MAJOR ARTICLE

Genetic Variation in *IL18R1* and *IL18* Genes and Inteferon γ ELISPOT Response to Smallpox Vaccination: An Unexpected Relationship

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Background. Genetic association studies demonstrated a role for cytokine proteins and cytokine or cytokine receptor gene polymorphisms in smallpox vaccine–induced adaptive immunity.

Methods. We examined the association of genetic polymorphisms with cellular (interferon [IFN] γ enzyme-linked immunospot assay [ELISPOT]) immune response to smallpox vaccine in 1076 immunized individuals.

Results. The majority of significant associations were discovered between single-nucleotide polymorphisms/haplotypes in *IL18R1* and *IL18* genes, in which we previously reported an association with vaccinia virus–induced neutralizing antibody titers in this study cohort. A functional coding *IL18R1* polymorphism (rs1035130/Phe251 Phe; $P = .01$) was significantly associated with an allele dose-related increase in IFN-γ production and was also associated with vaccinia-specific neutralizing antibody titers. Significant associations were also found between *IL18R1* haplotypes and variations in IFN-γ ELISPOT responses (global $P < .0001$).

Conclusions. Our data suggest the importance of variants in the *IL18R1* and *IL18* genetic loci for broad-based smallpox vaccine–induced adaptive immunity.

Keywords. Polymorphism; single nucleotide; smallpox vaccine; vaccinia virus; interleukin-18; interleukin-18 receptor alpha subunit; haplotypes; genetic predisposition to disease; interferon-gamma; viral vaccines; genetic variation; enzyme-linked immunospot assay; european continental ancestry group; African-Americans.

Live vaccinia virus–based smallpox vaccine (Dryvax; Acam) is an effective vaccine that induces a robust and long-lasting humoral and cell-mediated immune response [1, 2]. This adaptive immune response is characterized by a broad interindividual spectrum of vaccinia virus–specific antibody titers, secreted cytokine levels and frequencies of interferon (IFN) γ–producing T cells [3–6]. With regard to smallpox vaccine–induced immunity, a number of important factors, including environmental factors, can determine the response to vaccinia vaccination. Among these are genetically determined host factors (gene polymorphisms), which may determine whether or not a person will adequately respond to vaccination and/or develop experience adverse events (AEs).

Our recent study on genetic associations with humoral immune response to smallpox vaccine suggested an important effect of *IL18* and *IL18R1* genetic variants (single-nucleotide polymorphism [SNPs]) and haplotypes on the vaccinia-induced circulating humoral antibody production [7]. Polymorphism in the *IL18R1* gene has also been linked to smallpox vaccine–induced interleukin 1β, tumor necrosis factor α, and interleukin (IL)-2 secretion [8]. In a recent study of 1076 healthy armed forces members, vaccinia-induced IFN-γ and IL-2 were significantly correlated with neutralizing antibody titers after smallpox vaccination [9]. In another study, smallpox vaccine–induced AEs were found to be correlated with increased circulating concentrations of

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IFN-γ, tumor necrosis factor α, IL-2, IL-5, and IL-10 [4]. In addition to the results associated with cytokine production, it has been shown that quantification of vaccinia-specific T-cell responses (from IFN-γ–producing cells) in immunized persons is important for the characterization of smallpox vaccine–induced cellular immunity [3, 6, 10]. Furthermore, a candidate gene association study identified specific *IL1* and *IL18* gene haplotypes associated with the development of fever after receipt of smallpox vaccine [11]. These vaccinia immunogenetic studies suggest that cytokine proteins and cytokine gene polymorphisms play a role in smallpox vaccine–induced adaptive immunity and in the development of AEs after smallpox vaccination.

We hypothesized that variations in the adaptive cellular IFN-γ responses after smallpox vaccination are associated with specific genetic markers in host cytokine and cytokine receptor genes. The purpose of this study was to examine genetic associations between individual SNPs and SNP haplotypes in the cytokine and cytokine receptor genes and vaccinia-specific T-cell response by IFN-γ enzyme-linked immunospot assay (ELISPOT).

