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HLA supertypes and immune responses to measles–mumps–rubella viral vaccine: Findings and implications for vaccine design

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Abstract

Although the outcome of the immune response to measles–mumps–rubella (MMR) vaccination depends on multiple factors, elucidation of specific host genetic markers, such as HLA supertypes based on a shared sequence motif in the peptide-binding pockets of HLA molecules, is essential. We studied the association between measures of humoral and cellular immune responses and HLA supertypes among 346 children previously immunized with two doses of MMR. We found that HLA supertypes, such as A3, B7, B44, B58, B62, and DR may play a role in modulating immune responses to the measles and mumps components of MMR vaccine. This information may be of significant value in the engineering of potential epitope-based vaccines that are recognized by T cells restricted by human HLA supertype alleles.

Keywords: MMR vaccine; HLA supertypes; Immunity

1. Introduction

The immune response to the measles–mumps–rubella (MMR) vaccine depends upon the development of effective humoral and cell-mediated immune responses [1,2]. Although the outcome of the immune response to MMR vaccination depends on multiple factors, elucidation of specific host genetic markers, such as human leukocyte antigen (HLA) supertypes based on a shared sequence motif in the peptide-binding pockets of HLA molecules, would be advantageous for the engineering a potential new vaccine. We previously reported an association between HLA haplotypes and MMR vaccine-induced immunity [3]. However, the relationship between HLA supertypes and measures of MMR immune response is less well established.

Studies have demonstrated that the majority of HLA class I and class II molecules can be grouped in broad supertypes characterized by overlapping peptide-binding motifs and repertoires [4–6]. Although HLA molecules are highly polymorphic, there are shared epitopes with substantial overlap in peptide-binding capacity within groups of genetically related HLA alleles. Based on the structural similarities of these groups of HLA alleles and analysis of their peptide-binding specificity, the following class I supertype molecules have been proposed; HLA-A1, A2, A3, A24, B7, B27, B44, B58, and B62 supertype groups [7-9]. In the case of class II HLA molecules, Southwood et al. [6] identified a large set of HLA-DR molecules, which includes DRB1*0101, DRB1*0401, DRB1*0701, DRB5*0101, DRB1*0901, DRB1*1302, and DRB1*1501; characterized by overlapping peptide-binding repertoires. In addition, Ou et al. [10] described a novel categorization of HLA-DR alleles on the basis of function. Thus, HLA-DR molecules were grouped into functional categories on the basis of their ability to bind and present antigenic peptides to T cells, and to shape T cell repertoires and

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their association with susceptibility or resistance to various diseases [10]. The particular supertype (supermotif) within pocket 4 of HLA-DR includes DRB1*0301, DRB1*0401, DRB1*0402, DRB1*0404, DRB1*0405, DRB1*1101, DRB1*1201, DRB5*0101, and DRB1*1501 alleles [10].

Applying the concept of HLA supertypes to a novel MMR vaccine is attractive as HLA supertypes would narrow the search for antigenic peptides that will bind HLA alleles of a large proportion of the population (so-called "promiscuous peptides"). For our purposes, an important question arises. Are common class I and class II HLA supertypes associated with variations in MMR vaccine-induced immunity in healthy individuals? To answer this question, we sought to determine whether associations exist between HLA supertypes and the humoral and cellular immune status following two doses of MMR for each of the three vaccines, measles, mumps, and rubella. Further, we sought to assess associations between HLA supertypes and measles virus-specific IFN-γ and IL-4 cytokine levels in healthy individuals after two doses of the MMR vaccine.

2. Materials and methods

2.1. Study participants

Details of our study methods and subject identification have been published elsewhere [11]. Overall, we recruited a total of 346 children 12–18 years of age in Olmsted County, Minnesota. Study subjects (161 girls and 185 boys) had medical record documentation of receiving two doses of the MMR vaccine containing the Edmonston strain of measles virus (tissue culture infective dose $TCID_{50} \geq 1000$), the Jeryl Lynn B-strain of mump virus ($\geq$20,000 $TCID_{50}$), and the Wistar RA 27/3-strain of rubella virus ($\geq$1000 $TCID_{50}$) (Merck, West Point, PA, USA) at or after the age of 12 months. HLA and measles-specific cytokine data were available on 339 subjects. Mayo Clinic's Institutional Review Board approved the study. All study subjects or guardians provided written informed consent according to the guidelines of the Institutional Review Board of the Mayo Clinic. Younger subjects also provided written assent.

2.2. Antibody measurement

Quantitative levels of measles, mumps, and rubella IgG antibody titers for all serum specimens were determined by the Enzygnost (Dade Behring, Marburg, Germany) anti-measles-virus/IgG enzyme immunoassay (EIA) (sensitivity = 99.6%; specificity = 100%), anti-mumps-virus/IgG EIA (sensitivity = 95.4%; specificity = 93.7%), and anti-rubella-virus/IgG EIA (sensitivity = 100%; specificity = 98.5%), respectively, according to the manufacturer's instructions. The coefficients of variation in our laboratory for the measles, mumps and rubella assays were 3.8%, 4.1%, and 4.0%, respectively.

