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Human Leukocyte Antigen and Cytokine Receptor Gene Polymorphisms Associated With Heterogeneous Immune Responses to Mumps Viral Vaccine

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Abstract

OBJECTIVES—Mumps outbreaks continue to occur throughout the world, including in highly vaccinated populations. Vaccination against mumps has been successful; however, humoral and cellular immune responses to mumps vaccines vary significantly from person to person. We set out to assess whether HLA and cytokine gene polymorphisms are associated with variations in the immune response to mumps viral vaccine.

METHODS—To identify genetic factors that might contribute to variations in mumps vaccine–induced immune responses, we performed HLA genotyping in a group of 346 healthy schoolchildren (12–18 years of age) who previously received 2 doses of live mumps vaccine. Single-nucleotide polymorphisms (minor allele frequency of >5%) in cytokine and cytokine receptor genes were genotyped for a subset of 118 children.

RESULTS—Median values for mumps-specific antibody titers and lymphoproliferative stimulation indices were 729 IU/mL and 4.8, respectively. Girls demonstrated significantly higher mumps antibody titers than boys, indicating gender-linked genetic differences in humoral immune response. Significant associations were found between the HLA-DQB1*0303 alleles and lower mumps-specific antibody titers. An interesting finding was the association of several HLA class II alleles with mumps-specific lymphoproliferation. Alleles of the DRB1 (*0101, *0301, *0801, *1001, *1201, and *1302), DQA1 (*0101, *0105, *0401, and *0501), and DQB1 (*0201, *0402, and *0501) loci were associated with significant variations in lymphoproliferative immune responses to mumps vaccine. Additional associations were observed with single-nucleotide polymorphisms in the interleukin-10RA, interleukin-12RB1, and interleukin-12RB2 cytokine receptor genes. Minor alleles for 4 single-nucleotide polymorphisms within interleukin-10RA and interleukin-12RB genes were associated with variations in humoral and cellular immune responses to mumps vaccination.

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CONCLUSIONS—These data suggest the important role of HLA and immunoregulatory cytokine receptor gene polymorphisms in explaining variations in mumps vaccine-induced immune responses.

Keywords

mumps; mumps vaccine; immunity; cellular; antibodies; viral; lymphocyte proliferation; genes; HLA complex; single-nucleotide polymorphisms

What's Known on This Subject

Mumps outbreaks throughout the world reveal the continued susceptibility to mumps virus in highly vaccinated populations. Immune responses to mumps-containing vaccine may vary from person to person. Limited information exists regarding the genetic basis for variation in mumps vaccine-induced immune responses.

What This Study Adds

This study examined how genetic polymorphisms lead to individual and population variations in immune responses to mumps viral vaccine. Both HLA and cytokine receptor gene polymorphisms play an important role in variations in mumps-induced humoral and cellular immune responses after mumps immunization.

Despite the availability of an effective vaccine, outbreaks of mumps continue to occur throughout the world. Although primarily an acute, self-limited, nonsuppurative parotitis, mumps can lead to a variety of relatively frequent and potentially serious complications. In 2006, the United States experienced its largest outbreak of mumps since 1988, with nearly 3000 cases reported in at least 11 states.^{1,2} In the US outbreak, 63% of patients had received 1, 2, or more doses of measles-mumps-rubella (MMR) vaccine.¹ The underlying reason for this epidemic is unknown. The United Kingdom also experienced a recent mumps epidemic that peaked during 2005 with ~56 000 cases and a high attack rate among young adults,³ although the epidemiology behind the US (highly vaccinated population) and British (decline in vaccine use) mumps epidemics is different.⁴ The majority of mumps epidemics affect the unvaccinated^{5,6}; however, mumps epidemics have also occurred in highly vaccinated populations.^{7,8} It is not known exactly why mumps outbreaks are occurring, especially in highly vaccinated populations; therefore, understanding the genetic factors that might influence immunity to mumps vaccination could provide additional insight into mumps vaccine nonresponse.

