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Genetic Variants in Toll-Like Receptor 2 (TLR2), TLR4, TLR9, and FCγ Receptor II Are Associated with Antibody Response to Quadrivalent Meningococcal Conjugate Vaccine in HIV-Infected Youth

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This study examined the association of host genetic variants with the antibody response to the quadrivalent meningococcal conjugate vaccine (MCV4) in HIV-infected youth. Genetic variants associated with severity of meningococcal disease, including the IgG Fc receptor (FCγRII)-A484T, interleukin-10 (IL-10)-A1082G, -C819T, and -C627A, IL-4-C589T, mannose binding lectin-2 (MBL2)-A/O, -H/L, -P/Q, and -X/Y, toll-like receptor 2 (TLR2)-G2408A, TLR4-A12874G and -C13174T, and TLR9-T1237C and -T1486C were determined by real-time PCR (RT-PCR) for 271 HIV-infected subjects (median, 17 years). Response was defined as a ≥4-fold increase from entry in bactericidal antibody titers to each serogroup. Generalized estimating equation (GEE) models were used to evaluate the association of allelic variants with the immunologic response to all serogroups within each subject with and without adjusting for CD4 percentage and HIV viral load. At week 4, but not after, subjects with TLR2-2408-G/A versus -G/G genotypes and the TLR4-12874-A/A genotype were more likely to achieve a ≥4-fold increase overall in the four serogroups (unadjusted *P* of 0.006 and adjusted *P* of 0.008 and unadjusted *P* of 0.008 and adjusted *P* of 0.019, respectively). At week 28, the TLR9-1237 T allele was associated with enhanced antibody response (T allele versus C/C, unadjusted *P* of 0.014 and adjusted *P* of 0.009), which was maintained at week 72 (unadjusted and adjusted *P* of 0.008). At week 72, the FcγRII-131Arg allotype was associated with a ≥4-fold increase in antibody titer versus those with His/His (unadjusted *P* of 0.009; adjusted *P* of <0.001). These findings suggest that for HIV-infected youth, the initial antibody response to MCV4 is associated with variants in TLR2 and TLR4 while the long-term response is associated with genetic polymorphisms in TLR9 and FcγRIIa.

Neisseria meningitidis infections can be rapidly fatal as a result of an acute inflammatory response associated with sepsis or meningitis. Although the risk factors associated with serious and fatal disease are not fully known, recent studies support a combined role for variable virulence factors and genetic variation of the host immune system as important contributors to the pathogenesis of invasive meningococcal disease (1). The most common invasive meningococcal serogroups are A, B, C, W135, and Y. Of these five serogroups, the capsular polysaccharide of serogroup B is least immunogenic. Thus, the current quadrivalent meningococcal conjugate vaccine (MCV4) is targeted against the other four invasive *N. meningitidis* serogroups, A, C, Y, and W-135, with each capsular polysaccharide antigen individually conjugated to diphtheria toxoid protein. The vaccine is licensed for use in individuals 9 months to 55 years of age (2, 3).

Recently, we reported on a phase I/II trial of the safety and immunogenicity of MCV4 in youth infected with HIV (4, 5). Our findings demonstrated that although MCV4 is safe and immunogenic in HIV-infected youth, the response rates are lower than those in healthy recipients, particularly in those with advanced HIV disease, greater immunosuppression, and higher viral load.

Although it could have been anticipated that youth with lower $CD4^+$ lymphocyte counts would respond more poorly to the vaccine, the responses for the individual patient were not able to be predicted based solely on $CD4^+$ count and viral load. Several host genetic factors have been associated with the risk and severity of meningococcal disease (6–8). Of these host determinants, toll-like receptors (TLRs) have been identified to play a central role in the immune response to *N. meningitidis* (9). TLRs are a class of pattern recognition receptors that identify distinct microbial structures. To date, 10 TLRs have been identified in humans, with each recognizing different microbial structures (10). Polymorphisms in three TLRs have been associated with invasive meningococcal disease, including TLR2, which recognizes bacterial lipoproteins, TLR4, which recognizes lipopolysaccharide (LPS), and TLR9, which recognizes unmethylated CpG DNA present on both bacteria and viruses (8, 11–13).