2.3. Lymphoproliferation assays

Peripheral blood mononuclear cells (PBMC) were separated on Ficoll-Hypaque (Sigma, St. Louis, MO, USA) density gradients and resuspended in RPMI 1640 freezing media (Celox Laboratories Inc., St. Paul, MN, USA) containing 10% dimethyl sulfoxide (Sigma) and 20% heat-inactivated fetal calf serum (FCS), frozen at −80 °C, and stored in liquid nitrogen until cultured. The cellular immune status to MMR viruses was assessed using an *in vitro* [^{3}H]-thymidine incorporation assay as described [2,12]. Individual vaccine virus-specific T cell responses were measured by proliferation of PBMC (2×10^5 cells/well) incubated in the presence of live attenuated measles, mumps or rubella virus vaccine (Merck, 75 pfu/well of each vaccine virus). Results were expressed as antigen-specific stimulation indices (SI), defined as the ratio of the median counts per minute (cpm) of antigen-stimulated wells to the median cpm of unstimulated wells. An SI of three or higher was considered to be an indicator of a positive lymphoproliferarative response [13].

2.4. HLA typing

Details of HLA typing have been published elsewhere [11]. High molecular weight DNA was extracted from blood samples using the Puregene extraction kit (Gentra Systems Inc., Minneapolis, MN, USA) and used for polymerase chain reaction (PCR)—based high resolution HLA genotyping (Dynal Biotech, Brown Deer, WI, USA). Class I and class II four-digit molecular typing was performed for supertype classification. Specifics for HLA class I and class II DR supertypes classification based on a shared sequence motif in peptide-binding pockets of HLA molecules, have been described in detail elsewhere [5–7,10].

2.5. In vitro IFN-γ and IL-4 measles-specific cytokine assays

In vitro cytokine responses to measles virus were measured as previously described [14]. Briefly, for IFN-γ determination, PBMC were cultured for 6 days at a concentration of 2×10^5 cells/well with either medium or measles virus at a multiplicity of infection (moi) of 0.5 (the Edmonston B vaccine strain of measles virus stock at 1×10^7 plaque forming units [pfu]/ml). For IL-4 determination, the PBMC were cultured at a concentration of 4×10^5 cells/well. Cells were cultured in the presence of 2 μg/ml of monoclonal IL-4 receptor antibody (mAb) (R&D Systems, Minneapolis, MN, USA) with or without measles virus diluted in RPMI 1640 supplemented with 1% normal human sera (NHS) at a moi of 0.1 as previously described [15]. The secretion of IFN-γ and IL-4 was evaluated by a standard ELISA (OptiEIA Human IFN-γ and IL-4; PharMingen, San Diego, CA). Median background levels of IFN-γ and IL-4 cytokine production in cultures not stimulated with measles virus was subtracted from the median measles-induced responses to produce corrected secretion

values. The levels of sensitivity for the IFN-γ and IL-4 assays were 4.7 pg/ml and 7.8 pg/ml, respectively.

2.6. Statistical analysis

Eight outcomes were of primary interest: measles, mumps and rubella antibody titers (measured as IU/ml); measles, mumps and rubella lymphoproliferation (measured as SI); and IFN-γ and IL-4 supernatant cytokine responses to measles virus (measured as pg/ml). Data were descriptively summarized using frequencies and percentages for all categorical variables, and medians and interquartile ranges (IQRs) for all continuous variables. Associations of immune response with demographic and clinical variables of interest were assessed using analysis of variance methods. Due to data skewness, all *p*-values were calculated using transformed immune response values. We used simple log transformations for antibody and lymphoproliferation levels. Such transformations could not be used for cytokine secretion levels due to the possibility of zero or negative values (caused when unstimulated secretion levels equaled or exceeded stimulated secretion levels); for these variables rank transformations were used.

Measures of immune response associated with HLA loci were summarized on a supertype allele level. Each person contributed two observations to this descriptive analysis—one for each allele. Alleles were grouped for each locus by allele supertype and summarized using medians and interquartile ranges. Following the descriptive comparisons, associations were formally evaluated using linear regression analyses. In contrast to the descriptive comparisons, each subject contributed one observation to the regression analysis based on the individual genotype. Regression variables were created for each supertype allele and were coded as 0, 1, or 2, according to the number of copies of the allele that a subject carried. As mentioned above, transformed version of the original response values for each of the outcomes of interest were used as the dependent variables in these regression models. Global differences in immune response among all alleles of a given locus were carried out by simultaneously including all but one of the allele variables in a multivariate linear regression model.

Following these global tests, we examined individual supertype allele effects on immune 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. In these follow-up analyses, each supertype allele variable was included in separate linear regression analyses, effectively comparing degree to which the immune response variable was associated with genotypic dosage of the allele of interest.