In this regard, the HLA genes encode proteins expressed on antigen-presenting cells and are the mechanism by which processed antigenic peptides are presented to T cells and, hence, in part determine the ability of the person to respond immunologically to endogenous and exogenous mumps antigens.^{9,10} Binding of antigenic peptides occurs within the HLA peptide binding groove, and the repertoire of naturally processed peptides depends on HLA allele-specific polymorphisms.¹¹ As intercellular protein messengers, cytokines also play an important role in the regulation of both humoral and cellular immunity.^{12,13} Cytokines act on their target cells by binding specific membrane receptors (ie, cytokine receptors). On the basis of their 3-dimensional structure and activities, the receptors and their corresponding cytokines have been divided into several families.^{14,15} In recent years, the cytokine receptors have received special attention because of their characteristics and the important role of cytokine and cytokine receptor gene polymorphisms in inflammatory and infectious diseases.^{16,17} Because of the importance of cytokines in shaping immune responses, polymorphisms in cytokines or their receptors that alter cytokine levels or cytokine activity could significantly

influence the immune response to mumps virus. For example, by integrating interleukin (IL)-15 cytokine into the vaccinia virus strains, new smallpox vaccine candidates with greater immunogenicity, efficacy, and safety were developed.¹⁸

Few data are available regarding the influence of such immune response gene polymorphisms on mumps vaccine immune responses.^{19,20} Understanding genetic mechanisms for variation among individuals with regard to mumps antibody and lymphoproliferative responses may allow for better understanding of mumps immunity. We hypothesize that HLA and cytokine gene polymorphisms significantly influence the immune response to mumps viral vaccine. We sought to determine whether associations exist between individual HLA, cytokine, and cytokine receptor genes and mumps-specific humoral (immunoglobulin G [IgG] antibody titer) and cellular (lymphocyte proliferation) immune responses in patients after 2 doses of mumps-containing vaccine. We now report the first data regarding associations between HLA and cytokine receptor gene polymorphisms and immune responses after 2 doses of mumps vaccine.

METHODS

Study Patients and Immune Markers

Details of our study methods and patient identification have been published elsewhere.²¹ Briefly, we recruited schoolchildren ($n = 346$, 12–18 years of age) who were previously vaccinated with 2 doses of live attenuated MMR vaccine (Merck Research Laboratories, West Point, PA) that contained the Jeryl Lynn B strain of mump virus. The institutional review board of the Mayo Clinic approved the study, and we obtained parental permission (informed consent) and pediatric assent from the participants.

Mumps-specific humoral immunity was determined by measuring mumps-specific IgG titers using a whole-virus enzyme immunoassay (EIA; anti-parotitis virus/IgG EIA; Dade Behring, Marburg, Germany; sensitivity: 95.4%; specificity: 93.7%) for all patients as described previously.^{19,22} The limit of detection of the test was <230 of mumps IgG antibody titer. The cellular immune status to mumps vaccine was assessed by using an *in vitro* [³H]thymidine incorporation assay as previously described.²⁰ Results were expressed as antigen-specific stimulation indices (SIs), defined as the ratio of the median counts per minute of mumps vaccine-stimulated wells to the median counts per minute of unstimulated wells. An SI of ≥ 3 was considered to be a marker of a positive lymphoproliferative response, consistent with standard practice.²³

DNA Extraction and HLA Genotyping

Details of HLA typing have been published elsewhere.^{19,20} High molecular weight genomic DNA was extracted from blood samples by using the Puregene extraction kit (Gentra Systems Inc, Minneapolis, MN) and used for polymerase chain reaction (PCR)-based high-resolution HLA genotyping, including PCR with sequence-specific primers (Invitrogen, Brown Deer, WI). All class I and class II 4-digit molecular typing was performed with negative controls, and every 50th PCR was repeated for quality control.

Genotyping of Cytokine and Cytokine Receptor Gene Polymorphisms

Single-nucleotide polymorphisms (SNPs; minor allele frequency > 5%) in cytokine (IL-2, IL-4, IL-10, IL-12A, IL-12B, and γ interferon [IFN- γ]) and cytokine receptor (IL-2RA, IL-2RB, IL-4RA, IL-10RA, IL-10RB, IL-12RB1, IL-12RB2, and IFN- γ R) genes were genotyped on a subset of 118 patients selected from a previous study. Criteria for selection of patients for additional study were based on extreme (high or low) values of measles antibody and cellular immune response in an attempt to maximize the probability of finding an association, if 1 existed, by studying individuals at the 2 ends of the immunity spectrum. As described

previously, we used multiplex PCR and SNP analyses by means of the GenomeLab SNPstream platform (Beckman Coulter Inc, Fullerton, CA).²⁴ Genotyping for IL-10.G microsatellite and IFN- γ CA repeats polymorphisms were analyzed using an Applied Biosystems (Foster City, CA) 3100 DNA sequencer. A total of 58 SNPs that met Hardy-Weinberg equilibrium assumptions were examined.

