

Host Single-Nucleotide Polymorphisms and Altered Responses to Inactivated Influenza Vaccine

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We analyzed the relationship between host gene polymorphisms and responses in recipients of inactivated influenza vaccine, who were classified into poor, normal, or adverse response groups. The frequency of the mannose-binding lectin-2 codon 54 allele was significantly different among the 3 types of responders, with a decreased odds ratio for the development of poor or adverse responses ($P = .033$). There was no statistical relationship between responses and either tumor necrosis factor- α or interleukin (IL)-10 promoter polymorphisms among the 3 response groups. When poor and normal responses were combined, the -1082 A allele in the IL-10 promoter conferred a significantly decreased risk of the development of adverse responses ($P = .041$). These data indicate that host polymorphisms play a role in determining responses to influenza vaccine.

Widespread immunization has markedly reduced the incidence of certain infectious diseases. Wild-type poliomyelitis has been eradicated from the Western Hemisphere, and global eradication is planned. Vaccination against influenza has substantial health-related and economic benefits for both children and adults [1]. However, the poor immune responses seen in some vaccine recipients result in wild-type disease.

Despite the success of vaccines in preventing many infectious diseases, adverse events temporally associated with vaccine administration occur. Because vaccines are administered to large

populations, occasional serious adverse events, in addition to local pain and tenderness at the injection site, have been noted [2]. Vaccine responses are determined not only by the chemical nature of the antigen and the manner in which it is delivered but also by environmental factors and host genetic factors. Identifying the predictors of individual variability in immune responses to vaccination and the factors that contribute to an increased risk for adverse reactions would enhance our understanding of vaccine responses.

It has been demonstrated that host polymorphisms affect the development and progression of certain diseases and disorders by either encoding altered gene products or causing changes in transcriptional regulation. The mannose-binding lectin (MBL)-2 gene encodes a calcium-dependent protein that plays an important role in innate immunity; circulating MBL-2 levels are largely the result of several single-nucleotide polymorphisms (SNPs) in the exon 1 gene and promoter region. Variant MBL-2 alleles have been associated with increased susceptibility to several infections [3–5]. Tumor necrosis factor (TNF)- α and interleukin (IL)-10 are 2 important cytokines that are associated with the regulation of cellular immune responses. Several polymorphisms in their promoter regions have been shown to directly affect their gene transcription and are associated with the development and progression of autoimmune and infectious diseases [6, 7]. Furthermore, the imbalance in Th1/Th2 cytokine production may contribute to the adverse responses induced by vaccines when natural infection occurs [8].

The present study focused on 8 host SNPs in 3 immunogenetic genes: codons 52 (rs503078), 54 (rs1800450), and 57 (rs1800451) in the MBL-2 exon 1 gene; -238 (rs36152) and -308 (rs1800629) in the TNF- α promoter region; and -592 (rs1800872), -819 (rs1800871), and -1082 (rs1800896) in the IL-10 promoter region. Using immune responses and adverse events assessed in an earlier influenza vaccine trial that enrolled 5210 subjects [9], we defined poor and normal immune responses and the presence or absence of adverse events as the variables of interest. Archived serum specimens were retrieved, genomic DNA was extracted, and 8 SNP alleles were determined by either (1) polymerase chain reaction (PCR) followed by restriction fragment-length polymorphism (RFLP) analysis or (2) real-time allele-discrimination TaqMan PCR. Subjects who experienced either poor influenza virus-specific antibody responses or adverse events to vaccines were compared with those who had brisker immune responses and no adverse reactions.

Subjects, materials, and methods. A large number of healthy volunteers had been enrolled in a National Institutes

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of Health–funded, double-blind randomized controlled trial at Vanderbilt University to compare the safety, immunogenicity, and efficacy of standard trivalent inactivated influenza vaccine (H1N1, H3N2, and B) with experimental bivalent cold-adapted live attenuated vaccine (H1N1 and H3N2) in the 1990s [9]. Only recipients of the inactivated vaccine were included in the present study (Institutional Review Board approval number 990325). The recipients were categorized on the basis of their vaccine-induced responses into the following groups: (1) poor responders, defined as vaccine recipients with a prevaccination hemagglutination-inhibition (HI) antibody titer of <1:16 who achieved a <4-fold increase in HI titer to both the H1N1 and H3N2 vaccine components after the first vaccination; (2) adverse responders, defined as vaccine recipients with a temperature $\geq 38.3^{\circ}\text{C}$ after the first vaccination; and (3) normal responders, defined as vaccine recipients with a ≥ 4 -fold increase in HI antibody titer to both the H1N1 and H3N3 vaccine components and a temperature of $\leq 38.3^{\circ}\text{C}$ after the first vaccination. All subjects categorized as poor and adverse responders who had archived serum specimens available were included in the present study. Approximately the same number of subjects was selected from normal responders, who were matched for age, sex, and race to the poor and adverse responders.