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

3. Results

3.1. HLA supertypes in study subjects previously immunized with MMR

Table 1 lists the alleles confirmed to be part of the HLA class I and class II (DR) supertypes and their estimated frequencies in our study group. The majority of subjects were white (94%), and the median ages at first and second immunization were 15.6 months and 12.1 years, respectively. The most prevalent alleles in the study population were expressed in frequencies similar to the HLA subtype frequencies published elsewhere [6,7]. Molecular HLA typing of 346 study subjects yielded 104 HLA-A2 individuals (30.2%), reflecting A2 supertype expression in a largely white population [7]. In addition, the A3 supertype alleles were found in 29.5% of study subjects. It was noted that the most prevalent HLA-B supertype in this study population were B7 and B62, which were expressed in 28.2% and 25.7% of individuals, respectively. In regard to class II, approximately 47% of our study subjects carried common alleles of the main HLA-DR supertype (Table 1).

3.2. HLA supertypes and measles vaccine immune responses

Separate analyses were performed for each measure of humoral and cellular immunity. Median values for measles antibody levels and SI were 1556.0 IU/ml and 3.6, respectively (Table 2). The two global tests for association failed to show a statistically significant association between class I A supertypes and measles antibody levels and lymphocyte proliferation (*p*-value of 0.10 and 0.73, respectively). The global tests did, however, reveal a significant association between humoral immune responses to measles virus and class I B supertypes (*p*-value of 0.01). In particular, B7 supertype (median 1848 IU/ml; $p = 0.01$) was associated with higher measles-specific antibody levels. In contrast, the supertypes with the strongest associations with lower measles antibody levels included B44 (median 1337 IU/ml; $p = 0.03$) and B58 (median 1079 IU/ml; $p = 0.06$). No association was found with class I B supertypes and cellular immunity (global *p*-value = 0.41).

For the main (Southwood) class II HLA-DR supertype, which was initially characterized by overlapping peptide-binding repertoires (Southwood's et al. classification) [6], we found no associations with either the measles antibody or lymphoproliferation levels (global *p*-values of 0.29 and

Table 1
Frequency of the HLA supertypes in 346 study subjects[a]

HLA supertype[b]	Allele(s)	Number of alleles	% Allele	Frequency (total %)
A1				23.98
	*0101	111	16.04	
	*0102	1	0.14	
	*2501	16	2.31	
	*2601	16	2.31	
	*3201	22	3.18	
A2				30.18
	*0201	199	28.76	
	*0202	1	0.14	
	*0205	3	0.43	
	*0206	1	0.14	
	*0207	1	0.14	
	*6802	3	0.43	
	*6901	1	0.14	
A3				29.49
	*0301	108	15.61	
	*1101	30	4.34	
	*3101	19	2.75	
	*3301	4	0.58	
	*6801	43	6.21	
A24				11.99
	*2301	12	1.73	
	*2402	60	8.67	
	*2403	1	0.14	
	*3001	6	0.87	
	*3002	4	0.58	
B7				28.17
	*0702	83	11.99	
	*0704	2	0.29	
	*0705	1	0.14	
	*3501	38	5.49	
	*3502	3	0.43	
	*3503	18	2.6	
	*5101	31	4.48	
	*5301	2	0.29	
	*5501	15	2.17	
	*5601	2	0.29	
B27				10.54
	*1401	3	0.43	
	*1402	7	1.01	
	*1503	2	0.29	
	*1510	2	0.29	
	*1518	1	0.14	
	*2702	2	0.29	
	*2705	28	4.05	
	*3801	16	2.31	
	*3901	8	1.16	
	*3902	1	0.14	
	*4801	2	0.29	
	*7301	1	0.14	
B44				25.86
	*3701	5	0.72	
	*4001	44	6.36	
	*4101	1	0.14	
	*4402	73	10.55	
	*4403	27	3.9	
	*4501	7	1.01	
	*4901	15	2.17	
	*5001	7	1.01	
B58				4.03
	*1516	1	0.14	
	*1517	2	0.29	
	*5701	20	2.89	
	*5702	1	0.14	
	*5801	3	0.43	
	*5802	1	0.14	
B62				11.99
	*1301	2	0.29	
	*1302	17	2.46	
	*1501	52	7.51	
	*1502	1	0.14	
	*1506	2	0.29	
	*4601	3	0.43	
	*5201	6	0.87	
DRB1 (Southwood et al. [6] classification)				46.97
	*0101	55	7.95	
	*0401	62	8.96	
	*0701	73	10.55	
	*0901	11	1.59	
	*1302	36	5.2	
	*1501	88	12.72	
DRB1 (Ou et al. [10] classification)				47.70
	*0301	93	13.44	
	*0401	62	8.96	
	*0402	6	0.87	
	*0404	28	4.05	
	*0405	5	0.72	
	*1101	28	4.05	
	*1201	20	2.89	
	*1501	88	12.72	

[a] Each subject represented twice—once for each allele.
[b] Sette and Sidney [5,8], Southwood et al. [6], and Ou et al. [10].