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In addition to TLRs, numerous other genetic polymorphisms have been associated with the development and severity of meningococcal disease, including genes that affect innate and adaptive immunity and inflammation (7, 8, 14). In this study, we examined the association of polymorphisms in TLR2, -4, and -9, the IgG FC receptor (FCγRII), interleukin-10 (IL-10) (7, 8, 14), interleukin-4 (IL-4), and mannose binding lectin-2 (MBL2) with the response to a single dose and two doses of MCV4 in 11- to 24-year-old HIV-infected youth.

MATERIALS AND METHODS

P1065 study population and entry criteria. P1065 is a phase I/II study of the safety and immunogenicity of MCV4 in HIV-infected children and youth aged 2 to <25 years at study entry. To be eligible for the study, patients were required to be on a stable antiretroviral regimen or on no treatment for at least 90 days, have no personal or family history of Guillain-Barré syndrome, and have never received MCV4 or received the meningococcal polysaccharide vaccine within the last 2 years. (NB: No study participants had a history of having received a meningococcal vaccine prior to enrollment in the study.) For complete inclusion and exclusion criteria, see Siberry et al. (4).

Study design for the youth component of P1065. Subjects were stratified by CD4 percentage into the following three strata: <15%, 15% to 24%, and ≥25%. All subjects received the MCV4 vaccine at entry. At week 24, subjects who were still eligible received a second dose of MCV4 if their entry CD4 lymphocyte percentage (CD4%) was <15% (study group 2), while those in study group 1 (CD4% ≥ 15) were randomized to receive or not receive a second dose of MCV4. HIV plasma RNA load was quantified using PCR assays locally available at each research site. Serum was drawn for meningococcal assays at entry (prevaccine) and 4-, 24-, 28-, and 72-week visits. Peripheral blood mononuclear cells were obtained only for genetic testing from study participants aged 11 to 24 years. This study focuses on the association of genetic variants with the vaccine response at 4, 28, and 72 weeks.

All study participants and/or their legal guardians signed written informed consent to participate in this study. The research was approved by the University of California, San Diego, Institutional Review Board Human Research Protection Program.

Immunogenicity. An immune response to MCV4 was defined as a ≥4-fold rise in serum bactericidal antibody (SBA) against each meningococcal serogroup (A, C, W-135, and Y). A rabbit serum bactericidal assay (rSBA) against each serogroup was performed by Sanofi-Pasteur laboratories as previously described (15). Titers that were correlated with immunity were absolute rSBA titers, identified as follows: ≥1:128, immune; <1:8, susceptible; 1:8 to 1:64, indeterminate (4, 16, 17).

Detection of genetic variants. Genetic variants associated with the severity of meningococcal disease, including rs1801274 (FCγRII-A484G [His131Arg]), rs1800896 (IL-10-A1082G), rs1800871 (IL-10-C819T), rs1800872 (IL-10-C627A), rs2243250 (IL-4-C589T), rs5030737, rs1800450, rs1800451, rs11003125, rs7095891, rs7096206 (MBL2-A/O, -H/L, -P/Q, and -X/Y), rs5743708 (TLR-2-G2408A), rs4986790 (TLR-4-A12874G), rs4986791 (TLR-4-C13174T), rs5743836 (TLR-9-T1237C), and rs187084 (TLR-9-T1486C), were determined by real-time PCR. Primer and probe sequences used to detect each of the single nucleotide polymorphisms (SNPs) are available in Table S1 in the supplemental material.

Statistical methods. At weeks 4, 28, and 72, generalized estimating equation (GEE) models were used to evaluate the association of genetic variants with immunologic response to all four serogroups, accounting for the correlated responses from the serogroups in each subject. In the multivariate analyses, screening CD4% strata and entry HIV RNA were included in the GEE models as potential confounders. In the analyses of the immunologic responses at weeks 28 and 72, treatment arm (whether subjects received one or two doses of MCV4) was also included in the multivariate GEE models. For allelic variants with an overall significant effect in the GEE model, individual logistic regression models were fitted to evaluate the association of genetic variants with antibody responses to each serogroup. In the case of sparse data, an exact logistic regression model was used. No significant associations with antibody response were observed for the genetic variants IL-10-A1082G, -C819T, and -C627A, IL-4-C589T, TLR-4-A13174G, TLR-9-T1486C, and MBL2-A/O, -H/L, -P/Q, and -X/Y in either the unadjusted or adjusted analyses at week 4, 28, or 72. Only findings for the significant TLR and FcγRII genetic variants are discussed below.

