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Relationship between HLA Polymorphisms and Gamma Interferon and Interleukin-10 Cytokine Production in Healthy Individuals after Rubella Vaccination▽

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We studied the association between HLA alleles and rubella-specific gamma interferon (IFN-γ) (Th1) and interleukin-10 (IL-10) (Th2) cytokine responses among 106 healthy children (ages, 14 to 17 years) previously immunized with two doses of rubella vaccine. Antibody titers and cytokine responses to rubella vaccination were not sex or age dependent. Several class I HLA-A (*0201, *2402, *6801) alleles were significantly associated with rubella vaccine-induced IFN-γ secretion. Several class II HLA-DRB1 (*0101) and HLA-DQB1 (*0501) alleles were also suggestive of an association with IFN-γ secretion. Alleles with potential associations with rubella-specific IL-10 production included HLA-A (*0201, *6801), HLA-B (*4901), and HLA-DRB1 (*1302). The class I A*0201 and A*6801 alleles were associated with both IFN-γ and IL-10 secretion. These tentative associations need to be validated in larger studies with subjects of differing ethnicities. These results provide additional evidence that HLA genes may influence Th1- and Th2-specific cytokine response(s) following rubella immunization, which in turn can influence both cellular and humoral immune responses to rubella vaccination.

Rubella, a viral infection, is still a major health concern in developing countries, with approximately 100,000 cases of congenital rubella syndrome reported worldwide each year (3, 11, 38). Although rare in the United States, the disease exists elsewhere and is imported into developed countries. For this reason, it is important to continue to vaccinate children and women of childbearing age to induce protective immunity and prevent congenital rubella syndrome. The cost of containing and treating rubella cases in developing countries is high (39), and rubella cases continue to be brought into the United States (8, 9, 12).

The vaccine currently used in the United States and in other developed countries consists of the Wistar RA 27/3 strain, attenuated by cold adaptation and serial passage through human diploid cells (37). Immunogenicity trials reveal rates of seroconversion of 92 to 98% (17, 21, 40, 45). Efficacy and immunogenicity studies have shown failure rates of 3 to 14% (5, 16, 20, 40, 43), and borderline or subprotective levels of rubella antibody may exist in percentages ranging from 4 to 23% of vaccinees 6 to 16 years after rubella vaccination (10, 25, 31, 32). The molecular mechanism for this low immune response is unknown; therefore, it is important to understand the immunogenetic mechanisms underlying poor immune response to the vaccine.

Two main CD4 T-helper-cell patterns in response to viral stimulation have been recognized (27, 28). Type 1 helper T cells produce cytokines favoring T-cell-mediated immunity and are classically assessed by measuring gamma interferon (IFN-γ) (or interleukin-2 [IL-2]). Type 2 helper T cells produce cytokines that favor the development of a strong humoral (antibody) immune response and are classically assessed by measuring IL-4 or IL-10. Thus, IFN-γ and IL-10 (or IL-4) can serve as markers to determine whether cellular or humoral immune pathways are predominantly activated, or, alternately, fail to respond to stimulation by vaccination.

Human leukocyte antigen (HLA) genes play an important role in regulating the immune response to viral pathogens via the ability to process and present a repertoire of antigenic peptides to T cells (22, 23, 41). These activated T cells produce patterns of secreted cytokines, thereby being indirectly modulated by HLA molecules to generate a response specific to the stimulatory pathogen (7). Significant differences between these cytokine secretion profiles for peripheral blood mononuclear cells (PBMC) from HLA-B8, DR3-positive individuals and those for HLA-B8, DR3-negative individuals have been observed (7, 24). In fact, HLA-B8, DR3-positive individuals produced levels of IL-2, IL-5, and IFN-γ different from those produced by negative individuals in response to mitogenic PBMC stimulation with phytohemagglutinin (PHA) but produced similar quantities of IL-4, IL-6, and IL-10 (6, 7, 24). This suggests that the HLA-peptide complexes generated in response to vaccination or infection determine the pattern of secretion of cytokines of preferential interest and hence the outcome of the immune response. However, it is not clear whether rubella vaccination has an effect on cytokine production based on the HLA genotype of the host. We hypothesized

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that HLA genes significantly impact the immune response to rubella at the level of cytokine production. To test this hypothesis, we evaluated associations between HLA class I and class II alleles and IFN-γ and IL-10 cytokine production in response to rubella vaccination in healthy subjects.

