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Associations between race, sex and immune response variations to rubella vaccination in two independent cohorts

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Abstract

Introduction—Immune response variations after vaccination are influenced by host genetic factors and demographic variables, such as race, ethnicity and sex. The latter have not been systematically studied in regard to live rubella vaccine, but are of interest for developing next generation vaccines for diverse populations, for predicting immune responses after vaccination, and for better understanding the variables that impact immune response.

Methods—We assessed associations between demographic variables, including race, ethnicity and sex, and rubella-specific neutralizing antibody levels and secreted cytokines (IFN γ , IL-6) in two independent cohorts (1,994 subjects), using linear and linear mixed models approaches, and genetically defined racial and ethnic categorizations.

Results—Our replicated findings in two independent, large, racially diverse cohorts indicate that individuals of African descent have significantly higher rubella-specific neutralizing antibody levels compared to individuals of European descent and/or Hispanic ethnicity ($p < 0.001$).

Conclusion—Our study provides consistent evidence for racial/ethnic differences in humoral immune response following rubella vaccination.

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Competing Interests

Dr. Poland is the chair of a Safety Evaluation Committee for novel non-rubella 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. Drs. Poland and Ovsyannikova hold two patents related to vaccinia peptide research. 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.

Keywords

Race; Ethnicity; Sex; Antibodies; Cellular Immunity; Rubella vaccine; MMR; Continental Population Groups; Ethnic Groups; Sex; Antibodies; Immunity, Cellular; Rubella Vaccine; Measles-Mumps-Rubella Vaccine

Introduction

Developing more effective vaccines can be difficult due to a lack of data that explain inter-individual variations in immune responses following vaccination [1]. Elucidation of the underlying factors that cause these differences could lead to better vaccines and the ability to predict immune responses in certain populations and/or individuals [1-3]. Currently, several factors explain, in part, the observed heterogeneity in immune responses following vaccination, including genetic host determinants, such as polymorphisms in immune function-related genes, and demographic factors [4-6]. Data from the literature and our own published data suggest that vaccine-induced immune responses are considerably influenced by demographic and clinical variables such as age, sex, ethnicity and race [2, 6-10].

Previous studies have demonstrated that women have significantly higher humoral immune responses to vaccination than men, but limited and/or controversial data is available on cell-mediated immune responses after vaccination [2]. We have reported higher IFN γ ELISPOT responses in males versus females after smallpox vaccination [6]. In a population-based study of 346 schoolchildren following two doses of measles-mumps-rubella vaccination, we also demonstrated sex-related differences in rubella virus-specific and mumps virus-specific IgG antibody titers, but no significant differences in T cell-lymphoproliferative responses [5, 11, 12]. Demographic factors such as race and ethnicity are also known to influence susceptibility to infectious diseases (tuberculosis, dengue fever, HIV, smallpox) [13-15], and have recently been shown to be associated with immune response variations and adverse events following vaccination [6-10]. Race and ethnicity-based differences have not been systematically assessed following rubella vaccination, and their influence on the magnitude and longevity of neutralizing antibody levels and cellular immune responses (rubella-specific secreted cytokines) following vaccination is unclear.

We hypothesized that sex, race and ethnicity may significantly contribute to inter-individual immune response variations observed after live rubella vaccination and tested this hypothesis in two distinct racially diverse cohorts.

Methods

The methods described below are similar or identical to those published for our previous studies [6, 16-24].

Study participants

The study cohort was a large population-based sample of 2,221 healthy children, older adolescents, and healthy adults (age 11 to 40 years), consisting of Rochester, MN, and San Diego, CA, cohorts (1,145 and 1,076 subjects, respectively), with clinical and demographic characteristics previously reported [18, 24-26].

The Rochester cohort comprised a sample of 1,145 individuals (age 11 to 22 years) from three independent age-stratified random cohorts of healthy schoolchildren and adolescents (with written records of having received two doses of measles-mumps-rubella vaccine),

recruited between 2001 and 2009, from all socioeconomic strata from Olmsted County, MN, as previously described [5, 11, 16, 18, 27].

The replication (San Diego) cohort was recruited between 2006 and 2007, and comprised a sample of 1,076 healthy older adolescents and healthy adults (age 18 to 40 years) from armed forces personnel who participated in a smallpox immunization program at the Naval Health Research Center (NHRC) in San Diego, CA. Subject enrollment for this study has been previously described in detail [22, 25, 28]. As members of the U.S. military, these subjects represent a cross section of the U.S. population with proven vaccine-induced immunity to MMR, and documented receipt of MMR (rubella) vaccine. All subjects included in the current rubella vaccine gave an informed consent to use their samples in future vaccine studies. The Institutional Review Boards of the Mayo Clinic and NHRC approved the study, and written informed consent (for participation in rubella and/or future vaccine studies) was obtained from each subject described above, from the parents of all children who participated in the study, as well as written assent from age-appropriate participants.

