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Importance of HLA-DQ and HLA-DP polymorphisms in cytokine responses to naturally processed HLA-DR-derived measles virus peptides

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Abstract

We studied the association between class II human leukocyte antigen (HLA)-DRB1*0301 presented measles virus (MV) peptide-specific cytokine responses and DQB1 and DPB1 alleles among 313 individuals who received two doses of measles–mumps–rubella-II vaccine. The overall median IFN-γ secretion levels (first and third quartiles) for the 19-amino acid MV phosphoprotein (MV-P)- and 14-amino acid MV nucleoprotein (MV-N)-derived peptides were 27.7 pg/ml (1.8, 109.4) and 1.9 pg/ml (−6.2, 13.0), respectively; median IL-4 secretion levels were −0.6 pg/ml (−7.1, 6.2) and 2.4 pg/ml (−3.2, 9.3), respectively. Primary statistical analyses were adjusted for previously identified DRB1 associations. A marginally significant increase in the frequency of the DQB1*0604 ($p = 0.02$) allele was found among subjects who demonstrated detectable IL-4 levels to the MV-P peptide. Further, DPB1*0201 ($p = 0.02$) and DPB1*1301 ($p = 0.09$) alleles provided suggestive evidence of an association with MV-P-induced IL-4 secretion. Examination of IFN-γ responses to MV-P and MV-N indicated that none of the individual alleles of the DQB1 and DPB1 loci were associated with peptide-induced T cell response. An increase in the frequency of DPB1*0501 ($p = 0.01$) was found among subjects who failed to produce MV-N peptide-specific IL-4 responses. These data further confirm that HLA-DRB1 alleles are the major restriction molecules for MV-P and MV-N measles virus antigen presentation to T cells. We speculate that MV-P and MV-N peptides derived from DRB1*0301 could potentially be recognized in association with different HLA molecules, including DQB1 and DPB1; however, statistical adjustments for the effect of HLA-DR locus could potentially alter these genetic relationships. This concept provides important information supporting the use of promiscuous peptides in a peptide-based vaccine approach.

Keywords

Cytokines; HLA; Measles peptides

1. Introduction

The three major polymorphic families of functional human leukocyte antigen (HLA) class II antigens in humans are DR, DQ, and DP. Each of these molecules provides restriction determinants for the binding and presentation of antigenic peptides to CD4 + T helper cells,

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which allows for an immune response [1–3]. Accordingly, the T cell immune response is dependent upon antigen processing and presentation, HLA polymorphism, and T cell recognition.

Previously, we reported that HLA-DRB1 molecules contribute the main restriction determinants for antigen-specific T cell recognition of naturally processed measles virus (MV) class II epitopes derived from measles phosphoprotein (MV-P) and nucleoprotein (MV-N) [4]. We demonstrated a significant association of DRB1*0301 alleles with MV-P specific IFN-γ and IL-4 responses. In addition, the DRB1*1501 (DR2) and DRB1*1103 (DR5)/*1303 (DR6) alleles were associated with MV-N-induced IFN-γ and IL-4 secretion, respectively. Because of tight linkage disequilibrium between DR, DQ and/or DP alleles and the frequent sharing of epitopes among these HLA class II loci [5], the importance of non-DR class II molecules in MV peptide responses required further exploration.

Early research demonstrated that approximately 40% of the T cell response to complex protein antigens (purified protein derivative (PPD)-tuberculin, tetanus toxoid, and *Candida albicans* extract) was restricted by non-DR encoded class II molecules [6]. In this context, a conserved region of the major surface antigen (p190) of *Plasmodium falciparum merozoites* is recognized in association with many different HLA class II molecules, which suggests that HLA restriction would not be a major limitation in the development of a malaria subunit vaccine [7].

To further explore this concept of promiscuous HLA presentation, we studied the association between cytokine responses to HLA class II DRB1-derived peptides and DQB1 and DPB1 alleles among 313 healthy children who received two doses of measles vaccine to test whether these earlier observations are generalizable to other antigens such as measles virus peptides. We describe here the results of in vitro IFN-γ and IL-4 cytokine responses to MV and measles DRB1-derived peptides and report the results of the association between these cytokine responses and HLA class II DQB1 and DPB1 alleles.

2. Materials and methods

2.1. Study subjects

Our methods for subject identification and recruitment have been previously described [8]. In brief, study participants were enrolled as part of a larger stratified random sample to assess associations between HLA genes and the immune response to measles–mumps–rubella-II (MMR-II) vaccine (Merck Research, West Point, PA, USA) in healthy children and young adults in Rochester, Minnesota. To evaluate cytokine responses of vaccinated individuals to MV and measles-derived peptides, 313 subjects (12–18 years of age) were studied. Out of these 313 recruited individuals, seven subjects did not have IFN-γ cytokine values available and 29 subjects did not have IL-4 cytokine values available. Thus, the HLA and IFN-γ and IL-4 cytokine data were analyzed on 306 and 284 subjects, respectively. All enrolled subjects had been previously immunized with two doses of MMR-II vaccine containing the further attenuated Edmonston B strain of measles. The majority of individuals were Caucasians (92.6%) and the mean age at first and second vaccination was 18.0 months and 11.3 years, respectively. There were a total of 168 (53.7%) males and 145 (46.3%) females in the study. The mean time from the second MMR-II to blood draw was 4.7 years. Mayo Clinic's Institutional Review Board granted approval for the study, and peripheral blood samples were drawn after written informed consent was obtained from each subject and/or guardian.

2.2. Preparation of peripheral blood leukocytes

Peripheral blood mononuclear cells (PBMC) were separated from heparinized venous blood by Ficoll-Hypaque (Sigma, St. Louis, MO, USA) density gradient centrifugation. Cells were

washed in RPMI 1640 medium (Celox Laboratories Inc., St. Paul, MN, USA) supplemented with 100 μg/ml streptomycin, 100 U/ml penicillin and 8% heat-inactivated fetal calf serum (FCS, Hyclone, Logan, UT, USA), counted, and then resuspended in RPMI freezing media containing 10% dimethyl sulfoxide, frozen at −80 °C and stored in liquid nitrogen until cultured. No significant differences in cellular viability estimated by trypan blue exclusion were observed between the same PBMC samples obtained before and after their storage in liquid nitrogen.