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MATERIALS AND METHODS

Study population. We conducted a large, population-based, stratified, random sample study to assess associations between HLA genes and immune responses to measles-mumps-rubella (MMR) vaccine in healthy children and young adults in Olmsted County, MN. The study subjects ($n = 106$) were randomly selected from a previously recruited and described cohort of 346 children (ages, 12 to 18) enrolled in Minnesota Independent School District 535 (34). Limited PBMC availability did not allow us to study all 346 children. Mayo Clinic's Institutional Review Board approved the study, and written informed consent and/or assent was obtained from all study participants and their guardians. Each participant had written medical record documentation of receipt of two age-appropriate doses of MMR vaccine (Merck Research Laboratories, West Point, PA). There had been no circulating rubella virus in the community within the participants' lifetimes, suggesting that immune responses were due to vaccination alone.

Cell isolation and storage. PBMC were purified from heparinized blood by Ficoll-Hypaque (Sigma, St. Louis, MO) density gradient centrifugation as described previously (14). The viability of isolated cells ranged from 95 to 98%, as determined by trypan blue exclusion. The cells were aliquoted and cryopreserved in liquid nitrogen until future use.

Rubella IgG enzyme immunoassay. Whole blood (5 ml) was collected from each subject and allowed to clot, and serum was removed after centrifugation of the sample at $900 \times g$ at room temperature. Aliquots of each serum sample were stored at −80°C. A rubella virus-specific enzyme immunoassay (Enzygnost anti-rubella-virus/immunoglobulin G [IgG] enzyme immunoassay; Dade Behring Marburg GmbH, Marburg, Germany) was used to quantify the presence of rubella-specific IgG antibodies in all serum samples as previously described (2, 34). A negative cutoff of rubella virus-specific IgG antibody level of 4.0 IU/ml was used. The coefficient of variation for this assay was 4% in our laboratory.

IFN-γ and IL-10 ELISA. Enzyme-linked immunosorbent assays (ELISA) for the quantification of rubella vaccine virus-specific IFN-γ and IL-10 secretion were performed in our laboratory as previously described (15, 33), with slight modifications. Thawed PBMC were diluted to a final concentration of 2×10^6 cells/ml with RPMI 1640 culture medium containing 25 mM HEPES (Celox Laboratories Inc., St. Paul, MN) supplemented with 100 U/ml penicillin (Sigma, St. Louis, MO), 100 μg/ml streptomycin (Sigma), 1 mM sodium pyruvate (Invitrogen, Carlsbad, CA), and 0.2% bovine serum albumin (Sigma). PBMC (2×10^5/well) from each subject were added in triplicate to flat-bottom tissue culture plates and stimulated either with 35 PFU/ml of the Wistar RA 27/3 strain of the rubella vaccine virus (Meruvax, Merck & Co., Inc., West Point, PA) (18) diluted in RPMI 1640 culture medium supplemented with 20% normal human AB serum (Pel-Freez Clinical Systems, LLC, Brown Deer, WI) or with RPMI culture medium containing 20% normal human AB serum (Pel-Freez; negative control) and cultured for 5 days (34). ELISA were performed on cell culture supernatants according to the manufacturer's instructions (BD PharMingen, San Diego, CA). The optical density of each plate was measured at 450 nm by use of a microplate reader (Molecular Devices Corporation, Sunnyvale, CA), and the concentration of each cytokine secreted was determined from the standard curve included on each plate. Mean background levels of IFN-γ and IL-10 cytokine production in cultures not stimulated with rubella virus were subtracted from the mean rubella-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.

HLA class I and class II typing. Genomic DNA was extracted from frozen EDTA blood samples by use of a Puregene extraction kit following standard procedures (Gentra Systems Inc., Minneapolis, MN). HLA class I and class II typings were performed as described previously (34, 35). Briefly, the HLA class I A locus was typed using a SeCore HLA-A locus-sequencing kit. The HLA-B locus was typed using a reference strand conformation analysis multidye B locus kit, and the HLA-Cw locus typing was performed primarily using the Cw high-resolution sequence-specific primer (SSP) UniTray (Pel-Freez Clinical Systems, LLC).

Similarly, the HLA class II DRB1 locus was typed using the DRB1 reference strand conformation analysis and SSP UniTray typing kits, while the DQB1 and DPB1 loci were typed using locus-specific high-resolution SSP UniTray typing kits (Pel-Freez Clinical Systems, LLC). Any ambiguities were resolved using additional typing procedures, and the data were analyzed using Match Tools software, as described previously (34, 35). All assays were controlled for reproducibility by repeating every 50th PCR.