TABLE 1 Baseline characteristics[a]

Characteristic	Value
Age at study entry (yrs)	
Median	17
Mean (SD)	17.66 (3.96)
No. (%) aged 11 to 15 yrs	94 (35)
No. (%) aged 16 to 20 yrs	93 (34)
No. (%) aged 21 to 25 yrs	84 (31)
No. of males (%)	163 (60)
Race/ethnicity (no. [%])	
White non-Hispanic	34 (13)
Black non-Hispanic	118 (44)
Hispanic	112 (41)
Other	7 (2)
% on HAART at entry	69
$CD4^+$ count (cells/μl) at screening	
Median	504.5
Mean (SD)	556 (315)
CD4% at screening	
Median	24
Mean (SD)	25.7 (10.3)
No. (%) in group 2 with a CD4% of <15	26 (10)
No. (%) in group 1 with a CD4% of 15 to <25	112 (41)
No. (%) in group 1 with a CD4% of ≥25	133 (49)
$\log_{10}$ RNA at study entry	
Median	2.92
Mean (SD)	3.07 (1.26)
No. (%) with <400 copies/ml	126 (46)
No. (%) with 400 to 10,000 copies/ml	69 (25)
No. (%) with >10,000 copies/ml	76 (28)

[a] Total $n = 271$.

RESULTS

Study population. Of the 271 subjects who were included in the genetic testing from P1065, the median age at study entry was 17 years, 60% were male, 44% were black non-Hispanic, and 41% were Hispanic (Table 1). Of note, the race/ethnicity of the subjects enrolled into this study is representative of youth infected with HIV in the United States. A total of 90% were in group 1 with a screening CD4% of ≥15% (41% had a CD4% of 15 to 24% and 49% had a CD4% of ≥25), and 10% were in group 2 with a CD4% of <15. The median $CD4^+$ lymphocyte count and percentage were 505 and 24%, respectively. At study entry, the time of the first vaccination, 69% were receiving combination antiretroviral therapy; HIV viral load was <400 copies/ml in 46% of the subjects, and the median $\log_{10}$ HIV plasma RNA load was 2.92.

TABLE 2 TLR2, TLR4, TLR9, and FcγRII genotypes by race/ethnicity

SNP	Minor allele (frequency [%])	Genotype	No. (%) with each genotype in each group					*P* value
			Total ($n = 271$)	White non-Hispanic ($n = 34$)	Black non-Hispanic ($n = 118$)	Hispanic ($n = 112$)	Other ($n = 7$)	
TLR2-2408	G (1.66%)	G/G	262 (97)	31 (91)	115 (97)	109 (97)	7 (100)	0.282
		G/A	9 (3)	3 (9)	3 (3)	3 (3)	0 (0)	
TLR4-12874	G (6.27%)	A/A	239 (88)	30 (88)	102 (86)	100 (89)	7(100)	0.940
		A/G	30 (11)	4 (12)	15 (13)	11 (10)	0 (0)	
		G/G	2 (1)	0 (0)	1 (1)	1 (1)	0 (0)	
TLR9-1237	C (24.35%)	T/T	153 (56)	23 (68)	47 (40)	79 (71)	4 (57)	<0.001
		T/C	104 (38)	10 (29)	61 (52)	31 (28)	2 (29)	
		C/C	14 (5)	1 (3)	10 (8)	2 (2)	1 (14)	
FcγRII-484 (His131Arg)	A (50.00%)	A/A	63 (23)	12 (35)	23 (19)	28 (25)	0 (0)	0.341
		A/G	145 (54)	16 (47)	66 (56)	59 (53)	4 (57)	
		G/G	63 (23)	6 (18)	29 (25)	25 (22)	3 (43)	

