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Influence of HLA-DRB1 Alleles on Lymphoproliferative Responses to a Naturally Processed and Presented Measles Virus Phosphoprotein in Measles Immunized Individuals

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Abstract

Identification of stimulatory T-cell epitopes recognized by $CD4^+$ T lymphocytes is important for vaccine development. Our previous studies using mass spectrometry identified a naturally processed HLA class II restricted DRB1*0301 T cell epitope in the measles virus phosphoprotein, MV-P1 (residues 179-197). Here we provide lymphocyte proliferation data from peripheral blood mononuclear cells (PBMC) obtained from 131 HLA-DRB1*0301-positive and HLA-DRB1*0301-negative (HLA discordant) individuals previously immunized against measles and report that a single amino acid substitution in the MV-P1 T cell epitope can reduce T cell proliferation and $CD4^+$ T-cell recognition. Measles virus and measles peptide-specific lymphoproliferative responses and HLA-DRB1 allele associations reveal that the DRB1*0701 allele provided suggestive evidence of association with both measles virus ($p = 0.03$) and MV-P1 peptide ($p = 0.06$) lymphoproliferation. A marginally significant increase in the frequency of the *0301 allele ($p = 0.10$) was found among subjects who demonstrated low cellular responses to the measles virus. We found no associations between proliferation levels to the MV-P1 and MV-P2 peptides with *0301 alleles. We speculate that the glutamic acid at position 192 of the measles phosphoprotein is a critical immunogenicity factor and may influence the antigenicity of the naturally processed HLA class II MV-P1 epitope.

Keywords

measles; HLA class II epitopes; cellular immunity; measles peptides; T-cell recognition

INTRODUCTION

Human leukocyte antigen (HLA) genes are important determinants of genetic susceptibility to viral infections because of their antigen presenting function. In particular, the class II HLA molecules play a significant role in stimulating an immune response to measles virus (MV) by binding foreign peptides of extracellular origin and presenting them to $CD4^+$ T lymphocytes, resulting in cytokine production and T cell help for antibody production [1,2]. HLA-DRB1*0301 is a class II allele present in greater than 20% of the human population. Our previous work suggests that measles vaccine virus-induced humoral immune responses are associated with both HLA class I and class II genes [3,4]. Further, we have demonstrated that carriage of class II HLA-DRB1*03 alleles are associated with low levels of antibody after measles immunization [5]. Previously, we identified a naturally processed and presented peptide, ASDVETAEGGEIHELLRLQ, derived from a measles phosphoprotein (MV-P1,

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residues 179-197) and presented by class II HLA-DRB1*0301 molecules on measles-infected Epstein-Barr virus (EBV)-transformed B cells [6]. In addition, we described a measles phospho-protein peptide variant (MV-P2) obtained from a measles genome that differs only by one amino acid from the eluted MV-P1 peptide, a lysine (Lys or K) versus glutamic acid (Glu or E) in position 192. Although we did not observe this MV-P2 peptide in our analyses of naturally processed peptides expressed by HLA-DRB1*0301, we synthesized this peptide for further study.

It is becoming more apparent that virus-specific $CD4^+$ T cells play an important role in measles immune responses [7–9]. Only a few naturally processed HLA class I associated antigenic sites or cytotoxic T cells (CTL) epitopes on MV hemagglutinin (H), fusion (F) glycoprotein, matrix (M), and non-structural C proteins have been identified at the peptide level [10,11]. These measles class I epitopes may have important implications for the induction of antiviral T cell immunity [12]. However, relatively little is known about $CD4^+$ T-cell responses to naturally processed MV peptides presented by HLA class II molecules.

Cell-mediated immunity (CMI) is of crucial importance to measles immunity and can be assessed by measuring CTL, lymphoproliferative and cytokine responses [13]. Memory T lymphocyte proliferative responses to measles antigens have been reported in 100% of individuals who had natural measles infection and in approximately 60% to 90% of immunized children [14–16]. Although we have reported associations between low antibody levels and specific HLA alleles, associations between cellular (proliferative) immune responses elicited by MV and by naturally processed measles-derived peptides have not yet been identified.