TABLE 1. Immunologic characteristics of study subjects after rubella vaccination

Variable	No. of subjects	Median (IQR[a])	*P* value[c]
Rubella-specific IgG (IU/ml) for:			0.41
Both sexes	106	41.8 (23.3, 58.3)	
Males	58	39.21 (19.74, 56.69)	
Females	48	42.41 (23.90, 59.77)	
Rubella-specific IFN-γ and IL-10 secretion (pg/ml)[b]			
IFN-γ secretion for:			0.19
Both sexes	106	23.3 (−7.0, 91.3)	
Males	58	14.60 (−8.35, 76.83)	
Females	48	32.98 (0.98, 132.12)	
IL-10 secretion for:			0.99
Both sexes	106	291.0 (197.8, 355.6)	
Males	58	285.71 (202.49, 369.29)	
Females	48	294.90 (192.20, 350.72)	

[a] IQR, interquartile range (first quartile, third quartile).
[b] Rubella-stimulated median minus unstimulated median.
[c] *P* values compare distributions across gender by use of a Wilcoxon rank sum test.

Statistical analysis. Our statistical methods are similar to those in previously published reports (34, 35). Briefly, comparisons of immune responses across demographic variables of interest were carried out using Wilcoxon rank sum tests. We assessed pairwise linear associations of different immune response measures by use of Spearman correlation coefficients. Descriptive associations of cytokine response with HLA loci were evaluated on an allelic level, grouped for each locus by allele subtype, and summarized using medians and interquartile ranges. Associations were then more formally evaluated using linear regression analyses. In contrast to the descriptive comparisons, each subject contributed one observation to the regression analysis, with allelic variables coded as 0, 1, or 2, according to the number of copies of the allele that a subject carried. Alleles with fewer than five occurrences among all subjects were pooled into a category labeled "other." Due to data skewness, response values for each regression model were replaced with rank-transformed values. Global differences in cytokine response among all alleles within a given locus were assessed 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 global significance. Each allele variable was included in a separate linear regression analysis, effectively comparing cytokine response for the allele of interest against those of all other alleles combined.

All global and allelic analyses described above were adjusted for race, gender, age at enrollment, age at first MMR vaccination, and age at second MMR vaccination. All statistical tests were two sided, and all analyses were carried out using the SAS software system (SAS Institute, Inc., Cary, NC).

RESULTS

Study participants. A total of 106 healthy subjects, ranging in age from 14 to 17 years, were recruited into the study. The majority of study participants were white (93.4%). The median ages (first quartile, third quartile) at the first and second immunizations were 15.9 (15.2, 18.3) months and 12.2 (11.7, 12.6) years, respectively. There were a total of 48 (45.3%) females and 58 (54.7%) males in the study. The median value for circulating rubella IgG antibody level was 41.8 IU/ml (Table 1). Both female and male subjects demonstrated similar rubella

TABLE 2. HLA allelic associations with rubella virus vaccine-specific cytokine IFN-γ responses

Locus	Allele[a]	Allele count	IFN-γ secretion value (pg/ml)			Allele *P* value[b]
			Median	First (lower) quartile	Third (upper) quartile	
All loci	All alleles	212	23.28	−6.99	91.32	
HLA-A	*0101	34	23.89	−0.33	89.18	—
	*0201	63	30.79	0.35	117.12	**0.03**
	*0301	35	14.04	−1.58	41.86	—
	*1101	8	18.73	−13.36	39.95	—
	*2402	23	4.64	−34.33	55.81	**0.04**
	*2501	5	41.86	−34.05	61.23	—
	*3101	6	13.77	−20.2	64.88	—
	*3201	7	23.05	−160.4	99.29	—
	*6801	13	3.03	−51.54	62.78	**0.03**
	Other	18	93.78	32.48	268.31	—
HLA-B	*0702	23	16.33	−8.02	93.78	—
	*0801	22	11.9	−28.84	53.78	—
	*1302	6	12.57	−14.46	36.2	—
	*1501	20	11.13	−15.6	93.23	—
	*1801	7	49.93	−34.05	410.16	—
	*2705	10	34.35	2.6	91.76	—
	*3501	9	32.49	11.43	118.02	—
	*3801	7	199.9	−53.57	287.15	—
	*4001	14	70.86	−1.83	117.12	—
	*4402	25	12.36	−29.33	61.23	—
	*4403	9	36.2	11.43	93.78	—
	*4901	7	118.02	−9.32	265.56	—
	*5101	6	54.91	−1.58	122.61	—
	*5501	5	13.17	1.61	93.3	—
	*5701	5	53.22	27.86	189.05	0.07
	Other	37	23.5	2.6	55.59	—
HLA-Cw	*0102	9	76.34	3.04	181.36	—
	*0202	12	41.69	−4.17	105.77	—
	*0303	16	18.73	−31.58	90.23	—
	*0304	21	64.88	3.03	117.12	0.07
	*0401	22	28.39	3.04	99.29	—
	*0501	21	9.8	−29.33	55.81	—
	*0602	16	22.09	10.72	125.91	—
	*0701	30	12.86	−24.49	84.01	—
	*0702	27	9.8	−8.02	83.57	—
	*0704	5	1.61	−87.18	23.5	—
	*1203	16	51.55	−13.59	277.73	—
	*1601	5	23.05	11.43	36.2	—
	Other	12	32.98	12.48	39.66	—
HLA-DRB1	*0101	19	91.32	14.04	214.5	**0.03**
	*0301	24	12.86	−9.5	58.28	—
	*0401	23	13.17	−8.35	76.34	—
	*0404	8	15.33	−8.04	47.83	—
	*0701	22	30.67	11.43	53.78	—
	*0801	6	9.29	−1.58	30.79	—
	*0901	5	61.23	−29.37	76.83	—
	*1101	10	77.41	13.17	120.22	—
	*1104	5	199.9	16.33	410.16	—
	*1201	5	−8.02	−8.06	37.46	—
	*1301	18	9.9	−1.83	62.78	—
	*1302	11	64.88	−23.15	117.12	—
	*1401	6	69.56	23.5	118.02	—
	*1501	22	47.42	−20.2	268.31	—
	Other	28	8.5	−8.16	55.59	—
HLA-DQB1	*0201	27	12.36	−4.55	53.78	—
	*0202	17	33.48	11.43	49.93	—
	*0301	45	16.33	−8.02	93.3	—
	*0302	19	15.13	−8.06	61.23	—
	*0303	11	61.23	−29.37	99.29	—
	*0402	7	2.6	−23.9	15.98	—