Statistical Analysis

Data were summarized by using frequencies and percentages for categorical variables and medians and inter-quartile ranges (IQRs) for continuous variables. Plots of immune response by assay date identified an upward trend of cellular proliferation values over time. We fit polynomial linear regression models to evaluate this association and used the resulting models to recalibrate measures of cellular immune response. No recalibration was necessary for humoral immune response. Associations of immune response with demographic and clinical variables were assessed using analysis of variance methods. Because of data skewness, all *P* values were calculated on the basis of log-transformed values. Descriptive associations between immune response and HLA loci were obtained on an allelic level. Each person contributed 2 observations to these summaries, 1 for each allele. Alleles were grouped for each locus by subtype and summarized using medians and IQRs. After these descriptive evaluations, associations were more formally examined using linear regression analyses. In contrast to the descriptive comparisons, each patient contributed 1 observation to these analyses on the basis of an observed genotype. Regression variables were created for each allele and coded as 0, 1, or 2 according to the number of copies of the allele that a patient carried. Rare alleles, defined as those with fewer than 5 occurrences overall, were pooled into a category labeled "other." Original response values were again replaced with corresponding logarithmic values. Global differences in immune response among all alleles within a given locus were evaluated by simultaneously including all but 1 of the allele variables in a linear regression model. After these global tests, we examined individual allele effects. These series of tests were performed in the spirit of Fisher's protected least significant difference test; associations of individual alleles were not considered statistically significant when the overarching global gene test was nonsignificant.²⁵ Each allele variable was included in a separate linear regression analysis, effectively comparing immune response for the allele of interest against all other alleles combined. All analyses described were performed after adjustment for the following set of potential confounding variables: age, race, gender, age at first MMR, and age at second MMR. Cytokine and cytokine receptor SNPs from a subset of 118 patients were examined on a genotypic level, using 3 categories to describe each SNP: homozygous major allele, heterozygous, and homozygous minor allele. Associations with immune response were performed using analysis of covariance, accounting for the measles antibody and cellular immune response variables that were used to select the patients. Because of data skewness, all *P* values were calculated by using log-transformed values. All statistical tests were 2-sided, and all analyses were conducted by using the SAS system (SAS Institute, Inc, Cary, NC).

RESULTS

Characteristics of Study Patients

We examined 346 children who were enrolled in the study and ranged in age from 12 to 18 years (Table 1). Slightly more than half (53%) of our study population was male, and the majority (94%) of the patients were white. The median age of the patients at the time of the first mumps immunization was 15.6 months; the median age at the second mumps immunization was 12.1 years. Medians and IQRs for mumps antibody titers and lymphocyte proliferation (SIs) levels were 729 IU/mL (IQR: 411–1286 IU/mL) and 4.84 IU/mL (IQR: 2.43–9.23 IU/mL), respectively.²⁰ As shown in Table 1, we found no statistically significant difference across age for either the mumps antibody or lymphocyte proliferation immune

responses. Examination of mumps virus–specific antibody titers by EIA revealed that girls demonstrated significantly higher antibody titers than boys (median: 876 IU/mL vs 677 IU/mL; $P = .003$). We also found a statistically significant association between mumps antibody titers and time from second MMR to blood draw ($P = .05$).

Associations of Individual HLA Alleles With Mumps-Specific Antibody and Lymphoproliferative Responses After Mumps Vaccination

Separate analyses were performed for each measure of humoral and cell-mediated immunity. Global statistical tests revealed a significant association between the HLA-DQB1 locus ($P = .020$) and mumps IgG antibody titers (Table 2). In particular, allele DQB1*0303 (median 490.08 IU/mL; $P = .035$) was associated with lower mumps-specific antibody titers.