The objective of the present study was to investigate the T cell responses of previously immunized individuals to MV, to the naturally processed HLA class II MV-P1 epitope and to the MV-P2 peptide variant. Furthermore, we sought to determine if associations exist between measles virus, MV-P1 and MV-P2 specific lymphoproliferative responses and alleles of the HLA-DRB1 locus for subjects who had been vaccinated against measles.

MATERIALS AND METHODS

Study Patients

Details of patient identification and recruitment have been previously published [17]. Briefly, study participants were enrolled as part of a larger stratified random sample study to assess associations between HLA genes and immune response to measles-mumps-rubella-II (MMR-II) vaccine in healthy, school-age children and young adults (ages 12 to 18 years old). To evaluate the cellular responses to measles peptides, 131 subjects were used. In addition, a random subset of 43 individuals was tested for reactivity to a control measles fusion (F) peptide. All enrolled subjects had been previously immunized with two doses of MMR-II vaccine (Merck Research, West Point, PA, USA) containing the attenuated Edmonston strain of MV. All but four of these participants were Caucasian and all subjects resided in a geographic area where no wild type MV had circulated in the community during the patients' lifetimes. The Institutional Review Board of the Mayo Clinic granted approval for the study, and peripheral blood samples were drawn after informed consent was obtained from each patient. We also obtained written, informed consent from parents or guardians of all the subjects at the time of enrollment in the study.

HLA Typing

Genomic DNA was extracted from blood samples by conventional techniques using the Pyregene extraction kit (Gentra Systems Inc., Minneapolis, MN, USA). DNA was used for class II HLA-DRB1 allele typing using a high resolution DRB96 SSP (sequence-specific

primer) Unitray typing kit with the entire locus on a single tray (Pel-Freez Clinical Systems, LLC, Brown Deer, WI) [18]. Locus-specific primers were used to amplify the HLA-DRB1 locus and polymerase chain reaction (PCR) products were separated on 2% agarose gel and stained with ethidium bromide. Any ambiguities were resolved using the ABI DRB1 sequencing kit (Applied Biosystems, Foster City, CA, USA). All PCR amplifications were carried out in a GeneAmp PCR system 9600 (Perkin-ElmerCetus Instruments, Norwalk, CT). All reactions were run with negative controls and every 50th PCR reaction repeated for quality control.

Measles Vaccine Virus and Synthetic Peptides

Many of the details of peptide identification and peptide sequencing methodology have been previously published [6]. Measles vaccine (Attenuvax; Merck Inc.) containing 1000 median tissue culture infective doses ($TCID_{50}$) of the Edmonston strain of MV was used for lymphocyte proliferation assays. Measles peptides were synthesized by the Mayo Protein Core Facility (Rochester, Minnesota) using N-(9-fluorenyl)methoxycarbonyl protection chemistry and carbodiimide/N-hydroxybenzotriazole activation on a MPS 396 Multiple Peptide Synthesizer (Advanced Chemtech, Louisville, KY, USA). The following three peptides were utilized: (1) measles-derived naturally processed 19 amino acid MV-P1 peptide of the measles P protein, ASDVETAEGGEIHELLRLQ; (2) single amino acid substituted MV-P2 peptide, ASDVE-TAEGGEIHKLLRLQ, that differ only by one amino acid, a Lys (K) versus Glu (E) at position 192; (3) randomly chosen 14 amino acid control peptide of the MV F protein, PLRHQATTASSTKP (MV-F). The sequences for MV-P1 and MV-P2 are both identical as measles phosphoprotein in the NCBI nr database. MV-P1 and MV-P2 were each synthesized in order to confirm our identification of the naturally processed peptide from measles as MV-P1 and for use in understanding the role of amino acid sequence in inducing $CD4^+$ T-cell response.

