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# Race and Sex-Based Differences in Cytokine Immune Responses to Smallpox Vaccine in Healthy Individuals

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## Abstract

We assessed the effects of sex, race and ethnicity on smallpox vaccine-induced immune responses in 1,071 armed forces members after primary Dryvax® smallpox vaccination, including 790 males and 281 females; 580 Caucasians, 217 African-Americans, and 217 Hispanics. Analysis of vaccinia-specific cytokine responses revealed that Caucasians had higher total IFNγ ELISPOT responses (median 57 spot-forming units/SFUs per 200,000 cells, $p=0.01$) and $CD8^+$IFNγ ELISPOT responses (12 SFUs, $p<0.001$) than African-Americans (51 and 4 SFUs, respectively) and Hispanics (47 and 8 SFUs, respectively). Similarly, Caucasians secreted higher levels of vaccinia-specific IL-2 ($p=0.003$) and IFNα ($p<0.001$) compared to other racial/ethnic groups. Males had higher total IFNγ ELISPOT responses (median 55 SFUs) compared to females (41 SFUs, $p<0.001$). We observed statistically significant sex-related differences in the secretion of IL-2 ($p<0.001$), IL-1β ($p<0.001$) and IL-10 ($p=0.017$). These data suggest that vaccinia-specific cytokine responses following primary smallpox vaccination are significantly influenced by race and sex of vaccinees.

### Keywords

Smallpox Vaccine; Cytokine; Cellular Immunity; Race; Sex



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**Competing Interests**

Dr. Poland is the chair of a Safety Evaluation Committee for novel investigational vaccine trials being conducted by Merck Research Laboratories. Dr. Poland offers consultative advice on vaccine development to Merck & Co. Inc., CSL Biotherapies, Avianax, Sanofi Pasteur, Dynavax, Novartis Vaccines and Therapeutics, PAXVAX Inc, 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 been reviewed by the Mayo Clinic Conflict of Interest Review Board and was conducted in compliance with Mayo Clinic Conflict of Interest policies.

**MESH Keywords**

Smallpox Vaccine; Cytokines; Immunity, Cellular; Sex; Hispanics; African Americans; Whites

## Introduction

Demographic variables may influence susceptibility to infectious diseases, disease pathogenesis and clinical course, and immune responses to infectious agents and vaccines, and these effects are a result of the dynamic interaction of complex genetic and environmental factors. [1–11]. Sex-based differences in vaccine efficacy, adverse events, and humoral immune response after immunization have been reported for many viral vaccines, including measles-mumps-rubella (MMR), influenza, hepatitis A, hepatitis B, yellow fever, smallpox, rabies and human papillomavirus.[12] In most cases, women generate a more robust antibody response to vaccines than men, while data on sex-based differences in cellular immune responses remains controversial [6,9,12–19]. The effects of race and ethnicity on variations in vaccine-induced immunity, including cellular immunity, have not been comprehensively studied. In this population-based study of 1,071 individuals, we assessed the effects of sex and race/ethnicity on multiple measures of innate/inflammatory and adaptive immune responses following primary smallpox vaccination.

## Material and methods

### Study cohort

The methods described below are similar or identical to those published for our previous studies [17,20,21]. The recruitment of the study subjects and the demographic and vaccination data have been previously reported [17,20,21]. Briefly, all subjects (n=1,071) were recruited as participants in a smallpox immunization program at the Naval Health Research Center (NHRC) in San Diego, CA, and civilian healthcare worker smallpox immunization program at the Mayo Clinic in Rochester, MN. All subjects have received a single dose of Dryvax® smallpox vaccine (Wyeth Laboratories, Inc., Marietta, PA) within four years prior to enrollment in the study [17,20]. The Institutional Review Boards of NHRC and the Mayo Clinic approved the study, and written informed consent and demographic information was obtained from each subject at the time of enrollment.

