1-DPA1-DPB1				0.895
	*03-*05-*02-*01-*03	0.014	2.26	0.024	
	*04-*03-*03-*01-*03	0.020	1.94	0.053	
Class II	*11-*05-*03-*01-*04	0.041	-1.69	0.092	
	*13-*01-*06-*01-*04	0.041	-1.93	0.054	
	A-C-B				0.356
	*11-*04-*35	0.011	2.20	0.028	
	DRB1-DQA1-DQB1-DPA1-DPB1				0.091
	*04-*03-*03-*01-*02	0.016	2.86	0.004	
<i>IL-2</i> Class I	*04-*03-*03-*01-*04	0.098	3.06	0.002	
	*07-*02-*02-*01-*04	0.026	1.82	0.069	
	*13-*01-*06-*01-*03	0.013	-1.98	0.049	
	A-C-B				0.026
	*02-*03-*40	0.033	-2.20	0.028	
	*03-*03-*40	0.013	2.94	0.003	
<i>IL-12p40</i> Class I	*03-*07-*07	0.059	2.14	0.033	
	DRB1-DQA1-DQB1-DPA1-DPB1				0.371
	*03-*05-*02-*02-*01	0.037	-1.78	0.075	
	*07-*02-*03-*01-*04	0.014	2.27	0.024	
	*11-*05-*03-*01-*04	0.041	1.85	0.065	

Only haplotypes with estimated frequencies ≥ 0.01 and *p*-values ≤ 0.10 are presented. Haplotype effects estimated using a haplotype *t*-statistic that assesses associations of cytokine levels across haplotypes. Allele *p*-value compares haplotype of interest to all other haplotypes combined. Statistically significant findings (*p* < 0.05) are bolded. Analyses adjust for age at blood draw, gender, race, age at first rubella vaccine, age at second rubella vaccine, and cohort status.

Table 3
Associations between rubella virus-specific Th2 (IL-4, IL-5 and IL-10) cytokine responses and HLA haplotypes.

Cytokine/HLA locus	Haplotype	Frequency	Test statistic (haplotype <i>t</i> - statistic)	Allele <i>p</i> -value	Global <i>p</i> -value
<i>IL-4</i> Class I Class II	A-C-B				0.981
	DRB1-DQA1-DQB1-DPA1-DPB1				0.451
	*07-∗02-∗02-∗02-∗11	0.012	2.08	0.038	
<i>IL-5</i> Class I	*15-∗01-∗06-∗01-∗02	0.013	2.78	0.006	
	A-C-B				0.399
	*01-∗06-∗57	0.011	1.78	0.075	
Class II	*02-∗07-∗08	0.013	2.68	0.008	
	DRB1-DQA1-DQB1-DPA1-DPB1				0.106
	*07-∗02-∗02-∗01-∗04	0.026	1.95	0.052	
	*11-∗05-∗03-∗01-∗02	0.011	-2.99	0.003	
	*11-∗05-∗03-∗01-∗04	0.041	-1.79	0.075	
	*13-∗01-∗06-∗01-∗02	0.026	1.77	0.078	
<i>IL-10</i> Class I	A-C-B				0.557
	*01-∗07-∗07	0.011	1.96	0.050	
	DRB1-DQA1-DQB1-DPA1-DPB1				0.268
Class II	*03-∗05-∗02-∗01-∗02	0.011	2.03	0.043	

Only haplotypes with estimated frequencies ≥0.01 and *p*-values ≤0.10 are presented. Haplotype effects estimated using a haplotype *t*-statistic that assesses associations of cytokine levels across haplotypes. Allele *p*-value compares haplotype of interest to all other haplotypes combined. Statistically significant findings (*p* < 0.05) are bolded. Analyses adjust for age at blood draw, gender, race, age at first rubella vaccine, age at second rubella vaccine, and cohort status.

Table 4
Associations of rubella virus-specific innate/proinflammatory (TNF- α , GM-CSF and IL-6) cytokine responses with HLA haplotypes.