0.57, respectively). However, global tests revealed significant associations between humoral immunity to measles and the alternate (Ou) class II HLA-DR supertype (p-value of 0.02), which was primarily characterized on a functional basis (Ou's et al. classification) [10]. Indeed, this alternate DR supertype was associated with higher measles-induced humoral immune responses (median 1713 IU/ml; $p = 0.02$). We found no association with the Ou et al. [10] DR classification and cellular immunity (global p-value = 0.27).

3.3. HLA supertypes and mumps vaccine immune responses

The association between HLA supertypes and mumps-specific immune response profile following two doses of MMR immunization was also examined and the results are summarized in Table 3. Median values for mumps antibody levels and SI were 728.6 IU/ml and 4.8, respectively. The global tests for association failed to show a statistically significant association between mumps antibody and lymphoproliferation levels and class I A supertypes (p-value

Table 2
HLA supertype association with measles vaccine virus-induced humoral and cellular immunity

HLA locus	HLA supertype	Number of alleles	Measles virus antibody level				Measles virus-specific lymphoproliferation			
			Median, Ab value (IU/ml)	Lower quartile, Q1	Upper quartile, Q3	p-value[a]	Median, SI value	Lower quartile, Q1	Upper quartile, Q3	p-value[a]
Overall		692	1556	746	2677		3.63	2.13	6.17	
Class IA						**0.10**				0.73
	A1	166	1504.0	684.68	2555.4	0.77	3.24	1.81	6.32	0.16
	A2	209	1670.1	834.49	2401.9	0.57	3.75	2.27	6.15	0.66
	A3	204	1658.4	836.90	3063.9	0.12	3.67	2.08	6.00	0.63
	A24	83	1201.0	701.39	2419.7	0.14	3.87	2.49	6.45	0.62
	Other[b]	30	838.7	379.42	2421.1	**0.05**	3.80	3.21	6.17	0.96
Class IB						**0.01**				0.41
	B7	195	1848.4	971.29	2986.8	**0.01**	3.79	2.13	6.45	0.35
	B27	73	1395.2	659.77	2335.4	0.33	3.87	2.53	6.43	0.50
	B44	179	1337.2	680.61	2389.5	**0.03**	3.45	2.03	5.80	0.10
	B58	28	1079.9	508.91	2273.5	**0.06**	3.36	2.53	6.42	0.86
	B62	83	1563.1	675.30	2952.6	0.98	4.03	2.17	7.12	0.21
	Other[b]	134	1583.7	784.51	2917.6	0.39	3.25	1.83	5.64	0.31
Class II DR (Southwood et al. [6] classification)						0.29				0.57
	Main DR	325	1602.1	765.09	2719.3	0.29	3.49	2.07	6.32	0.57
	Other[b]	367	1481.9	737.27	2633.1	0.29	3.73	2.13	6.11	0.57
Class II DR (Ou et al. [10] classification)						**0.02**				0.27
	Main DR	330	1713.6	765.09	2969.8	**0.02**	3.49	2.04	5.80	0.27
	Other[b]	362	1420.4	745.81	2389.5	**0.02**	3.79	2.34	6.45	0.27

Q1 and Q3 represent the first and third quartiles, respectively; suggestive findings ($p \leq 0.10$) are shown in bold; abbreviation: SI, stimulation indices.

[a] Linear regression analysis. Due to data skewness, p-values were based on log-transformed data. Analyses adjust for age at blood draw, gender, race, age at first MMR, and age at second MMR.

[b] "Other" includes the following HLA-A alleles: *0302, *2608, *2901, *2902, *3206, *6601, *6803; HLA-B alleles: *0706, *0714, *0801, *1522, *1525, *1801, *1803, *3504, * 3508, *3523, *3906, *4002, *4102, *4201, *4404, *4405, *4701, *4803; HLA-DRB1 (Southwood et al. classification) *0102, 0103, *0301, *0302, *0402, *0403, *0404, *0405, *0407, *0408, *0801, *0802, *0803, *0804, *1001, *1101, *1102, *1103, *1104, *1106, *1111, *1119,*1201, *1202, *1208, *1301, *1303, *1304, *1305, *1310, *1315, *1401, *1405, *1406, *1410, *1424, *1502, *1503, *1601, *1602; and HLA-DRB1 (Ou et al. classification) *0101, *0102, *0103, *0302, *0403, *0407, *0408, *0701, *0801, *0802, *0803, *0804, *0901, *1001, *1102, *1103, *1104, *1106, *1111, *1119, *1202, *1208, *1301, *1302, *1303, *1304, *1305, *1310, *1315, *1401, *1405, *1406, *1410, *1424, *1502, *1503, *1601,*1602, respectively.