Genomic DNA was extracted from serum by use of a QIAamp blood kit (Qiagen), in accordance with the manufacturer's instructions [5]. DNA extracted from 200 μL of serum ranged from 150 to 1000 ng (on the basis of measurement of optical density at 260 nm), and ~ 20 ng of DNA was used in

PCR amplification. PCR was used to amplify a 119-bp region of exon 1 that contains codons 52, 54, and 57 and to detect and determine the alleles in these codons. Polymorphisms were determined by RFLP analysis through the digestion of PCR products with the restriction enzymes *BanI*, *MboII*, and *MluI* (New England Biolabs), followed by separation on 4% gel with ethidium bromide staining [10]. A real-time allele-discrimination PCR assay was used to detect and discriminate alleles –238 and –308 SNP in the TNF- α promoter region and –592, –819, and –1082 SNP in the IL-10 promoter region, by use of the ABI PRISM 7700 Sequence Detection System (Applied Biosystems). PCR amplification was performed by denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 92°C for 15 s, and then annealing and extension at 62°C for 1 min. After PCR, the genotype of each sample was attributed automatically by measuring allele-specific fluorescence with the ABI PRISM 7900 Sequence Detection System, by use of the SDS software for allelic discrimination (version 2.2.2; Applied Biosystems). Nucleotide sequences of primers and fluorophore TaqMan MGB probes were designed using Primer Express software (version 1.5; Applied Biosystems) (for TNF- α –238, 5'-AAATCAGTCAGTGGCCCAGAA-3', 5'-TCATTCAACCAGC-GGAAAAC-3', 5'-FAM-CTCCCTGCTCCGATT-MGB-3', and 5'-VIC-CTCCCTGCTCTGATT-MGB-3'; for TNF- α –308, 5'-GAAATGGAGGCAATAGGTTTTGAG-3', 5'-GTAGGACCCT-GGAGGCTGAAC-3', 5'-FAM-CCGTCCCCATGCC-MGB-3', and 5'-VIC-CCGTCCCTCATGCC-MGB-3'; for IL-10 –592, 5'-AGCTGAAGAGGTGGAAACATGTG-3', 5'-CAAGCAGCCC-

Table 1. Genotype frequency and odds ratios (ORs) for mannose-binding lectin (MBL)–2 single-nucleotide polymorphisms (SNPs) and altered immune responses to inactivated influenza vaccine.

Loci, allele	Poor responders (n = 99)	Normal responders (n = 101)	Adverse responders (n = 98)	Overall <i>P</i> ^a	OR (95% CI) ^b	
					Poor vs. normal	Adverse vs. normal
52				.062		
C/C	80	72	83			
C/T	19	29	15			
T/T	0	0	0			
54				.033		
G/G	79	67	79		1.0	1.0
G/A	20	32	17		0.499 (0.263–0.947)	0.474 (0.248–0.907)
A/A	0	2	2			
57				NA		
G/G	99	101	98			
G/A	0	0	0			
A/A	0	0	0			

NOTE. CI, confidence interval; NA, not applicable.

^a Overall *P* values were computed using multinomial logistic regression in which the 3 categories of responses were taken into account.

^b ORs were calculated only for loci with a significant overall *P* value. The risk of developing an abnormal response (either poor and adverse) was estimated by an OR, which was calculated using the wild-type allele as the reference group (OR of 1) and with the heterozygous and homozygous variants combined.

TTCCATTTACT-3', 5'-FAM-CCGCCTGTCCTGT-MGB-3', and 5'-VIC-CCCGCCTGTACTGT-MGB-3'; for IL-10 -819, 5'-AGGGTGAGGAAACCAAATTCTCA-3', 5'-CCATGACCCCT-ACCGTCTCTATT-3', 5'-FAM-TGATGTAACATCTCTGTGC-MGB-3', and 5'-VIC-TGATGTAATATCTCTGTGCCT-MGB-3'; and for IL-10 -1082, 5'-CACACACAAATCCAAGACAAC-ACTAC-3', 5'-GGGTGGAAGAAGTTGAAATAACAAG-3', 5'-FAM-TACTTCCCCCTCCC-MGB-3', and 5'-VIC-TACTTCC-CCTTCCC-MGB-3').