2.3. IFN-γ and IL-4 supernatant cytokine levels after measles or synthetic measles peptide stimulation

Peripheral blood lymphocytes from 313 individuals were tested in a cytokine secretion assay with either the synthetic measles peptides or the virus. Details of our in vitro cytokine secretion assay have been reported elsewhere [4]. In brief, cryopreserved PBMC were thawed in a 37 °C water bath and then washed twice with 10 × volume of complete RPMI 1640 media supplemented with 10% FCS at 700 rpm for 5 min. The final cell pellet was resuspended in RPMI media containing 100 U/ml penicillin, 100 μg/ml streptomycin (Sigma) and supplemented with 5% normal human AB sera (NHS, Irvin Scientific, Santa Ana, CA, USA). The Edmonston B vaccine strain of measles virus was cultured in Vero cells in OPTI-MEM reduced serum medium (Invitrogen, Carlsbad, CA, USA), supplemented with 5% FCS (virus stocks of 2×10^7 plaque-forming units [pfu]/ml). The following naturally processed, DRB1*0301-bound synthetic peptides were used: (i) a 19 amino acid (aa) peptide derived from the phosphoprotein (MV-P, residues 179–197), ASDVETAEGGEIHELLRLQ and (ii) a 14 aa peptide derived from measles nucleoprotein (MV-N, residues 372–385), SAGKVSSTLASELG. These peptides were isolated from HLA-DRB1 molecules after human cell (PBMC) culture infection [4,9].

For IFN-γ determination, thawed PBMC were cultured at a concentration of 2×10^5 in RPMI containing 5% NHS with or without measles peptides (10 μg/well) and MV at a multiplicity of infection (moi) of 0.5 for 6 days. For IL-4 determination in cell culture supernatants, thawed PBMC were cultured at a concentration of 4×10^5 in RPMI media, supplemented with 5% NHS in the presence of 2 μg/ml of IL-4 receptor antibody (R&D Systems, Minneapolis, MN, USA) with or without synthetic measles peptides (10 μg/well) or MV at a moi of 0.1 for 6 days, as previously described [10]. Cell culture supernatants were collected in a volume of 150 μl/well for both IFN-γ and IL-4 and were frozen at −80 °C. The culture supernatants were assayed using a standard ELISA kit (OptiEIA Human IFN-γ and IL-4) (PharMingen, San Diego, CA, USA) at a dilution of 1:1 in phosphate-buffered saline (PBS) containing 10% FCS according to the manufacturer's instructions. ELISA plates (Immulon-4) (Dynex Technologies Inc., Chantilly, VA, USA) were coated with capture IFN-γ or IL-4 monoclonal antibody (mAb). The antibody-coated plates were incubated with diluted supernatant samples for 2 h followed by incubation with biotinylated mouse anti-human IFN-γ or IL-4 conjugated to avidin-horseradish peroxidase. The absorbance of the product was read at 450 nm using a microplate reader (Molecular Devices, Sunnyvale, CA, USA). The IFN-γ and IL-4 concentration of each test sample was calculated by reference to the standard curve. The levels of sensitivity for the IFN-γ and IL-4 assays were 4 pg/ml and 7 pg/ml, respectively. Unstimulated and antigen-stimulated secretion measurements for IFN-γ were performed in triplicate, while IL-4 was performed in duplicate. Individual-specific values were then summarized by taking means of the duplicate or triplicate values. Mean background levels of IFN-γ and IL-4 cytokine production in cultures not stimulated with measles peptides or MV were subtracted from the mean antigen-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.

2.4. HLA class II typing

Genomic DNA was extracted from blood samples by conventional techniques using the Puregene DNA extraction kit (Gentra Systems Inc., Minneapolis, MN, USA) and used for class II HLA allele typing. High-resolution sequence-specific primers (SSP) and reference-strand conformation analysis (RSCA) DRB1, DQB1 SSP and DPB1 SSP Unitray® typing kits with the entire locus on a single tray were utilized (Dynal Biotech, Brown Deer, WI, USA). Polymerase chain reaction (PCR) was followed by AmbiSolv™ primer mixes when needed. Any ambiguities were resolved using the ABI PRISM sequencing kits (Applied Biosystems, Foster City, CA, USA). All PCR amplificates were separated on an ABI 377 and analyzed using MatchTools software. All reactions were run with negative controls and every 50th PCR reaction was repeated for quality control.

2.5. Statistical analysis

The following outcomes were of primary interest: two sets of in vitro cytokine production (both IFN-γ and IL-4), each induced separately by three stimuli (live MV, MV-P, or MV-N peptides). Data were descriptively summarized using frequencies and percentages for all categorical variables, medians, and inter-quartile ranges for all continuous variables. We assessed pairwise linkage disequilibrium of the HLA-DQ and -DP loci with the HLA-DR locus using methods outlined by Lewontin [11].

Descriptive associations of the continuously distributed cytokine secretion values with HLA-DQ and HLA-DP alleles were evaluated on an allelic level. Each person contributed two observations to this descriptive analysis—one for each allele. Alleles were grouped by HLA type and summarized using medians and inter-quartile ranges. Following the descriptive comparisons, associations were more formally evaluated using linear 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. Rare alleles, defined as occurring fewer than five times among all subjects, were pooled into a category labeled “other.” Due to data skewness, the original secretion values were replaced with corresponding rank values. Global differences in cytokine secretion levels among all alleles were first 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 allele effects on cytokine secretion levels. This 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 univariate linear regression analysis, effectively comparing secretion levels for the allele of interest against all other alleles combined.

Primary global and allelic regression analyses included the design variable as a covariate. Subsequent analyses adjusted for the effects of the following potential confounding variables: age, race, gender, age at the first MMR-II and age at the second MMR-II vaccination. Furthermore, it is possible that linkage disequilibrium of DQ and DP alleles with the HLA-DR locus could confound the observed DQ and DP associations; that is, any observed findings for the DQ and DP alleles may in fact represent associations with DR alleles that are in linkage disequilibrium with DQ and DP. To assess this possibility, we ran a third set of regression analyses, which included the set of DR alleles as potential confounding variables. All *p*-values less than 0.05 were considered significant; however, *p*-values between 0.05 and 0.10 were considered marginally significant. We have treated the results as exploratory and worthy of replication in further studies. 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. Cytokine responses of study subjects

Overall, in vitro cytokine secretion levels for IFN-γ ($n = 306$) and IL-4 ($n = 284$) were assessed from the peripheral blood lymphocytes of subjects. The MV antigen was able to induce strong recall measles-specific IFN-γ (median response, 66.8 pg/ml) and IL-4-specific responses (median response, 10.3 pg/ml). Likewise, the overall median IFN-γ secretion levels for MV-P and MV-N peptides were 27.7 pg/ml and 1.9 pg/ml, respectively; and the median IL-4 secretion levels were −0.6 pg/ml and 2.4 pg/ml, respectively. In addition, MV-, MV-P-, and MV-N-induced detectable IFN-γ responses were found in approximately 88%, 76%, and 56% of the 306 subjects, respectively. We observed detectable IL-4 cytokine responses to MV, MV-P, and MV-N antigens in approximately 86%, 47%, and 63% of the 284 individuals, respectively. As expected, assessment of pairwise linkage disequilibrium revealed strong linkage between HLA-DRB1 and both HLA-DQB1 and HLA-DPB1 loci ($p < 0.001$ for each).