Preparation of Peripheral Blood Leukocytes and T-Cell Proliferation Assay

Details of our *in vitro* lymphoproliferation assay have been reported elsewhere [19]. In brief, peripheral blood mononuclear cells (PBMC) were separated from heparinized blood by Ficoll-Hypaque (Sigma, St. Louis, MO, USA) density gradient centrifugation and washed in RPMI 1640 medium (Celox Laboratories, Inc., St. Paul, MN, USA) supplemented with 2 mM L-glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin, and 8% fetal calf serum (Life Technologies, Gaithersburg, MD, USA). Measles virus, MV-P1, MV-P2, and MV-F specific T-cell responses were measures by proliferation of PBMC (2×10^5) incubated in RPMI-1640 medium, supplemented with 5% autologous sera, with live attenuated MV (50 pfu/ml, positive control) or synthetic peptides (20 μg/well) compared with unstimulated cell control wells. Phytohemagglutinin (PHA, 5 μg/ml) was used to assess cell vitality. Only assays in which PBMC responded to PHA were accepted. T lymphocyte proliferation was measured after 4 days by [^{3}H]-tritiated thy-midine uptake. Cells were then harvested onto glass fiber filters, using a 96-well harvesting system (Skatron Instruments, Lier, Norway). The amount of incorporated radioactivity was determined by a liquid scintillation counter (Packard Instrument Company, Boston, MA, USA). We used six replicates of counts per minute (cpm) values for unstimulated cells, and three replicates each for T cells stimulated with peptides and live measles vaccine. For each subject, median cpm were calculated for unstimulated cells, as well as for cells stimulated with MV-P1, MV-P2, MV-F, and measles. Results were then 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 control wells. Stimulation indices of 2 or higher were considered to represent significant responses [20,21]. A $SI > 2$ was arbitrarily selected prior to the study as an indication of the presence of reactive peptide specific memory T cells, and $SI < 2$ as an indicator of the lack of memory T lymphocytes to measles-derived peptides [22].

Statistical Analysis

Three outcomes were of primary interest: T-cell proliferation (as measured by stimulation indices) induced separately by live MV, the MV-P1 peptide, and the MV-P2 peptide variant. Data were descriptively summarized using frequencies and percentages for all categorical variables, and medians and ranges for all continuous variables. To summarize the association of the three outcome variables with each other, we used Wilcoxon signed rank tests and Spearman rank correlation coefficients (on the original continuously-distributed variables), as well as cross-tabulations with sensitivity estimates (on the categorized stimulation index values). For the latter, measles-induced lymphoproliferation was used as the gold standard.

Descriptive associations of the categorized stimulation indices with HLA-DR alleles were evaluated on an allelic level. Each person contributed two observations to this descriptive analysis–one for each allele. Alleles were grouped by DR status and summarized using frequencies and percents. Following the descriptive comparisons, associations were more formally evaluated using logistic regression analyses. In contrast to the descriptive comparisons, each subject contributed one observation to the regression analysis, based on his or her genotype. Regression variables were created for each allele and were coded as 0, 1, or 2, according to the number of copies of the allele that a subject carried. Thus, allelic odds ratios can be interpreted as the estimated increase in the odds of a high lymphoproliferative response for each additional copy of the allele of interest possessed by an individual. Rare alleles, defined as those with fewer than five occurrences among all subjects, were pooled into a category labeled “other”. Global differences in stimulation indices among all alleles were first carried out via likelihood ratio tests by simultaneously including all but one of the allele variables in a multivariate logistic regression model. Following these global tests, we examined individual allele effects on stimulation indices. This series of 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 global significance. Each allele variable was included in a separate univariate logistic regression analysis, effectively comparing lymphoproliferation levels for the allele of interest against all other alleles combined. All global and univariate regression analyses included the design variable, age, as a covariate. All statistical tests were two-sided, and all analyses were carried out using the SAS software system (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Cellular Responses of Vaccinated Individuals to Measles Virus and Measles Peptides

Measles virus, MV-P1 peptide, MV-P2 peptide variant, and the randomly chosen control MV-F peptide were tested for the ability to elicit in vitro recall lymphoproliferative responses in the subjects’ PBMC after stimulation with MV ($n = 131$), MV-P1 ($n = 131$), MV-P2 ($n = 130$), or MV-F ($n = 43$). Stimulation of PBMC with measles resulted in significant MV-specific T-cell responses. Measles virus stimulation indices (median SI 4.7, range 0.5–30.5) were higher than MV-P1 peptide (median 1.7, range 0.5–20.3, p value Wilcoxon signed rank test < 0.001) or single amino acid substituted MV-P2 peptide stimulation indices (median 1.2, range 0.4–16.2, p value < 0.001). Lymphoproliferative responses observed in the subjects’ PBMC after stimulation with MV or with peptides revealed a positive correlation of MV-stimulated lymphoproliferative responses with MV-P1 peptide and MV-P2 peptide variant SIs (Spearman correlation coefficients = 0.40 [$p < 0.001$] and 0.25 [$p < 0.005$], respectively) across all subjects, indicating comparative T cell responses for both forms of peptides.