Cytokine/HLA locus	Haplotype	Frequency	Test statistic (haplotype <i>t</i> - statistic)	Allele <i>p</i> -value	Global <i>p</i> -value
<i>TNF-α</i> Class I	A-C-B				0.231
	*24-*07.*07	0.017	1.78	0.075	
	*29-*16.*44	0.022	2.38	0.017	
	DRB1-DQA1-DQB1-DPA1-DPB1				0.547
Class II	*07-*02.*03.*01-.*04	0.014	-1.65	0.100	
	*11-*05.*03.*01-.*04	0.041	-1.83	0.067	
	*13-*01.*06.*01-.*03	0.013	1.90	0.057	
	DRB1-DQA1-DQB1-DPA1-DPB1				0.823
<i>GM-CSF</i> Class I	A-C-B				0.968
Class II	DRB1-DQA1-DQB1-DPA1-DPB1				0.270
<i>IL-6</i> Class I	A-C-B				
Class II	*01-*06.*57	0.011	-1.94	0.053	
	*24-*07.*07	0.017	2.58	0.010	
	DRB1-DQA1-DQB1-DPA1-DPB1				0.916
	*03-*05.*02.*01-.*04	0.048	-2.20	0.028	

Only haplotypes with estimated frequencies ≥ 0.01 and *p*-values ≤ 0.10 are presented. Haplotype effects estimated using a haplotype *t*-statistic that assesses associations of cytokine levels across haplotypes. Allele *p*-value compares haplotype of interest to all other haplotypes combined. Statistically significant findings (*p* < 0.05) are bolded. Analyses adjust for age at blood draw, gender, race, age at first rubella vaccine, age at second rubella vaccine, and cohort status.

Table 5
HLA class I supertype associations with rubella virus-induced T cell (ELISPOT) responses.

ELISPOT/HLA locus	HLA supertype	Number of alleles	Median response (SFC 1 × 10 ⁶ cells)	Lower quartile, Q1	Upper Quartile, Q3	p-value ^a	Global p-value ^a
<i>IFN-γ</i> ELISPOT Class IA	Overall	1438	-20	-60	0		0.826
	A1	336				0.549	
	A2	437				0.586	
	A3	410				0.991	
	A24	185				0.552	
	Other ^b	82				0.366	
							0.776
Class IB	B7	424				0.212	
	B27	156				0.657	
	B44	374				0.259	
	B58	53				0.879	
	B62	162				0.540	
	Other ^b	281				0.870	
	Overall	1450	5	-35	45		0.003
<i>IL-10</i> ELISPOT Class IA	A1	336	0	-40	45	0.002	
	A2	437	10	-25	50	0.004	
	A3	410				0.730	
	A24	185				0.494	
	Other ^b	82				0.116	
							0.629
	B7	424				0.761	
Class IB	B27	156				0.488	
	B44	374				0.165	
	B58	53				0.358	
	B62	162				0.932	
	Other ^b	281				0.353	

ELISPOT/HLA locus	HLA supertype	Number of alleles	Median response (SFC 1×10^6 cells)	Lower quartile, Q1	Upper Quartile, Q3	<i>p</i> -value ^a	Global <i>p</i> -value ^a
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Q1 and Q3 represent the first (25th percentile) and third (75th percentile) quartiles, respectively. SFC: spot-forming cells per 1×10^6 T cells. Statistically significant findings ($p < 0.05$) are bolded.

^aLinear regression analysis. Due to data skewness, *p*-values were based on inverse cumulative normal transformation. Analyses adjust for age at blood draw, gender, race, age at first rubella vaccine, age at second rubella vaccine, and cohort status.

^b“Other” includes the following HLA-A alleles: *0217, *0302, *2407, *2608, *2901, *2902, *3004, *3206, *3303, *3401, *6601, *6803, *7401; HLA-B alleles: *0706, *0714, *0801, *1507, *1511, *1522, *1525, *1801, *1803, *1813, *3504, *3508, *3511, *3512, *3523, *3542, *3906, *4002, *4102, *4201, *4427, *4404, *4405, *4701, *4803, *5703, respectively.

HLA class I supertype associations with rubella virus-induced Th1 (IFN- γ , IL-2 and IL-12p40) cytokine immune responses.