Distribution of TLR2, TLR4, TLR9, and FcγRII genotypes by gender and race/ethnicity. The distribution of TLR and FcγRII genotypes did not differ by gender (data not shown). TLR2-2408-G/A, TLR4-12874-A/G, and FcγRII-His131Arg were equally distributed among white non-Hispanics, black non-Hispanics, and Hispanics (Table 2). No subjects were found to be TLR2-2408-A/A. For TLR9-1237, the TT variant was less-commonly found in black non-Hispanic subjects than in the other racial groups.

Association of TLR2, TLR4, TLR9, and FcγRII genetic variants with the week-4 serum bactericidal antibody response to MCV4 using a GEE model. At study entry, 61% of the 271 participated subjects had an rSBA titer of ≥1:128 to at least one serogroup. However, only 11% exhibited immunity to serogroup C, while the greatest preexisting protective immunity was found to be 41% to serogroup A (Table 3). At week 4, 92% of subjects had any rSBA titer of ≥1:128 to at least one group (A, 85%; C, 55%; W-135, 73%; Y, 73%) and 89% of subjects had a ≥4-fold rise in rBSA in at least one serogroup (A, 69%; C, 51%; W-135, 72%; Y, 62%).

The initial analysis examined the associations between each of the 4 serogroups contained within MCV4 at week 4 and genetic variants of TLR2, -4, and -9 and FcγRII. In univariate analyses, the TLR4-12874-A/A genotype, compared to the A/G and G/G genotypes, was significantly associated with a greater likelihood of achieving a ≥4-fold rise in antibody titer to serogroup C (A/A versus A/G and G/G; odds ratio [OR] = 2.73 [95% confidence interval {CI}, 1.24 to 6.02], $P = 0.013$) and serogroup Y (A/A versus A/G and G/G; OR = 2.80 [95% CI, 1.31 to 5.95], $P = 0.008$). These comparisons remained significant when controlling for baseline CD4% and plasma HIV RNA (serogroup C, adjusted OR [AOR] = 2.57 [95% CI, 1.11 to 5.92], $P = 0.027$; serogroup Y, AOR = 2.62 [95% CI, 1.18 to 5.82], $P = 0.018$). While there were no statistically significant associations found with serogroups A and W, the odds ratios were in the same direction (1.42 and 1.48, respectively), suggesting that there may be an association between TLR4-A12874G genetic variants and the overall response to MCV4 (see Table S2 in the supplemental material). For TLR2-2408, all the subjects with genotype G/A had a ≥4-fold increase in the rSBA titer at week 4 for serogroups Y and W. The exact logistic regression showed that the association was significant, with the presence of the A allele in genotype G/A being associated with a greater likelihood of immunologic response than genotype G/G for serogroup Y ($P = 0.028$); the result remained significant ($P = 0.030$) after controlling for baseline CD4% and RNA.

To test the hypothesis that genetic variants in TLR2 and TLR4 were associated with the overall response to MCV4 at week 4, a GEE model which estimates the overall response of the allelic variants to the four serogroups, accounting for the within-subject correlation between the serogroups, was used (Table 4). In unadjusted analyses, subjects with the TLR2-2408-G/A genotype were more likely to achieve a ≥4-fold increase overall in the four serogroups than those with the G/G variant (OR = 5.74 [95% CI, 1.93 to 17.03], $P = 0.006$). This difference was maintained when controlling for CD4% and HIV plasma RNA (OR = 5.93 [95% CI, 2.18 to 16.13], $P = 0.008$). Similarly, TLR4-12874-A/A homozygotes were more likely to achieve a ≥4-fold increase overall in the four serogroups than the A/G or G/G genotype (OR = 2.08 [95% CI, 1.27 to 3.40], $P = 0.008$, in the unadjusted analysis; AOR = 1.83 [95% CI, 1.14 to 2.93], $P = 0.019$, after controlling for CD4% and HIV RNA). No significant association was found for TLR9-T1237C or FcγRII-A484G.

