measles vaccination is needed. In addition, these data also imply that measles infection occurring after vaccination could be related to a suboptimal innate or CMI response.

Variation in measles-specific CMI responses has been associated with multiple genetic factors, including HLA gene polymorphisms and single-nucleotide polymorphisms (SNP) in cytokine, cytokine receptor, and innate immunity genes (9,11,41,42). These data also imply that variations in measles-specific innate/inflammatory and CMI responses may also be associated with demographic variables in a given population; thus offering a macro-scale view of variations in measles vaccine-induced innate/inflammatory and CMI responses. These data could potentially be used to create improved vaccines that are designed to upregulate key innate/inflammatory or CMI pathways in an effort to generate a protective immune response in a greater proportion of the population following measles vaccination (49,50).

To expand and test the hypothesis for gender and racial differences in measles-specific cytokine responses, we examined associations between innate/inflammatory and CMI responses following two doses of measles-mumps-rubella (MMR) vaccine and demographic or clinical variables. Specifically, a large population-based study was conducted (n = 764) to determine measles-specific immune responses in children and young adults 11–22 years of age vaccinated with two doses of MMR vaccine. Associations between gender, race, or ethnicity, and cytokine measures (secreted IL-2, IL-6, IL-10, IFN-a, IFN-c, IFN-k1, TNF-a, as well as IFN-c ELISPOT responses) were examined to determine if demographic variables were significantly associated with measles vaccine-induced innate/inflammatory and CMI responses. We reported such associations with humoral immunity in a previous article (23), and herein focus on markers of cell-mediated immunity.

Methods

Study subjects

The study cohort was comprised of a combined sample of 821 subjects from two independent, age-stratified random cohorts of healthy schoolchildren and young adults from all socioeconomic strata in Rochester, Minnesota, from which 764 were eligible for the current study (23). Between December 2006 and August 2007, 440 healthy children were enrolled (age 11–19 y) in Rochester, MN (cohort 1) as previously reported (7,20,36,38). Three-hundred ninety-six parents agreed to allow their children to take part in this study, and from these 396 children we obtained blood samples. From November 2008 through September 2009, an additional 381 healthy children and young adults were enrolled (age 11–22 y) in Rochester, Minnesota (cohort 2), and we also obtained blood samples from these 381 children (22,23,39,40).

Individuals born outside the United States were excluded from the study, and all participants received two and only two documented doses of MMR (Merck, Whitehouse Station, NJ) vaccine containing the attenuated Edmonston strain of MV (50% tissue culture infective dose of ≥1000). Additionally, no known circulating wild MV was observed in the community since the earliest year of birth for any subject. From the 821 study subjects who were enrolled, self-declared demographic data and immune measures were available for 764 subjects, who composed the final sample population. The institutional review board (IRB) of the Mayo Clinic approved the study, and written informed consent was obtained from the parents of all children who participated in the study, as well as written informed consent from age-appropriate children and young adults.

Isolation of peripheral blood mononuclear cells (PBMCs)

One hundred milliliters of whole blood was collected from each participant in Cell Preparation Tube with Sodium Citrate (CPT ; BD, Franklin Lakes, NJ) tubes, and PBMCs were isolated within 24 h according to manufacturer's protocol. Isolated PBMCs were resuspended at a concentration of 1×10^7 cells/mL in RPMI 1640 media containing L-glutamine (Invitrogen, Carlsbad, CA) supplemented with 10% dimethyl sulfoxide (DMSO; Protide Pharmaceuticals, St. Paul, MN) and 20% fetal calf serum (FCS; Hyclone, Logan, UT), frozen overnight at - 80 C in a controlled-rate freezing container, and transferred to liquid nitrogen for storage until use.

Measurement of IFN-c-secreting cells

The IFN-c cytokine response was assessed using commercially available IFN-c ELISPOT kits from R&D Systems (Minneapolis, MN) as previously described (51). Briefly, an ELISPOT plate pre-coated with anti-human IFN-c antibody (Ab) was blocked for 20 min with RPMI culture medium supplemented with 5% FCS. One aliquot of 5×10^6 cryopreserved PBMCs was thawed, counted, and resuspended in RPMI culture medium supplemented with 5% FCS. Seven wells were plated with 2×10^5 PBMCs per well for each subject; three wells were supplemented with live MV (Edmonston strain of MV, MOI 0.5); three wells were supplemented with culture medium to serve as negative controls, and one well was supplemented with 5 lg/mL of PHA-P to serve as a positive control. Any subject negative for PHA-P was repeated. The plates were incubated at 37 C in 5% CO_2 for precisely 42 h. After incubation, the plates were developed according to the manufacturer's protocol. The plates were read with an ImmunoSpot S4 Pro Analyzer from C.T.L. (Cleveland, OH). Counting parameters were optimized to precisely and accurately count all ELISPOT plates. Several plates were assessed to optimize the counting parameters, including sensitivity, spot size thresholds, background hue, and spot separation (51). Once the counting parameters were established, these parameters were used to count all ELISPOT plates, ensuring that spots were counted consistently across all subjects. Quality control was performed by a single individual for each plate to manually eliminate any spurious spots resulting from debris or overdeveloped regions.