Soluble immunocolorimetric neutralization assay for quantification of rubella virus-specific neutralizing antibodies (sICNA)

We quantified rubella-specific neutralizing antibody titers (i.e., functional antibodies relevant to protection) as our major immune response outcome. We used a modified version of the CDC (Centers for Disease Control and Prevention, Atlanta, GA) immunocolorimetric-based neutralization method, which was optimized to a high-throughput microformat [24, 26]. Heat-inactivated sera were serially diluted in two-fold, in triplicate for each dilution, beginning from 1:12.5 through 1:100, using a diluent: phosphate-buffered saline (PBS, pH 7.4), supplemented with 1% fetal bovine serum (FBS) (final volume 30 ! L per dilution). Rubella virus stock (vaccine virus HPV77) was diluted to a concentration of 1.2×10^3 plaque-forming units (PFU)/mL, and was added (30 ! L) to an equal volume of diluted serum (or diluent as in the case of virus-only control). The plate was incubated for 1.5 hour at 37°C, 5% CO₂. Fifty microliters of each mixture were used to inoculate confluent Vero cell monolayers (in flat-bottom 96-well plates) and the cells were incubated for 1 hour at 37°C, 5% CO₂. After the incubation period, DMEM supplemented with 5% FBS and 50 ! g/mL Gentamicin (Gibco; Invitrogen, Carlsbad, CA) was added to each well and the plate was further incubated for 72 hours at 37°C, 5% CO₂. For development, plates were washed once with PBS and fixed with cold methanol for 10 minutes. PBS supplemented with 5% skim milk (Difco; Becton-Dickinson, Franklin Lakes, NJ) and 0.1% Tween 20 (blotto) was added for 30 minutes for blocking. Rubella monoclonal antibody targeting the E1 glycoprotein (CDC, Atlanta, GA) was diluted in blotto to a concentration of 5 µg/mL and added to each well for 30 minutes. Plates were washed three times with PBS supplemented with 0.05% Tween 20 (PBS-T). Goat anti-mouse HRP-conjugated detection antibody (Invitrogen, CA) was diluted to 0.5 µg/mL in blotto and added to each well for 30 minutes. After the detection antibody was added, plates were washed again. Aqueous NeA-Blue Tetramethylbenzidine/TMB substrate solution (Clinical Science Products, Mansfield, MA) was added for 10 minutes and the reaction was stopped using 0.5M sulfuric acid. Optical density (OD) values were measured by spectrophotometry at 450 nm. Each assay contained the following controls: virus-only control (no serum); uninfected control (no serum or virus); and two reference sera (CDC anti-rubella human serum reference preparation IS2153, CDC, Atlanta, GA; and a seronegative serum RP-011 panel member 1, Biomex GmbH, Heidelberg, Germany). The neutralization titer was calculated as the highest dilution at which the input virus signal was reduced by at least 50% within the dilution series (NT₅₀). The Loess method of statistical interpolation was used to estimate final neutralization titers (NT₅₀) from observed values and refine quantitative estimates [24, 26]. The resultant sICNA assay

results were in good agreement with the rubella-specific Beckman Coulter's Access® Rubella IgG chemiluminescent immunoassay results, as evaluated in 732 subjects with both assessments (Spearman correlation coefficient 0.76, data not shown) [26]. The intra-class correlation coefficient (ICC) based on log-transformed estimates from repeated NT₅₀ measurements was 0.89, which demonstrates a high degree of reproducibility in the sICNA assay [26] [24].

Rubella-specific cytokine secretion

The levels of secreted cytokines (IFN γ , IL-6) were measured following stimulation of PBMC cultures with live rubella virus (W-Therien strain of rubella virus, a gift from Dr. Teryl Frey, Georgia State University, Atlanta, GA), using optimized MOI and incubation times depending on the specific cytokine measured, as previously described [20]. Rubella virus-specific IFN γ and IL-6 secretion levels were used in our analysis as measures of rubella-specific Th1/proinflammatory response because these classical Th1/proinflammatory cytokines were detectable in our cohorts. Th2 cytokines and/or other important cytokines were hardly detectable in our study subjects, as published previously [20], and therefore were not used.

Statistical methods

Race and ethnicity resolution—The purpose of the work presented here was to compare measures of immune response to rubella immunization among individuals of distinct racial and ethnic groups. In order to refine the grouping of individual into racial/ethnic groups, we utilized genetic data available from study participants who had been genotyped using genome-wide SNP arrays. Within each study group independently, we selected SNPs with >99% call rates, with inter-SNP distances of at least 100 kb, from those SNPs genotyped on the genome-wide arrays. We performed principal components analyses with these SNPs using the approach implemented in *Eigenstrat* software [29]. The resulting principal components reflecting genetic similarity between subjects were used to classify individuals into racial/ethnic groups using a clustering approach similar to that incorporated in the *Structure* software [30]. This approach has been described in prior reports of genetic associations with smallpox immune responses in the San Diego cohort [6, 21-23, 29]. Because these racial/ethnic groups were defined using genetic data, it is important to note that our classification of ethnicity reflects differences in ancestry, but not the cultural and/or other (other than genetic) differences that more completely define ethnic groups.