3.2. HLA associations with measles virus-induced cytokine responses

The associations between HLA-DQB1 and HLA-DPB1 loci and measles-specific IFN-γ and IL-4 responses are shown in Table 1. After adjusting for the effects of the DRB1 locus, global tests of significance failed to reveal significant associations between the DQB1 and DPB1 alleles with measles-specific IFN-γ secretion levels. The alleles with suggestive associations with measles-specific IFN-γ response in MMR-II immunized subjects included DQB1*0201 (median 25.9 pg/ml, $p = 0.02$), DQB1*0202 (median 45.1 pg/ml, $p = 0.07$), DQB1*0303 (median 22.9 pg/ml, $p = 0.02$), DQB1*0502 (median 175.3 pg/ml, $p = 0.08$), and DQB1*0602 (median 123.5 pg/ml, $p = 0.003$). However, these apparent associations were no longer significant after adjustment for the effect of HLA-DRB1 locus. In contrast, the DQB1*0604 (median 48.9 pg/ml, adjusted $p = 0.06$) allele was associated with lower measles-specific IFN-γ levels and remained stable after statistical adjustment for DRB1. Our subsequent exploratory analyses by allele for the HLA-DPB1 locus and IFN-γ secretion suggested a potential association with alleles DPB1*0101 (median 49.2 pg/ml, $p = 0.08$), DPB1*0402 (median 113.4 pg/ml, $p = 0.07$), and DPB1*0601 (median 257.2 pg/ml, $p = 0.02$). Again, after statistical adjustments for DRB1, only the DPB1*0601 (adjusted $p = 0.03$) allele was associated with high levels of MV-specific IFN-γ secretion. Furthermore, adjustments for HLA-DRB1 led to the finding of a suggestive association of the DPB1*0201 (median 42.3 pg/ml, adjusted $p = 0.09$) allele with lower measles-induced IFN-γ secretion. However, these results should be viewed with caution due to the lack of significant global tests.

Separate analyses were also performed for class II DQB1 and DPB1 loci and MV-specific IL-4 secretion. The two global tests (Table 1) failed to reveal statistically significant associations with IL-4 levels and the DQB1 and DPB1 loci. When examining individual alleles in an exploratory fashion, alleles DQB1*0202 (median 4.9 pg/ml, $p = 0.05$) and DQB1*0603 (median 12.1 pg/ml, adjusted $p = 0.09$) approached statistical significance and were marginally associated with lower and higher IL-4 response to measles, respectively. However, adjustments for DRB1 led to the dismissal of the DQB1*0202 allele association. Conversely, we found suggestive associations with the DPB1*0501 (median 4.2 pg/ml, adjusted $p = 0.06$) allele and measles-specific lower IL-4 immune response. These associations should again be interpreted with caution, however, due to the lack of significant global tests.

3.3. Associations between DQB1 and DPB1 alleles and recall peptide-specific cytokine responses

The association between class II (HLA-DQB1 and HLA-DPB1) loci and MV-P and MV-N peptide-specific cytokine responses were assessed and the results are summarized in Tables 2 and 3. Overall, very low secretion levels of IL-4 for MV-P peptide and low IFN-γ and IL-4

secretion levels for MV-N peptide were observed. The global test revealed no significant association of the DQB1 and DPB1 alleles with MV-P and MV-N peptide-specific IFN-γ and IL-4 cytokine secretion levels. Examination of IFN-γ responses to the MV-P peptide indicated that none of the individual alleles of the DQB1 and DPB1 loci were associated with an MV-P-induced IFN-γ T cell response (Table 2). A marginally significant increase in the frequency of the DQB1*0201 (median 2.6 pg/ml, $p = 0.06$) and DPB1*0201 (median 1.7 pg/ml, $p = 0.05$) alleles was found among subjects who demonstrated measurable IL-4 levels to the MV-P peptide. However, further analyses revealed that only DPB1*0201 alleles were associated with an IL-4 response to MV-P after adjustments for the HLA-DRB1 locus (adjusted $p = 0.02$). In fact, adjustments for HLA-DRB1 led to the finding of involvement of several other HLA markers: DQB1*0604 (median 2.1 pg/ml, adjusted $p = 0.02$) and DPB1*1301 (median −2.1 pg/ml, adjusted $p = 0.09$). The DQB1*0604 allele was marginally associated with a higher IL-4 response to MV-P, whereas the DPB1*1301 allele was marginally associated with a lower MV-P peptide-specific IL-4 secretion. The group of “other” alleles (HLA-DQB1) was also found to be statistically significant. However, these associations should be interpreted with caution due to the non-significance of global tests.

For the MV-N peptide (Table 3), the DQB1*0602 (median 4.7 pg/ml, $p = 0.03$) allele was associated with MV-N-induced IFN-γ response; however, adjustments for DRB1 led to the dismissal of this association. By examining the DPB1 alleles individually, we found an increase in the frequency of the DPB1*0501 allele (median −3.1 pg/ml, $p = 0.005$) among subjects who failed to produce MV-N peptide-specific IL-4 responses. Adjustments for the DRB1 locus did not change this strong relationship with DPB1*0501 allele (adjusted $p = 0.01$). However, these associations require further confirmation in larger studies and should be interpreted with caution due to the absence of significant global tests. In addition to the age-adjusted analyses presented above, we ran multivariate logistic regression analyses, accounting for the effects of gender, race, age at first administration of the MMR-II vaccine, and age at the second administration of the vaccine. These results were virtually identical to the age-adjusted results shown in Tables 1–3.