Measurement of secreted cytokines

Enzyme-linked immunosorbent assays (ELISAs) were performed to measure the level of seven (IL-2, IL-6, IL-10, IFN-a, IFN-c, IFN-k1, and TNF-a) cytokines secreted by PBMCs following *in vitro* stimulation with live MV (Edmonston strain of MV) as previously described (37). Briefly, 1.5×10^7 cryopreserved PBMCs were thawed, counted, and resuspended in RPMI culture medium supplemented with 5% FCS. Eleven wells on three 96-well round-bottom plates were plated with 2×10^5 cells/well. Five wells were

supplemented with MV (the MOI is dependent on the cytokine, as described below), the wells were supplemented with RPMI culture medium with 5% FCS to serve as negative controls, and one well was supplemented with PHA-P to serve as a positive control. Any subject negative for PHA-P was repeated. The MOI and incubation time for each cytokine was as follows: IL-2, MOI 0.5, 48 h; IL-6, MOI 1.0, 72 h; IL-10, MOI 0.5, 48 h; IFN-a, MOI 1.0, 24 h; IFN-c, MOI 1.0, 72 h; IFN-k1, MOI 1.0, 72 h; TNF-a, MOI 1.0, 24 h, as previously optimized (37,44). After incubation, cell-free supernatants were harvested from each plate, transferred to a 96-well round-bottom plate, and frozen at - 80 C until analysis. Cytokine levels were measured with commercial kits according to the manufacturers' instructions. IL-2, IL-6, IL-10, IFN- c, and TNF-a were measured using commercial kits from BD Biosciences (San Jose, CA), IFN-a was measured using commercial kits from Mabtech (Cincinnati, OH), and IFN-k1 was measured using commercial kits from R&D Systems. Cytokine concentrations were determined by measuring absorbance at 450 nm correlated with a standard curve created by performing serial dilutions of the manufacturers' reference standard.

Other cytokines, such as IL-4, IL-5, IL-12, and IL-17, were not quantified in this study, due to the negligible amounts of these cytokines detected in PBMC cultures (stimulated with MV) in our previous studies (11,21).

Statistical analysis

The statistical methods described herein are similar to those reported in previous articles (7,23,53). The comparisons of interest in this report are differences between patient-level demographic data and cellular immune measures of response to measles vaccination. The demographic characteristics were descriptively summarized across individuals using frequencies and percentages for categorical variables, and medians and interquartile ranges (IQRs) for continuous variables. A total of eight measures were assessed, as described above. Assessments of cytokine secretion for each of the measured cytokines resulted in three recorded values per outcome prior to stimulation with MV, and three values post-stimulation. In contrast, the ELISPOT assessments resulted in three recorded values pre-stimulation and three post-stimulation. For summary purposes, a single response measurement per individual was obtained for each outcome by subtracting the median of the unstimulated values from the median of the stimulated values. These single per-individual results were descriptively summarized across individuals using medians and interquartile ranges. Associations between each of the demographic or clinical variables (age, gender, race and ethnicity, recruitment cohort, and timing of immunization relative to recruitment), and each of the measures of innate and CMI were assessed using linear mixed models approaches on all of the repeated measurements (both stimulated and unstimulated) obtained for each immune measure on each subject. These approaches are similar to ANOVA, but allow for the analysis of repeated measurements made on each subject by accounting for the degree to which the measurements are correlated within a person. In these models, the data were rank-transformed to meet modeling assumptions, and tested for associations between demographic characteristics and an MV stimulation effect by assessing the interaction between a variable indicating stimulation status, and a variable indicating demographic group membership, while controlling for within-subject correlations. In addition to performing tests of significance for each measure individually, assessments were performed in which each of the demographic variables was adjusted for all others. Analyses were carried out using the SAS version 9 (SAS Institute, Inc., Cary, NC) software system.

Results

Study population characteristics

The median age of participants at the time of enrollment was 15 years (IQR 13.0–17.0), as previously reported (23). The median age at first measles vaccination was 15 months (IQR 15.0–16.0), the median age at second measles vaccination was 5.0 years (IQR 4.0–6.0), and the median time from second measles vaccination to enrollment in the study was 7.4 years (IQR 5.6–9.2) (23).