Cytokine/HLA locus		HLA supertype	Number of alleles	Median secretion value (pg/ml)	Lower Quartile, Q1 (pg/ml)	Upper Quartile, Q3 (pg/ml)	p-value ^a	Global p-value ^a
IFN- γ Class IA	Overall		1426	8.53	2.97	23.41		0.865
	A1		330				0.334	
	A2		433				0.370	
	A3		398				0.948	
	A24		183				0.961	
	Other ^b		82				0.952	
	Class IB							0.881
IL-2 Class IA	B7		417				0.369	
	B27		154				0.785	
	B44		370				0.520	
	B58		51				0.436	
	B62		159				0.619	
	Other ^b		275				0.846	
	Overall		1426	17.59	7.73	30.48		0.203
	Class IA							
	A1		330				0.226	
	A2		433				0.427	
	A3		398	18.92	8.42	31.40	0.017	
	A24		183				0.718	
	Other ^b		82				0.686	
	Class IB							0.557
	B7		417				0.378	
	B27		154				0.553	
	B44		370				0.537	
	B58		51				0.418	
	B62		159				0.164	
	Other ^b		275				0.535	

Cytokine/HLA locus	HLA supertype	Number of alleles	Median secretion value (pg/ml)	Lower Quartile, Q1 (pg/ml)	Upper Quartile, Q3 (pg/ml)	<i>p</i> -value ^a	Global <i>p</i> -value ^a
<i>IL-12p40</i> Class IA	Overall	1422	0.00	-7.15	7.17		0.633
	A1	329				0.656	
	A2	432				0.752	
	A3	396				0.644	
	A24	183				0.111	
	Other ^b	82				0.964	
Class IB							0.593
	B7	415				0.148	
	B27	154				0.628	
	B44	369				0.889	
	B58	51				0.567	
	B62	159				0.778	
	Other ^b	274				0.149	

Q1 and Q3 represent the first (25th percentile) and third (75th percentile) quartiles, respectively. Statistically significant findings ($p < 0.05$) are bolded.

^aLinear regression analysis. *p*-values for cytokine levels were based on inverse cumulative normal transformation. Analyses adjust for age at blood draw, gender, race, age at first rubella vaccine, age at second rubella vaccine, and cohort status.

^b“Other” includes the following HLA-A alleles: *0217, *0302, *2407, *2608, *2901, *2902, *3206, *3303, *3401, *6601, *6803, *7401, *6803, *7401, *0706, *0714, *0801, *1507, *1511, *1522, *1525, *1801, *1803, *1813, *3504, *3508, *3511, *3512, *3523, *3542, *3906, *4002, *4102, *4201, *4427, *4404, *4405, *4701, *4803, *5703, respectively.

HLA class I supertype associations with rubella virus-induced Th2 (IL-4, IL-5 and IL-10) cytokine immune responses.

Cytokine/HLA locus		HLA supertype	Number of alleles	Median secretion value (pg/ml)	Lower Quartile, Q1 (pg/ml)	Upper Quartile, Q3 (pg/ml)	p-value ^a	Global p-value ^a
IL-4	Class IA	Overall	1382	0.30	-0.31	0.96		0.971
		A1	317				0.831	
		A2	424				0.817	
		A3	386				0.862	
		A24	177				0.816	
		Other ^b	78				0.531	
	Class IB							0.868
IL-5	Class IA	B7	402				0.765	
		B27	151				0.419	
		B44	356				0.554	
		B58	50				0.473	
		B62	156				0.491	
		Other ^b	267				0.994	
	Class IB	Overall	1382	0.47	0.00	1.09		0.261
		A1	317				0.573	
		A2	424				0.290	
		A3	386	0.45	0.00	0.95	0.060	
		A24	177				0.190	
		Other ^b	78				0.531	
		B7	402				0.384	0.550
		B27	151				0.110	
		B44	356				0.440	
		B58	50				0.459	
		B62	156				0.834	
		Other ^b	267				0.687	

Cytokine/HLA locus	HLA supertype	Number of alleles	Median secretion value (pg/ml)	Lower Quartile, Q1 (pg/ml)	Upper Quartile, Q3 (pg/ml)	<i>p</i> -value ^a	Global <i>p</i> -value ^a
<i>IL-10</i> Class IA	Overall	1426	4.20	2.29	6.69		0.140
	A1	330				0.115	
	A2	433				0.874	
	A3	398	4.01	1.91	6.60	0.022	
	A24	183				0.532	
	Other ^b	82				0.309	
Class IB							0.575
	B7	417				0.859	
	B27	154				0.634	
	B44	370				0.323	
	B58	51				0.897	
	B62	159	4.13	2.44	6.17	0.079	
	Other ^b	275				0.821	

Q1 and Q3 represent the first (25th percentile) and third (75th percentile) quartiles, respectively. Statistically significant finding ($p < 0.05$) is bolded.