The global tests of significance revealed significant associations between lymphoproliferation levels to mumps and the HLA-B, HLA-DRB1, HLA-DQA1, and HLA-DQB1 loci (global $P = .056$, $P < .001$, $P = .001$, and $P = .001$, respectively; Table 3). Alleles B*1302 (median SI: 12.84; $P = .017$), B*3701 (median SI: 13.26; $P = .015$), and B*3801 (median SI: 9.52; $P = .007$) were associated with higher lymphoproliferation to mumps. Alleles DRB1*0101 (median SI: 7.07; $P = .002$), DRB1*0701 (median SI: 7.07; $P = .057$), and DRB1*1001 (median SI: 8.57; $P = .038$) were also associated with higher mumps-specific lymphoproliferative responses. In contrast, lower cellular immune responses were associated with several HLA-DRB1 alleles, including DRB1*0301 (median SI: 3.69; $P = .016$), DRB1*0801 (median SI: 2.59; $P = .021$), DRB1*1201 (median SI: 2.95; $P = .040$), and DRB1*1302 (median SI: 2.48; $P = .012$). For the HLA-DQA1 locus (global $P < .001$), we found significant associations between higher mumps vaccine–specific lymphoproliferation and the alleles DQA1*0101 (median SI: 7.03; $P = .003$) and DQA1*0105 (median SI: 8.58; $P = .040$); however, DQA1*0401 (median SI of: 2.59; $P = .003$) and DQA1*0501 (median SI: 3.56; $P = .010$) alleles both were associated with lower mumps-induced lymphoproliferative immune responses. Furthermore, the DQB1*0501 (median SI: 7.08; $P < .001$) allele was significantly associated with higher lymphoproliferation to mumps virus. Finally, we found that lower lymphoproliferation to mumps was associated with DQB1*0201 (median SI: 3.69; $P = .041$) and DQB1*0402 (median SI: 2.59; $P = .024$) alleles (Table 3). The entire spectrum of associations between class I and class II HLA alleles and mumps virus–specific humoral and cellular immune responses in the population cohort analyzed in this study are available (Tables 6–9, which are published as supporting information on www.pediatrics.org/content/full/121/5/FirstPageNo).

Associations Between SNPs in Cytokine and Cytokine Receptor Genes With Mumps-Specific Antibody and Lymphoproliferative Responses After Mumps Vaccination

We also examined associations between measures of mumps immunity and cytokine and cytokine receptor gene SNPs in a subgroup of 118 patients. We found significant associations between certain SNPs in cytokine receptor gene IL-12RB ($P \leq .05$) and mumps-specific IgG antibody titers (Table 4). The presence of minor allele T for intronic SNP rs2201584 ($P = .05$) within the IL-12RB2 gene was associated with an allele dose-related decrease in mumps antibody titers. In contrast, the minor allele for intronic SNP rs372889 ($P = .03$) within the IL-12RB1 gene was associated with an allele dose-related increase in mumps-specific antibody titers. A genotype variant for another intronic SNP (rs1870063; $P = .01$) in the IL-12RB1 gene also demonstrated significant associations with variations in mumps antibody titers; however, no allele dose-related response was observed in our study cohort.

Minor allele A for rs1870063 ($P < .01$) in the IL-12RB1 gene was also associated with higher lymphoproliferative response to mumps virus in an allele dose-related manner (Table 5). In contrast, minor allele A of rs372889 ($P = .02$) within the IL-12RB1 gene was associated with lower mumps-induced lymphoproliferative responses. Heterozygous genotype TC for an

intronic SNP rs2508454 (SI: 5.83 vs 4.25 homozygous major genotype CC; $P = .05$) within the IL-10RA gene demonstrated a significant association with a higher mumps-specific lymphoproliferative response; however, we could not confirm an allele dose-related effect for this particular SNP because of the lack of minor allele variant TT in this modest-sized study cohort.