Demographic characteristics

Self-declared demographic data were used for all demographic analyses (Table 1). The sample population was relatively balanced in terms of gender, with 55.9% males and 44.1% females. The majority, 80.6%, of the sample population indicated that they were of Caucasian descent. Likewise, 97.1% of study participants were Not Hispanic or Latino.

Detection of CMI responses

A wide range of secreted cytokines were detected in PBMC culture supernatants following *in vitro* stimulation with live MV as shown in Table 2. Strong interferon and proinflammatory cytokine responses were primarily detected. Specifically, we detected robust secretion of IFN- a, high secretion levels of IFN-c, moderate secretion of IFN-k1, and slight secretion of TNF-a. In addition, robust secretion of IL-6, moderate secretion of IL-2, and slight secretion of IL-10 was detected.

The frequencies of IFN-c-secreting cells were measured following MV stimulation using ELISPOT assays. Specifically, 726 IFN-c ELISPOT assays were performed, and the median detection of measles-specific IFN- c-secreting cells

Table 1. Demographic Characteristics of the Study Cohort

Variable	Result
Gender, n (%)	
Male	427 (55.9)
Female	337 (44.1)
Race, n (%)	
Caucasian	616 (80.6)
African-American	89 (11.7)
Asian, Hawaiian, Pacific Islander	22 (2.9)
Other, multiple or unknown	37 (4.8)
Ethnicity, n (%)	
Not Hispanic or Latino	742 (97.1)
Hispanic or Latino	15 (2.0)
Unknown	7 (0.9)

Table 2. Secreted Cytokine Levels of the Study Cohort

Cytokine	Assays performed (n)	Median (IQR) pg/mL
IL-2	758	37.5 (20.5–64.4)
IL-6	756	354.6 (248.5–461.4)
IL-10	759	18.3 (11.3–28.5)
IFN-a	753	550.9 (272.6–1031.2)
IFN-c	756	67.4 (35.2–120.5)
IFN-k1	757	34.1 (14.1–68.2)
TNF-a	751	13.7 (9.2–18.8)

IQR, interquartile range.

was 36.0 spot-forming units (sfu) per 200,000 cells (IQR 13.0–69.0 sfu) as previously reported (23).

Associations between CMI responses and demographic data

Associations between demographic data and measles-specific CMI responses were examined to determine whether gender, race, or ethnicity were associated with variations in measles-specific CMI measures.

Several immune outcomes significantly varied by gender as outlined in Table 3. Females had significantly higher levels of secreted TNF-a, IL-6, and IFN-a ($p < 0.001$, $p < 0.002$, and $p < 0.04$, respectively).

In addition, the levels of several secreted cytokines varied significantly by self-declared race (Table 4). Specifically, Caucasians had significantly higher levels of IFN- k1, IL-10, IL-2, TNF-a, IL-6, and IFN-a ($p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.003$, $p < 0.01$, and $p < 0.02$, respectively) compared to all other racial groups combined (Caucasians versus non-Caucasians). Additionally, Caucasians had a greater number of IFN-c-secreting cells compared to other racial groups ($p < 0.001$).

Ethnicity was not significantly associated with variations in measles-specific CMI measures (data not shown).

Associations between CMI responses and clinical data

In addition, the number of detectable measles-specific IFN-c-secreting cells was significantly associated with age at last vaccination (<14 mo median = 30.0 sfu, IQR 11.0–69.0 sfu; 15 mo median = 40 sfu, IQR 16.5–83.5 sfu; >16 mo median = 30.5 sfu, IQR 10.0–65.0 sfu; $p < 0.001$).

Table 3. Variation in Measles-Specific CMI Responses by Gender

CMI measure	Gender	Median (IQR)[a] pg/mL	p Value[b]	p Value[c]
TNF-a	Male	13.5 (8.8–18.7)	<0.001	<0.001
	Female	14.0 (9.6–19.4)		
IL-6	Male	339.1 (235.4–446.6)	0.002	0.002
	Female	378.7 (269.5–480.9)		
IFN-a	Male	523.0 (254.0–888.6)	0.07	0.04
	Female	609.6 (284.4–1093.7)		

[a]IQR, interquartile range.
[b]Univariate p value.
[c]Multivariate p value, adjusting for all other demographic and clinical variables.
CMI, cell-mediated immune.