As defined in the Materials and Methods section, a lymphoproliferative response was considered positive (stimulatory effect) if the SI was greater than 2.0. According to this criterion, 107 of 131 (82%) subjects had MV stimulation indices greater than 2, indicating that

MV contains multiple T helper lymphocyte epitopes and were recognized by PBMC derived from most of the individuals. Likewise, recall measles-derived MV-P1 lymphoproliferative responses were detected in 41% (53/131) of the subjects, suggesting MV-P1 recognition by memory T cells from previously immunized subjects. In contrast, the single amino acid substituted MV-P2 peptide was recognized in only 17% (22/130) of subjects. Among 107 subjects who responded to measles, 50 also responded to the MV-P1 peptide (sensitivity = 47%) and 21 responded to the MV-P2 peptide variant (sensitivity = 20%). Among the 52 subjects who responded to the MV-P1 peptide, 12 (23%) also responded to MV-P2-modified peptide, suggesting that MV-P1 and MV-P2 peptides possibly share common epitopes. The recall lymphoproliferative responses elicited by MV and peptides were antigen dose dependent, with optimal doses of peptides between 15 and 20 μg/2 × 10^5 PBMC. Finally, lymphoproliferative responses to the randomly chosen control MV-F peptide were quite low overall (median 1, range 0–3), although 5 (11%) of 43 subjects had SI > 2. Age and sex of the study subjects did not affect antigen-specific lymphoproliferative responses (data not shown).

Occurrence of HLA-DRB1 Alleles in Study Patients Previously Immunized Against Measles

HLA allelic frequencies in this study population were determined after molecular HLA typing. It was noted that the most prevalent alleles in this study population were HLA-DRB1*0301, *1501, *0401, and *0701 which were expressed in 14.5%, 13.0%, 11.5%, and 11.5% individuals, respectively (Table 1).

Associations Between HLA-DRB1 Alleles and Lymphoproliferative Responses to Measles Virus and Measles-Derived Peptides

The association between class II HLA-DRB1 alleles and lymphocyte proliferation response to measles and measles peptides was examined using logistic regression analysis. Tables 2, 3, and 4 present the results of the logistic regression analysis of association with measles, MV-P1 and MV-P2-modified peptides and individual comparison of HLA-DRB1 alleles across the lymphocyte proliferation status. Global tests revealed no significant associations of HLA-DRB1 alleles with measles, MV-P1 and MV-P2 peptide variant specific lymphoproliferative responses. However, univariate analyses (Table 2) revealed a marginally significant ($p = 0.10$) increase in the frequency of the *0301 allele among subjects who demonstrated low SI levels to MV (22.9 %) compared to those with significant levels (SI > 2) of MV specific lymphoproliferative response (12.6%, odds ratio [OR] 0.50; 95% confidence interval [CI] 0.22–1.15). In other DRB1 alleles, we found a significant ($p = 0.03$) decrease in the frequency of the DRB1*0701 allele among subjects who responded (9.3%) compared to those with low (SI > 2) MV specific lymphoproliferative responses (20.8%, OR 0.40; CI 0.18–0.92).

Measles-derived P1 peptide specific cellular responses and HLA-DRB1 alleles associations are summarized in Table 3. We found no associations between MV-P1 peptide with HLA-DRB1*0301 alleles. However, the frequency of *0701 alleles (OR, 0.40; CI 0.19–1.05, $p = 0.06$) was also lower in subjects with significant MV-P1 specific lymphoproliferative responses (6.6%) compared to individuals with low SI levels to the MV-P1 peptide (14.7%). There were no other strong associations (except for the DRB1*0701 allele) between the MV-P1 specific lymphoproliferation and the frequency of other alleles; however, these associations should be interpreted with caution due to the small sample size and due to the absence of significant global tests. Examination of the lymphoproliferative responses to MV-P2 peptide variant indicated that none of the alleles of the HLA-DRB1 locus were associated with MV-P2 peptide variant T cell recognition (Table 4).