TLR9 and FcγRII, but not TLR2 and TLR4, genetic variants are associated with immunogenicity at weeks 28 and 72. Of 221 subjects who had serum bactericidal antibody measured at week

TABLE 3 rSBA titers by serogroup

Wk	rSBA titer	No. (%) with each titer in each serogroup				
		Any serogroup	A	C	W-135	Y
0	≥1:128	166 (61)	110 (41)	31 (11)	41 (15)	96 (35)
4	≥1:128	248 (92)	231 (85)	148 (55)	197 (73)	198 (73)
	≥4-fold increase[a]	241 (89)	186 (69)	139 (51)	195 (72)	168 (62)
28	≥1:128	198 (90)	167 (76)	95 (43)	165 (75)	165 (75)
	≥4-fold increase[a]	199 (90)	124 (56)	100 (45)	161 (73)	137 (62)
72	≥1:128	157 (71)	119 (54)	53 (24)	116 (52)	127 (57)
	≥4-fold increase[a]	152 (69)	76 (34)	54 (24)	115 (52)	105 (48)

[a] Compared to entry titers.

TABLE 4 GEE model fit to the week-4 response (≥4-fold increase) for the four serogroups in each subject by TLR-2, -4, and -9 and FcγRII[a]

Genotype	Comparison	Odds ratio (95% CI)	*P* value	Adjusted odds ratio (95% CI)	*P* value
TLR2-G2408A	G/A vs G/G	5.74 (1.93 to 17.03)	0.006	5.93 (2.18 to 16.13)	0.008
TLR4-A12874G	A/A vs A/G+G/G	2.08 (1.27 to 3.40)	0.008	1.83 (1.14 to 2.93)	0.019
TLR9-T1237C	T/T vs C/C	1.73 (0.82 to 3.66)	0.186	2.58 (1.02 to 6.52)	0.083
	T/C vs C/C	2.12 (0.98 to 4.59)	0.091	3.00 (1.17 to 7.68)	0.052
	T/T+T/C vs C/C	1.88 (0.90 to 3.91)	0.133	2.74 (1.10 to 6.85)	0.068
FcγRII-A484G (His131Arg)	G/G vs A/A	1.07 (0.62 to 1.84)	0.820	1.44 (0.84 to 2.46)	0.175
	A/G vs A/A	1.04 (0.69 to 1.58)	0.848	1.25 (0.83 to 1.89)	0.295
	G/G+A/G vs A/A	1.05 (0.70 to 1.56)	0.816	1.30 (0.88 to 1.93)	0.191

[a] Unadjusted and adjusted for CD4 T lymphocyte percentage strata and HIV RNA groups.

28 (17 subjects were in group 2, who received the second dose, and 204 subjects were in group 1, in which 102 subjects received the second dose and another 102 subjects did not), 90% had protective antibody levels (titers ≥ 1:128) to at least one serogroup (A, 76%; C, 43%; W-135, 75%; Y, 75%). Also, 90% of the subjects had a ≥4-fold SBA titer rise from study entry to at least one serogroup (A, 56%; C, 45%; W-135, 73%; Y, 62%). We used the GEE model to estimate the overall antibody response of the allelic variants to the four serogroups at week 28. TLR2 and TLR4 genotypes were no longer associated with the antibody response (Table 5); additionally, none of the serogroup-specific antibody responses was associated with TLR2 or TLR4. In further contrast to the findings at week 4, the TLR9-1237 T allele was associated with an enhanced antibody response (T/T versus C/C, OR = 2.12 [95% CI, 1.24 to 3.64], $P = 0.020$; T/C versus C/C, OR = 2.47 [95% CI, 1.37 to 4.45], $P = 0.011$; and T/T+T/C versus C/C, OR = 2.25 [95% CI, 1.33 to 3.78], $P = 0.014$) in the GEE analysis. After controlling for CD4%, HIV RNA, and treatment arm, the results remain significant with T/T versus C/C (AOR = 2.31 [95% CI, 1.28 to 4.17], $P = 0.021$), T/C versus C/C (AOR = 3.29 [95% CI, 1.79 to 6.06], $P = 0.004$), and T/T+T/C versus C/C (AOR = 2.64 [95% CI, 1.51 to 4.60], $P = 0.009$). In the individual logistic regressions for each specific serogroup, significant associations were related to serogroups W-135 and Y, with odds ratios from the other serogroups going in the same direction.