Table 3
HLA supertype association with mumps vaccine virus-induced humoral and cellular immunity

HLA locus	HLA supertype	Number of alleles	Mumps virus antibody level				Mumps virus-specific lymphoproliferation			
			Median, Ab value (IU/ml)	Lower quartile, Q1	Upper quartile, Q3	p-value[a]	Median, SI value	Lower quartile, Q1	Upper quartile, Q3	p-value[a]
Overall		692	728.64	410.91	1286.20		4.84	2.43	9.23	
Class IA						0.15				0.43
	A1	166	684.34	395.43	1222.0	0.35	4.51	2.00	9.26	0.50
	A2	209	696.93	396.95	1263.8	0.79	4.73	2.31	8.75	0.22
	A3	204	854.18	419.73	1484.0	**0.07**	4.87	2.57	8.85	0.42
	A24	83	684.25	478.38	1459.5	0.86	5.78	2.93	10.44	0.15
	Other[b]	30	492.35	342.47	1082.7	0.06	4.61	2.29	10.57	0.97
Class IB						0.61				**0.08**
	B7	195	822.09	436.93	1386.7	0.16	4.88	2.62	8.50	0.71
	B27	73	734.35	385.31	1597.3	0.55	4.97	2.92	9.56	0.36
	B44	179	711.25	396.95	1226.7	0.16	4.73	2.28	9.11	0.34
	B58	28	782.09	361.49	1458.6	0.85	4.76	3.50	8.39	0.32
	B62	83	608.21	395.38	1395.4	1.00	6.08	3.46	12.84	**0.02**
	Other[b]	134	652.78	414.23	1282.7	0.50	3.65	1.98	8.73	**0.07**
Class II DR (Southwood et al. [6] classification)						0.12				0.14
	Main DR	325	684.44	363.52	1188.8	0.12	5.42	2.80	9.32	0.14
	Other[b]	367	774.60	447.57	1413.2	0.12	4.20	2.26	8.73	0.14
Class II DR (Ou et al. [10] classification)						0.13				**<0.001**
	Main DR	330	773.04	446.84		0.13	4.21	2.19	7.85	**<0.001**
	Other[b]	362	697.77	390.95		0.13	5.18	2.69	11.35	**<0.001**

Q1 and Q3 represent the first and third quartiles, respectively; suggestive findings ($p \leq 0.10$) are shown in bold; abbreviation: SI: stimulation indices.

[a] Linear regression analysis. Due to data skewness, p-values were based on log-transformed data. Analyses adjust for age at blood draw, gender, race, age at first MMR, and age at second MMR.

[b] "Other" includes the following HLA-A alleles: *0302, *2608, *2901, *2902, *3206, *6601, *6803; HLA-B alleles: *0706, *0714, *0801, *1522, *1525, *1801, *1803, *3504, * 3508, *3523, *3906, *4002, *4102, *4201, *4404, *4405, *4701, *4803; HLA-DRB1 (Southwood et al. classification) *0102, 0103, *0301, *0302, *0402, *0403, *0404, *0405, *0407, *0408, *0801, *0802, *0803, *0804, *1001, *1101, *1102, *1103, *1104, *1106, *1111, *1119,*1201, *1202, *1208, *1301, *1303, *1304, *1305, *1310, *1315, *1401, *1405, *1406, *1410, *1424, *1502, *1503, *1601, *1602; and HLA-DRB1 (Ou et al. classification) *0101, *0102, *0103, *0302, *0403, *0407, *0408, *0701, *0801, *0802, *0803, *0804, *0901, *1001, *1102, *1103, *1104, *1106, *1111, *1119, *1202, *1208, *1301, *1302, *1303, *1304, *1305, *1310, *1315, *1401, *1405, *1406, *1410, *1424, *1502, *1503, *1601,*1602, respectively.

of 0.15 and 0.43, respectively). However, when examining class I A supertypes individually, the A3 supertype (median 854.2 IU/ml; $p = 0.07$) approached statistical significance with higher mumps antibody levels.

Further, the global tests for association failed to demonstrate a statistically significant association between mumps antibodies and the class I B supertypes (p-value of 0.61). However, the global tests revealed a marginally significant association between cellular immune responses to mumps virus and class I B supertype (p-value of 0.08). In particular, the B62 supertype (SI 6.1; $p = 0.02$) was marginally associated with higher mumps-specific lymphoproliferation after accounting for the global p-value.

We found no associations with the class II DR supertype based on the Southwood et al. [6] classification, and mumps antibody and lymphoproliferation levels (global p-value of 0.12 and 0.14, respectively). Interestingly, the class II DR supertype, based on the Ou et al. [10] classification (global p-value of <0.001), was associated with significantly lower mumps-specific lymphoproliferation (SI 4.2; $p < 0.001$).