### Cytokine immune measures

Twelve vaccinia-specific Th1, Th2 and innate/inflammatory cytokines were quantified using commercial ELISA kits (BD Pharmingen) in peripheral blood mononuclear cell (PBMC) cultures after *in vitro* stimulation with inactivated vaccinia virus (NYCBOH strain) at optimal (for each cytokine) multiplicity of infection/MOI and incubation periods, as previously reported [21].The frequencies of IFNγ-positive cells (in spot-forming units/SFUs) were measured in PBMC cultures using commercial total IFNγ and $CD8^{+}$IFNγ ELISPOT kits (R&D Systems, Minneapolis, MN) following the manufacturer's specifications, with good measurement reproducibility (intraclass correlation coefficients/ICCs comparing multiple observations per subject of 0.94 and 0.85 for stimulated and unstimulated values, respectively) [21,22].

### Statistical analysis

The statistical methods described herein are similar or identical to those published for our previous studies [20–24]. Study participants were asked to indicate their race and ethnicity via a questionnaire. Since many individuals indicated “Other”, “Unknown”, or “Don’t Know” race and/or ethnicity on the survey, and because of the availability of genome-wide data obtained from Illumina HumHap550/650 arrays, we verified the declared racial and ethnic categorizations with observed genetic data. We also used the genetic data to categorize subjects (having chosen: “More than one race,” “Other,” or “Don’t Know”) into racial/ethnic groups. Our approach for racial/ethnic classification based on genetic data has been reported in detail elsewhere [20,23,24]. Briefly, we identified a collection of SNPs (n=22,863 SNPs) not in linkage disequilibrium with one another and applied a principal components approach to quantify population genetic similarity among the study subjects [25]. We clustered these results with known racial and ethnic data and were able to classify participants into three major groups: Caucasian race, African race, and Hispanic ethnicity. Subjects not clustering unambiguously into one of these three groups were retained as another genetic group labeled “Other.” The sex of the study participants was identified based on self-declaration and verified with genetic data, using the PLINK genome-wide association study (GWAS) to compute the inbreeding coefficients on the X chromosome. This assessment identified one individual as an XXY male (Klinefelter’s syndrome), who was excluded from the study [24]. Summaries of the measured immune response outcomes were obtained from per-subject medians of the replicates measured by the assays. Statistical comparisons were made using linear mixed models approaches that modeled all of the measured replicates as the outcome while accounting for within-subject correlations. Analyses adjusted for sex and race (where needed), quartiles of age at enrollment, quartiles of time since vaccination to blood draw, quartiles of time from blood draw to assay, shipping temperature of the sample, and season when the sample was shipped. In order to ensure that statistical modeling assumptions were met, we applied inverse-normal transformations to the outcome data prior to performing statistical tests. Statistical analyses were performed in the SAS statistical software system (Cary, NC).

## Results and Discussion

The demographics of the study population and all immune response variables have been previously reported [17,20,21]. The median age at enrollment was 24 years (inter-quartile range/IQR, 22–27) and the median time since immunization to blood draw was 15.3 months (IQR, 9–34) [21]. The study cohort consisted of 790 males (74%) and 281 females [21]. The four major race/ethnicity groups based on the genomic data were: Caucasian (n=580); African-American (n=217); Hispanic (n=217); and other (n=57), including Asian, American Indian, Alaskan Native, Native Hawaiian, other Pacific Islander and subjects with unknown race/ethnicity. As previously reported, we observed a strong concordance between self-declared and genetic race/ethnicity for study participants with clear self-declaration [23,24]. The cell mediated immunity (CMI) variables for the study population (cytokine responses in response to vaccinia virus stimulation) have been previously described in detail and were characterized by a Th1-biased cytokine immune response pattern and a strong innate/proinflammatory cytokine response [21,22]. The median total IFNγ ELISPOT response for

the overall cohort was 52 SFUs (IQR, 24–88) and the median $CD8^+IFN\gamma$ ELISPOT response was 10 SFUs (IQR, −2 to 27) [21]. The median levels for secreted IFNγ, IL-2, IL-10, IFNα and IL-1β were 290 pg/mL, 16 pg/mL, 3 pg/mL, 63 pg/mL and 51 pg/mL, respectively, as previously reported [21].