Discussion

Race and gender have been associated with variations in immune response following viral vaccination with multiple pathogens, including measles (4,8,13,17,28,31,32,46). However, most measles studies have focused on racial and gender associations with humoral immunity (as the neutralizing antibodies are an accepted correlate of protection), rather than innate/inflammatory or CMI measures of immune response. In this study, measles-specific innate/inflammatory and CMI responses were examined in a healthy cohort of children and young adults who had received their second dose of MMR 7.4 y (median) earlier.

A Th-1-dominant cytokine response in concert with a robust innate/inflammatory cytokine secretion pattern was detected in PBMC cultures stimulated with live MV. Early studies examining CMI responses in children who had been vaccinated with measles vaccine suggested that a Th-2-like cytokine secretion pattern, driven primarily by the production of IL-4, dominated the CMI response to MV (19,55). However, more recent work has suggested that a Th-1-like cytokine secretion pattern, driven by IFN-c secretion, dominates the response to measles vaccination in children and young adults after two doses of MMR vaccine (16,24,43).

Negligible amounts of the classical Th-2 cytokines IL-4 and IL-5 were detected in our previous studies of MV-stimulated PBMC culture supernatants (12,21), thus IL-10 rather than these cytokines was measured in the current study, as IL-10 plays a critical role in inhibiting the activity of Th-1 cells, natural killer (NK) cells, and macrophages (3,15). Additionally, other studies have detected IL-10 secretion in PBMC cultures of individuals previously vaccinated with MV (3,15,33). In this study, we observed strong measles-specific secretion of IFN- c, and high numbers of IFN-c-secreting cells with only slight secretion of IL-10 (Table 1), which suggests a Th-1-biased recall immune response 7.4 years following the second MMR vaccination. In addition, this conclusion is further strengthened by the detection of moderate secretion of IFN-k1, a cytokine that has been shown to induce a Th-1 bias (25,26), highlighting the important role of CMI in developing a robust, persistent immune response following measles vaccination.

Gender differences have also been associated with differences in measles antibody titers. A study of children and adults that had previously been vaccinated with MV containing vaccine from Catalonia, Spain (<15 years of age) found that females have significantly higher measles-specific IgG titers following vaccination compared to age-matched males (13). No studies have shown gender associations with measles-specific CMI responses.

Males have been shown to have a greater CMI response (higher stimulation index [SI] in lymphoproliferation) to rubella virus at 2 and 4 week post-MMR vaccination, and to varicella zoster virus (higher frequencies of VZV-specific $CD4^+$ T cells in healthy naturally immune adults) compared to females; thus it is feasible that CMI responses to measles vaccine also vary by gender (28,32). By assessing measles-specific cytokine responses 7.4 years following the last immunization, our study found that females secreted greater

Table 4. Variation in Measles-Specific CMI Responses by Race

CMI measure	Race	Median (IQR) [a]	p Value[b]	p Value[c]
IFN-k1	Caucasian	39.2 (16.6–86.3) pg/mL	<0.001	<0.001
	Non-Caucasian	20.1 (5.4–48.8) pg/mL		
IL-10	Caucasian	19.0 (12.0–28.6) pg/mL	<0.001	<0.001
	Non-Caucasian	14.5 (8.3–25.6) pg/mL		
IL-2	Caucasian	40.1 (21.6–66.7) pg/mL	<0.001	<0.001
	Non-Caucasian	30.2 (14.6–47.5) pg/mL		
IFN-c-secreting cells	Caucasian	37.0 (15.1–67.0) sfu [d]	0.005	<0.001
	Non-Caucasian	30.0 (11.0–62.0) sfu [d]		
TNF-a	Caucasian	13.9 (9.6–28) pg/mL	<0.001	0.003
	Non-Caucasian	12.9 (9.6–24) pg/mL		
IFN-a	Caucasian	592.7 (284.1–1073.6) pg/mL	0.02	0.01
	Non-Caucasian	441.6 (246.4–784.6) pg/mL		
IL-6	Caucasian	354.9 (248.4–568.7) pg/mL	0.02	0.02
	Non-Caucasian	353.2 (254.0–476.3) pg/mL		

[a]IQR, interquartile range.
[b]Univariate p value.
[c]Multivariate p value, adjusting for all other demographic and clinical variables.
[d]Spot forming units per 200,000 cells.

in vitro levels of innate/inflammatory cytokines (IL-6, TNF- a, and IFN-a) compared to males.