DISCUSSION

Previous evidence exists for a genetic influence on mumps vaccine immune responses. For example, a study of 100 twin pairs consisting of 45 pairs of monozygotic twins and 55 pairs of dizygotic twins raised together found evidence of a significant heritable association with mumps vaccine antibody levels.²⁶ In that study, 38.8% (95% 1-sided confidence interval: 1.60%) of the total variability in mumps IgG antibody levels could be attributed to genetic effects.^{26,27} Hyöty et al²⁸ evaluated HLA association and antibody response to mumps virus after immunization in a small study population of 25 patients. They demonstrated that the heightened IgA response to mumps after vaccination in seropositive patients was significantly associated with HLA DR3 and DR4 antigens; however, no HLA associations were observed with IgG or IgM antibody levels in response to mumps vaccination.²⁸ Our own population-based study assessing associations between HLA genes and immune responses to MMR vaccine in healthy children and young adults demonstrated that the HLA A*26-Cw*12-B*38 haplotype was associated with both higher mumps-specific humoral ($P = .007$) and cellular ($P = .01$) immune responses after 2 doses of vaccine.¹⁹ The haplotypes with the strongest associations with mumps lymphoproliferation were DRB1*03-DQB1*02-DPB1*04 ($P = .006$) and DRB1*01-DQB1*05-DPB1*04 ($P = .008$). In this study, the DRB1*0101, DRB1*0301, DQB1*0201, and DQB1*0501 alleles were also associated with significant variations in lymphoproliferative immune responses to mumps vaccine. We also found that the class II HLA-DR supertype ($P < .001$) was associated with lower mumps-specific lymphoproliferation.²⁰ Homozygosity at increasing numbers of HLA loci was associated with lower mumps antibody ($P = .02$) and lymphoproliferation ($P = .04$) levels.²⁹ In addition, homozygosity at HLA class IA was associated with a lower lymphoproliferative response compared with heterozygosity at the same locus ($P = .04$).²⁹ These results further suggest a role for HLA molecules in influencing mumps vaccine-induced immune response.^{30,31}

Our multivariate linear regression analyses demonstrated an association of class II DQB1*0303 with humoral immune response to mumps vaccination, as reflected by the lower antibody titers in DQB1*0303-positive individuals. In addition, we observed suggestive associations with the expression of class I B*1302, B*3701, and B*3801 alleles and higher mumps vaccine cellular immune responses. An interesting finding was the association of several class II HLA alleles with mumps-specific lymphoproliferative responses. Class II DRB1*0301, DRB1*0801, DRB1*1201, DRB1*1302, DQA1*0401, DQA1*0501, DQB1*0201, and DQB1*0402 alleles were significantly associated with lower mumps-specific lymphoproliferation, whereas DRB1*0101, DRB1*0701, DRB1*1001, DQA1*0101, DQA1*0105, and DQB1*0501 alleles were associated with higher mumps vaccine lymphoproliferative responses. These data might suggest that cellular immune responses to mumps vaccine could be restricted or at least influenced by class II DR and DQ molecules, although additional studies are required to prove this. Our experiment-wise type I error rate was controlled by focusing only on global tests of significance. Even so, we are cognizant of multiple testing issues; therefore, attempts to replicate these data in future studies should be conducted. Together, these findings are consistent with the important role that HLA molecules play in the process of immune recognition and activation.³² The associations between HLA and mumps vaccine-induced immune responses do not fully explain the genetic influence on mumps immunity, so we performed gene polymorphism association study testing 58 SNPs.

There is considerable cross-regulation of humoral and cellular immune responses by cytokines and their receptors and polymorphisms that influence cytokine and cytokine receptor levels, which can have an impact on these responses. In this study, associations between mumps antibody titers and lymphoproliferation and SNPs in the cytokine and corresponding receptor genes were determined in a selected group of 118 study patients. These patients were originally selected on the basis of measles antibody and cellular immune response variables.²⁴ This issue can potentially affect our study results, because it is not known whether patients' mumps-specific immune responses systematically differ from their measles immune responses. We accounted for the sampling design in the analysis phase by including the measles antibody and cellular response variables as covariates in resulting regression models; however, it is possible that some level of residual confounding may remain. Significant associations were observed for 4 SNPs in the IL-10RA, IL-12RB1, and IL-12RB2 cytokine receptor genes, implicating a role for IL-10R and IL-12R in immune responses to mumps vaccine. We recognize that associations with some rare SNPs may have been missed as a result of our modest sample size. Furthermore, the possibility of associations by chance also cannot be dismissed because of the number of statistical tests. Hence, the cytokine receptor gene effects reported here should be confirmed in other populations. This information may provide additional understanding of the functional HLA and cytokine receptor gene polymorphisms in vaccine-induced immunity and could point to novel mechanisms and targeted therapeutics.