The purpose of this report is to explicitly analyze and report the data based on race/ethnicity and sex of the study participants. Our adjusted statistical analysis revealed significant associations between race/ethnicity and vaccinia virus-specific CMI outcomes following primary smallpox immunization. Subjects of European descent (Caucasians) had significantly higher total IFNγ ELISPOT response and $CD8^+IFN\gamma$ ELISPOT response (median 57 SFUs per 200,000 cells [IQR, 28–95] and 12 SFUs [IQR, 0–32], respectively) than subjects of African descent (median 51 SFUs [IQR, 23–84] and 4 SFUs [IQR, −7 to 18], respectively) or subjects of Hispanic ethnicity (median 47 SFUs [IQR, 17–76] and 8 SFUs [IQR, −2 to 20], respectively) ($p=0.01$ and $p<0.001$, Table 1). Similarly, we detected higher secreted levels of vaccinia-specific IFNγ in Caucasians (median 315 pg/mL) than African-Americans (271 pg/mL) and Hispanics (267 pg/mL) ($p=0.09$, data not presented). Although the differences in secreted IFNγ did not reach statistical significance, the trend is apparent and likely limited due to the limited sample size of some of the analyzed groups. In concert with these observations, Caucasians had also significantly higher levels of secreted IL-2 (37 pg/mL) and IFNα (97 pg/mL) compared to all other groups ($p=0.003$ and $p<0.001$, Table 1).

Ethnicity and race-specific data on infectious disease susceptibility and clinical course, and/or differences in immune responses to pathogens and vaccines is limited in the literature, and the underlying mechanisms for the reported observations are still unknown [5,11,26–28]. Although the observed statistically significant effects (cytokine response differences) in our study are relatively small and there is no known correlate of protection for vaccinia-specific cell-mediated immunity, cellular immunity contributes to overall immune protection and resolution of poxvirus infections [27]. Further studies are warranted to determine how and if these CMI response differences are biologically important and/or are related to protection and disease susceptibility in different racial/ethnic groups.

Differences in lymphoproliferative responses to influenza vaccination have been reported in elderly Caucasians and Latino (Hispanics) subjects versus elderly African-American subjects, and the latter demonstrated a differential lack of cell-mediated influenza vaccine-specific immune response [27]. Statistically significant differences in measles antibody seroprevalence and/or measles antibody titers were also reported between African-Americans and Caucasians [29] and between Innu/Inuit and Caucasians [30]. Interestingly, we have previously reported that Caucasians had significantly higher measles-specific total IFNγ ELISPOT responses than non-Caucasians (including African-American, Asian, Hawaiian, Pacific Islander and Hispanic subjects) and also higher levels of measles vaccine-specific secreted IL-2 and IFNα (and IFNλ1, IL-10, TNFα, IL-6) [28], a result that is in concert with our current findings after smallpox vaccine. These consistent findings point to a more general pattern of vaccine-induced cellular immune response in different racial/ethnic groups. The underlying mechanisms for the observed differences in CMI response to vaccines between populations are still not delineated, but might be due in part to differences

in allele frequencies (polymorphisms) in cytokine and other immune genes with implications for immune regulation and function [31]. Both common and race-specific genetic associations have been reported between single nucleotide polymorphisms in cytokine and cytokine receptor genes and variations in smallpox vaccine-induced humoral and cellular immunity [20,23], which suggests that differences in immune response may vary by race. We have also previously reported on the role of HLA in the genetic control of immune response following smallpox vaccination (including cytokine responses) [32]. It is important to note that in order to predict the population substructure (genetically assign race/ethnicity), we used SNPs that spanned the entire genome and, as expected, a handful of them are in the HLA region and within other important immune genes (six SNPs are annotated as being "HLA," but none of which are coding SNPs). However, the number of these SNPs is very small relative to the 22,863 total SNPs. Because of this, these markers only contribute a very small component to the estimation of genetic groupings and the overall predicted population substructure.