Of the 131 patients, only 4 described themselves as non-Caucasian. Since allele distribution can differ drastically across race and ethnicity, we ran additional sensitivity analyses excluding these four subjects. Results were nearly identical to those presented in Tables 2–4 (not shown).

DISCUSSION

There is a major interest in defining T- and B-cell epitopes recognized by $CD4^+$ T cells involved in immune responses to measles immunization. Although $CD4^+$ helper T cells recognizing different portions of the MV proteins have been reported, T cell responses to measles HLA class I and class II synthetic peptides corresponding to sequences of measles proteins are imprecise and not well characterized [23–26]. In addition, in measles patients and measles vaccine recipients only a few immunodominant T cell epitopes of MV antigens have been defined and mapped [23,24]. Therefore, additional studies are needed to identify other MV sequences containing important T cell epitopes. The purpose of this study was to analyze the lymphoproliferative responses of fresh PBMC of previously immunized HLA-DRB1*0301-positive and HLA-DRB1*0301-negative (HLA discordant) individuals to MV, to a naturally processed MV-derived peptide from the 70 kD phosphoprotein and to a measles peptide variant, and to determine if associations exist between MV and measles peptide specific lymphoproliferative responses and class II HLA-DRB1 alleles. Our evaluation of measles specific T lymphocyte proliferative responses to live attenuated measles vaccine virus showed that evidence of cellular immunity (SI > 2) was detected in 82% of all study subjects. In contrast, CMI responses to single MV-P1 and MV-P2 epitopes were detected in 41% and 17% of the individuals, respectively. The finding that an identified MV-P1 peptide, eluted from a nonresponder associated HLA-DRB1*03 allele, was antigenic for recall lymphoproliferative responses in this study population is significant. This high frequency of proliferation is likely attributed to the fact that MV-P1 peptide was naturally processed and presented by DRB1*0301 alleles and is capable of inducing peptide specific recall immune response in the context of multiple HLA-DRB1 molecules.

Using PBMC from previously immunized subjects, we demonstrated that the MV-P2 peptide variant significantly affects *in vitro* T-cell proliferation. Our data suggest that single amino acid changes at critical residues of measles-derived peptide could diminish T cell proliferation and activation. Possible explanations include the changes in the binding affinity of the 19-mer MV-P2 peptide variant to HLA-DRB1 class II molecules from subjects PBMC and their ability to be recognized by specific T cell receptors [21,27]. Wang *et al.* [28] recently reported that naturally occurring single amino acid variants of the Th1 epitope of hepatitis C virus (HCV) could modulate in vitro T cell responses by both escaping $CD4^+$ T cell recognition and modulation of cytokine production. However, the role of altered binding affinity of HCV variant epitopes was not investigated in this study.

The MV-P2 peptide variant differed from the naturally processed and presented MV-P1 peptide only by one amino acid, a lysine (K) versus glutamic acid (E) at a position 192. Since these two amino acids vary significantly in the shape, charge, and overall size of their side chains, it is logical to presume that this amino acid variation may have significant effects on the overall antigenicity of the MV peptides [29,30]. Glutamic acid is a relatively small amino acid with an acidic carboxyl (COO^-) group side chain – very different from lysine, with its longer side chain containing a basic amino (NH_3^+) group. We hypothesize that the glutamic acid at position 192 of the measles phosphoprotein is a critical factor that influences the antigenicity of the HLA class II MV-P1 epitope.

We have demonstrated that PBMC expressing the HLA-DRB1*0701 allele induced weak lymphoproliferative responses among antigen-specific T cells to either measles or synthetic measles-derived MV-P1 peptide. In addition, subjects who demonstrated positive recall lymphoproliferative responses to MV were less likely to carry the HLA-DRB1*0301 allele compared to the fraction of individuals who exhibited low measles-specific lymphoproliferative responses. These results should be viewed with some caution as they exist in the absence of significant global tests. However, our results are in agreement with previous

studies examining the association between HLA-DRB1 alleles and humoral nonresponsiveness to another viral vaccine, hepatitis B vaccine. Other investigators have noted a significant increase in the frequency of HLA-DR3 and/or -DR7 alleles among poor responders to vaccine [31,32]. In addition, the excess prevalence of HLA-DR7 alleles was observed in patients with chronic persistent infection with hepatitis B virus [33]. However, we did not observe associations between MV-P1 peptide and MV-P2 peptide variant and *0301 allele, which was previously demonstrated to be important in antibody response to measles vaccine virus [5].