Prospective effects of cytokine genes encoding IL-10 and IL-12 merit particular attention, because the use of recombinant cytokines (and cytokine receptors) as vaccine adjuvants may offer a mechanism whereby the magnitude and phenotype of the immune response to vaccination can be altered. Both IL-10 and IL-12 are key immunoregulatory cytokines. Signal transducers and activators of transcription 3 (STATs) and small amounts of STAT1 transcription factors are activated during IL-10 signaling.¹⁵ It has been reported that mumps virus V protein prevents responses to IL-6 and viral cytoplasmic tyrosine kinase signals and can induce apoptosis in STAT3-dependent multiple myeloma cells.³³ Blocking the receptor for IL-10 improves antimycobacterial chemotherapy and response to a mycobacterial subunit vaccination.³⁴ In addition, IL-10 inhibits Th1 cytokine production and expression of CD80 and CD86 molecules by antigen-presenting cells.³⁵ The heterodimeric proinflammatory cytokine IL-12 represents a functional bridge between innate resistance and antigen-specific adaptive immune response.³⁶ The important role of IL-12 and the IL-12R β 2 subunit in the generation of pathogenic auto-reactive Th1 cells has been reported.³⁷ Regulation of the IL-12 receptor β 2 chain has also been suggested as a molecular switch in determining T-cell phenotype.³⁶

Polymorphisms in both coding and noncoding regions of cytokine genes can affect multiple aspects of cytokine biology, such as transcriptional activity, protein production, receptor binding, direct interactions with viral proteins, and functional activity.^{16,38,39} Cytokine receptor polymorphisms can similarly affect cytokine function.^{16,40,41} Polymorphisms that affect functional activity are known for all of the cytokines and their respective receptors. For instance, SNPs at the IL-12B promoter (heterozygous L/S genotype) are associated with nonresponsiveness to hepatitis B virus vaccine in North American adolescents.⁴² Similarly, it was shown that cytokine polymorphisms play a role in susceptibility to ultraviolet B-induced modulation of immune responses after hepatitis B virus vaccination, and exposure to ultraviolet B significantly suppressed antibody responses to hepatitis B vaccine in individuals with the minor variant for the IL-1 β (+3953) polymorphism.⁴³ Associations between disease severity and cytokine gene polymorphisms have been found for many other viral pathogens, including respiratory syncytial virus, hepatitis C virus, human immunodeficiency virus, human T-cell leukemia virus type 1, and parvovirus B19.^{44–48} Our data suggest that certain SNPs in IL-10RA and IL-12RB genes are associated with variations in immune responses to mumps vaccination. Understanding the genetic factors that might influence lower immunity to mumps

vaccination by modulating the cytokine microenvironment of the host may provide additional insight into mumps vaccine-induced immune responses and possible vaccine/therapeutic approaches.

We also found that median titers of mumps-specific antibodies were significantly higher in girls than in boys ($P = .003$) after 2 doses of mumps vaccine. Similar gender differences in the humoral (antibody) response to live measles and rubella vaccine in young adults were previously described.^{49,50} In some observational studies, girls have had higher rates of mumps despite similar rates of vaccination^{5,51}; however, not all outbreaks demonstrate this association.⁵² Gender differences may represent social or behavioral differences in disease exposure or gender-linked genetic differences in immune response.⁴⁹

CONCLUSIONS

Our results indicate associations between HLA and cytokine receptor gene polymorphisms in variations of mumps vaccine-induced immune responses and may provide insight into the factors that result in low immune responses after mumps immunization and mumps outbreaks. These genetic associations may partially explain the genetic mechanistic component for vaccine-induced immune response heterogeneity among individuals and between populations.