Among the 198 subjects who had week-72 antibody titers, the proportion of subjects having protective antibody levels to at least one serogroup dropped to 71% (A, 54%; C, 24%; W-135, 52%; Y, 57%), and only 69% (A, 34%; C, 24%; W-135, 52%; Y, 48%) had a ≥4-fold SBA titer rise to at least one serogroup compared to study entry. Similar to the findings at week 28, the GEE model showed that subjects with the TLR9-1237 T allele remained more likely to have maintained a ≥4-fold antibody response than those with the C/C genotype (T/T versus C/C, OR = 3.15 [95% CI, 1.54 to 6.44], $P = 0.008$; T/C versus C/C, OR = 3.25 [95% CI, 1.53 to 6.90], $P = 0.009$; and T/T+T/C versus C/C, OR = 3.19 [95% CI, 1.58 to 6.90], $P = 0.008$). The results remained significant when adjusted for CD4%, HIV RNA, and treatment arm (Table 6). The individual logistic regressions showed that there was still a significant association present for serogroups W-135 and Y, with odds ratios from other serogroups in the same direction.

In addition to the findings for TLR9 at week 72, the GEE model showed that FcγRII polymorphisms were associated with the overall antibody response to immunization at this time point, such that study participants with the FcγRII-131Arg allotype were more likely to have a ≥4-fold antibody response than those with the His/His genotype. The odds ratios (both unadjusted and adjusted for CD4%, viral load, and treatment arm) were significant for the comparisons of Arg/Arg versus His/His, Arg/His versus His/His, and Arg/Arg+Arg/His versus His/His (Table 6). Individual logistic regression results demonstrated that this significant association was due mainly to the association between FcγRII and vaccine response in serogroups A and Y, with the effects from serogroups C and W-135 going in the same direction.

DISCUSSION

Although an increasing literature supports the necessity of critical interactions between the innate and adaptive immune systems for the control of microbial pathogens, including *N. meningitidis*, to our knowledge, the findings reported here are the first to examine the impact of host genetic variation on the response to the quadrivalent meningococcal conjugate vaccine. Although these findings are specific to HIV-infected youth, it is likely that host genetic factors are also associated with responses to MCV4 in non-HIV-infected individuals. Unfortunately, we did not have access to a cohort of healthy children immunized with MCV4 to determine if

TABLE 5 GEE model fit to the week-28 response (≥4-fold increase) for the four serogroups in each subject by TLR-2, -4, and -9 and FcγRII[a]

Genotype	Comparison	Odds ratio (95% CI)	*P* value	Adjusted odds ratio (95% CI)	*P* value
TLR2-G2408A	G/A vs G/G	0.85 (0.43 to 1.68)	0.621	1.00 (0.42 to 2.36)	0.998
TLR4-A12874G	A/A vs A/G+G/G	1.64 (0.98 to 2.76)	0.084	1.52 (0.96 to 2.41)	0.091
TLR9-T1237C	T/T vs C/C	2.12 (1.24 to 3.64)	0.020	2.31 (1.28 to 4.17)	0.021
	T/C vs C/C	2.47 (1.37 to 4.45)	0.011	3.29 (1.79 to 6.06)	0.004
	T/T+T/C vs C/C	2.25 (1.33 to 3.78)	0.014	2.64 (1.51 to 4.60)	0.009
FcγRII-A484G (His131Arg)	G/G vs A/A	1.34 (0.75 to 2.38)	0.323	1.68 (0.97 to 2.93)	0.072
	A/G vs A/A	1.18 (0.76 to 1.85)	0.455	1.59 (1.01 to 2.50)	0.050
	G/G+A/G vs A/A	1.23 (0.80 to 1.90)	0.348	1.62 (1.05 to 2.49)	0.036

[a] Unadjusted and adjusted for CD4% and HIV RNA group.