This study suggests that females may have a more robust antiviral response to MV via secretion of innate/inflammatory cytokines upon MV recognition. The difference in median TNF-a secretion between males and females was negligible, although statistically significant. Although there was a statistically significant variation between females and males for some of the CMI measures, it is unlikely that these findings are clinically or biologically significant. The differences in both median IL-6 and IFN-a secretion between males and females (39.6 pg/mL and 86.6 pg/mL, respectively) were much larger. These translate into approximately a 10% and 14% increase in cytokine production, respectively. Although these findings might be biologically relevant, studies that monitor physiological changes with respect to cytokine levels are warranted to ascertain the biological/clinical relevance of the observed differential cytokine secretion by gender. Additionally, larger studies *in vivo* that monitor the kinetics of cytokine responses following measles vaccination (or measles infection) are also needed to validate these observations. Further, the age group studied in this study ranges from pre-pubescent to adolescent (age 11–22 years). It is known that cytokine responses may be modulated by sex hormones, which could potentially influence the results demonstrated in this study.

IL-6, a proinflammatory cytokine, and IFN- a, a type I interferon, are both essential to initiating an adaptive immune response. Other studies have shown that females have a higher measles-specific IgG titer compared to males (13). Thus we can speculate that the increased levels of some measles-specific cytokines secreted by females in response to MV stimulation may explain why females had higher antibody titers following measles vaccination compared to males.

Race and ethnicity have also been associated with both humoral and CMI responses to MV. A study of Innu, Inuit, and Caucasian schoolchildren from Newfoundland, Canada, found that Innu and Inuit children had a significantly higher measles seropositive rate, as well as higher measles antibody titers, following vaccination with a single dose of MMR vaccine as compared to Caucasian children (46). Additionally, a study of measles antibody seroprevalence in the U.S. from 1999–2004 found that non-Hispanic blacks had a significantly higher seroprevalence of measles antibodies compared to non-Hispanic whites (although the authors suggest that this might be due to natural measles infection), and that Mexican Americans had a significantly lower seroprevalence of measles antibodies than non-Hispanic Whites and non-Hispanic Blacks (31). Genetic factors, including SNPs in cytokine and cytokine receptor genes, have also been significantly associated with measles-induced immunity in Caucasians and Somali African-Americans (8,10).

The race-specific innate/inflammatory and CMI cytokine immune response data from this study are interesting due to the wide range of cytokines that were differentially expressed in Caucasians as compared to other races combined. Caucasians secreted significantly greater amounts of the immunosuppressive cytokine IL-10 (with pleiotropic effects in immunoregulation and inflammation), as well as significantly greater amounts of multiple immunostimulating cytokines, such as antiviral interferons, IFN-a, TNF-a, and IFN-k1, and the proinflammatory cytokine IL-6. However, similarly to the gender-specific analysis, it is possible that some of the race-specific statistically significant associations have limited clinical or biological significance, due to the minimal differences seen among median secretion levels between Caucasians and all other races combined. Future studies designed to look specifically for dose-related physiological changes of a given cytokine over time, in response to MV stimulation or *in vivo*, could offer a clearer picture of the precise clinical relevance of these data.

Interestingly, our data demonstrated an upregulation of both immunosuppressive CMI and immunostimulating innate cytokines in Caucasians. Previous studies have associated IL-10 with immunosuppression and impaired T-cell responses (3,15,33); however, these data indicate that Caucasians had increased expression of innate stimulating

cytokines in addition to increased IL-10 expression. In part, undersampling of minority races in this study could explain these observations. However, at a macro level an interesting observation can be drawn from these data. Caucasians had increased expression of both immunostimulatory and immunosuppressive cytokines following measles vaccination. It is possible that the immunosuppressive properties of IL-10 are being balanced by the significantly higher expression of immunostimulatory cytokines such that Caucasians still generate a successful immune response following measles vaccination. This idea also implies that different races might have differential activation of biological pathways essential to generating an immune response. To fully understand the implications of differential cytokine production patterns, a systems biology approach that combines immunologic data with high-dimensional laboratory assays and clinical data sets could use pathway analysis to explain the impact of race-specific differential cytokine expression following MV stimulation.

This study had multiple strengths, including a large study population and the use of a wide range of measles-specific innate/inflammatory and CMI markers to immunologically characterize the study population. Additionally, the study population consisted of a random sample of children and young adults 11–22 years of age. The time and detail spent to recruit a random sample population was designed to increase confidence that the outcomes derived from the data would be relevant. The sample population was balanced in terms of gender distribution, but had limited racial and ethnic diversity. This limited the statistical power to associate small variations in measles-specific CMI responses with race or ethnicity. Additionally, this study relied on self-declared race information rather than genetic determinants to assign demographic information. Using principal component analysis with high-density SNP typing to determine racial background could allow for more precise subject classification. In the future, enriching the sample population with racial and ethnic minorities would increase the ability to elucidate race- and ethnicity-specific associations with measles-specific CMI measures. Additionally, sampling the study population prior to, and at multiple time points after, vaccination (which was not feasible in our study) could provide a better understanding of measles-specific innate/inflammatory and adaptive immune responses, as well as the kinetics of cytokine responses following vaccination.