As outlined, sex-based dissimilarities in humoral immunity post-vaccination (including smallpox vaccine) are already well established [12]. In the current study, we performed a comprehensive analysis of 10 markers of smallpox vaccine-induced cellular immunity (including detectable secreted cytokines and two ELISPOT measures) in order to understand the sex-based effects on vaccinia-specific cellular immunity. Our results demonstrate that men had significantly higher total IFNγ ELISPOT responses (median 55 SFUs [IQR, 27–95]) than women (median 41 SFUs [IQR, 17–70], $p<0.001$, Table 1) and higher levels secretion of the proinflammatory cytokine IL-1β ($p<0.001$, Table 1), while women had higher secretion of vaccinia-specific IL-2 and IL-10 ($p<0.001$ and $p=0.017$, respectively, Table 1). While these findings are not yet validated in other vaccine studies (smallpox and/or other viral vaccines [12]) and the clinical/biological implications of the observed differences are unclear, our data suggest a sex bias in the cellular measures of smallpox vaccine-induced immunity that might relate to vaccine efficacy and/or adverse events following immunization. The biological mechanisms underlying the observed sexual dimorphism in immune response (including innate, humoral and cellular immunity) are complex and involve the multifaceted influence of sex steroids (17β-oestradiol, progesterone, and testosterone) on immune function including activation and/or suppression of the activity of immune cells. Sex hormones bind to their specific receptors (expressed on multiple lymphoid cells) and differentially influence various cell-signaling pathways (such as NF-κB and IRF) leading to the production of chemokines and cytokines [12]. Sex chromosomal gene effects (such as polymorphisms in immune function-related genes and regulatory miRNAs on the X chromosome) have also been implicated in sex differences in disease pathogenesis and vaccine-induced immunity [12].

The strengths of our study include a large, racially diverse cohort with documented primary smallpox immunization and vaccine "take," precise demographic and genetic (genome-wide genotyping) data for reliable racial/ethnic classification, and comprehensive immune profiling, and statistical analysis adjusting for all other covariates. The limitations include the lack of replication in an independent cohort, which is currently in progress.

In conclusion, our results highlight the importance of demographic variables, such as race, ethnicity and sex, in immune response inter-individual variations following smallpox immunization. Such findings will fuel efforts to enhance our understanding of how smallpox vaccine-induced immune responses are generated in diverse populations.