We have demonstrated that a naturally occurring MV epitope can efficiently elicit or stimulate recall immune responses in previously immunized individuals in the context of multiple HLA-DRB1 molecules. Further, we have demonstrated that a measles epitope variant was capable of modifying cellular immune responses to a single naturally processed measles peptide sequence, and that the glutamic acid at position 192 of the measles structural phosphoprotein is critical for the antigenicity of this naturally processed HLA class II MV peptide. We found that HLA-DRB1*0701 allele is over-represented in the group of individuals who demonstrated low lymphoproliferative responses to measles and measles-derived MV-P1 peptide and therefore may be regarded as a factor influencing cellular immune responses. Our study of immune responses to naturally processed and presented T cell epitopes should provide the experimental framework for the development of improved vaccines against measles.

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ABBREVIATIONS

CMI
cell-mediated immunity

CI
confidence interval

CPM
counts per minute

CTL
cytotoxic T cells

EBV
Epstein-Barr virus

F
fusion

Glu E
glutamic acid

H
hemagglutinin

HLA
human leukocyte antigen

Lys or K
lysine

M
matrix

MMR-II
measles-mumps-rubella II

MV
measles virus

MV-P1
naturally processed and presented peptide, ASDVETAEGGEIHELLRLQ

MV-P2
measles phosphoprotein peptide variant

OR
odds ratio

PBMC
peripheral blood mononuclear cells

SI
stimulation indices

TABLE 1

HLA-DRB1 allelic frequency of the 131 study patients[a]

HLA-DRB1 locus	Allele	Number of alleles	Percent of allele subtype	Percent of HLA-DRB1 locus allele type
DR1	DRB1*0101	20	7.63	8.4
	DRB1*0102	2	0.76	
DR103	DRB1*0103	2	0.76	0.8
DR2	DRB1*1501	34	12.98	14.5
	DRB1*1601	3	1.15	
	DRB1*1602	1	0.38	
DR3	DRB1*0301	38	14.50	14.9
	DRB1*0302	1	0.38	
DR4	DRB1*0401	30	11.45	17.6
	DRB1*0402	3	1.15	
	DRB1*0404	8	3.05	
	DRB1*0405	2	0.76	
	DRB1*0407	2	0.76	
	DRB1*0408	1	0.38	
DR5	DRB1*1101	11	4.20	9.9
	DRB1*1102	1	0.38	
	DRB1*1103	2	0.76	
	DRB1*1104	5	1.91	
	DRB1*1201	7	2.67	
DR6	DRB1*1301	14	5.34	16.0
	DRB1*1302	16	6.11	
	DRB1*1303	2	0.76	
	DRB1*1304	1	0.38	
	DRB1*1310	1	0.38	
	DRB1*1401	6	2.29	
	DRB1*1405	1	0.38	
	DRB1*1406	1	0.38	
DR7	DRB1*0701	30	11.45	11.4
DR8	DRB1*0801	9	3.44	4.2
	DRB1*0803	2	0.76	
DR9	DRB1*0901	4	1.53	1.5
DR10	DRB1*1001	2	0.76	0.8

[a]Each patient represented twice–once for each allele.

TABLE 2

HLA-DRB1 allelic associations with MV-specific lymphoproliferative responses

	Allele counts, lymphoproliferation (SI < 2)		Allele counts, lymphoproliferation (SI > 2)		Odds of stimulation index positivity		Locus *p* value[c]	Global *p* value[b,c]
	Number	Percent	Number	Percent	OR[a]	95% CI		
HLA-DRB1 locus								
DR1	4	8.33	18	8.41	0.93	(0.28, 3.07)	0.90	0.16
DR2	5	10.42	33	15.42	1.61	(0.62, 4.22)	0.33	
*0301	11	22.92	27	12.62	0.50	(0.22, 1.15)	0.10	
DR4	5	10.42	41	19.16	1.93	(0.66, 5.66)	0.23	
DR5	3	6.25	23	10.75	2.04	(0.56, 7.38)	0.28	
DR6	5	10.42	37	17.29	1.85	(0.66, 5.17)	0.24	
DR7	10	20.83	20	9.35	0.40	(0.18, 0.92)	0.03	
DR8	2	4.17	9	4.21	1.21	(0.23, 6.29)	0.82	
Other DR alleles[d]	3	6.25	6	2.80	0.36	(0.07, 1.74)	0.20	

[a] Odds ratio (OR) estimating increase in odds of positivity for each copy of allele of interest, relative to all other alleles pooled together.