In summary, Th-1-dominated cytokine patterns, as well as important immunostimulating cytokines, were predominantly detected, highlighting the importance of innate/inflammatory and CMI responses in generating a successful measles immune response. The humoral immune responses of this cohort have been described previously, with no reported seronegative individuals, and 91.1% with antibody concentrations above the protective threshold (210 mIU/mL, corresponding to a PRMN titer of 120) (23). Finally, we observed that innate/inflammatory and CMI responses to MV vary significantly by gender and race, advancing our understanding of inter-individual and subgroup variations in immune responses to measles vaccination. Delineating mechanisms that induce variations in the immune response to MV could facilitate the development of the next generation of vaccines that increase a host's immune responses following measles vaccination.

Acknowledgments

We would like to thank all of the children and parents who participated in this study. We would like to acknowledge and thank the laboratory staff, statisticians, and nurses at the Mayo Clinic Vaccine Research Group for their invaluable assistance with this study, as well as Megan O'Byrne for her immense help with statistical analysis. This project was supported by award numbers AI33144 and AI48793 (which recently received a MERIT Award) from the National Institute of Allergy and Infectious Diseases (NIAID), and 5UL1RR024150-03 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), and the NIH Roadmap for Medical Research. The content is solely the responsibility of the authors and does not necessarily represent the official views of NIAID or the NIH.

Author Disclosure Statement

Dr. Poland is the chair of a safety evaluation committee for novel non-measles vaccines undergoing clinical studies by Merck Research Laboratories. Dr. Jacobson recently served on a Safety Review Committee for a post-licensure study conducted by Kaiser-Permanente concerning Gardasil HPV vaccine funded by Merck & Co. The other authors declare that they have no conflict of interest.