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## References

1. Thio CL, Thomas DL, Goedert JJ, Vlahov D, Nelson KE, Hilgartner MW, O'Brien SJ, Karacki P, Marti D, Astemborski J, Carrington M. Racial differences in HLA class II associations with hepatitis C virus outcomes. J Infect Dis. 2001; 184:16–21. [PubMed: 11398104]
2. Mayr FB, Spiel AO, Leitner JM, Firbas C, Kliegel T, Jilma B. Ethnic differences in plasma levels of interleukin-8 (IL-8) and granulocyte colony stimulating factor (G-CSF). Transl Res. 2007; 149:10–14. [PubMed: 17196517]
3. Hoffmann SC, Stanley EM, Cox ED, DiMercurio BS, Koziol DE, Harlan DM, Kirk AD, Blair PJ. Ethnicity greatly influences cytokine gene polymorphism distribution. Am J Transplant. 2002; 2:560–567. [PubMed: 12118901]
4. Zabaleta J, Schneider BG, Ryckman K, Hooper PF, Camargo MC, Piazuelo MB, Sierra RA, Fontham ET, Correa P, Williams SM, Ochoa AC. Ethnic differences in cytokine gene polymorphisms: potential implications for cancer development. Cancer Immunol Immunother. 2008; 57:107–114. [PubMed: 17618436]
5. de la C Sierra B, Kouri G, Guzmán MG. Race: a risk factor for dengue hemorrhagic fever. Arch Virol. 2007; 152:533–542. [PubMed: 17106622]
6. Dhiman N, Ovsyannikova IG, Vierkant RA, Pankratz VS, Jacobson RM, Poland GA. Associations between cytokine/cytokine receptor SNPs and humoral immunity to measles, mumps and rubella in a Somali population. Tissue Antigens. 2008; 72:211–220. [PubMed: 18715339]
7. Dhiman N, Haralambieva IH, Vierkant RA, Pankratz VS, Ryan E, Jacobson RM, Ovsyannikova IG, Poland GA. Predominant inflammatory cytokine secretion pattern in response to two doses of live rubella vaccine in health vaccinees. Cytokine. 2010; 50:24–29. [PubMed: 20117947]
8. Fares A. Racial diferences in susceptibility to infection by Mycobacterium tuberculosis. Tropical Medicine and Public Health. 2012; 5:307–312.
9. Green MS, Shohat T, Lerman Y, Cohen D, Slepon R, Duvdevani P, Varsano N, Dagan R, Mendelson E. Sex differences in the humoral antibody response to live measles vaccine in young adults. Int J Epidemiol. 1994; 23:1078–1081. [PubMed: 7860159]
10. Marriott I, Huet-Hudson YM. Sexual dimorphism in innate immune responses to infectious organisms. Immunol Res. 2006; 34:177–192. [PubMed: 16891670]
11. Fish EN. The X-files in immunity: sex-based differences predispose immune responses. Nat Rev Immunol. 2008; 8:737–744. [PubMed: 18728636]
12. Klein SL, Jedlicka A, Pekosz A. The Xs and Y of immune responses to viral vaccines. Lancet Infect Dis. 2010; 10:338–349. [PubMed: 20417416]
13. Klein NP, Holmes TH, Sharp MA, Heineman TC, Schleiss MR, Bernstein DI, Kemble G, Arvin AM, Dekker CL. Variability and gender differences in memory T cell immunity to varicella-zoster virus in healthy adults. Vaccine. 2006; 24:5913–5918. [PubMed: 16759768]