MATERIALS AND METHODS

Study Subjects

Details of this study's recruitment and study subjects have been provided elsewhere [5–8, 10, 12]. Briefly, we enrolled 1076 healthy subjects (aged 18–40 years). All study subjects had been immunized with a single dose of Dryvax vaccine (Wyeth Laboratories) between 2002 and 2006. A total of 1056 subjects, 580 white and 217 African American, participated in this study. All subjects had a documented vaccine "take" at the vaccination site after immunization. The institutional review boards of both the Mayo Clinic (Rochester, Minnesota) and Naval Health Research Center (San Diego, California) granted permission for the study, and written informed consent was obtained from each subject.

IFN-γ ELISPOT

Our description of the ELISPOT that measures vaccinia-specific IFN-γ in vitro production by $CD4^+$ and $CD8^+$ T cells (ELISPOT kits; R&D Systems) is similar to those we published elsewhere [6, 10, 13]. Briefly, peripheral blood mononuclear cells were stimulated with inactivated vaccinia virus (NYCBOH strain) at a multiplicity of infection of 5 for 24 hours. Plate scanning and spot counting were performed on an ImmunoSpot S4 Pro-Analyzer using ImmunoSpot software, version 4.0 (Cellular Technology). Outcomes are expressed as spot-forming cells (SFCs) per 200 000 peripheral blood mononuclear cells. ELISPOT counts were successfully obtained for all subjects in all replicate assessments, except for 6. In these subjects, all 3 counts were available from the stimulated wells, but only 2 were successfully measured from the unstimulated wells.

SNP Genotyping

The genotyping methods we used for this study were identical to those published elsewhere [7, 8]. We selected tag SNPs within the 32 candidate cytokine and cytokine receptor genes, and 10 kb upstream and downstream of them, using the linkage disequilibrium (LD) tag SNP selection approach from the HapMap Phase II (http://www.hapmap.org), Seattle SNPs (http://pga.mbt.washington.edu/), NIEHS SNPs (http://egp.gs.washington.edu/), and NCBI (http://www.ncbi.nlm.nih.gov/projects/SNP/) databases. The selected 785 candidate SNPs were genotyped using 2 custom-designed 384-plex Illumina GoldenGate assays (Illumina), TaqMan custom assays (Applied Biosystems), and pyrosequencing. After removal of failed SNPs and DNA samples, 701 SNPs were used for analysis. We genotyped 1076 subjects for 785 SNPs in candidate cytokine genes (*IL2, IFNG, IL12A, IL12B, IL4, IL6, IL10, IL18, IL1B, TNFA, IFNA*, and *IFNB*) and their corresponding receptor genes. A total of 701 SNPs met all genotyping quality assessments and were used for analysis in 1056 subjects. The Illumina genotyping success rate was 89% (701 SNPs yielded genotyping data for analysis of 785 SNPs), and the study sample success rate was 98% (1056 samples yielded genotyping data out of 1076) [7, 8]. Subjects were excluded on the basis of inadequate or poor DNA quality (n = 5) or low call rates (<0.95; n = 15) [7, 8].

Statistical Methods

Medians were used to summarize the ELISPOT data across groups of study subjects. As repeated measurements were obtained for each subject, the subject-specific median was used as a single per-individual response measure in these summaries. Associations between IFN-γ ELISPOT responses and each of the genotyped SNPs in the candidate genes were assessed in all recruited subjects and also within white and African American subgroups, using linear mixed models regression to account for the repeated assessments of ELISPOT counts per subject. An inverse-normal transformation was applied to the data to ensure that modeling assumptions were met. In these analyses, we modeled each SNP genotype using a covariate representing the number of copies of the minor allele carried by each individual, and we adjusted for covariates that could have an impact on observed ELISPOT counts (see footnote to Table 1). We computed *Q* values, which estimate the probability that an observed *P* value is a false-positive [14, 15], and we identified SNP associations as meriting future consideration if their *Q* values were <0.5.