References

1. Advisory Committee on Immunization Practices: Recommended Immunization Schedules for Persons Aged 0 Through 18 Years—United States, 2010. MMWR 2010;58: 1–4.
2. Anders JF, Jacobson RM, Poland GA, Jacobsen SJ, and Wollan PC: Secondary failure rates of measles vaccines: a metaanalysis of published studies. Pediatr Infect Dis J 1996;15:626–36.
3. Brooks DG, Trifilo MJ, Edelmann KH, Teyton L, McGavern DB, and Oldstone MB: Interleukin-10 determines viral clearance or persistence in vivo. Nat Med 2006;12:1301–9.
4. Christensen PE, Schmidt H, Jensen O, Bang HO, Andersen V, and Jordal B: An epidemic of measles in Southern Greenland, 1951. Measles in virgin soil. I. Acta Med Scand 1953;144:313–22.
5. Cohen BJ, Audet S, Andrews N, and Beeler J: Plaque reduction neutralization test for measles antibodies: Description of a standardised laboratory method for use in immunogenicity studies of aerosol vaccination. Vaccine 2007;26:59–66.
6. Cohen BJ, Parry RP, Doblas D, et al.: Measles immunity testing: comparison of two measles IgG ELISAs with plaque reduction neutralisation assay. J Virol Methods 2006;131: 209–12.
7. Dhiman N, Haralambieva IH, Vierkant RA, et al.: Predominant inflammatory cytokine secretion pattern in response to two doses of live rubella vaccine in health vaccinees. Cytokine 2010;50:240–9.
8. Dhiman N, Ovsyannikova IG, Cunningham JM, et al.: Associations between measles vaccine immunity and single nucleotide polymorphisms in cytokine and cytokine receptor genes. J Infect Dis 2007;195:216–9.
9. Dhiman N, Ovsyannikova IG, Vierkant RA, Pankratz VS, Jacobson RM, and 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.
10. Dhiman N, Ovsyannikova IG, Vierkant RA, Pankratz VS, Jacobson RM, and 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.
11. Dhiman N, Ovsyannikova IG, Vierkant RA, et al.: Associations between SNPs in toll-like receptors and related intracellular signaling molecules and immune responses to measles vaccine: preliminary results. Vaccine 2008;26:1731–1736.
12. Dhiman N, Ovsyannikova IG, Vierkant RA, et al.: Associations between SNPs in toll-like receptors and related intracellular signaling molecules and immune responses to measles vaccine: preliminary results. Vaccine 2008;26:1731–1736.
13. Dominguez A, Plans P, Costa J, et al.: 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.
14. Elliman D, and Sengupta N: Measles. Curr Opin Infect Dis 2005;18:229–234.
15. Fiorentino DF, Zlotnik A, Vieira P, et al.: IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. J Immunol 1991;146:3444–3451.
16. Gans HA, Maldonado Y, Yasukawa LL, et al.: IL-12, IFN-gamma, and T cell proliferation to measles in immunized infants. J Immunol 1999;162:5569–5575.
17. Gardner EM, Gonzalez EW, Nogusa S, and Murasko DM: Age-related changes in the immune response to influenza vaccination in a racially diverse, healthy elderly population. Vaccine 2006;24:1609–1614.
18. Good RA, and Zak SJ: Disturbances in gamma globulin synthesis as "experiments of nature." Pediatrics 1956;18:109–149.
19. Griffin DE, and Ward BJ: Differential CD4 T cell activation in measles. J Infect Dis 1993;168:275–281.
20. Haralambieva IH, Dhiman N, Ovsyannikova IG, et al.: 2′–5′ Oligoadenylate synthetase single-nucleotide polymorphisms and haplotypes are associated with variations in immune responses to rubella vaccine. Hum Immunol 2010;71:383–391.
21. Haralambieva IH, Ovsyannikova IG, Dhiman N, Vierkant RA, Jacobson RM, and Poland GA: Differential cellular immune responses to wild-type and attenuated Edmonston tag measles virus strains are primarily defined by the viral phosphoprotein gene. J Med Virol 2010;82:1966–1975.
22. Haralambieva IH, Ovsyannikova IG, Kennedy RB, et al.: Associations between single nucleotide polymorphisms and haplotypes in cytokine and cytokine receptor genes and immunity to measles vaccination. Vaccine 2011.
23. Haralambieva IH, Ovsyannikova IG, O'Byrne M, Pankratz VS, Jacobson RM, and Poland GA: A large observational study to concurrently assess persistence of measles specific B-cell and T-cell immunity in individuals following two doses of MMR vaccine. Vaccine 2011;29:4485–4491.
24. Howe RC, Dhiman N, Ovsyannikova IG, and Poland GA: Induction of CD4 T cell proliferation and Th1-like cytokine responses in vitro to measles virus. Clin Exp Immunol 2005;140:333–342.
25. Jordan WJ, Eskdale J, Boniotto M, Rodia M, Kellner D, and Gallagher G: Modulation of the human cytokine response by interferon lambda-1 (IFN-lambda1/IL-29). Genes Immun 2007;8:13–20.
26. Jordan WJ, Eskdale J, Srinivas S, et al.: Human interferon lambda-1 (IFN-lambda1/IL-29) modulates the Th1/Th2 response. Genes Immun 2007;8:254–261.
27. Katz SL: A vaccine-preventable infectious disease kills half a million children annually. J Infect Dis 2005;192:1679–1680.
28. Klein NP, Holmes TH, Sharp MA, et al.: Variability and gender differences in memory T cell immunity to varicella-zoster virus in healthy adults. Vaccine 2006;24:5913–5918.
29. Lee KY, Lee HS, Hur JK, Kang JH, and Lee BC: The changing epidemiology of hospitalized pediatric patients in three measles outbreaks. J Infect 2007;54:167–172.
30. Markowitz LE, Preblud SR, Fine PEM, and Orenstein WA: Duration of live measles vaccine-induced immunity. Pediatr Infect Dis J 1990;9:101–110.
31. McQuillan GM, Kruszon-Moran D, Hyde TB, Forghani B, Bellini W, and Dayan GH: Seroprevalence of measles antibody in the US population, 1999–2004. J Infect Dis 2007;196:1459–1464.
32. Mitchell LA: Sex differences in antibody- and cell-mediated immune response to rubella re-immunisation. J Med Microbiol 1999;48:1075–1080.
33. Moss WJ, Ota MO, and Griffin DE: Measles: immune suppression and immune responses. Int J Biochem Cell Biol 2004;36:1380–1385.
34. Orenstein WA, Herrmann K, Albrecht P, et al.: Immunity against measles and rubella in Massachusetts schoolchildren. Develop Biol Standard 1986;65:75–83.
35. Otten M, Kezaala R, Fall A, et al.: Public-health impact of accelerated measles control in the WHO African Region 2000–03. Lancet 2005;366:832–839.
36. Ovsyannikova IG, Dhiman N, Haralambieva IH, et al.: Rubella vaccine-induced cellular immunity: evidence of associations with polymorphisms in the toll-like, vitamin A and D receptors, and innate immune response genes. Hum Genet 2010;127:207–221.
37. Ovsyannikova IG, Dhiman N, Jacobson RM, Vierkant RA, Pankratz VS, and Poland GA: HLA homozygosity does not adversely effect measles vaccine-induced cytokine responses. Virology 2007;364:87–94.
38. Ovsyannikova IG, Haralambieva IH, Dhiman N, et al.: Polymorphisms in the vitamin A receptor and innate immunity genes influence the antibody response to rubella vaccination. J Infect Dis 2010;201:207–213.
39. Ovsyannikova IG, Haralambieva IH, Vierkant RA, O'Byrne MM, Jacobson RM, and Poland GA: The association of CD46, SLAM, and CD209 cellular receptor gene SNPs with variations in measles vaccine-induced immune responses—A replication study and examination of novel polymorphisms. Hum Hered 2011.
40. Ovsyannikova IG, Haralambieva IH, Vierkant RA, Pankratz VS, and Poland GA: The role of polymorphisms in toll-like receptors and their associated intracellular signaling genes in measles vaccine immunity. Hum Genet 2011;130:547–561.
41. Ovsyannikova IG, Jacobson RM, Ryan JE, Vierkant RA, Pankratz VS, and Poland GA: Human leukocyte antigen and interleukin 2, 10 and 12p40 cytokine responses to measles: Is there evidence of the HLA effect? Cytokine 2006;36:173–179.
42. Ovsyannikova IG, Jacobson RM, Vierkant RA, Pankratz SV, Jacobsen SJ, and Poland GA: Associations between human leukocyte antigen (HLA) alleles and very high levels of measles antibody following vaccination. Vaccine 2004;22:1914–1920.
43. Ovsyannikova IG, Reid KC, Jacobson RM, Oberg AL, Klee GG, and Poland GA: Cytokine production patterns and