4. Discussion

Collectively, this study demonstrates the biological relevance of HLA-DRB1*0301 presented measles virus peptides and the importance of identification of measles promiscuous peptides that could make ideal targets for peptide-based vaccine development. We assessed IFN-γ and IL-4 cytokine responses to measles and to naturally processed measles HLA-DR-presented peptides, corresponding to 19-aa and 14-aa-long peptides derived from measles phosphoprotein (ASDVETAEGGEIHELLRLQ) and nucleoprotein (SAGKVSSTLASELG), respectively. We were interested in assessing IFN-γ and IL-4 production since the Th1 signature (IFN-γ) cytokine is identified during the early phases of MV infection, and the Th2 signature (IL-4) cytokine, predominates as the infection progresses [12,13]. In this study, peripheral blood lymphocytes from 88% and 86% of our subjects proliferated and secreted IFN-γ and IL-4 cytokines, respectively, in response to the measles virus. Peripheral blood lymphocytes from 76% (IFN-γ) and 47% (IL-4) of individuals responded to MV-P, and 56% (IFN-γ) and 63% (IL-4) of individuals responded to MV-N peptides, respectively. However, measles-specific cytokine responses were usually higher than MV-P or MV-N peptide-specific IFN-γ and IL-4 levels. The ability of these measles peptides to generate cell-mediated immune responses and stimulate cytokine production allowed us to further explore the concept of promiscuous T cell recognition of immunodominant antigenic peptides with a wide range of class II HLA molecules [14–16].

Previously, we reported associations between some HLA-DRB1 alleles and cytokine responses to the MV-P and MV-N peptides [4]. For the MV-P peptide, the allele with the strongest

association with both IFN-γ ($p = 0.02$) and IL-4 ($p = 0.03$) secretion was DRB1*0301. For the MV-N peptide, the alleles with the strongest associations with IFN-γ and IL-4 production were DRB1*1501 ($p = 0.04$) and DRB1*1103/*1303 ($p = 0.01$), respectively, suggesting that MV-N is a promiscuous peptide [4]. These data support the concept that HLA-DR-derived measles peptides are not completely restricted, and can potentially be presented by other class II alleles. Thus, we ran a series of regression analyses adjusting for the effects of the DRB1 locus. Associations of DQB1 and DPB1 alleles with cytokine secretion were substantially attenuated after adjustment for DRB1. For example, the statistically significant global association (*p*-value 0.001) between measles-specific IFN-γ and DQB1 disappeared (global *p*-value 0.33), and many of the other global and allele-specific associations also disappeared or attenuated. This confirms the notion that HLA-DRB1 are the major restriction molecules for measles-derived antigen presentation to T cells and that "the immunogenicity and protective efficacy of candidate vaccines need to be defined in the context of HLA-DR polymorphisms" [17]. Importantly, both genetic and non-genetic factors were used for statistical adjustment in all our analyses.

A marginally significant increase in the frequency of the DQB1*0604 alleles were found among subjects who demonstrated the highest IL-4 levels after MV-P peptide stimulation. The DPB1*0201 and DPB1*1301 alleles were marginally associated with MV-P-induced IL-4 secretion, suggesting that PBMC expressing the DPB1*0201 and DPB1*1301 alleles may induce recall Th2-like cytokine responses among previously primed measles-specific T lymphocytes. In addition, we found an increase in the frequency of the DPB1*0501 allele among subjects who failed to produce MV-N-specific IL-4 responses. Generally, genetic associations between MV-N peptide-specific IL-4 levels and HLA class II DQB1 and DPB1 alleles were not as strong as those observed for IL-4 responses to the MV-P peptide. After examining the associations between DQB1 and DPB1 loci and measles peptide-specific cytokine responses, we found that none of the alleles of the DQ and DP loci were associated with MV-P and MV-N peptide-induced IFN-γ T cell responses. These data suggest that a variety of HLA alleles (and other genetic factors) may play a role in determining the level of cytokine responses after antigen stimulation. However, these results should be viewed cautiously due to multiple testing issues. Although we have observed limited associations between DQ and DP loci and MV-derived peptide-specific cytokine secretion levels, other candidate genes, such as those that encode immunoregulatory cytokines and cytokine receptors, may also influence these immune responses. Further studies are necessary in this regard, and are planned.

There are limitations to our study. 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 IFN-γ secretion and the pooled group of "other" alleles for HLA-DQB1 highlights this possibility. We also recognize that secreted cytokine values cannot truly be negative and that negative values are a reflection of measurement variability. Additionally, it is difficult to detect secreted IL-4 levels in culture supernatants stimulated in vitro with measles and measles-derived peptides using conventional ELISA techniques [10]. In order to detect IL-4 secretion after 6 days in cell culture, anti-IL-4 receptor-blocking antibody was used in order to inhibit IL-4 uptake by various blood cells. Further, low measles-specific precursor T cell frequency in the peripheral circulation might have also resulted in low cytokine production by measles-reactive cells [18].

It has also been reported that linkage disequilibrium exists between DR and its neighbor loci, DQ and DP [6]. Sierpe et al. [19] showed that linkage disequilibrium between DRB1 and DQA1 is very strong, much stronger than the linkage disequilibrium between DQA1 and DQB1 loci. For example, all DR4 subtypes (*0401–*0408) carry the DQA1*0301 allele, and almost all subtypes of DR11 and DR12 carry the DQA1 subtype DQA1*0501 [19]. In the relationship

between DRB1 and DQB1 loci, there is linkage disequilibrium between DRB1*0301 and DQB1*0201, DRB1*1101–*1104 and DQB1*0301, and DRB1*1303 and DQB1*0301. There is an exception with the DRB1*0407 allele, which is found exclusively on the same haplotype as DQB1*0301 rather than DQB1*0302 [19]. In spite of the generally held belief that there is no linkage disequilibrium between DRB1 and DPB1 loci, a number of significant linkage disequilibria between DR7 and DPB1*1101 and DPB1*1701 have been reported [19]. In fact, the relationship between DRB1*0301 (DR3) and DPB1*0101 has been described [20]. Recently, it was also reported that alleles DRB1*1501 (DR2) and DRB1*0401 are in significant linkage disequilibrium with DPB1*0401. In fact, our study found strong evidence of linkage disequilibrium between the DR, DQ and DP loci. Upon adjustment for DRB1, some of the DQB1- and DPB1-related associations with cytokine secretion disappeared or attenuated substantially. Thus, HLA-DR restricted responses appear to play a significant role in the cellular immune recall response following measles immunization. Future studies, perhaps based on HLA haplotypes, may be needed to further resolve these issues.