Associations between haplotypes in the *IL18R1* gene and IFN-γ ELISPOT responses were tested by extracting covariates representing the expected haplotype dosage for additive haplotype effects in each individual using the haplo.design function implemented in the Haplo.Stats R package (http://cran.r-project.org/web/packages/haplo.stats/index.html), and using these expected dosage variables [16] in linear mixed models that

Table 1. SNPs Associated With Vaccinia-Specific IFN-γ ELISPOT Responses in the Study Cohort[a]

Gene	SNP ID	Location	Genotype[b]	Subjects With Genotype, No.	IFN-γ, SFCs/2 × 10^5 Cells, Median (IQR)[c]	*P* Value[d]
IL6	rs2069827	Promoter (−1427)	CC	923	51 (23–87)	.0004
			CA	117	63 (33–97)	
			AA	3	122 (48–150)	
IL18R1	rs6749014	Intron (boundary)	GG	307	48 (22–92)	.005
			GA	497	55 (28–89)	
			AA	238	48 (22–84)	
IL18R1	rs4851004	Intron	GG	307	48 (22–92)	.005
			GA	495	55 (28–90)	
			AA	241	48 (22–84)	
IL18R1	rs6706002	Intron	AA	307	48 (22–92)	.005
			AG	496	55 (28–89)	
			GG	239	48 (22–84)	
IL18R1	rs1420096	Intron (boundary)	GG	307	48 (22–91)	.005
			GA	491	55 (28–90)	
			AA	239	48 (22–84)	
IL18R1	rs7584093	Intron	GG	309	49 (24–91)	.005
			GA	490	54 (27–89)	
			AA	242	48 (22–84)	
IL18R1	rs1420094	3′ Intergenic	GG	307	48 (22–92)	.005
			GA	497	55 (28–89)	
			AA	240	48 (22–84)	
IL2RB	rs2235330	Intron (boundary)	AA	671	52 (22–85)	.005
			AG	311	54 (27–93)	
			GG	57	51 (32–91)	
IL18R1	rs6758936	Intron	GG	308	48 (23–91)	.005
			GA	493	55 (27–89)	
			AA	241	49 (22–84)	
IL18R1	rs3213732	Intron	AA	308	49 (24–91)	.006
			AG	493	55 (27–89)	
			GG	242	48 (22–84)	
IL18R1	rs7556917	G/GC/T/TT (insertion)	CC	311	49 (22–92)	.006
			CA	476	54 (28–89)	
			AA	235	48 (22–83)	
IL18R1	rs10204757	Intron	AA	307	49 (24–91)	.006
			AC	493	55 (27–89)	
			CC	242	48 (22–84)	
IL18R1	rs2287033	Intron	AA	307	48 (22–92)	.006
			AG	495	55 (27–90)	
			GG	239	48 (22–84)	
IL18R1	rs2041739	Intron	GG	310	48 (22–91)	.007
			GA	493	55 (28–89)	
			AA	239	49 (22–85)	
IL18R1	rs11465641	Intron	AA	309	49 (24–91)	.007
			AC	493	55 (27–89)	
			CC	240	48 (22–84)	
IL10	rs6697497	3′ UTR	GG	1013	52 (24–88)	.008
			GA	27	60 (32–111)	
			AA	2	91 (60–123)	
IL18R1	rs4851570[e]	Intron	AA	594	51 (24–82)	.011
			AG	374	53 (25–95)	
			GG	76	53 (25–98)	

Table 1 continued.

Gene	SNP ID	Location	Genotype[b]	Subjects With Genotype, No.	IFN-γ, SFCs/2 × 10^5 Cells, Median (IQR)[c]	*P* Value[d]
TNFRS1A	rs505844	Intron	AA	647	48 (22–83)	.012
			AG	336	58 (31–95)	
			GG	54	66 (32–105)	
IL18R1	rs1035130[e]	Synonymous (F251F)	GG	594	51 (24–82)	.013
			GA	372	53 (25–95)	
			AA	78	57 (25–98)	
IL18R1	rs3771172	Intron	GG	620	51 (24–82)	.014
			GA	352	52 (24–93)	
			AA	72	61 (30–99)	

Abbreviations: A, adenine; C, cytosine; ELISPOT, enzyme-linked immunospot assay; G, guanine; IFN-γ, interferon γ; IQR, interquartile range; SFCs, spot-forming cells; SNP, single-nucleotide polymorphism.