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TABLE 2—*Continued*

Locus	Allele[a]	Allele count	IFN-γ secretion value (pg/ml)			Allele *P* value[b]
			Median	First (lower) quartile	Third (upper) quartile	
	*0501	26	91.54	14.04	222.75	**0.03**
	*0503	6	69.56	23.5	118.02	—
	*0602	26	44.64	−29.33	144.06	—
	*0603	16	9.9	−1.71	58.03	—
	Other	12	33.62	−32.5	103.15	—
HLA-DPB1	*0101	17	14.04	−0.33	62.78	—
	*0201	21	49.93	2.6	87.16	—
	*0301	16	52.76	6.77	99.48	—
	*0401	91	23.5	−8.35	89.18	—
	*0402	29	15.17	−8.35	144.06	—
	*0601	5	19.23	4.64	64.88	—
	Other	33	23.05	−1.58	99.29	—

[a] "Other" includes the following: HLA-A alleles *0102, *0202, *0205, *0206, *0207, *0302, *2301, *2403, *2601, *2608, *2902, *3001, *3002, *3206, *3301, *6601, *6802, *6803, and *6901; HLA-B alleles *0704, *0705, *0706, *0714, *1301, *1401, *1402, *1502, *1503, *1506, *1510, *1516, *1517, *1518, *1522, *1525, *1803, *2702, *3502, *3503, *3504, *3508, *3523, *3701, *3901, *3902, *3906, *4002, *4101, *4201, *4404, *4405, *4501, *4601, *4701, *4801, *4803, *5201, *5301, *5601, *5702, *5801, *5802, and *7301; HLA-Cw alleles *0302, *0403, *0406, *0604, *0706, *0710, *0712, *0801, *1202, *1402, *1502, *1505, *1602, *1604, *1701, *1703, and *1802; HLA-DRB1 alleles *0102, *0103, *0302, *0402, *0403, *0405, *0407,*0408, *0802, *0803, *0804, *1001, *1102, *1103, *1105, *1106, *1111, *1119, *1202, *1208, *1303, *1304, *1305, *1310, *1315, *1405, *1406, *1410, *1424, *1502, *1503, *1601, and *1602; HLA-DQB1 alleles *0203, *0304, *0306, *0502,*0601, *0604, *0608, *0609, and *0614; and HLA-DPB1 alleles *0501,*0901, *1001, *1101, *1301, *1401, *1501,*1601, 1701,*1901, *2001, *2101, *2301, *2401, *2601, *3001, *5001, *5101, *6301, and *6601.