3.4. HLA supertypes and rubella vaccine immune responses

Associations between HLA supertypes and measures of humoral and cellular immune responses to rubella virus are presented in Table 4. Median values for rubella antibody levels and SI were 38.6 IU/ml and 2.3, respectively. We found no associations with any of the HLA-A, B or DR supertypes and either rubella antibody or cellular immune status. This suggests that HLA supertypes may have limited associations with humoral and cellular immune responses to rubella vaccine.

3.5. HLA supertypes and measles vaccine IFN-γ and IL-4 cytokine immune responses

The association between HLA supertypes and measles-specific cytokine Th1/Th2 immune response profile following two doses of live viral MMR immunization was also examined and the results are summarized in Table 5. Median values for measles virus-specific IFN-γ and IL-4 cytokines were 40.7 pg/ml (IQR 8.1 pg/ml–176.7 pg/ml) and 9.7 pg/ml (IQR 2.8 pg/ml–24.3 pg/ml), respectively. The global tests revealed a marginally significant association between IFN-γ secretion and class I A and B supertypes (p-values of 0.10 and 0.11, respectively). Supertypes A3 (median 53.8 pg/ml; $p = 0.01$), B7 (median 49.9 pg/ml; $n = 0.09$) and B62 (median 61.6 pg/ml; $p = 0.07$) were marginally associated with higher measles-virus induced IFN-γ secretion levels, whereas B44 (median 28.6 pg/ml; $p = 0.06$) was marginally associated with lower IFN-γ levels. The global tests for association failed to show a statistically significant association between measles IL-4 secretion levels and class I A and B supertypes (p-value of 0.34 and 0.68, respectively). When examining class I A supertypes individually, the A3 supertype (median 14.1 pg/ml; $p = 0.04$) appeared to be marginally associated with higher measles-specific IL-4 responses. Finally, we found no associations with any of the DR supertypes (by either Southwood et al. or Ou et al. classifications) [6,10] and either measles virus-induced IFN-γ or IL-4 production.

4. Discussion

The present study is an effort to describe HLA association with MMR-induced humoral and cellular immune responses on the basis of shared sequence specificity (motif) in the peptide-binding pockets of HLA molecules. Whereas our previous studies described associations of individual HLA alleles with antibody and lymphoproliferation levels following MMR vaccine [3,11,16], this study showed that HLA supertypes were also associated with immune responses to measles and mumps viruses following two doses of MMR vaccine.

Our findings demonstrate that among our study subjects, the supertype B44 is strongly associated with lower measles vaccine-specific antibodies. Furthermore, the least frequent HLA supertype, B58, was also associated with lower measles-induced antibodies. These two observations are important as both point towards prospective mechanistic approaches to understanding poor immune responses following measles vaccine in otherwise healthy individuals. The most common HLA supertypes, B7 and DR (Ou et al. classification), were associated with higher measles antibody response in our cohort. Indeed, HLA-DR molecules are known to be the main restriction determinants for antigen-specific T cell recognition and are predominant alleles worldwide in the human population. In the case of the B7 supertype, it has been shown that approximately 20% of the super-motif bearing peptides are cross-reactive peptides [17]. Thus, these B7, B44, and B58 supertype molecules could be considered important immune response HLA supertypes to the measles virus component of the MMR vaccine. In regards to mumps vaccine, we found that the B62 supertype was suggestive of association with mumps-specific higher lymphoproliferation; however, the DR supertype (Ou et al. classification) was significantly associated with lower mumps-specific cellular immune responses after MMR vaccine. In contrast, genetic associations between rubella vaccine immune responses and HLA supertypes were not as strong as those observed for measles and mumps, suggesting that HLA molecules may be less effective in the presentation of rubella cross-reactive peptides than for measles and mump. Finally, the A3, B7, B44 and B62 supertypes demonstrated suggestive association with variations in measles-induced IFN-γ immune responses, confirming our previous observations that HLA polymorphisms play a crucial role in modulating humoral and cellular immune responses to measles virus. This information will be helpful in developing potential epitope-based MMR vaccines that are recognized by T cells restricted by human HLA supertype alleles. “Just as pharmacogenetics has suggested ways

Table 4
HLA supertype association with rubella vaccine-induced humoral and cellular immunity