[a] Analyses were adjusted for sex and race of study participants, age at blood collection (quartiles), time from smallpox immunization to blood collection (quartiles), time from blood collection to assay (quartiles), shipping temperature of the sample (frozen or ambient), and time of year when the sample was shipped (warm weather months [April–September] vs cold weather months [October–March]).

[b] Values are presented as homozygous major allele/heterozygous/homozygous minor allele.

[c] Values represent simple medians (IQRs) of the raw data, not the modeled estimates. We show these values because of our use of an inverse-normal data transformation. Because of this transformation and our need to adjust for a number of covariates, and because of the high variability of the ELISPOT counts, the medians may not match the modeled ordinal genotypic trend, even though the model fit was adequate.

[d] Repeated-measures linear regression modeling approach.

[e] The SNPs rs4851570 and rs1035130 are in linkage disequilibrium ($r^2 = 1$).

paralleled those used for the single-SNP tests. A global test of significance was obtained for all modeled haplotypes, followed by individual per-haplotype tests. Analyses were conducted in the SAS, version 9.2, and R, version 2.14, (http://www.r-project.org/) statistical software packages.

RESULTS

Associations Between SNPs in Candidate Genes and IFN-γ ELISPOT Responses

The median for vaccinia-specific IFN-γ ELISPOT counts for all subjects was 52 SFCs/200 000 cells (IQR, 24–88). In the combined cohort (n = 1056), we estimated *Q* values and found 20 SNPs with a <50% chance of false-positive associations with IFN-γ ELISPOT responses ($P < .015$), as shown in Table 1. Of these, 16 SNPs (80%) were located in the gene encoding IL18R1. One genetic variant was a coding synonymous *IL18R1* SNP rs1035130 (Phe251Phe; $P = .01$; in LD with intronic rs4851570; $r^2 = 1$) associated with an increase in IFN-γ ELISPOT response.

Similarly, in the white cohort (n = 580), we found 20 significant associations (at $P < .015$) between genetic variants in cytokine or cytokine receptor genes and variations in IFN-γ ELISPOT responses (Table 2). Of these, 13 associations (65%) were identified with SNPs in the *IL18R1* gene and these associations were consistent with the overall cohort findings. In addition, an *IL6* promoter polymorphism (rs2069827, 51 vs 122 SFCs, $P = .0004$ for the overall cohort and 56 vs 122 SFCs, $P = .001$ for white) was significantly associated with a 2-fold allele dose-related increase in IFN-γ ELISPOT counts.

In the African American cohort (n = 217), 3 genetic variants in the promoter and intronic regions of the *IL10* (rs3024503; $P = .007$) and *IL18* (rs5744280 [$P = .014$] and rs3882891 [$P = .015$; $r^2 \geq 0.38$]) genes, respectively, exhibited an allele dose-response relationship with IFN-γ response. No association was found between *IL18R1*gene polymorphisms and IFN-γ ELISPOT responses among African Americans (Supplementary Table 1).

Associations Between *IL18R1* Haplotypes and IFN-γ ELISPOT Responses

As shown in Tables 3 and 4, we identified 5 haplotypes (2 in the combined cohort and 3 in white) in the *IL18R1* gene that were significantly associated with variations in IFN-γ ELISPOT responses (global $P < .0001$). Two specific haplotypes based on the 16 and 14 *IL18R1* SNPs (GAAAAGCCGGAAAAGA; *t* statistic, −5.39; $P < .0001$) and (GAGAAGCCGAAAGA; *t* statistic, −5.04; $P < .0001$) were associated with reduced vaccinia-specific IFN-γ responses in the combined cohort and in whites, respectively. Likewise, *IL18R1* haplotypes AGGCGAAAAAGGGGAG (overall cohort; *t* statistic, 4.63; $P < .0001$) and AGACGAAAA GGGAG (whites; *t* statistic, 4.20; $P < .0001$) demonstrated a significant haplotype-level association for high IFN-γ ELISPOT counts.