Statistical analysis—The comparisons of primary interest in this report were potential differences in rubella-specific immune responses among the major racial/ethnic groups defined by genome-wide data. Demographic features and immune measures were summarized within the two study cohorts using counts and percentages, or medians and interquartile ranges (25th and 75th percentiles), for qualitative or quantitative variables, respectively. For measures of cytokine secretion, the difference between the median values from the rubella-virus stimulated and unstimulated assay results was computed for each individual before summarizing within groups. We additionally summarized immune response by genetically defined race/ethnicity within each study cohort. We compared humoral immune responses among race/ethnic groups within each of the two study cohorts using linear models approaches. In these models, we used log₂-transformed NT₅₀ values in order to meet modeling assumptions and tested for differences among groups while adjusting for sex, age at enrollment, vaccination history (age at most recent immunization and time since last immunization to blood draw), and batch/run. We compared cytokine secretion of IL-6 and IFN γ among race/ethnic groups within each study cohort using linear mixed effects models, which incorporated all assay measures measured in triplicate by stimulation status while accounting for within-person correlations. In these analyses, we

used inverse-normal transformations in order to meet modeling assumptions, and adjusted for the same covariates as in the comparisons of antibody responses

Results

Genetic classification of the study subjects

As summarized above, and as previously described, we used a principal components approach to capture genetic differences among populations and define racial/ethnic groupings based on the observed clustering [6, 21-23, 29]. This approach allowed us to correctly classify additional subjects with unclear self-declaration (for race/ethnicity), and increased the power of the analyses [6, 23]. Based on the genome-wide data, we were able to classify study subjects into several major groups (for each cohort), as illustrated in Figure 1: Caucasians; African-Americans (consisting of African-Americans and African-Americans admixed); Somali (genetically distinct from African-Americans); and “Other” for the Rochester cohort; and Caucasians, African-Americans, Hispanics and “Other” for the San Diego cohort.

Demographic and immune variables of the study population

The demographic and immune variables of the study population (n=1,994) are summarized in Table 1. We have previously characterized in detail these variables for the discovery (Rochester) and replication (San Diego) cohorts [11, 16, 18, 22, 25, 28, 31-33]. Out of the discovery (Rochester) cohort, 1,052 subjects were successfully genotyped, met all inclusion, exclusion and QC criteria, had rubella immune outcome data available, and were included in the final analysis, of which 474 (45.1%) were females. The genetically defined groupings were: Caucasians 897 (85.3%); African-Americans/African-Americans admixed 62 (5.9%); and Somali 35 (3.3%). Out of the replication cohort (San Diego), 942 subjects were successfully genotyped, met all inclusion, exclusion and QC criteria, and had rubella immune outcome data available, and were included in the final analysis, of which 259 (27.5%) were females. The genetically defined groupings were: Caucasians 506 (53.7%); African-Americans 190 (20.2%); and Hispanics 198 (21.0%). The median interpolated neutralization titer for the Rochester cohort was 57 NT₅₀, and the median interpolated neutralization titer for the San Diego cohort was 66 NT₅₀ (Table 1). The proportion of subjects with low (below 1:25) interpolated neutralization titers after rubella vaccination for our study was 11.8% for the Rochester cohort and 5.6% for the San Diego cohort, 6.4 years (median) later for the Rochester cohort and 3 years (median) later for the San Diego cohort.

Associations between race and ethnicity with rubella vaccine-induced immune responses

Our analysis in the Rochester cohort indicates that both groups of subjects of African descent (African-Americans/African-Americans admixed and Somali) have significantly higher rubella-specific neutralizing antibody levels than subjects of European descent (Caucasians) (p = 0.0007, Table 2). The median neutralizing antibody titer for the Somali group is 118.6 NT₅₀, more than twice the median neutralizing antibody titer for the Caucasian group (55.4 NT₅₀), while the median neutralizing antibody titer for the African-American group is 77.3 NT₅₀. Consistent with this finding, our analysis in the San Diego cohort demonstrates that African-Americans also have significantly higher rubella-specific NT₅₀ titers (86.2 NT₅₀) compared to Caucasians (61.9 NT₅₀) and Hispanics (61.2 NT₅₀) (p! 0.0001, Table 2).