TABLE 6 GEE model fit to the week-72 response (≥4-fold increase) for the four serogroups in each subject by TLR-2, -4, and -9 and FcγRII[a]

Genotype	Comparison	Odds ratio (95% CI)	*P* value	Adjusted odds ratio (95% CI)	*P* value
TLR2-G2408A	G/A vs G/G	0.85 (0.50 to 1.45)	0.479	0.72 (0.41 to 1.25)	0.242
TLR4-A12874G	A/A vs A/G+G/G	1.43 (0.72 to 2.84)	0.320	1.21 (0.62 to 2.33)	0.591
TLR9-T1237C	T/T vs C/C	3.15 (1.54 to 6.44)	0.008	3.82 (1.72 to 8.47)	0.012
	T/C vs C/C	3.25 (1.53 to 6.90)	0.009	4.93 (2.19 to 11.12)	0.004
	T/T+T/C vs C/C	3.19 (1.58 to 6.90)	0.008	4.18 (1.94 to 9.03)	0.008
FcγRII-A484G (His131Arg)	G/G vs A/A	2.06 (1.15 to 3.71)	0.017	3.16 (1.76 to 5.67)	<0.001
	A/G vs A/A	1.77 (1.09 to 2.89)	0.022	2.58 (1.61 to 4.14)	<0.001
	G/G+A/G vs A/A	1.87 (1.17 to 2.97)	0.009	2.75 (1.74 to 4.35)	<0.001

[a] Unadjusted and adjusted for CD4% and HIV RNA group.

our findings are unique to HIV-infected children or can be generalized to all children immunized with the vaccine.

Our findings are somewhat surprising given the product description of Menactra, which consists of meningococcal serogroup A, C, Y, and W-135 capsular polysaccharide antigens individually conjugated to diphtheria toxoid protein (Sanofi-Pasteur product insert). In theory, the outer membrane protein and lipopolysaccharide of *N. meningitidis* should not be present in the vaccine and should not induce a signal through TLRs. The polysaccharide components of the vaccine are extracted from *N. meningitidis* and purified by centrifugation, detergent precipitation, alcohol precipitation, solvent extraction, and diafiltration. The diphtheria toxoid protein is purified by ammonium sulfate fractionation and diafiltration. In fact, when 20 μl of the Menactra vaccine was diluted 1:10 and assessed for TLR stimulation by assessing NF-κB activation in HEK293 cells expressing a given TLR, only a small amount of TLR4 stimulation was observed (InVivoGen) (data not shown). Thus, the amount of stimulation through TLR2 and TLR9 is below the level of detection of signaling assays (0.31 μg/ml; InVivoGen). However, given the low sensitivity of these assays, it is possible that TLR signaling may be occurring and not detected through standard assays. Of note, Moore et al. have recently identified that SNPs in TLR3 and CD44 genes are associated with persistence of antibody to serogroup C meningococcal conjugate vaccine in a cohort of healthy children aged 6 to 12 years (18). TLR3 recognizes double-stranded RNA through the N-terminal ectodomains; thus, how TLR3 might alter long-term antibody response to meningococcal vaccines is similarly unclear. Although in our study, we did not look at TLR3 variants, it is of interest that we also identified an association of TLRs with the antibody response to meningococcal conjugate immunization. Another possible explanation for the association between genetic variants within TLRs and response to conjugate meningococcal vaccination is that the TLR genetic variants identified may be in linkage disequilibrium with an unidentified gene(s) that is the actual functional association with the response to MCV4.

TLRs are an important first line of defense, with their primary function being the detection of the invading pathogen, resulting in activation of innate immune cells to clear the infection. In addition, TLRs play an important role in the initiation of the adaptive immune response during acute infection as well as following immunization (19–21). Dendritic cells are thought to be critical in linking the innate immune response to adaptive immunity (22). Recently, Finsen et al. examined the role of TLR2 and TLR4 in the immune response to *N. meningitidis* outer membrane vesicle (OMV) vaccine (19). Interestingly, they found that the immune response to the OMV vaccine was not impaired in mice deficient in TLR2 ($TLR2^{-/-}$) and, in fact, appeared to have an increase in antibody response. In contrast, the immune response in TLR4-deficient mice ($TLR4^{-/-}$) was severely impaired. Similarly, when the same group examined the antibody response to the whole-cell *Bordetella pertussis* vaccine, they again found that whereas TLR4 was critical to the immune response, the response of TLR2-deficient mice was unaffected.