Studies show that DR and DQ loci contribute the main restriction determinants for antigen-presenting cells [6,21,22]. Hickman et al. [23] report that human lymphoproliferative responses to measles nucleoprotein (N) and to a series of 32 overlapping MV-N synthetic peptides (17–21 residues in length) were inhibited by antibodies specific for the HLA-DR, -DQ and -DP alleles, suggesting that DR, and possibly DQ and DP alleles present these MV-N peptides to T cells. Further, mAb specific for HLA-DR resulted in approximately 50% inhibition of lymphoproliferative responses to MV-N protein [23]. Demotz et al. [24] demonstrated that DRB1*1103-restricted T cell clones specific for MV-N could be generated and mAb against HLA-DR could completely block the lymphoproliferative response to this peptide, although HLA-DP and HLA-DQ mAb did not prevent proliferation. However, the generation of MV nucleoprotein-specific T cells only occurred after stimulating PBMC with CNBr-digested N protein and was not studied following immunization. Isolated N-specific T cell clones do present in the context of HLA-DR, but it is unknown whether DR-specific mAb blocks the response to MV following immunization. Our own data demonstrate that after stimulation with 10 μg/ml of HLA-DR and HLA-DQ specific mAb, lymphocyte proliferation in response to measles stimulation was inhibited by approximately 71% and 32%, respectively, in subjects two weeks post MMR-II immunization [25]. This suggests that a significant proportion of the cellular response to measles in vitro is HLA-DR restricted and to a lesser extent HLA-DQ restricted [25]. This information is intriguing for the design of immunization strategies using peptide-based vaccines in the genetically outbred human population.

In conclusion, our data suggest that while the HLA-DRB1 locus is the major restriction molecule for measles virus antigen presentation, HLA-DRB1 measles-derived peptides can also be recognized in association with different HLA class II molecules, including the HLA-DQB1 and HLA-DPB1 alleles. We observed that cytokine responses to measles peptides are dominated by the effect of the HLA-DR allele-related polymorphisms. This information provides further support for the importance of identifying and sequencing of measles-derived peptides presented by other HLA-DQ and HLA-DP alleles. In turn, the identification of such promiscuous class II viral peptides could be useful in the development of a peptide-based measles vaccine.

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References

1. Eckels DD, Lake P, Lamb JR, et al. SB-restricted presentation of influenza and herpes simplex virus antigens to human T-lymphocyte clones. Nature 1983;301(5902):716–8. [PubMed: 6186920]
2. Germain RN. Modern concepts in immune recognition and lymphocyte activation: relevance for the development of useful vaccines. Int J Technol Assess Health Care 1994;10:81–92. [PubMed: 8157465]
3. Germain RN. The biochemistry and cell biology of antigen presentation by MHC Class I and Class II Molecules. Ann NY Acad Sci 1998;754:114–25. [PubMed: 7625645]
4. Ovsyannikova IG, Johnson KL, Muddiman DC, Vierkant RA, Poland GA. Identification and characterization of novel, naturally processed measles virus class II HLA-DRB1 peptides. J Virol 2004;78(1):42–51. [PubMed: 14671086]
5. Eckels DD, Zeevi A, Beatty PG, et al. ASHI Workshop Summary Report of the Science and Education Subcommittee: structural and functional relationships of human class II MHC molecules. Hum Immunol 1986;15(1):68–74. [PubMed: 2419286]
6. Mellins E, Woelfel M, Pious D. Importance of HLA-DQ and -DP restriction elements in T-cell responses to soluble antigens: mutational analysis. Hum Immunol 1987;18(3):211–23. [PubMed: 3032874]
7. Guttinger M, Romagnoli P, Vandel L, et al. HLA polymorphism and T cell recognition of a conserved region of p190, a malaria vaccine candidate. Int Immunol 1991;3(9):899–906. [PubMed: 1718405]
8. Ovsyannikova IG, Jacobson RM, Vierkant RA, Jacobsen SJ, Pankratz VS, Poland GA. The contribution of HLA class I antigens in immune status following two doses of rubella vaccination. Hum Immunol 2004;65:1506–15. [PubMed: 15603879]
9. Ovsyannikova IG, Johnson KL, Naylor S, Muddiman DC, Poland GA. Naturally processed measles virus peptide eluted from class II HLA-DRB1*03 recognized by T lymphocytes from human blood. Virology 2003;312(2):495–506. [PubMed: 12919753]
10. Dhiman N, Ovsyannikova IG, Howe RC, Ryan JE, Jacobson RM, Poland GA. Interleukin-4 induced by measles virus and measles-derived peptides as measured by IL-4 receptor-blocking ELISA. J Immunol Methods 2004;287(1–2):217–25. [PubMed: 15099769]
11. Lewontin RC. The interaction of selection and linkage. II. Optimum models. Genetics 1964;50:757–82. [PubMed: 14221879]
12. Griffin DE, Ward BJ. Differential CD4 T cell activation in measles. J Infect Dis 1993;168(2):275–81. [PubMed: 8101549]
13. Ward BJ, Griffin DE. Changes in cytokine production after measles virus vaccination: predominant production of IL-4 suggests induction of a Th2 response. Clin Immunol Immunopathol 1993;67:171–7. [PubMed: 8519092]
14. Panina-Bordignon P, Tan A, Termijtelen AAM, Demotz S, Corradin G, Lanzavecchia A. Universally immunogenic T cell epitopes: promiscuous binding to human MHC class II and promiscuous recognition by T cells. Eur J Immunol 1989;79:2237–42. [PubMed: 2481588]
15. Partidos CD, Steward MW. Prediction and identification of a T cell epitope in the fusion protein of measles virus immunodominant in mice and humans. J Gen Virol 1990;71:2099–105. [PubMed: 2212993]
16. Sette A, Vitiello A, Farness P, et al. Random association between the peptide repertoire of A2.1 class I and several different DR class II molecules. J Immunol 1991;147(11):3893–900. [PubMed: 1658152]
17. Taneja V, David CS. HLA transgenic mice as humanized mouse models of disease and immunity. J Clin Invest 1998;101:921–6. [PubMed: 9486959]
18. Ovsyannikova IG, Dhiman N, Jacobson RM, Vierkant RA, Poland GA. Frequency of measles virus-specific CD4+ and CD8+ T cells in subjects seronegative or highly seropositive for measles vaccine. Clin Diag Lab Immunol 2003;10(3):411–6.
19. Sierpe, EAG.; Keller, E.; Bettinotti, MDLP., et al. Proceedings of the Eleventh International Histocompatibility Workshop and Conference. W3.7, Oligotyping with DRB generic amplified DNA and oligonucleotides 0–25. In: Tsuji, K.; Aizawa, M.; Sasazuki, T., editors. HLA. Oxford: Oxford University Press. 1991. 1992. p. 454-7.