[b] Linear regression analysis was done to study allelic associations. Due to data skewness, *P* values were based in rank-transformed data. Analyses were adjusted for age at blood draw, gender, race, age at first MMR, and age at second MMR. *P* values of ≤0.05 are shown in bold. —, *P* values of >0.10 have been omitted. The global *P* values for HLA-A, HLA-B, HLA-Cw, HLA-DRB1, HLA-DQB1, and HLA-DPB1 loci were 0.03, 0.60, 0.53, 0.42, 0.29, and 0.85, respectively.

antibody responses (median values of 42.4 IU/ml versus 39.2 IU/ml, respectively); females demonstrated slightly higher antibody levels than males, but this difference was not statistically significant ($P = 0.41$). All study subjects were antibody seropositive after receiving two doses of the rubella vaccine. Rubella virus vaccine was able to induce a recall rubella-specific in vitro IFN-γ and IL-10 cytokine response from PBMC of previously immunized subjects. Median values (first quartile, third quartile) for rubella-specific IFN-γ and IL-10 cytokines were 23.3 (−7.0, 91.3) pg/ml and 291.0 (197.8, 355.6) pg/ml, respectively. Cytokine responses to rubella vaccination were not sex or age dependent (median IFN-γ levels, 33.0 pg/ml for females versus 14.6 pg/ml for males [$P = 0.19$]; median IL-10 levels, 294.9 pg/ml for females versus 285.7 pg/ml for males [$P = 0.99$]). Rubella-specific IFN-γ and IL-10 responses were detected in 71% and 99% of subjects, respectively. The linear correlation between rubella-specific IFN-γ and IL-10 secreted cytokines was 0.26 based on rank-transformed data ($P = 0.007$). Finally, we did not find any correlation between antibody levels to rubella and IFN-γ ($r = 0.10$, $P = 0.32$) and IL-10 ($r = 0.02$, $P = 0.86$) cytokines.

Associations between HLA alleles and IFN-γ and IL-10 responses. The association between class I and class II HLA loci and rubella virus-specific IFN-γ (Th1) cytokine secretion is shown in Table 2. Global statistical tests revealed significant associations with the HLA-A locus ($P = 0.03$) but did not reveal a statistically significant association between IFN-γ levels and the HLA-B, HLA-Cw, HLA-DRB1, HLA-DQB1, and HLA-DPB1 loci (*P* values of 0.60, 0.53, 0.42, 0.29, and 0.85, respectively). When alleles were examined individually, HLA associations with higher rubella-specific IFN-γ responses could be potentially attributed to several HLA alleles, including A*0201 (median, 30.79 pg/ml; $P = 0.03$), B*5701 (median, 53.22 pg/ml; $P = 0.07$), Cw*0304 (median, 64.88 pg/ml; $P = 0.07$), DRB1*0101 (median, 91.32 pg/ml; $P = 0.03$), and DQB1*0501 (median, 91.54 pg/ml; $P = 0.03$). Conversely, A*2402 (median, 4.64 pg/ml; $P = 0.04$) and A*6801 (median, 3.03 pg/ml; $P = 0.03$) alleles were both associated with lower IFN-γ secretion. When the associations were analyzed at the two-digit specificity level, global tests revealed a significant association between the IFN-γ secretion level in response to rubella antigens and the HLA-DQB1 locus ($P = 0.05$). In particular, the DQB1*05 allele (median, 89.18 pg/ml; $P = 0.02$) was significantly associated with increased IFN-γ secretion.

The association between class I and class II HLA loci and rubella-specific IL-10 (Th2) cytokine secretion was also examined, and the results are summarized in Table 3. Global tests failed to find a statistically significant association with IL-10 cytokine responses and class I (HLA-A, HLA-B, and HLA-Cw [*P* values of 0.29, 0.39, and 0.54, respectively]) and class II (HLA-DRB1, HLA-DQB1, and HLA-DPB1 [*P* values of 0.16, 0.13, and 0.85, respectively]) loci. However, univariate analyses identified potential associations between higher IL-10 responses and alleles A*0201 (median, 335.88 pg/ml; $P = 0.004$), B*4901 (median, 403.42, $P = 0.004$), Cw*0102 (median, 374.61; $P = 0.06$), and DRB1*1302 (median, 344.49 pg/ml; $P = 0.03$). In addition, we found suggestive associations between lower rubella vaccine-specific IL-10 secretion and the alleles A*6801 (median, 269.18; $P = 0.004$), Cw*0701 (median, 288.55 pg/ml; $P = 0.08$), DRB1*0701 (median, 246.80 pg/ml; $P = 0.07$), and DQB1*0202 (median, 259.87 pg/ml; $P = 0.06$). However, these associations should be interpreted with caution due to the nonsignificance of the global tests. Interestingly, the group of "other" alleles (HLA-B and HLA-DQB1) also was found to be statistically significant. No associations were found with HLA-DPB1 alleles and either IFN-γ or IL-10 rubella-induced secretion.