Table 2. SNPs Associated With Vaccinia-Specific IFN-γ ELISPOT Responses in White Subjects[a]

Gene	SNP ID	Location	Genotype[b]	Subjects With Genotype, No.	IFN-γ, SFCs/2 × 10^5 Cells, Median (IQR)[c]	*P* Value[d]
IL6	rs2069827	Promoter (−1427)	CC	472	56 (28–93)	.001
			CA	87	68 (36–120)	
			AA	3	122 (48–150)	
TNF	rs1800610	Intron	GG	459	58 (30–98)	.002
			GA	99	51 (25–83)	
			AA	4	48 (21–85)	
IL2RG	rs11574625	Intron (boundary)	AA	477	60 (30–100)	.004
			AG	35	31 (15–61)	
			GG	50	55 (27–82)	
IL2RB	rs12160547	Intron	AA	561	58 (29–95)	.006
			AG	1	145 (145–145)	
			GG	0	. . .[e]	
IL2RB	rs3218333	3′ UTR	AA	559	58 (29–95)	.006
			AG	1	145 (145–145)	
			GG	0	. . .[e]	
IL18R1	rs6749014	Intron (boundary)	GG	159	63 (35–102)	.008
			GA	295	59 (28–96)	
			AA	108	49 (25–81)	
IL18R1	rs6758936	Intron	GG	159	63 (35–102)	.009
			GA	295	59 (28–96)	
			AA	108	50 (25–84)	
IL18R1	rs3213732	Intron	AA	15929	63 (35–102)	.0099
			AG	5	59 (28–96)	
			GG	109	49 (25–82)	
IL18R1	rs10204757	Intron	AA	159	63 (35–102)	.0099
			AC	259	59 (28–96)	
			CC	109	49 (25–82)	
IL18R1	rs6706002	Intron	AA	159	63 (35–102)	.0099
			AG	295	59 (28–96)	
			GG	109	49 (25–82)	
IL18R1	rs4851004	Intron	GG	159	63 (35–102)	.0099
			GA	295	59 (28–96)	
			AA	109	49 (25–82)	
IL18R1	rs1420094	3′ Intergenic	GG	159	63 (35–102)	.0099
			GA	295	59 (28–96)	
			AA	109	49 (25–82)	
IL18R1	rs7584093	Intron	GG	159	63 (35–102)	.010
			GA	293	58 (28–95)	
			AA	109	49 (25–82)	
IL18R1	rs3771172	Intron	GG	286	55 (29–87)	.011
			GA	229	64 (28–108)	
			AA	48	54 (35–101)	
IL18R1	rs7556917	G/GC/T/TT (Insertion)	CC	161	63 (35–101)	.012
			CA	284	58 (28–95)	
			AA	105	52 (25–82)	
IL18R1	rs11465641	Intron	AA	159	63 (35–102)	.012
			AC	295	59 (28–96)	
			CC	108	49 (25–84)	
IL18R1	rs2287033	Intron	AA	159	63 (35–102)	.012
			AG	294	59 (28–96)	
			GG	108	49 (25–84)	

Table 2 continued.

Gene	SNP ID	Location	Genotype[b]	Subjects With Genotype, No.	IFN-γ, SFCs/2 × 10^5 Cells, Median (IQR)[c]	*P* Value[d]
IL18R1	rs1420096	Intron (boundary)	GG	158	63 (35–100)	.013
			GA	293	60 (29–96)	
			AA	109	49 (25–82)	
TNF	rs1799724	Promoter (−1038)	CC	457	58 (30–98)	.013
			CT	100	51 (26–84)	
			TT	5	70 (26–87)	
IL18R1	rs2080289	Intron	GG	286	55 (29–89)	.015
			GA	252	63 (28–101)	
			AA	22	74 (42–145)	

Abbreviations: A, adenine; C, cytosine; ELISPOT, enzyme-linked immunospot assay; G, guanine; IFN-γ, interferon γ; IQR, interquartile range; SFCs, spot-forming cells; SNP, single-nucleotide polymorphism; T, thymine.