[b] Logistic regression analysis, accounting for the design variable age.

[c] Likelihood ratio test.

[d] Other includes the following DRB1 alleles: DRB1*0103, DRB1*0302, DRB1*0901, and DRB1*1001.

Abbreviations: CI = confidence interval; HLA = human leukocyte antigen; MV = measles virus; OR = odds ratio; SI = stimulation indices.

TABLE 3

HLA-DRB1 allelic associations with measles-derived MV-P1 peptide-specific lymphoproliferative responses

	Allele counts, lymphoproliferation (SI < 2)		Allele counts, lymphoproliferation (SI > 2)		Odds of stimulation index positivity		Locus *p* value[b]	Global *p* value[b,c]
	Number	Percent	Number	Percent	OR[a]	95% CI		
HLA-DRB1 locus								
DR1	11	7.05	11	10.38	1.69	(0.68, 4.21)	0.26	0.50
DR2	19	12.18	19	17.92	1.51	(0.78, 2.92)	0.22	
*0301	25	16.03	13	12.26	0.70	(0.33, 1.46)	0.34	
DR4	27	17.31	19	17.92	1.09	(0.54, 2.24)	0.80	
DR5	14	8.97	12	11.32	1.32	(0.57, 3.06)	0.52	
DR6	28	17.95	14	13.21	0.69	(0.34, 1.39)	0.29	
DR7	23	14.74	7	6.60	0.44	(0.19, 1.05)	0.06	
DR8	5	3.21	6	5.66	1.76	(0.48, 6.22)	0.40	
Other DR alleles[d]	4	2.56	5	4.72	1.96	(0.47, 8.14)	0.35	

[a] Odds ratio (OR) estimating increase in odds of positivity for each copy of allele of interest, relative to all other alleles pooled together.

[b] Logistic regression analysis, accounting for the design variable age.

[c] Likelihood ratio test.

[d] Other includes the following DRB1 alleles: DRB1*0103, DRB1*0302, DRB1*0901, and DRB1*1001.

Abbreviations: CI = confidence interval; HLA = human leukocyte antigen; MV-P2 = naturally processed and presented peptide; SI = stimulation indices.

TABLE 4

HLA-DRB1 allelic associations with MV-P2 peptide variant specific lymphoproliferative responses

	Allele counts, lymphoproliferation (SI < 2)		Allele counts, lymphoproliferation (SI > 2)		Odds of stimulation index positivity		Locus *p* value[b]	Global *p* value[b,c]
	Number	Percent	Number	Percent	OR[a]	95% CI		
HLA-DRB1 locus								
DR1	18	8.33	4	9.09	1.10	(0.34, 3.53)	0.87	0.65
DR2	28	12.96	8	18.18	1.68	(0.72, 3.94)	0.23	
*0301	34	15.74	4	9.09	0.55	(0.18, 1.70)	0.30	
DR4	36	16.67	10	22.73	1.48	(0.60, 3.68)	0.39	
DR5	20	9.26	6	13.64	1.54	(0.55, 4.30)	0.41	
DR6	36	16.67	6	13.64	0.77	(0.29, 2.00)	0.58	
DR7	27	12.50	3	6.82	0.57	(0.17, 1.93)	0.36	
DR8	9	4.17	2	4.55	1.04	(0.20, 5.47)	0.96	
Other DR alleles[d]	8	3.70	1	2.27	0.37	(0.04, 3.28)	0.37	

[a] Odds ratio (OR) estimating increase in odds of positivity for each copy of allele of interest, relative to all other alleles pooled together.

[b] Logistic regression analysis, accounting for the design variable age.

[c] Likelihood ratio test.

[d] Other includes the following DRB1 alleles: DRB1*0103, DRB1*0302, DRB1*0901, and DRB1*1001.

Abbreviations: CI = confidence interval; HLA = human leukocyte antigen; MV-P2 = measles phosphoprotein variant; SI = stimulation indices.