14. Mitchell LA. Sex differences in antibody- and cell-mediated immune response to rubella re-immunisation. J Med Microbiol. 1999; 48:1075–1080. [PubMed: 10591160]
15. Dominguez A, Plans P, Costa J, Torner N, Cardenosa N, Batalla J, Plasencia A, Salleras L. Seroprevalence of measles, rubella, and mumps antibodies in Catalonia, Spain: results of a cross-sectional study. Eur J Clin Microbiol Infect Dis. 2006; 25:310–317. [PubMed: 16786377]
16. Ovsyannikova IG, Jacobson RM, Dhiman N, Vierkant RA, Pankratz VS, Poland GA. Human leukocyte antigen and cytokine receptor gene polymorphisms associated with heterogeneous immune responses to mumps viral vaccine. Pediatrics. 2008; 121:e1091–e1099. [PubMed: 18450852]
17. Kennedy R, Ovsyannikova IG, Pankratz VS, Vierkant RA, Jacobson RM, Ryan MAK, Poland GA. Gender effects on humoral immune response to smallpox vaccine. Vaccine. 2008; 27:3319–3323. [PubMed: 19200827]
18. Cook IF. Sexual dimorphism of humoral immunity with human vaccines. Vaccine. 2008; 26:3551–3555. [PubMed: 18524433]
19. Atabani S, Landucci G, Steward MW, Whittle H, Tilles JG, Forthal DN. Sex-associated differences in the antibody-dependent cellular cytotoxicity antibody response to measles vaccines. Clin Diagn Lab Immunol. 2000; 7:111–113. [PubMed: 10618288]
20. Haralambieva IH, Ovsyannikova IG, Dhiman N, Kennedy RB, O'Byrne M, Pankrantz VS, Jacobson RM, Poland GA. Common SNPs/Haplotypes in IL18R1 and IL18 Genes are Associated with Variations in Humoral Immunity to Smallpox Vaccination in Caucasians and African-Americans. J Infect Dis. 2011; 204:433–441. [PubMed: 21742843]
21. Umlauf BJ, Ovsyannikova IG, Haralambieva IH, Kennedy RB, Vierkant RA, Pankrantz VS, Jacobson RM, Poland GA. Correlations Between Vaccinia-Specific Immune Responses Within a Cohort of Armed Forces Members. Viral Immunol. 2011; 24:415–420. [PubMed: 21958369]
22. Haralambieva IH, Oberg AL, Dhiman N, Ovsyannikova IG, Kennedy RB, Grill DE, Jacobson RM, Poland GA. High-dimensional gene expression profiling studies in high and low responders to primary smallpox vaccination. J Infect Dis. 2012; 206:1512–1520. [PubMed: 22949304]
23. Ovsyannikova IG, Haralambieva IH, Kennedy RB, Pankratz VS, Vierkant RA, Jacobson RM, Poland GA. Impact of cytokine and cytokine receptor gene polymorphisms on cellular immunity after smallpox vaccination. Gene. 2012; 510:59–65. [PubMed: 23009887]
24. Kennedy RB, Ovsyannikova IG, Pankratz VS, Haralambieva IH, Vierkant RA, Jacobson RM, Poland GA. Genome-wide genetic associations with IFNgamma response to smallpox vaccine. Hum Genet. 2012; 131:1433–1451. [PubMed: 22661280]
25. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 2006; 38:904–909. [PubMed: 16862161]
26. Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, Hunsperger E, Kroeger A, Margolis HS, Martinez E, Nathan MB, Pelegrino JL, Simmons C, Yoksan S, Peeling RW. Dengue: a continuing global threat. Nat Rev Microbiol. 2010; 8:S7–S16. [PubMed: 21079655]
27. Gardner EM, Gonzalez EW, Nogusa S, Murasko DM. Age-related changes in the immune response to influenza vaccination in a racially diverse, healthy elderly population. Vaccine. 2006; 24:1609–1614. [PubMed: 16260072]
28. Umlauf BJ, Haralambieva IH, Ovsyannikova IG, Kennedy RB, Pankratz VS, Jacobson RM, Poland GA. Associations between demographic variables and multiple measles-specific cell-mediated immune responses following measles vaccination. Viral Immunol. 2012; 25:29–36. [PubMed: 22239234]
29. McQuillan GM, Kruszon-Moran D, Hyde TB, Forghani B, Bellini W, Dayan GH. Seroprevalence of measles antibody in the US population, 1999–2004. J Infect Dis. 2007; 196:1459–1464. [PubMed: 18008224]
30. Poland GA, Jacobson RM, Colbourne SA, Thampy AM, Lipsky JJ, Wollan PC, Roberts P, Jacobsen SJ. Measles antibody seroprevalence rates among immunized Inuit, Innu and Caucasian subjects. Vaccine. 1999; 17:1525–1531. [PubMed: 10195789]

31. Van Dyke AL, Cote ML, Wenzlaff AS, Land S, Schwartz AG. Cytokine SNPs: Comparison of allele frequencies by race and implications for future studies. Cytokine. 2009; 46:236–244. [PubMed: 19356949]

32. Ovsyannikova IG, Vierkant RA, Pankratz VS, Jacobson RM, Poland GA. Human leukocyte antigen genotypes in the genetic control of adaptive immune responses to smallpox vaccine. J Infect Dis. 2011; 203:1546–1555. [PubMed: 21592983]