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Abbreviations

MMR	measles-mumps-rubella
IL	interleukin
IgG	immunoglobulin G
EIA	enzyme immunoassay
SI	stimulation index
PCR	polymerase chain reaction
SNP	single-nucleotide polymorphism
IFN-γ	γ interferon

IQR

interquartile range

STAT

signal transducer and activator of transcription

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Comparison of Selected Demographic and Clinic Variables With Lymphoproliferation and Antibody Titers to Mumps Vaccine

Variable	n	Lymphoproliferation, SI		Antibody Titer, IU/mL	
		Median (IQR ^a)	P ^b	Median (IQR ^d)	P ^b
Age at enrollment, y			.060		.920
12	53	4.86 (2.02–8.99)		696 (462–1543)	
13	47	3.74 (1.97–7.31)		698 (346–1718)	
14	53	5.34 (2.62–8.75)		776 (435–1239)	
15	48	4.22 (2.49–9.83)		708 (418–1227)	
16	48	5.02 (3.57–13.76)		703 (472–1425)	
17	49	4.79 (2.51–7.47)		647 (344–1183)	
18	48	6.10 (2.86–12.27)		740 (434–1118)	
Gender			.240		.003 ^c
Male	185	4.97 (2.53–10.63)		677 (385–1082)	
Female	161	4.57 (2.28–8.49)		876 (455–1519)	
Race			.720		.220
White, non-Hispanic	323	4.88 (2.43–9.26)		704 (406–1269)	
Other	23	3.74 (2.29–8.99)		1226 (474–1681)	
Age at first MMR, mo			.090		.280
≤15.12	84	6.21 (2.70–13.11)		641 (347–1233)	
15.13–15.61	90	4.05 (2.02–7.50)		795 (471–1237)	
15.62–17.45	85	4.44 (2.69–8.49)		793 (477–1597)	
>17.45	87	4.86 (2.51–8.75)		711 (369–1502)	
Age at second MMR, y			.670		.490
≤11.50	86	5.07 (2.22–10.57)		734 (417–1524)	
11.51–12.07	87	4.37 (2.06–8.47)		649 (386–1273)	
12.08–12.56	86	5.62 (2.31–9.57)		662 (395–1082)	
>12.56	87	4.82 (2.93–8.73)		818 (446–1413)	
Time from second MMR to blood draw, y			.350		.050 ^c
≤2.79	87	4.20 (2.00–7.31)		927 (461–1504)	
2.80–4.68	86	4.57 (2.53–10.54)		834 (446–1465)	
4.69–6.07	86	5.03 (2.92–9.26)		643 (344–1039)	

Variable	<i>n</i>	Lymphoproliferation, SI		Antibody Titer, IU/mL	
		Median (IQR ^a)	<i>P</i> ^b	Median (IQR ^a)	<i>P</i> ^b
>6.07	87	5.93 (2.25–10.75)		687 (432–1243)	
Time from first to second MMR, y					
≤9.9	86	5.21 (2.45–10.57)	.400	687 (395–1459)	.370
10.0–10.7	87	4.59 (2.09–8.69)		618 (385–1383)	
10.8–11.2	87	4.12 (2.12–8.85)		798 (351–1243)	
>11.2	86	4.92 (2.93–9.26)		809 (550–1287)	

^aFirst quartile to third quartile.

^bAnalysis of variance. Because of data skewness, *P* values were calculated on the basis of log-transformed data.

^cStatistically significant.

TABLE 2
HLA Allelic Associations With Mumps-Specific Antibody Response After Mumps Immunization

Locus	Allele	Allele Counts	Median Antibody Titer, IU/mL	First quartile	Third quartile	P ^d
HLA-A	Overall	692	729	411	1286	.566 ^b
HLA-B						.109 ^b
HLA-Cw						.890 ^b
HLA-DRB1						.207 ^b
HLA-DQA1						.450 ^b
HLA-DQB1						.020 ^{b,d}
HLA-DPA1	*0303	24	490	327	1101	.035 ^c
HLA-DPB1						.160 ^b
						.955 ^b

^a Linear regression analysis. Because of 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. Allele-specific *P* values are presented only when the global test and the allele-specific test reached statistical significance (*P* ≤ .05). Analyses are based on 346 patients.

^b Based on global tests of significance, simultaneously including all but 1 of the allele variables in a multivariate regression model.

^c Allele specific, comparing the allele of interest with all other alleles combined.

^d Statistically significant global test.