Differences in age, sex, and race among the poor, normal, and adverse responders were examined using EpiInfo software (version 6; Centers for Disease Control and Prevention). Because very few subjects were determined to be homozygous for all 8 tested SNPs, all alleles with heterozygous and homozygous mutations were combined for statistical analysis. A multinomial logistic regression was performed for a global test in which the 3 response groups were analyzed simultaneously to obtain an overall *P* value for the 3 groups. Unconditional univariate logistic regression was used to test the association between host polymorphisms and varied vaccine-induced responses as well as to estimate odds ratios (ORs) and 95% confidence intervals (CIs). The wild type of the SNPs was treated as the reference category for the OR calculation. The probability of the logistic regression was modeled for an abnormal response (either poor or adverse) for MBL-2 and for an adverse response for TNF- α and IL-10. All *P* values presented in this report are 2-sided, and *P* \leq .05 was considered to indicate statistical significance. All of the calculations were done using SAS software (version 9.1; SAS Institute).

Results. Vaccinees enrolled in the parent vaccine trial who had received inactivated influenza vaccine and who fell into 1 of the 3 above-defined response categories were re-reviewed. All poor and adverse responders with available archived serum specimens were included. Approximately the same number of subjects was selected for the normal response group, who were matched by age, sex, and race with the other 2 groups. A total of 298 subjects were included in the present study—99, 101, and 98 in the poor, normal, and adverse response groups, respectively. No significant differences were noted in age (mean \pm SD, 30.6 \pm 14.2, 30.9 \pm 14.3, and 32.3 \pm 14.9 years), sex (proportion male, 45.5%, 48.5%, and 49.0%), or race (proportion white, 94.9%, 92.1%, and 92.9%) among the poor, normal, and adverse response groups, respectively.

We first analyzed the 3 SNPs in the MBL-2 exon 1 gene; the frequency of each allele in codons 52, 54, and 57 is listed in table 1. The differences in each allele among the poor, normal, and adverse responders were analyzed by multinomial logistic regression. There were no significant differences in codon 52 and 57 alleles; however, a significant difference in allele frequency in codon 54 was detected ($\chi^2 = 6.81$; *P* = .033). An unconditional univariate logistic regression analysis indicated

Table 2. Frequencies of tumor necrosis factor (TNF)- α and interleukin (IL)-10 promoter single-nucleotide polymorphisms (SNPs) and inactivated influenza vaccine-induced adverse responses.

Cytokine, loci, allele	Poor and normal responders (n = 200)	Adverse responders (n = 98)	OR (95% CI), poor and normal vs. adverse ^a	<i>P</i>
TNF-α				
-238				
G/G	174	91	1.0	
G/A	25	7	0.515 (0.215–1.232)	.136
A/A	1	0		
-308				
G/G	158	73	1.0	
G/A	41	21	1.288 (0.730–2.272)	.561
A/A	1	4		
IL-10				
-592				
C/C	101	41	1.0	
C/A	89	51	1.418 (0.871–2.310)	.160
A/A	10	6		
-819				
C/C	103	51	1.0	
C/T	86	41	0.979 (0.603–1.587)	.930
T/T	11	6		
-1082				
G/G	38	29	1.0	
G/A	98	36	0.558 (0.319–0.976)	.041
A/A	64	33		

NOTE. Subjects with poor and normal responses were combined as a baseline to further study the relationship between promoter SNPs and adverse responses. CI, confidence interval; OR, odds ratio.

^a The risk of developing of an adverse response was estimated by an OR, which was calculated using the wild-type allele as the reference group (OR of 1) and with the heterozygous and homozygous variants combined.

that the G→A polymorphism in the codon 54 allele was related to a decreased risk for the development of either a poor (OR, 0.499 [95% CI, 0.263–0.947]) or adverse (OR, 0.474 [95% CI, 0.248–0.907]) response compared with the normal responders (table 1). These results suggest that the MBL-2 codon 54 allele polymorphism is independently associated with poor and adverse responses to influenza vaccination.

Two host SNPs in the TNF- α (-238 and -308) and 3 in the IL-10 (-592, -819, and -1082) promoter regions were determined by real-time allele-discrimination TaqMan PCR. The multinomial logistic regression analysis did not discern any significant differences in allele frequency among the poor, normal, and adverse responders (table 2). We then focused on testing our hypothesis that imbalanced Th1/Th2 cytokine production is associated with an adverse response, indicated by a temperature \geq 38.4°C. We combined the subjects in the poor and normal response groups and compared them with adverse

responders by unconditional univariate logistic regression analysis. In comparison to the poor/normal response group, the G→A polymorphism in the IL-10 promoter -1082 allele indicated a significantly decreased risk for the development of adverse responses (OR, 0.558 [95% CI, 0.319–0.976]) (table 2). These data suggest that IL-10 promoter polymorphisms may be associated with adverse systemic responses to influenza vaccine.