HLA locus	HLA supertype	Number of alleles	Rubella virus antibody level				Rubella virus-specific lymphoproliferation			
			Median, Ab value (IU/ml)	Lower quartile, Q1	Upper quartile, Q3	p-value[a]	Median, SI value	Lower quartile, Q1	Upper quartile, Q3	p-value[a]
Overall		692	38.63	21.81	61.11		2.29	1.53	3.63	
Class IA						0.63				0.84
	A1	166	41.62	21.68	61.80	0.52	2.04	1.30	3.67	0.25
	A2	209	39.47	23.21	65.72	0.28	2.27	1.52	3.69	0.93
	A3	204	35.02	21.40	57.14	0.18	2.37	1.58	3.63	0.53
	A24	83	37.49	19.83	65.72	0.69	2.69	1.79	3.60	0.70
	Other[b]	30	35.98	22.96	51.17	0.90	2.24	1.85	3.44	0.91
Class IB						0.72				0.76
	B7	195	36.11	22.59	60.89	0.57	2.46	1.58	3.86	0.16
	B27	73	35.26	19.86	60.48	0.18	2.08	1.53	3.52	0.75
	B44	179	39.19	23.25	61.80	0.38	2.04	1.33	3.53	0.26
	B58	28	40.30	19.67	69.93	0.72	2.54	1.46	3.59	0.83
	B62	83	39.65	18.73	60.30	0.47	2.56	1.68	3.70	0.83
	Other[b]	134	42.61	20.23	60.89	0.91	2.27	1.40	3.51	0.63
Class II DR (Southwood et al. [6] classification)						0.64				0.12
	Main DR	325	38.18	22.19	60.30	0.64	2.16	1.49	3.52	0.12
	Other[b]	367	38.97	21.40	61.77	0.64	2.41	1.53	3.74	0.12
Class II DR (Ou et al. [10] classification)						0.57				0.24
	Main DR	330	40.72	20.86	62.29	0.57	2.26	1.53	3.44	0.24
	Other[b]	362	36.38	22.19	58.94	0.57	2.32	1.53	3.90	0.24

Q1 and Q3 represent the first and third quartiles, respectively; suggestive findings ($p \leq 0.10$) are shown in bold; abbreviation: SI: stimulation indices.

[a] Linear regression analysis. Due to data skewness, p-values were based on log-transformed data. Analyses adjust for age at blood draw, gender, race, age at first MMR, and age at second MMR.

[b] "Other" includes the following HLA-A alleles: *0302, *2608, *2901, *2902, *3206, *6601, *6803; HLA-B alleles: *0706, *0714, *0801, *1522, *1525, *1801, *1803, *3504, * 3508, *3523, *3906, *4002, *4102, *4201, *4404, *4405, *4701, *4803; HLA-DRB1 (Southwood et al. classification) *0102, 0103, *0301, *0302, *0402, *0403, *0404, *0405, *0407, *0408, *0801, *0802, *0803, *0804, *1001, *1101, *1102, *1103, *1104, *1106, *1111, *1119,*1201, *1202, *1208, *1301, *1303, *1304, *1305, *1310, *1315, *1401, *1405, *1406, *1410, *1424, *1502, *1503, *1601, *1602; and HLA-DRB1 (Ou et al. classification) *0101, *0102, *0103, *0302, *0403, *0407, *0408, *0701, *0801, *0802, *0803, *0804, *0901, *1001, *1102, *1103, *1104, *1106, *1111, *1119, *1202, *1208, *1301, *1302, *1303, *1304, *1305, *1310, *1315, *1401, *1405, *1406, *1410, *1424, *1502, *1503, *1601,*1602, respectively.

Table 5
HLA supertype association with measles vaccine-induced IFN-γ and IL-4 cytokine immune responses

HLA locus	HLA supertype	Number of alleles	Measles virus-specific IFN-γ secretion[a]				Measles virus-specific IL-4 secretion[a]			
			Median secretion value (pg/ml)	Lower quartile, Q1 (pg/ml)	Upper quartile Q3 (pg/ml)	*p*-value[b]	Median secretion value (pg/ml)	Lower quartile, Q1 (pg/ml)	Upper quartile Q3 (pg/ml)	*p*-value[b]
Overall		678	40.73	8.11	176.68		9.71	2.77	24.30	
Class IA						**0.10**				0.34
	A1	163	34.30	5.66	133.48	0.30	8.92	4.29	22.00	0.76
	A2	205	35.79	6.63	132.65	0.18	9.10	1.50	24.91	0.13
	A3	198	53.80	12.44	226.80	**0.01**	14.06	4.64	25.63	**0.04**
	A24	82	38.54	8.41	230.20	0.69	9.10	1.83	24.30	0.84
	Other[c]	30	25.86	9.64	97.13	0.31	8.36	1.87	21.46	0.83
Class IB						0.11				0.68
	B7	190	49.86	12.84	176.68	**0.09**	10.82	2.05	25.95	0.83
	B27	73	36.30	9.84	230.20	0.92	13.86	2.30	30.34	0.30
	B44	175	28.62	5.80	148.81	**0.06**	8.56	1.87	20.01	0.24
	B58	26	38.13	10.80	195.79	0.57	13.77	3.54	25.66	0.72
	B62	81	61.65	14.44	244.03	**0.07**	7.29	1.83	26.69	0.41
	Other[c]	133	33.25	3.37	154.13	0.20	10.48	4.91	22.56	0.46
Class II DR (Southwood et al. [6] classification)						0.69				0.18
	Main DR	316	42.57	8.02	167.68	0.69	9.59	1.87	22.75	0.18
	Other[c]	362	37.77	8.41	195.79	0.69	9.96	3.83	25.95	0.18
Class II DR (Ou et al. [10] classification)						0.48				0.47
	Main DR	324	43.51	8.00	197.08	0.48	9.98	4.44	23.09	0.47
	Other[c]	354	39.32	8.41	168.24	0.48	9.29	1.50	25.34	0.47

Q1 and Q3 represent the first and third quartiles, respectively; suggestive findings ($p \leq 0.10$) are shown in bold.