TABLE 3. HLA allelic associations with rubella virus vaccine-specific cytokine IL-10 responses

Locus	Allele[a]	Allele count	IL-10 secretion value (pg/ml)			Allele *P* value[b]
			Median	First (lower) quartile	Third (upper) quartile	
All loci	All alleles	212	291.04	197.84	355.59	
HLA-A	*0101	34	282.33	196.28	392.48	—
	*0201	63	335.88	224.89	377.8	**0.004**
	*0301	35	293.53	185.92	349.71	—
	*1101	8	326.36	253.49	352.29	—
	*2402	23	259.87	160.9	329.16	—
	*2501	5	185.92	168.34	233.72	—
	*3101	6	313.37	202.49	343.33	—
	*3201	7	283.32	188.33	391.53	—
	*6801	13	269.18	197.84	282.97	**0.004**
	Other	18	300.83	275.56	338.23	—
HLA-B	*0702	23	296.25	269.18	352.08	—
	*0801	22	280.36	193.36	374.61	—
	*1302	6	266.83	112.96	369.29	—
	*1501	20	299.22	195.6	363.29	—
	*1801	7	271.53	168.34	293.79	—
	*2705	10	278	202.49	374.61	—
	*3501	9	267.37	212.75	361.24	—
	*3801	7	329.16	258.1	377.8	—
	*4001	14	300.15	271.62	344.49	—
	*4402	25	262.84	193.88	343.33	—
	*4403	9	289.06	260.88	305.41	—
	*4901	7	403.42	338.1	447.18	**0.004**
	*5101	6	338.27	305.62	392.48	—
	*5501	5	278.38	193.88	283.32	—
	*5701	5	282.33	167.87	403.42	—
	Other	37	288.09	185.92	335.88	0.06
HLA-Cw	*0102	9	374.61	333.22	458.65	0.06
	*0202	12	242.88	178.65	305.31	—
	*0303	16	288.17	195.86	363.29	—
	*0304	21	304.29	271.62	350.94	—
	*0401	22	309.11	212.75	391.53	—
	*0501	21	262.84	196.28	343.33	—
	*0602	16	310.68	212.99	411.96	—
	*0701	30	288.55	212.75	401.47	0.08
	*0702	27	293.79	174.02	349.71	—
	*0704	5	304.29	197.84	333.82	—
	*1203	16	311.35	222.01	374.4	—
	*1601	5	267.37	142.21	296.25	—
	Other	12	273.98	164.06	340.78	—
HLA-DRB1	*0101	19	293.02	212.75	374.61	—
	*0301	24	280.36	191.94	361.73	—
	*0401	23	275.56	168.34	355.59	—
	*0404	8	324.95	265.11	350.72	—
	*0701	22	246.8	154.81	296.25	0.07
	*0801	6	248.28	145.45	269.18	—
	*0901	5	352.08	196.28	374.57	—
	*1101	10	332.52	215.6	391.53	—
	*1104	5	370.99	271.53	424.53	—
	*1201	5	224.89	188.33	288.09	0.10
	*1301	18	284.55	193.88	329.16	—
	*1302	11	344.49	282.97	429.63	**0.03**
	*1401	6	319.05	293.79	392.48	—
	*1501	22	294.78	271.53	352.08	—
	Other	28	312.07	205.9	392.75	—
HLA-DQB1	*0201	27	278.38	188.33	348.85	—
	*0202	17	259.87	145.45	293.79	0.06
	*0301	45	279.5	196.28	370.99	—
	*0302	19	324.66	262.84	350.94	—
	*0303	11	333.22	196.28	391.53	—

Continued on following page

TABLE 3—*Continued*

Locus	Allele[a]	Allele count	IL-10 secretion value (pg/ml)			Allele *P* value[b]
			Median	First (lower) quartile	Third (upper) quartile	
	*0402	7	260.88	145.45	305.62	—
	*0501	26	294.52	212.75	374.61	—
	*0503	6	319.05	293.79	392.48	—
	*0602	26	296.14	271.53	361.24	—
	*0603	16	274.17	183.95	321.83	—
	Other	12	340.19	277.88	457.27	0.04
HLA-DPB1	*0101	17	306.87	254.02	374.61	—
	*0201	21	293.79	215.6	399.09	—
	*0301	16	333.22	290.94	347.1	—
	*0401	91	288.09	193.36	355.59	—
	*0402	29	275.56	193.88	370.99	—
	*0601	5	282.97	254.02	344.49	—
	Other	33	289.06	197.84	352.08	—