^aLinear regression analysis. *p*-values for cytokine levels were based on inverse cumulative normal transformation. Analyses adjust for age at blood draw, gender, race, age at first rubella vaccine, age at second rubella vaccine, and cohort status.

^b“Other” includes the following HLA-A alleles: *0217, *0302, *2407, *2608, *2901, *2902, *3004, *3206, *3303, *3401, *6601, *6803, *7401; HLA-B alleles: *0706, *0714, *0801, *1507, *1511, *1522, *1525, *1801, *1803, *1813, *3504, *3508, *3511, *3512, *3523, *3542, *3906, *4002, *4102, *4201, *4427, *4404, *4405, *4701, *4803, *5703, respectively.

Table 8

HLA class I supertype associations with rubella virus-induced innate/proinflammatory (TNF- α , GM-CSF and IL-6) cytokine immune responses.

Cytokine/HLA locus	HLA supertype	Number of alleles	Median secretion value (pg/ml)	Lower Quartile, Q1 (pg/ml)	Upper Quartile, Q3 (pg/ml)	p-value ^a	Global p-value ^d
TNF- α Class IA	Overall	1426	29.74	-7.00	89.23		0.010
	A1	330				0.275	
	A2	433				0.938	
	A3	398	25.64	-15.44	77.79	0.015	
	A24	183				0.950	
	Other ^b	82	42.65	-1.40	109.24	0.004	
							0.850
Class IB	B7	417				0.657	
	B27	154				0.359	
	B44	370				0.907	
	B58	51				0.874	
	B62	159				0.383	
	Other ^b	275				0.480	
	Overall	1422	28.04	23.56	32.55		0.210
GM-CSF Class IA	A1	329				0.796	
	A2	432				0.433	
	A3	396				0.167	
	A24	183	28.48	24.01	32.90	0.052	
	Other ^b	82				0.375	
							0.881
	B7	415				0.254	
Class IB	B27	154				0.479	
	B44	369				0.622	
	B58	51				0.670	
	B62	159				0.866	

Cytokine/HLA locus	HLA supertype	Number of alleles	Median secretion value (pg/ml)	Lower Quartile, Q1 (pg/ml)	Upper Quartile, Q3 (pg/ml)	<i>p</i> -value ^a	Global <i>p</i> -value ^a
<i>IL-6</i> Class IA	Other ^b	274				0.921	
	Overall	1426	3680.99	3159.97	4051.96		0.272
	A1	330				0.459	
	A2	433	3718.26	3204.13	4069.96	0.030	
	A3	398				0.192	
	A24	183				0.721	
	Other ^b	82				0.877	
Class IB							0.143
	B7	417	3630.54	3083.48	4000.90	0.041	
	B27	154				0.214	
	B44	370				0.219	
	B58	51	3735.30	2974.14	4116.66	0.107	
	B62	159				0.759	
	Other ^b	275				0.566	

Q1 and Q3 represent the first (25th percentile) and third (75th percentile) quartiles, respectively. Statistically significant findings ($p < 0.05$) are bolded.

^aLinear regression analysis. *p*-values for cytokine levels were based on inverse cumulative normal transformation. Analyses adjust for age at blood draw, gender, race, age at first rubella vaccine, age at second rubella vaccine, and cohort status.

^b“Other” includes the following HLA-A alleles: *0217, *0302, *2407, *2608, *2901, *2902, *3004, *3206, *3303, *3401, *6601, *6803, *7401; HLA-B alleles: *0706, *0714, *0801, *1507, *1511, *1522, *1525, *1801, *1803, *1813, *3504, *3508, *3511, *3512, *3523, *3542, *3906, *4002, *4102, *4201, *4427, *4404, *4405, *4701, *4803, *5703, respectively.