The findings in the current study are consistent with the observations of Finsen et al. (19) in that the genetic variant leading to diminished TLR4 expression was associated with an impaired antibody response at 4 weeks after the vaccination. Additionally, our finding that a variant of TLR2 which leads to decreased expression is associated with an enhanced antibody response is consistent with the Finsen observations in TLR2-deficient mice. Thus, it would appear that the initial antibody response to the MCV4 vaccine is associated with TLR4 signaling and less dependent on, or perhaps even inversely associated with, TLR2 activation.

Whereas the initial antibody response to the MCV4 vaccine was associated with TLR2 and TLR4 genotypes, sustained increases in antibody at weeks 28 and 72 were associated with genetic variants in TLR9 and FcγRII. Of note, the sustained antibody responses associated with the TLR9 and FcγRII genetic variants were observed in study participants who received only a single dose of MCV4 as well as those who received two doses of the vaccine (data not shown). Thus, although an association with an anamnestic antibody response cannot be excluded, the persistence of specific antibody would appear to be related to the induction of memory B cells. In this regard, activation of B cells through TLR9 has been shown to trigger differentiation into $CD138^{+}$ plasma cells and to increase TLR9 expression. In addition to its role in the differentiation of transitional B cells to IgM memory-like B cells and IgM-secreting plasma cells, TLR9 signaling combined with engagement of TLR7 also induces naive B cells to proliferate and differentiate into IgG-producing cells (23–25). Bernasconi et al. have proposed a model of serological memory in which short-term serological memory is a consequence of antigen-dependent B cell activation and lasts for a few months (26). In contrast, long-term serological memory is the result of antigen-independent polyclonal activation and differentiation of memory B cells. This antigen-independent phase is hypothesized as being, at least in part, dependent on CpG signaling through TLR9, resulting in the long-term persistence of pathogen-specific memory B cells.

The His131Arg amino acid change in FcγRII has functional significance and has been associated with autoimmune diseases as

well as susceptibility to bacterial infections (27). His131Arg is located in the IgG-binding site of FcγRIIa 18, and the amino acid change alters the ability of the receptor to bind to IgG2 and IgG3, with only 131His capable of binding IgG2. These alterations also affect the phagocytic capacity of polymorphonuclear leukocytes. Whereas the 131Arg variant has weaker binding affinity of IgG, less-effective phagocytosis, and a lower capacity for immune activation, the His131 allotype has more-effective phagocytosis and is thought to be associated with hyperactivation of immune cells. Our finding that the antibody response to the MCV4 vaccine at 72 weeks is enhanced in persons with the 131Arg genotype suggests that the vaccine, although immunogenic, elicits a greater antibody response in recipients with the FcγRIIa 131 allotype who are unable to bind IgG2. It is interesting to note that in studies of healthy individuals, the ratio of IgG1 to IgG2 antibody responses varies among vaccine recipients, with a subgroup of vaccinees making consistently higher IgG2 responses to all 4 components (28). To our knowledge, the association of the IgG subclass response with the FcγRII allelic variants has not been examined. However, although the impact of the FcγRIIa-Arg131His genetic variants on the severity of meningococcal disease remains controversial (7), Platonov et al. found that for children older than 5 years of age, the Arg131 allotype was associated with a greater risk of severe meningococcal disease (29).

In summary, our findings suggest that for HIV-infected youth, the antibody response to MCV4 is affected by host genetic variation. Whereas the initial response to the vaccine is associated with variants in TLR2 and TLR4, the sustained response is affected by genetic polymorphisms in TLR9 and FcγRIIa. Although these studies were performed only in HIV-infected vaccine recipients, it is likely that the response in healthy, uninfected children will also be affected by host genetic variation. These findings have the potential to help guide approaches to improve the short- and long-term antibody responses to meningococcal vaccines.

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