[a] Analyses were adjusted for subject sex, subject age at blood collection (quartiles); time from smallpox immunization to blood collection (quartiles), time from blood collection to assay (quartiles), shipping temperature of the sample (frozen or ambient), and time of year when the sample was shipped (warm weather months [April–September] vs cold weather months [October–March]).

[b] Values are presented as homozygous major allele/heterozygous/homozygous minor allele.

[c] Values reflect simple medians (IQRs) of the raw data, not the modeled estimates. We show these values because of our use of an inverse-normal data transformation. Because of this transformation and our need to adjust for a number of covariates, and because of the high variability of the ELISPOT counts, the medians may not match the modeled ordinal genotypic trend, even though the model fit was adequate.

[d] Repeated-measures linear regression modeling approach.

[e] No subjects with genotype.

DISCUSSION

By analyzing 701 candidate gene SNPs in 1056 subjects, which included 580 whites and 217 African Americans, we found 23 significant associations (20 and 3 associations for whites and African Americans, respectively) between SNPs and haplotypes and IFN-γ ELISPOT responses after smallpox vaccination. In our study, the majority of these significant associations were observed between SNPs in the coding, regulatory, and intronic regions of the interleukin 18 receptor alpha chain, *IL18R1*, and *IL18* genes and variations in vaccinia-specific T-cell response by IFN-γ ELISPOT. We identified a functional coding synonymous *IL18R1* polymorphism (rs1035130/Phe251Phe; in LD with 5 intronic SNPs; $r^2 \geq 0.92$) that was significantly associated with an allele dose-related increase (12%) in IFN-γ ELISPOT responses in the combined cohort of subjects. This SNP (Phe251 Phe) is located in the third immunoglobulinlike domain in transmembrane IL18R1, which is critical for interleukin 18 (IL-18) binding to its receptor (ie, *IL18R1*) and IL-18–induced IFN-γ activity [16]. Notably, this coding rs1035130 and several promoter and intronic SNPs (n = 15) in the *IL18R1* gene that were previously associated with vaccinia-specific neutralizing antibody titers in the combined cohort ($P < .001$), whites ($P = .005$), and African Americans ($P = .02$) [7] were also associated with IFN-γ ELISPOT responses ($P = .01$) in the combined cohort of subjects in this study. The association of rs1035130 with IFN-γ ELISPOT and antibody responses can also be explained by LD with other SNPs. In whites, we also identified a specific *IL18R1* haplotype (AGACGAAAAGGGAG) that includes rs2080289 (in LD with the coding rs1035130; $r^2 = 1$), in which a G→A substitution was

Table 3. *IL18R1* Gene Haplotype Associations With Vaccinia-Specific IFN-γ ELISPOT Responses in the Study Cohort

Allele[a]	Frequency	Test Statistic (Haplotype *t* Statistic)[b]	Allele *P* Value	Global *P* Value
GGGCGAAAGAAGGGAG	0.280370341	0.62	.5331	
GGGCGAAAAAGGGGAG	0.018751575	1.08	.2801	<.0001
GAAAAGCCGGAAAAGA	0.459881582	−5.39	<.0001	
AGGCGAAAAAGGGGAG	0.231306574	4.63	<.0001	

Abbreviations: A, adenine; C, cytosine; ELISPOT, enzyme-linked immunospot assay; G, guanine; IFN-γ, interferon γ; T, thymine.

[a] *IL18R1* genetic variants from left to right: rs3771172, rs6758936, rs2041739, rs7556917, rs7584093, rs3213732, rs10204757, rs11465641, rs1035130, rs6706002, rs4851570, rs6749014, rs4851004, rs1420096, rs2287033, and rs1420094.