20. Heward J, Gough SC. Genetic susceptibility to the development of autoimmune disease. Clin Sci (Lond) 1997;93(6):479–91. [PubMed: 9497784]

21. Kappes D, Strominger JL. Human class II major histocompatibility complex genes and proteins. Annu Rev Biochem 1988;57:991–1028. [PubMed: 3140715]

22. Thorsby E, Berle E, Nousiainen H. HLA-D region molecules restrict proliferative T cell responses to antigen. Immunol Rev 1982;66:39–56. [PubMed: 6182089]

23. Hickman CJ, Khan AS, Rota PA, Bellini WJ. Use of synthetic peptides to identify measles nucleoprotein T-cell epitopes in vaccinated and naturally infected humans. Virology 1997;235:386–97. [PubMed: 9281519]

24. Demotz S, Ammerlaan W, Fournier P, Muller CP, Barbey C. Processing of the DRB1*1103-restricted measles virus nucleoprotein determinant 185–199 in the endosomal compartment. Clin Exp Immunol 1998;114:228–35. [PubMed: 9822281]

25. Phelan DM, Ovsyannikova IG, Poland GA. HLA-DR/DQ specific monoclonal antibodies block lympholiferative response to measles vaccine in vitro. Mol Aspects Viral Immun 2001;222:64. [abstract]

Table 1

HLA class II allelic associations with whole measles virus-specific cytokine[a] responses

HLA locus	Number of alleles	IFN-γ			IL-4			
		Median secretion value (Q1, Q3) pg/ml	Allele *p*-value[b,c]	HLA-DR-adjusted *p*-value[d]	Number of alleles	Median secretion value (Q1, Q3) pg/ml	Allele *p*-value[b,c]	HLA-DR-adjusted *p*-value[d]
Overall	612	66.8 (12.7, 231.9)			568	10.3 (3.9, 24.9)		
HLA-DQB1								
*0201	82	25.9 (8.7, 120.8)	**0.02**	0.61	77	8.9 (5.1, 23.9)	0.93	0.65
*0202	47	45.1 (3.4, 112.8)	**0.07**	0.91	44	4.9 (0.9, 21.9)	**0.05**	0.89
*0301	117	66.8 (13.6, 248.5)	0.55	0.16	110	10.2 (4.0, 25.6)	0.64	0.37
*0302	61	75.3 (18.6, 281.1)	0.31	0.27	55	12.2 (4.0, 32.0)	0.61	0.84
*0303	24	22.9 (0.1, 96.3)	**0.02**	0.45	20	7.3 (0.7, 20.9)	0.20	0.22
*0402	17	27.3 (8.3, 68.8)	0.11	0.36	15	9.7 (4.4, 33.5)	0.57	0.74
*0501	70	73.2 (14.5, 196.8)	0.93	0.99	71	14.3 (4.3, 24.8)	0.88	0.58
*0502	10	175.3 (52.9, 227.8)	**0.08**	0.29	7	11.1 (7.5, 17.9)	0.87	0.74
*0503	15	67.1 (18.7, 198.1)	0.96	0.93	13	8.1 (0.6, 18.5)	0.34	0.48
*0602	85	123.5 (42.6, 325.7)	**0.003**	0.28	77	15.5 (3.8, 25.3)	0.57	0.83
*0603	41	86.6 (12.6, 301.4)	0.46	0.82	42	12.1 (4.5, 30.5)	0.29	**0.09**
*0604	30	48.9 (−0.6, 224.4)	0.21	**0.06**	26	8.6 (4.3, 18.5)	0.47	0.63
All other alleles[e]	13	214.7(39.5, 320.4)	0.08	0.08	11	17.7 (8.7, 30.4)	0.17	0.21
HLA-DPB1								
*0101	46	49.2 (8.1, 119.1)	**0.08**	0.63	45	11.4 (5.9, 24.5)	0.63	0.38
*0201	74	42.3 (12.4, 172.4)	0.13	**0.09**	67	13.0 (4.5, 26.0)	0.57	0.55
*0301	65	54.3 (11.3, 306.7)	0.88	0.98	58	15.8 (4.7, 31.2)	0.20	0.20
*0401	233	73.6 (13.5, 231.9)	0.47	0.70	213	9.7 (2.8, 23.1)	0.42	0.22
*0402	74	113.4 (13.0, 323.5)	**0.07**	0.12	74	11.2 (1.4, 30.4)	0.63	0.84
*0501	18	62.8 (16.7, 246.1)	0.91	0.98	16	4.2 (0.6, 14.2)	0.11	**0.06**
*0601	13	257.2 (72.0, 379.0)	**0.02**	**0.03**	11	15.8 (7.3, 31.2)	0.22	0.22
*1101	11	45.1 (2.8, 112.8)	0.16	0.91	10	8.0 (1.9, 24.3)	0.57	0.76
*1301	13	27.4 (13.0, 118.0)	0.31	0.24	13	5.5 (0.1, 11.1)	0.14	0.27
*1401	11	80.8 (15.7, 275.7)	0.79	0.99	11	12.4 (1.0, 17.3)	0.83	0.74
*1501	7	45.1 (17.8, 232.4)	0.80	0.89	6	22.7 (4.7, 29.1)	0.45	0.56
*1701	7	129.6 (1.7, 217.3)	0.94	0.96	7	19.6 (1.8, 29.7)	0.49	0.34
All other alleles[e]	40	66.6 (17.2, 215.4)	0.86	0.32	37	7.8 (4.4, 15.8)	0.42	0.60

Q1, Q3 represent the first and third quartiles, respectively.

Age-adjusted global *p* values are as follows: HLA-DQB1 IFN-γ, 0.001; HLA-DPB1 IFN-γ, 0.17; HLA-DQB1 IL-4, 0.58; HLA-DPB1 IL-4, 0.58. Age and HLA-DRB1-adjusted global *P* values are as follows: HLA-DQB1 IFN-γ, 0.33; HLA-DPB1 IFN-γ, 0.47; HLA-DQB1 IL-4, 0.75; HLA-DPB1 IL-4, 0.53. Suggestive findings ($p < 0.10$) are shown in bold.

[a] Mean value of antigen stimulated cells minus mean value of control cells.

[b] Linear regression analysis, accounting for the design variable age. Genotypes were modeled as ordinal variables with values ranging from 0 to 2, reflecting the number of copies possessed by an individual. Due to data skewness, all secretion values were rank-transformed.

[c] Comparing genotype of interest to all other genotypes combined.

[d] Analyses adjust for age and the HLA-DRB1*01, *02, *03, *04, *05, *06, *07, *08, and *09 alleles.