TABLE 3
HLA Allelic Associations With Mumps-Specific Lymphoproliferative Response After Mumps Immunization

Locus	Allele	Allele counts	Median, SI value	First Quartile	Third Quartile	P ^d
HLA-A	Overall	692	4.84	2.43	9.23	.127 ^b
HLA-B	*1302	17	12.84	4.56	17.00	.056 ^b
	*3701	5	13.26	9.48	24.32	.015 ^c
	*3801	16	9.52	4.96	13.75	.007 ^c
HLA-Cw						.414 ^b
HLA-DRB1	*0101	55	7.07	3.92	12.95	.001 ^{b,d}
	*0301	94	3.69	1.98	6.91	.002 ^c
	*0701	73	7.07	3.54	11.63	.016 ^c
	*0801	22	2.59	1.65	10.63	.057 ^c
	*1001	6	8.57	7.33	23.58	.021 ^c
	*1201	20	2.95	1.89	4.66	.038 ^c
	*1302	36	2.48	1.68	6.95	.040 ^c
HLA-DQA1	*0101	65	7.03	3.62	12.95	.012 ^c
	*0105	6	8.57	7.33	23.58	<.001 ^{b,d}
	*0401	24	2.59	1.24	4.81	.003 ^c
	*0501	94	3.56	1.95	6.91	.010 ^c
HLA-DQB1	*0201	104	3.69	1.99	7.22	.001 ^{b,d}
	*0402	18	2.59	1.31	10.63	.041 ^c
	*0501	79	7.08	3.92	14.30	.024 ^c
HLA-DPA1						<.001 ^c
HLA-DPB1						.110 ^b
						.750 ^b

^a Linear regression analysis. Because of 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. Allele-specific *P* values are presented only when the global test and the allele-specific test reached statistical significance ($P \leq .05$). Analyses are based on 346 patients.

^b Based on global tests of significance, simultaneously including all but 1 of the allele variables in a multivariate regression model.

^c Allele specific, comparing the allele of interest with all other alleles combined.

^d Statistically significant global test.

TABLE 4
Cytokine Receptor SNPs Associated With Mumps-Specific Antibody Response After Mumps Immunization

Cytokine Receptor	SNP ^a	Genotype	n	Median Antibody Titer, IU/mL (IQR)	P ^b
IL-12RB2	rs2201584 ^c	CC	84	877 (425–1470)	.05
		TC	29	527 (296–876)	
		TT	5	261 (247–697)	
IL-12RB1	rs372889 ^c	GG	30	688 (364–1187)	.03
		AG	58	670 (361–1040)	
		AA	29	876 (447–1592)	
IL-12RB1	rs1870063	GG	40	838 (400–1637)	.01
		GA	59	621 (355–1040)	
		AA	18	864 (462–1269)	

C indicates cytosine; T, thymine; G, guanine; A, adenine.

^a A total of 58 SNPs were examined; only those found to be statistically significant ($P \leq .05$) were included.

^b P values were based on log-transformed data using analysis of covariance, adjusting for measles antibody and lymphoproliferative response, to account for the original sampling of the individuals. Analyses are based on a subset of 118 patients.

^c SNP demonstrated an allele dose-related response.

TABLE 5
Cytokine Receptor SNPs Associated With Mumps-Specific Lymphoproliferative Response After Mumps Immunization

Cytokine Receptor	SNP ^a	Genotype	n	SI Value, Median (IQR)	<i>P</i> ^b
IL-12RB1	rs1870063 ^c	GG	40	4.58 (2.48–10.33)	<.01
		GA	59	4.73 (1.87–11.63)	
		AA	18	6.23 (3.92–9.00)	
IL-12RB1	rs372889 ^c	GG	30	5.24 (1.99–9.00)	.02
		AG	58	4.86 (2.00–12.95)	
IL-10RA	rs2508454	AA	29	4.59 (2.98–9.32)	.05
		CC	44	4.25 (2.04–8.26)	
		TC	69	5.83 (2.92–13.63)	
		TT	0	—	

C indicates cytosine; T, thymine; G, guanine; A, adenine.

^a A total of 58 SNPs were examined; only those found to be statistically significant ($P \leq .05$) were included.

^b *P* values were based on log-transformed data by using analysis of covariance, adjusting for measles antibody and lymphoproliferative response, to account for the original sampling of the individuals. Analyses are based on a subset of 118 patients.

^c SNP demonstrated an allele dose-related response.