[b] Haplotype effects were calculated using the haplotype *t* statistic, which shows the direction and magnitude of the estimated haplotypic effect on the IFN-γ ELISPOT measure. Allele *P* values reflect comparison of individual haplotypes with all other haplotypes combined. Statistical significance was defined as $P < .05$.

Table 4. *IL18R1* Gene Haplotype Associations With Vaccinia-Specific IFN-γ ELISPOT Responses in White Subjects

Allele[a]	Frequency	Test Statistic (Haplotype *t* Statistic)	Allele *P* Value	Global *P* Value
GGGCGAAAAGGGAG	0.254657349	0.35	.7298	
GGACGAAAAGGGAG	0.004138499	0.82	.4133	
GAGAAGCCGAAAGA	0.45163355	−5.04	<.0001	<.0001
AGGCGAAAAGGGAG	0.026272206	2.00	.0452	
AGACGAAAAGGGAG	0.258648173	4.20	<.0001	

Abbreviations: A, adenine; C, cytosine; ELISPOT, enzyme-linked immunospot assay; G, guanine; IFN-γ, interferon γ; T, thymine.

[a] *IL18R1* genetic variants from left to right: rs3771172, rs6758936, rs2080289, rs7556917, rs7584093, rs3213732, rs10204757, rs11465641, rs6706002, rs6749014, rs4851004, rs1420096, rs2287033, and rs1420094.

[b] Haplotype effects were calculated using the haplotype *t* statistic, which shows the direction and magnitude of the estimated haplotypic effect on the IFN-γ ELISPOT measure. Allele *P* values reflect comparison of individual haplotypes with all other haplotypes combined. Statistical significance was defined as $P < .05$.

associated with both higher IFN-γ ELISPOT and antibody responses to smallpox vaccine, indicating the potential functional significance of these SNPs (rs2080289 and rs1035130) [7]. One possible mechanism for *IL18R1* SNP associations with both IFN-γ ELISPOT and antibody responses may be the influence of *IL18/IL18R1*–modulated IFN-γ and interleukin 4/interleukin 13 secretion by T-helper (Th) 1 and 2 cells, natural killer cells, monocytes, and macrophages and concomitant regulation of IFN-γ ELISPOT and antibody production after vaccinia virus stimulation [17]. This is consistent with previous findings that control of vaccine-induced immunity is most likely mediated by multigenic interactions, pathways, and networks (ie, "immune response network") [18].

Key proinflammatory cytokines IL-18 (similarly known as IFN-γ–inducing factor) and IFN-γ, along with the *IL18R1* and IL18RAP receptors, play a significant part in the control of innate and adaptive immunity. Receptor *IL18R1* belongs to the interleukin 1 receptor family, is expressed in Th1 cells, binds IL-18, and is important for IL18–mediated signal transduction [19]. IL-18 is secreted by antigen-presenting cells, stimulates IFN-γ synthesis and is involved in the synergistic activation of interleukin 12 to promote a Th1-mediated immune response [19]. Type II IFN, IFN-γ, plays a central role in host protection against viral infections, including poxviruses, and mediates antiviral activity via the phosphorylation of STAT1 in the T and natural killer cells and macrophages [20]. Other evidence demonstrates that human poxviruses and poxvirus-encoded proteins, such as soluble IL-18–binding protein, can suppress IL-18–induced IFN-γ secretion [20]. Though *IL18R1/IL18RAP* and *IL18* genetic variants have recently been found to be linked to smallpox vaccine humoral immune response [7] and several diseases, such as inflammatory bowel disease [21], herpes virus seropositivity, and extrinsic asthma [22, 23], little is known about their impact on vaccine-induced cellular IFN-γ ELISPOT responses.