antibody response to measles vaccine. Vaccine 2003;21:3946–3953.
44. Ovsyannikova IG, Ryan JE, Vierkant RA, Pankratz SV, Jacobson RM, and Poland GA: Immunologic significance of HLA class I genes in measles virus-specific IFN-gamma and IL-4 cytokine immune responses. Immunogenetics 2005;57:828–836.
45. Poland GA, and Jacobson RM: Failure to reach the goal of measles elimination. Apparent paradox of measles infections in immunized persons. Arch Intern Med 1994;154:1815–1820.
46. Poland GA, Jacobson RM, Colbourne SA, et al.: Measles antibody seroprevalence rates among immunized Inuit, Innu and Caucasian subjects. Vaccine 1999;17:1525–1531.
47. Poland GA, Jacobson RM, Schaid D, Moore SB, and Jacobsen SJ: HLA alleles and measles vaccine-induced antibody levels: evidence of a significant association. Vaccine 1998;16: 1869–1871.
48. Poland GA, Jacobson RM, Thampy AM, et al.: Measles reimmunization in children seronegative after initial immunization. JAMA 1997;277:1156–1158.
49. Poland GA, Ovsyannikova IG, and Jacobson RM: Application of pharmacogenomics to vaccines. Pharmacogenomics 2009;10:837–852.
50. Poland GA, Ovsyannikova IG, Jacobson RM, and Smith DI: Heterogeneity in vaccine immune response: the role of immunogenetics and the emerging field of vaccinomics. Clin Pharmacol Ther 2007;82:653–664.
51. Ryan JE, Ovsyannikova IG, and Poland GA: Detection of measles virus-specific interferon-gamma-secreting T-cells by ELISPOT. Methods Mol Biol 2005;302:207–218.
52. Sheppeard V, Forssman B, Ferson MJ, et al.: Vaccine failures and vaccine effectiveness in children during measles outbreaks in New South Wales, March–May 2006. Commun Dis Intell 2009;33:216–226.
53. Tosh PK, Kennedy RB, Vierkant RA, Jacobson RM, and Poland GA: Correlation between rubella antibody levels and cytokine measures of cell-mediated immunity. Viral Immunol 2009;22:451–456.
54. Vartdal F, Gaudernack G, Funderud S, et al.: HLA class I and II typing using cells positively selected from blood by immunomagnetic isolation—a fast and reliable technique. Tissue Antigens 1986;28:301–312.
55. Ward BJ, and Griffin DE: Changes in cytokine production after measles virus vaccination: predominant production of IL-4 suggests induction of a Th2 response. Clin Immunol Immunopathol 1993;67:171–177.
56. Watson JC, Hadler SC, Dykewicz CA, Reef S, and Phillips L: Measles, mumps, and rubella—vaccine use and strategies for elimination of measles, rubella, and congenital rubella syndrome and control of mumps: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 1998;47:1–57.

Address correspondence to:
Dr. Gregory A. Poland
Mayo Clinic
Guggenheim 611C
200 First Street S.W.
Rochester, MN 55905

E-mail: poland.gregory@mayo.edu

Received July 19, 1011; accepted October 12, 2011.