[a] "Other" includes the following: HLA-A alleles *0102, *0202, *0205, *0206, *0207, *0302, *2301, *2403, *2601, *2608, *2902, *3001, *3002, *3206, *3301, *6601, *6802, *6803, and *6901; HLA-B alleles *0704, *0705, *0706, *0714, *1301, *1401, *1402, *1502, *1503, *1506, *1510, *1516, *1517, *1518, *1522, *1525, *1803, *2702, *3502, *3503, *3504, *3508, *3523, *3701, *3901, *3902, *3906, *4002, *4101, *4201, *4404, *4405, *4501, *4601, *4701, *4801, *4803, *5201, *5301, *5601, *5702, *5801, *5802, and *7301; HLA-Cw alleles *0302, *0403, *0406, *0604, *0706, *0710, *0712, *0801, *1202, *1402, *1502, *1505, *1602, *1604, *1701, *1703, and *1802; HLA-DRB1 alleles *0102, *0103, *0302, *0402, *0403, *0405, *0407,*0408, *0802, *0803, *0804, *1001, *1102, *1103, *1105, *1106, *1111, *1119, *1202, *1208, *1303, *1304, *1305, *1310, *1315, *1405, *1406, *1410, *1424, *1502, *1503, *1601, and *1602; HLA-DQB1 alleles *0203, *0304, *0306, *0502,*0601, *0604, *0608, *0609, and *0614; and HLA-DPB1 alleles *0501,*0901, *1001, *1101, *1301, *1401, *1501,*1601, 1701,*1901, *2001, *2101, *2301, *2401, *2601, *3001, *5001, *5101, *6301, and *6601.

[b] Linear regression analysis was done to study allelic associations. Due to data skewness, *P* values were based in rank-transformed data. Analyses were adjusted for age at blood draw, gender, race, age at first MMR, and age at second MMR. *P* values of ≤0.05 are shown in bold. —, *P* values of >0.10 have been omitted. The global *P* values for HLA-A, HLA-B, HLA-Cw, HLA-DRB1, HLA-DQB1, and HLA-DPB1 loci were 0.29, 0.39, 0.54, 0.16, 0.13, and 0.85, respectively.

DISCUSSION

This report provides additional data relevant to the associations between HLA polymorphisms and rubella virus vaccine-specific IFN-γ and IL-10 cytokine immune responses following rubella vaccination in healthy children. We demonstrate that HLA alleles and rubella-specific IFN-γ and IL-10 cytokine immune responses play an important role when evaluating immune responses to rubella vaccination. The present study also revealed that rubella-specific IgG antibody titers and cytokine responses to rubella vaccination were not sex or age dependent.

Little is known about associations between HLA genes and rubella vaccine-induced Th1 and Th2 cytokine production. Early papers suggested that the production of IFN-γ by human peripheral lymphocytes stimulated with rubella virus did not require the addition of macrophages and helper/inducer T cells were the main producers of IFN-γ (29). Others demonstrated the ability of rubella virus to replicate in both unstimulated and PHA-stimulated human lymphocytes as well as in macrophages and lymphocyte subpopulations (44). Recent studies demonstrated transient increases in serum levels of IL-10 in children with acute rubella infection (1) and increased production of anti-inflammatory IL-10 on day 30 after rubella vaccination (42). These changes were accompanied by significant reductions in plasma IFN-γ and a profound decrease in the lymphocyte response to PHA (42).

The outcome of the immune response to rubella is regulated by a number of molecules, including the HLA and cytokine production by T cells. These cytokine secretion profiles can be considered as either Th1 responses promoting cellular immunity or Th2 responses promoting humoral immunity (27, 28). The proinflammatory cytokine IFN-γ plays a key role in T-cell proliferation and differentiation and macrophage activation and is known to be an inducer of HLA molecule surface expression and an enhancer of cell-mediated immunity (19, 36, 46). Likewise, human IL-10 is known to be an important anti-inflammatory cytokine which stimulates growth of B cells and secretion of IgG (4). Functionally, IL-10 inhibits HLA class II and CD80 expression, antigen-presenting cell functions, and cytokine production of Th1 and Th2 cells (4, 13).

Numerous studies have demonstrated HLA regulation of cytokine production and, hence, the type of the immune response in both humans and mice (7). Our previous studies demonstrated associations between rubella humoral and cellular immune responses following rubella vaccination and HLA genes in immune response variation (34, 35). Further, Mitchell et al. (26) demonstrated that certain HLA-DR2 (*1501, *1601) and HLA-DR5 (*1101/*1104, *1201) alleles may influence susceptibility to the development of joint manifestations after the administration of live attenuated rubella vaccine viruses. However, there have been no studies of HLA class I and class II associations with cytokine outcomes after rubella virus exposure, such as vaccination.