[a] Mean value of measles virus stimulated cells minus value of control cells.

[b] Linear regression analysis. Due to data skewness, *p*-values were based on rank-transformed data. Analyses adjust for age at blood draw, gender, race, age at first MMR, and age at second MMR.

[c] "Other" includes the following HLA-A alleles: *0302, *2608, *2901, *2902, *3206, *6601, *6803; HLA-B alleles: *0706, *0714, *0801, *1522, *1525, *1801, *1803, *3504, * 3508, *3523, *3906, *4002, *4102, *4201, *4404, *4405, *4701, *4803; HLA-DRB1 (Southwood et al. classification) *0102, 0103, *0301, *0302, *0402, *0403, *0404, *0405, *0407, *0408, *0801, *0802, *0803, *0804, *1001, *1101, *1102, *1103, *1104, *1106, *1111, *1119,*1201, *1202, *1208, *1301, *1303, *1304, *1305, *1310, *1315, *1401, *1405, *1406, *1410, *1424, *1502, *1503, *1601, *1602; and HLA-DRB1 (Ou et al. classification) *0101, *0102, *0103, *0302, *0403, *0407, *0408, *0701, *0801, *0802, *0803, *0804, *0901, *1001, *1102, *1103, *1104, *1106, *1111, *1119, *1202, *1208, *1301, *1302, *1303, *1304, *1305, *1310, *1315, *1401, *1405, *1406, *1410, *1424, *1502, *1503, *1601,*1602, respectively.

of designing drugs to minimize population variability, understanding mechanisms of immunogenetic variation may lead to new vaccines designed specifically to minimize immunogenetically based vaccine failure" [18].

We examined associations of four HLA supertype loci with each of eight different immune response outcomes, resulting in a total of 32 global tests of significance; thus, multiple testing issues exist. It is noteworthy that among the 32 tests, we found six significant at the alpha = 0.10 level and three significant at the alpha = 0.05 level, in each case about twice as many as that expected by chance alone. This further reinforces the existence of an HLA-based genetic component to vaccine immunity.

Identification of alleles with overlapping peptide-binding specificities significantly facilitated epitope identification and epitope-based vaccine studies. These studies led to the demonstration of the association of specific HLA supertypes with pathogen susceptibility, chronic viral disease progression and immunity [19–23]. However, the high degree of HLA polymorphism complicated an epitope-based approach to novel vaccine development. In humans, there are more than 1000 documented alleles encoding class I cell surface glycoprotein molecules that present peptides derived from intracellular protein processing [24–26]. Previous studies have identified sets of HLA molecules or supertypes with similar specificities, and the peptide ligand specificity of numerous class I A and class I B molecules has been described as well [5,8,27,28]. It was shown that several unrelated HLA molecules are also characterized by similar specificities in terms of the main anchor residues of their peptide ligands [17]. Indeed, 50–60% of all known HLA-A and HLA-B alleles can be classified in one of four major HLA binding supertypes, such as A2, A3, B7, and B44 [27]. Sidney et al. [27] noted that "HLA binding supertypes and supermotifs demonstrate that MHC polymorphism may be functionally more limited than generally thought". In the case of class II HLA molecules, 50–80% of common alleles can be classified in a single DR supertype, depending on the ethnicities considered [6]. Thus, HLA supertypes and supermotifs offer a practical classification for the many different HLA alleles that overlap in their peptide-binding specificities worldwide in the human population [19].

The association of HLA supertypes with variations in immune response to MMR vaccine has particular significance with respect to vaccine design, since, irrespective of ethnicity, the frequency of some class I supertypes is as high as 35–55% of the general population [27]. For example, Bertoni et al. identified highly conserved hepatitis B virus (HBV)-derived peptides that bind with high affinity to various HLA class I alleles from the A2, A3, or B7 supertypes, and are recognized by cytotoxic T lymphocytes (CTL) in patients with acute hepatitis [23]. This study indicated that a large fraction of the patient population with HBV infection can be potentially covered by a synthetic peptide vaccine with only a few peptide specificities [23].

The results presented here validate the notion of HLA supertypes at the biological level by illustrating that the presence of certain HLA supertype alleles with similar peptide-binding motif may influence the outcome of the immune response to the measles and mumps components of the MMR vaccine. In addition, the present study may have practical implications for new vaccine design strategies with peptide-based vaccines. Viral MMR epitopes, which can be naturally processed and presented by various alleles of an HLA supertype – especially if they are shown to play a role in the immune response to vaccination – could form important elements for the development of novel HLA-based vaccines to be used in diverse ethnic populations.

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