Even though the strongest association was found for *IL6* promoter rs2069827 (both in the overall cohort and among whites) and *IL10* intronic rs3024503 (in African Americans), the majority of associations were discovered in the *IL18R1* and *IL18* genes. We found that the occurrence of specific genetic polymorphisms in the *IL18R1* genes were consistently associated (13 SNPs) with antigen-specific IFN-γ ELISPOT responses among both the overall cohort and white subjects. These also include the intronic *IL18R1* rs6758936 and tagged SNPs rs6749014, rs3213732, rs10204757, rs6706002, rs4851004, rs11465641, rs7584093, and rs1420096 (in strong LD; $r^2 \geq 0.99$) in the *IL18R1* gene cluster region that have been associated elsewhere with vaccinia virus–induced neutralizing antibody titers in the overall cohort and among whites [7]. Again, because *IL18R1/IL18* is known to induce synthesis and release of IFN-γ, interleukin 4, and interleukin 13, and to regulate the function of antigen-presenting cells, it is possible that variation in the *IL18R1/IL18* genes may play a role in the induction of both vaccine-induced humoral and cellular adaptive immunity.

An interesting observation in our study is the association of the intronic *IL18* SNP rs5744280 (in LD with rs3882891; $r^2 \geq 0.38$) with an allele dose-related decrease in IFN-γ ELISPOT response (38% decrease; $P = .01$) in the African American cohort. This specific *IL18* polymorphism rs5744280 has also been associated elsewhere with an allele dose-related reduction in vaccinia neutralizing antibody levels in whites (18% decrease; $P = .02$) and among African Americans (21% decrease; $P = .01$) [7], and it demonstrated a genome-wide association ($P = 3.9 \times 10^{-7}$) with variations in the IL-18 plasma levels among whites in the study by He et al [24]. We speculate that *IL18* polymorphism rs5744280 may be of functional relevance or in LD with a functional polymorphism that may modulate expression and production of IL-18 protein. Likewise, Stanley et al [11] observed an association between 3 *IL18* haplotypes, which include rs3882891 polymorphism (also associated with an allele dose-related 22% reduction in IFN-γ ELISPOT response in our study), that were associated with a reduced risk for fever (GGAATACAGGTGA haplotype) and also with an augmented susceptibility to fever (GGAATACAGATGA and AGCATACTGATGA haplotypes) after smallpox vaccination. These results

suggest the significance of the *IL18* gene polymorphism in smallpox vaccine–induced immunity and susceptibility to AEs. Our recent genome-wide association study analysis with vaccinia-specific cell-mediated immunity ($CD8^+$ IFN-γ ELISPOT) demonstrated that 3 of these cytokine receptor genetic variants (rs4851004, rs1420094, and rs2287033) were replicated, with genome-wide significance levels of $P = .0119$, $P = .0125$, and $P = .0125$, respectively, in whites [10].

To our knowledge, this is the first report of that associations between *IL18R1* and *IL18* gene polymorphisms and smallpox vaccine–induced IFN-γ ELISPOT responses. Our findings indicate that *IL18R1* and *IL18* gene SNPs, and *IL18R1* haplotypes may be important regulators of smallpox vaccine–induced IFN-γ immune responses. Further work is necessary to determine whether our results pertain to other racial or ethnic groups. Because we cannot exclude the probability of false-positive associations, replication and functional studies of causal polymorphisms in large independent cohorts are necessary to confirm these findings. Our data also suggest the possibility that common *IL18R1, IL18,* and other genetic polymorphisms may be linked to the control of vaccine-induced adaptive immunity (humoral and cell-mediated immunity) and inflammation (the development of AEs) in individuals after smallpox vaccination. Understanding molecular mechanisms behind these associations may help improve smallpox vaccines and will add to our knowledge of vaccine-induced immune responses.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. G. A. P. is the chair of a safety evaluation committee for nonsmallpox investigational vaccine trials being conducted by Merck Research Laboratories and offers consultative advice on vaccine development to Merck, CSL Biotherapies, Avianax, Sanofi Pasteur, Dynavax, Novartis Vaccines and Therapeutics, PAXVAX, and Emergent Biosolutions. These activities have been reviewed by the Mayo Clinic Conflict of Interest Review Board and are conducted in compliance with Mayo Clinic conflict of interest policies. This research has also been reviewed by the Mayo Clinic Conflict of Interest Review Board and was conducted in compliance with Mayo Clinic Conflict of Interest policies. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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