In our study, multivariate linear regression modeling of associations between HLA class I and class II antigen groups and cytokine secretion suggested that IFN-γ secretion was significantly associated with class I HLA-A*0201, A*2402, and A*6801 alleles. In addition, suggestive associations were observed with class I HLA-B*5701 and HLA-Cw*0304 alleles and with class II HLA-DRB1*0101 and HLA-DQB1*0501 alleles. We also found associations between specific class I A*0201, A*6801, B*4901, Cw*0102, and Cw*0701 alleles and class II DRB1*0701, DRB1*1302, and DQB1*0202 alleles and IL-10 secretion. These data highlight the critical importance of examining T-cell cytokine responses to rubella virus antigens and the need to define such responses in rubella-immunized

and -infected individuals from diverse genetic backgrounds. Importantly, two class I alleles, HLA-A*0201 and A*6801, were associated with both IFN-γ and IL-10 secretion following rubella vaccination. Specifically, A*0201 was associated with higher rubella-specific IFN-γ and IL-10 responses. In contrast, A*6801 was associated with lower levels of rubella-induced IFN-γ and IL-10 production. These findings suggest that specific HLA molecules may significantly influence cytokine responses to antigenic stimulation and hence immune outcomes. Though HLA genes have been associated with cytokine secretion levels, single-nucleotide polymorphisms in either the IFN-γ or IL-10 genes, or cytokine receptor genes, or other genes may also influence immune responses after rubella vaccination.

We found a potential association between rubella-specific IL-10 secretion and the DRB1*1302 allele ($P = 0.03$); however, the DRB1*1302 allele was not associated with rubella antibody levels in our previous work (34, 35). In this study, rubella antibody levels also lacked any correlation with measures of IL-10 ($r = 0.02$, $P = 0.86$) cytokine secretion. Similar disagreement in association between antibody levels to measles virus and IL-4 ($r = 0.04$, $P = 0.45$) secretion and measures of CD4 T-cell immunity was found in other studies (15, 30). The complex interplay between genetic HLA polymorphisms, secreted cytokines, and measures of humoral and cellular immune responses to rubella vaccine currently precludes a validated model to explain these associations.

A strength of our study is the stratified random sample of our well-characterized cohort of children used to test our hypothesis. School rosters in Minnesota Independent School District 535 were used as the sampling frame from which an age-stratified random sample of healthy children from all socioeconomic strata was obtained. Also, the humoral and cellular immune responses observed were solely due to vaccination, as these subjects had not encountered the wild-type rubella virus in their lifetimes. There are a few limitations to our study. First, associations with some rare HLA alleles may have been missed due to our modest sample size. Second, due to the number of statistical tests, the possibility of associations by chance alone cannot be dismissed. The fact that we found significant associations with IL-10 secretion and the pooled group of "other" alleles for HLA-B and HLA-DQB1 highlights this possibility. Thus, our findings should be confirmed in large studies with subjects of differing ethnicities.

Our previous work demonstrated that levels of rubella-specific IgG antibody were higher in females than in males ($P = 0.02$) (34, 35). In the present study, females also demonstrated higher antibody levels than males, but this difference was not statistically significant ($P = 0.41$). The discrepancy between our gender associations in the original study and in the current study is partially due to less power but also partially due to a smaller effect size: 42.4 IU/ml for females and 39.2 IU/ml for males in the current study, compared to 42.4 IU/ml and 33.9 IU/ml, respectively, in the previous study (34, 35). In the original study, we examined 346 children following two doses of rubella vaccine; in the current study, we examined a group of 106 children after two doses of rubella vaccine. Since our study group was moderately small, it is important that our results be confirmed with a larger study population.

In conclusion, our study results provide data suggesting that certain class I and class II HLA alleles may influence the variability of rubella-induced IFN-γ and IL-10 cytokine immune responses following the administration of live attenuated rubella vaccine viruses. In turn, these Th1- and Th2-like cytokine responses may impact the outcome of both cellular and humoral immune responses to rubella virus vaccine or infection. Further work is needed to better understand the complex interplay between cytokines and the role of HLA gene polymorphisms in the outcome of rubella immunity among populations with various HLA allelic distributions.

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G.A.P. chairs a DMSB for clinical trials of new vaccines for Merck, but none of these trials involve rubella vaccines. G.A.P. and R.M.J. have performed Merck-sponsored trials of non-MMR vaccines. All other authors report no potential conflicts of interest.

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