Discussion. In humans, the immune response to vaccination is heterogeneous despite the use of a constant formulation, route of administration, and dosage. Although the majority of vaccinees generate brisk immune responses with no adverse reactions, ~5% experience either hyporesponsiveness or adverse events [2]. The present study explored the possibility that host gene polymorphisms influence inactivated influenza vaccine-induced immune responses by comparing the frequencies of 8 SNPs in the MBL-2 gene and in the TNF- α and IL-10 promoter regions among different groups.

We found a significant difference in allele frequency in the MBL-2 codon 54 among the poor, normal, and adverse responders, suggesting that the allele polymorphism is independently associated with poor and adverse responses to influenza vaccination. MBL-2 is a member of the collectin family and is important in the initiation of the lectin pathway of complement activation and in opsonization [11]. The variant alleles in the MBL-2 gene are associated with MBL-2 deficiency, especially in individuals homozygous for the variant alleles [3]. Host polymorphisms can affect the development and progression of certain diseases and disorders by encoding altered gene products, resulting in poor immune responses. The variant codon 54 allele is more prevalent than codons 52 and 57 in white populations. Individuals with allele A in codon 54 demonstrate an even lower MBL-2 protein concentration than individuals with the 52 T allele, and the MBL-2 protein produced is incapable of activating the classic complement pathway [12]. Of the 3 polymorphisms within the MBL-2 gene, codon 54 has independently been found to be associated with increased susceptibility to infection [4, 13].

When natural disease occurs after receipt of inactivated vaccine, Th1/Th2 cytokine imbalances have been demonstrated for respiratory syncytial virus infection and measles [8]. Inflammatory responses must be finely tuned: too strong a response produces adverse events after vaccination, whereas too weak a response attenuates the immune responses. The Th1-like TNF- α is a potent immunomodulator and proinflammatory cytokine that has been implicated in the pathogenesis and development of various infectious diseases. In contrast, the Th2-like IL-10 is a potent anti-inflammatory cytokine that plays a role in down-regulating cell-mediated and cytotoxic inflammatory responses. Several polymorphisms in the promoter regions of the

genes for these 2 cytokines have been reported that affect the transcriptional regulation of the 2 genes. IL-10 responses to influenza vaccination have been reported to vary significantly among age groups and vaccines [8]. We hypothesized that SNPs in promoter regions result in imbalanced Th1/Th2 cytokine production, which leads to the occurrence of adverse events after the administration of inactivated influenza vaccine. Although a multivariate analysis did not show that the allele frequencies of 5 SNPs in the TNF- α and IL-10 promoter regions among the poor, normal, and adverse response groups altered responses, a specific analysis between adverse and nonadverse (poor or normal) responses indicated that the -1082 G→A polymorphism in the IL-10 promoter region conferred a significantly decreased risk for the development of adverse responses. Previous studies have indicated that the G→A SNP at -1082 is important in IL-10 regulation; homozygous individuals (G/G) had higher IL-10 expression after in vitro stimulation [11]. The SNP at -1082 has been associated with increased susceptibility of infection, severity of illness, organ dysfunction, and mortality [14, 15]. These findings support the present data by suggesting that the -1082 allele polymorphism in the IL-10 promoter region may be associated with adverse responses induced by influenza vaccine.

By use of genetic sequencing of the human genome, scientists are relating polymorphisms in various host gene alleles to variable host immune responses to infectious agents and vaccines. Because most previous vaccine trials have focused on humoral antibody responses, archival serum specimens are available for study. Several earlier studies as well as the present one have demonstrated that substantial quantities of human genomic DNA are present in such clinical samples as serum and cerebrospinal fluid [5]. With appropriate approval by institutional review boards, retrospective studies using the large quantity of archived serum specimens from previous vaccine trials and prospective studies can be conducted to assess many genetic factors.

There were several limitations to the present study, including (1) the small quantities of DNA available from serum specimens, (2) only 1 adverse event (fever) was assessed, and (3) a small number of host SNPs and not the entire genome were assessed. In spite of these limitations; however, this study suggests that additional studies should be conducted to confirm these findings.

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