[e] "Other" includes the following HLA-DQB1 alleles: *0203 (*n* = 2), *0304 (*n* = 1), *0306 (*n* = 2), *0601 (*n* = 4), *0608 (*n* = 1), *0609 (*n* = 1), *0614 (*n* = 2); and HLA-DPB1 alleles: *0202 (*n* = 4), *0901 (*n* = 6), *1001 (*n* = 6), *1601 (*n* = 4), *1901 (*n* = 4), *2001 (*n* = 3), *2101 (*n* = 1), *2301 (*n* = 3), *2401 (*n* = 1), *2601 (*n* = 2), *3001 (*n* = 1), *5001 (*n* = 1), *5101 (*n* = 1), *6301 (*n* = 1), *6601 (*n* = 2), respectively.

Table 2

HLA class II allelic associations with naturally processed measles virus-derived P peptide-specific cytokine[a] responses

HLA locus	Number of alleles	IFN-γ			IL-4			
		Median secretion value (Q1, Q3) pg/ml	Allele *p*-value[b,c]	HLA-DR-adjusted *p*-value[d]	Number of alleles	Median secretion value (Q1, Q3) pg/ml	Allele *p*-value[b,c]	HLA-DR-adjusted *p*-value[d]
Overall	612	27.7 (1.8, 109.4)			568	−0.6 (−7.1, 6.2)		
HLA-DQB1								
*0201	82	29.0 (6.9, 111.8)	0.31	0.14	77	2.6 (−4.6, 10.7)	**0.06**	0.80
*0202	47	15.7 (−3.0, 78.6)	0.28	0.92	44	−0.7 (−7.0, 6.3)	0.69	0.58
*0301	117	23.5 (−2.9, 95.7)	0.36	0.98	110	−0.6 (−7.8, 4.9)	0.42	0.58
*0302	61	39.6 (5.7, 121.8)	0.30	0.52	55	0.8 (−6.4, 7.9)	0.41	0.81
*0303	24	27.6 (3.2, 174.8)	0.67	0.57	20	−2.3 (−9.1, 9.9)	0.80	0.44
*0402	17	23.9 (3.3, 55.5)	0.51	0.59	15	−1.4 (−8.0, 17.9)	0.85	0.52
*0501	70	46.6 (3.5, 106.8)	0.30	0.28	71	−0.9 (−7.3, 5.3)	0.60	0.24
*0502	10	74.6 (−1.6, 157.3)	0.74	0.50	7	−0.4 (−6.6, 7.8)	0.68	0.56
*0503	15	17.4 (0.7, 70.2)	0.61	0.76	13	−1.2 (−7.2, −0.2)	0.44	0.81
*0602	85	23.9 (−3.0, 122.2)	0.70	0.33	77	−0.7 (−9.3, 5.8)	0.50	0.59
*0603	41	33.5 (−5.9, 65.3)	0.20	0.17	42	−3.0 (−9.5, 2.4)	0.10	0.65
*0604	30	13.6 (−2.9, 142.1)	0.58	0.59	26	2.1 (−5.7, 11.1)	0.29	**0.02**
All other alleles[e]	13	114.8 (17.6, 15.0)	0.04	0.02	11	0.8 (−16.2,15.0)	0.96	0.84
HLA-DPB1								
*0101	46	26.9 (11.9, 111.8)	0.27	0.81	45	−1.2 (−8.7, 11.3)	0.82	0.46
*0201	74	32.9 (1.9, 111.8)	0.53	0.28	67	1.7 (−4.1, 11.1)	**0.05**	**0.02**
*0301	65	25.5 (3.5, 121.8)	0.78	0.81	58	−0.7 (−8.9, 7.4)	0.63	0.48
*0401	233	27.4 (−0.7, 106.8)	0.36	0.32	213	0.2 (−7.2, 6.3)	0.80	0.63
*0402	74	25 (−0.3, 114.8)	0.61	0.64	74	−1.7 (−8.5, 5.6)	0.17	0.28
*0501	18	29.9 (0.1, 106.8)	0.95	1.00	16	−2.6 (−7.8, 2.2)	0.25	0.21
*0601	13	14.6 (5.0, 50.9)	0.65	0.54	11	0.3 (−4.6, 4.6)	0.66	0.68
*1101	11	9.9 (−5.7, 29.6)	0.18	0.23	10	0.5 (−2.7, 12.4)	0.33	0.64
*1301	13	28.0 (6.6, 132.3)	0.98	0.61	13	−2.1 (−8.7, 0)	0.10	**0.09**
*1401	11	29.3 (−5.4, 127.2)	0.95	0.86	11	5.3 (−2.0, 13.5)	0.27	0.17
*1501	7	7.6 (−4.7, 164.2)	0.87	0.88	6	4.9 (−1.0, 17.9)	0.28	0.36
*1701	7	17.4 (0.1, 78.6)	0.97	0.75	7	−0.6 (−6.1, 6.3)	1.00	0.82
All other alleles[e]	40	44.4 (3.2, 148.0)	0.27	0.23	37	−2.9 (−5.5, 4.7)	0.35	0.44

Q1, Q3 represent the first and third quartiles, respectively.

Age-adjusted global *p* values are as follows: HLA-DQB1 IFN-γ, 0.46; HLA-DPB1 IFN-γ 0.93; HLA-DQB1 IL-4, 0.70; HLA-DPB1 IL-4, 0.36. Age and HLA-DRB1-adjusted global *p* values are as follows: HLA-DQB1 IFN-γ, 0.18; HLA-DPB1 IFN-γ, 0.95; HLA-DQB1 IL-4, 0.49; HLA-DPB1 IL-4, 0.28. Suggestive findings ($p < 0.10$) are shown in bold.

[a] Mean value of antigen stimulated cells minus mean value of control cells.

[b] Linear regression analysis, accounting for the design variable age. Genotypes were modeled as ordinal variables with values ranging from 0 to 2, reflecting the number of copies possessed by an individual. Due to data skewness, all secretion values were rank-transformed.

[c] Comparing genotype of interest to all other genotypes combined.

[d] Analyses adjust for age and the HLA-DRB1*01, *02, *03, *04, *05, *06, *07, *08, and *09 alleles.

[e] "Other" includes the following HLA-DQB1 alleles: *0203 (*n* = 2), *0304 (*n* = 1), *0306 (*n* = 2), *0601 (*n* = 4), *0608 (*n* = 1), *0609 (*n* = 1), *0614 (*n* = 2); and HLA-DPB1 alleles: *0202 (*n* = 4), *0901 (*n* = 6), *1001 (*n* = 6), *1601 (*n* = 4), *1901 (*n* = 4), *2001 (*n* = 3), *2101 (*n* = 1), *2301 (*n* = 3), *2401 (*n* = 1), *2601 (*n* = 2), *3001 (*n* = 1), *5001 (*n* = 1), *5101 (*n* = 1), *6301 (*n* = 1), *6601 (*n* = 2), respectively.