**Table 1**

Associations between demographic variables and vaccinia-specific cytokine responses in vaccinees after primary smallpox vaccination

| Outcome | Variable | N | Mean | Standard Deviation | Median | 25% Quartile | 75% Quartile | Adjusted p-value |
|---|---|---|---|---|---|---|---|---|
| Total IFNγ ELISPOT | AA | 213 | 61.30 | 60.84 | 51.00 | 23.00 | 84.00 | 0.010 |
| | Cauc | 573 | 66.77 | 60.17 | 57.00 | 28.00 | 95.00 | |
| | Hisp | 216 | 50.93 | 46.49 | 47.00 | 17.00 | 76.00 | |
| | Other | 56 | 43.84 | 44.22 | 29.50 | 15.00 | 71.00 | |
| $CD8^+$IFNγ ELISPOT | AA | 205 | 9.11 | 38.51 | 4.00 | −7.00 | 18.00 | <.001 |
| | Cauc | 542 | 17.83 | 34.19 | 12.00 | 0.00 | 32.00 | |
| | Hisp | 200 | 13.52 | 47.01 | 8.00 | −2.00 | 19.50 | |
| | Other | 55 | 17.71 | 59.91 | 9.00 | −3.00 | 28.00 | |
| Secreted IL-2 | AA | 185 | 19.90 | 32.97 | 14.97 | 1.16 | 32.93 | 0.003 |
| | Cauc | 465 | 27.55 | 37.31 | 18.61 | 5.19 | 38.30 | |
| | Hisp | 174 | 22.71 | 31.46 | 14.26 | 4.72 | 34.52 | |
| | Other | 47 | 18.98 | 36.96 | 13.77 | 0.70 | 31.53 | |
| Secreted IFNα | AA | 209 | 76.02 | 85.98 | 49.33 | 12.05 | 120.53 | <.001 |
| | Cauc | 560 | 94.87 | 97.44 | 67.52 | 22.56 | 143.02 | |
| | Hisp | 212 | 83.59 | 83.43 | 63.79 | 13.33 | 132.32 | |
| | Other | 57 | 71.47 | 86.17 | 54.57 | 3.04 | 83.60 | |
| Total IFNγ ELISPOT | Male | 784 | 66.19 | 60.87 | 55.00 | 27.00 | 95.00 | <.001 |
| | Female | 274 | 47.01 | 43.20 | 41.00 | 17.00 | 70.00 | |
| Secreted IL-1β | Male | 664 | 116.08 | 194.72 | 57.26 | 27.17 | 144.82 | <.001 |
| | Female | 257 | 77.82 | 122.01 | 40.14 | 21.97 | 78.81 | |
| Secreted IL-2 | Male | 624 | 24.27 | 35.40 | 15.85 | 3.54 | 37.38 | <.001 |
| | Female | 247 | 25.05 | 35.45 | 18.52 | 4.21 | 34.78 | |
| Secreted IL-10 | Male | 735 | 9.83 | 32.27 | 2.22 | −0.56 | 10.45 | 0.017 |
| | Female | 268 | 14.18 | 28.46 | 4.63 | 0.30 | 18.01 | |

Only statistically significant findings ($p<0.05$) are presented.

Analyses are adjusted for sex and race (where needed); age at enrollment/blood draw (quartiles); time since vaccination to blood draw (quartiles); time from blood draw to assay (quartiles); shipping temperature of the sample (ambient or frozen); and season when the sample was shipped (April-September vs. October-March).

Race/ethnicity abbreviations are as follows: AA – African-American; Cauc – Caucasian; Hisp- Hispanic; Other - Asian, American Indian, Alaskan Native, Native Hawaiian, other Pacific Islander or a subject with unknown race/ethnicity.

Cytokine response is defined as the subject-specific median vaccinia virus-stimulated response (measured in triplicate) minus the median unstimulated response (also measured in triplicate). Results are presented in pg/mL for secreted cytokines and as IFNγ-positive SFUs per $2 \times 10^5$ cells (for the total IFNγ ELISPOT assay) or IFNγ-positive SFUs per $5 \times 10^5$ cells (for the $CD8^+$IFNγ ELISPOT assay).