Table 3

HLA class II allelic associations with naturally processed measles virus-derived N peptide-specific cytokine[a] responses

HLA locus	Number of alleles	IFN-γ			IL-4			
		Median secretion value (Q1, Q3) pg/ml	Allele *p*-value[b,c]	HLA-DR-adjusted *p*-value[d]	Number of alleles	Median secretion value (Q1, Q3) pg/ml	Allele *p*-value[b,c]	HLA-DR-adjusted *p*-value[d]
Overall	612	1.9 (−6.2, 13.0)			568	2.4 (−3.2, 9.3)		
HLA-DQB1								
*0201	82	2.2 (−5.4, 9.3)	0.50	0.52	77	2.3 (−1.2, 13.4)	0.36	0.15
*0202	47	−2.6 (−7.8, 14.0)	0.29	0.89	44	0.5 (−3.5, 6.8)	0.39	0.14
*0301	117	0.6 (−8.6, 14.7)	0.43	0.91	110	4.5 (−1.4, 11.3)	0.24	0.39
*0302	61	2.8 (−6.2, 10.4)	0.78	0.64	55	1.7 (−3.9, 8.8)	0.82	0.50
*0303	24	4.9 (−3.6, 13.8)	0.41	0.40	20	−0.1 (−7.5, 10.2)	0.47	0.68
*0402	17	4.2 (−2.8, 10.3)	0.43	0.88	15	1.9 (0.1, 9.0)	0.50	0.34
*0501	70	−1.3 (−6.1, 10.9)	0.45	0.44	71	2.1 (−3.1, 6.8)	0.24	0.38
*0502	10	2.1 (−7.5, 41.3)	0.62	0.69	7	−0.6 (−3.5, 9.9)	0.88	0.71
*0503	15	3.8 (−10.1, 10.2)	0.39	0.25	13	3.7 (−3.1, 12.2)	0.90	0.61
*0602	85	4.7 (−4.5, 35.2)	**0.03**	0.20	77	3.3 (−3.5, 9.9)	0.84	0.91
*0603	41	4.7 (−3.8, 12.8)	0.61	0.73	42	3.1 (−4.3, 6.2)	0.39	0.92
*0604	30	−2.2 (−12.3, 17.0)	0.34	0.18	26	3.8 (−2.0, 12.7)	0.62	0.17
All other alleles[e]	13	7.6 (0.2, 71.5)	0.10	0.22	11	8.4 (−5.4, 19.2)	0.28	0.17
HLA-DPB1								
*0101	46	2.5 (−4.5, 10.8)	0.92	0.64	45	1.8 (−3.3, 6.7)	0.49	0.35
*0201	74	0.3 (−9.0, 10.5)	0.20	0.19	67	4.8 (−2.4, 11.1)	0.46	0.47
*0301	65	1.8 (−5.5, 9.3)	0.31	0.18	58	4.3 (−1.8, 11.6)	0.26	0.21
*0401	233	2.3 (−5.5, 13.4)	0.75	0.99	213	2.8 (−3.1, 8.8)	0.73	0.67
*0402	74	2.9 (−6.1, 18.4)	0.32	0.23	74	3.7 (−2.1, 11.3)	0.69	0.83
*0501	18	7.5 (−4.5, 13.0)	0.50	0.63	16	−3.1 (−7.5, 1.9)	**0.005**	**0.01**
*0601	13	−4.6 (−17.6, 9.7)	0.13	0.19	11	5.2 (−0.3, 18.3)	0.31	0.32
*1101	11	0.4 (−3.9, 19.1)	0.82	0.42	10	−0.9 (−3.1, 6.8)	0.55	0.50
*1301	13	0.6 (−2.6, 14.7)	0.91	0.71	13	1.9 (−0.5, 9.4)	0.98	0.95
*1401	11	11.6 (−4.5, 28.4)	0.27	0.24	11	7.6 (1.6, 11.1)	0.27	0.15
*1501	7	9.2 (−8.2, 80.5)	0.26	0.17	6	6.9 (−1.5, 19.2)	0.30	0.38
*1701	7	4.7 (−2.5, 10.6)	0.53	0.44	7	1.9 (−6.7, 28.1)	0.64	0.62
All other alleles[e]	40	−0.7 (−7.6, 18.0)	0.77	0.70	37	0.4 (−5.4, 6.8)	0.25	0.38

Q1, Q3 represent the first and third quartiles, respectively.

Age-adjusted global *p* values are as follows: HLA-DQB1 IFN-γ, 0.30; HLA-DPB1 IFN-γ 0.70; HLA-DQB1 IL-4, 0.87; HLA-DPB1 IL-4, 0.25. Age and HLA-DRB1-adjusted global *p* values are as follows: HLA-DQB1 IFN-γ, 0.55; HLA-DPB1 IFN-γ, 0.55; HLA-DQB1 IL-4, 0.18; HLA-DPB1 IL-4, 0.21. Suggestive findings ($p < 0.10$) are shown in bold.

[a] Mean value of antigen stimulated cells minus mean value of control cells.

[b] Linear regression analysis, accounting for the design variable age. Genotypes were modeled as ordinal variables with values ranging from 0 to 2, reflecting the number of copies possessed by an individual. Due to data skewness, all secretion values were rank-transformed.

[c] Comparing genotype of interest to all other genotypes combined.

[d] Analyses adjust for age and the HLA-DRB1*01, *02, *03, *04, *05, *06, *07, *08, and *09 alleles.

[e] "Other" includes the following HLA-DQB1 alleles: *0203 (*n* = 2), *0304 (*n* = 1), *0306 (*n* = 2), *0601 (*n* = 4), *0608 (*n* = 1), *0609 (*n* = 1), *0614 (*n* = 2); and HLA-DPB1 alleles: *0202 (*n* = 4), *0901 (*n* = 6), *1001 (*n* = 6), *1601 (*n* = 4), *1901 (*n* = 4), *2001 (*n* = 3), *2101 (*n* = 1), *2301 (*n* = 3), *2401 (*n* = 1), *2601 (*n* = 2), *3001 (*n* = 1), *5001 (*n* = 1), *5101 (*n* = 1), *6301 (*n* = 1), *6601 (*n* = 2), respectively.