Associations between other variables and rubella vaccine-induced immune responses

Association analysis between antibody levels, cytokine secretion and sex revealed no statistically significant findings consistent between the two cohorts (Table 3). The data

indicates that statistically significant associations between rubella-specific IL-6 secretion and sex exist in both cohorts, however the direction of the observed response (higher/lower depending on sex) is not consistent between the two analyses (Table 3). Similarly, we did not observe consistent associations between other variables and rubella-specific immune response after vaccination (Supplementary Table 1).

Discussion

Multiple variables, including genetic, demographic, immunological and environmental factors, influence the development and longevity of immune responses after vaccination. It is known that genetic determinants play an important role in regulating immune responses after infection and/or vaccination. We have previously shown that polymorphisms in HLA, cytokine and cytokine receptor genes, antiviral effector genes, Toll-like receptor genes, vitamin A and D receptor genes, and other immune genes contribute to immune response variability after rubella vaccination [16, 18].

Here we report the replicated findings from an in-depth analysis of rubella-specific neutralizing antibodies and secreted cytokines, assessed explicitly by race, ethnicity, sex and age in two large, distinct racially diverse cohorts. Our results clearly demonstrate significant differences in the neutralizing antibody responses 2-6 years (median) after rubella vaccination in different racial groups with consistently higher titers observed in individuals of African descent, compared to individuals of European descent and/or Hispanic ethnicity. Our findings of higher neutralizing antibody levels in individuals of African descent post-rubella immunization are in line with, and may be related to, the higher immunoglobulin concentrations in blacks compared to whites (i.e., total IgG, IgG1, IgG2, IgM and IgA), which is largely attributed to genetic differences [34, 35]. At present, not much is known about the mechanisms and demographic factors accounting for immune response heterogeneity following vaccination. Large vaccine studies are often confounded by the lack of reliable racial/ethnic classification, lack of pre-immunization history (information about previous pathogen exposures and/or vaccinations), or other confounding variables, such as age, nutritional, economic, cultural, social, and environmental factors [36]. Still, several reports from the literature support our findings for disparity in antibody responses post vaccination between different racial/ethnic groups. A study involving 505 human immunodeficiency virus (HIV) glycoprotein/gp120 recipients reports higher serum neutralizing antibodies in African-Americans compared to Caucasians, although the observed differences were dependent on the vaccine formulation [36]. A large U.S. seroprevalence study (1999-2004), with a representative sample of the U.S. population (16,049 participants, 6-49 years), found significantly higher measles antibody seroprevalence rates in non-Hispanic black subjects compared to non-Hispanic white subjects and Mexican Americans [8], which might be attributed to genetics and/or other factors (vaccination history, measles exposures). Immunization with pertussis vaccine elicited two-fold higher humoral immune response in black infants compared to white infants, with racial differences in the occurrence and/or reporting of adverse reactions [37]. We have also reported higher measles-specific antibody seroprevalence and antibody titers in Innu and Inuit schoolchildren (n=253) compared to Caucasian schoolchildren (n=353) of northern Newfoundland, Canada, following a single dose of measles vaccine [10]. Disparities in vaccine-induced serologic responses were also reported between Caucasians, African-Americans and/or other racial/ethnic groups (in infants, children and adults) for yellow fever vaccine, Haemophilus influenzae type b polysaccharide vaccine, Neisseria meningitidis outer membrane protein conjugate vaccine and diphtheria toxoid vaccine [38-41].

Analyzing vaccinia virus-specific cytokine responses following primary smallpox vaccination in 1,071 armed forces vaccine recipients, we demonstrated that Caucasians overall had significantly higher cell-mediated immune response (IFN γ -producing cells, secreted IFN α and IL-2) compared to African-Americans and Hispanics [6]. Consistent with the above finding, severe adverse events (myopericarditis) following smallpox vaccination are observed primarily in males of self-reported Caucasian race [7]. Similarly, we found higher measles-specific IFN γ ELISPOT responses and higher cytokine secretion in Caucasians compared to non-Caucasians (African-Americans, Hispanics and other racial/ethnic groups) following two doses of measles-mumps-rubella vaccination [4]. Collectively, our own previous findings with measles and smallpox vaccines, and the literature provide consistent evidence for race-specific bias in humoral and cell-mediated immune responses following immunization.

The contributing factors and immunologic mechanisms behind these observations are still unclear, but the knowledge gained in this field may advance the development and design of better vaccines and vaccination strategies for diverse populations, including racial/ethnic groups with suboptimal immune response post-vaccination, and assist in better understanding the development of immune responses following vaccination. The diverse genetic background (HLA, Km/Gm immunoglobulin allotypes, other immune genes) of the studied racial/ethnic groups may, at least in part, explain the differences noted in host response across different races and ethnicities, in addition to other factors (e.g., differential pathogen exposure and/or vaccination history). Racial differences in host variation and allele frequencies (polymorphisms) in immune function-related genes (HLA, cytokine and cytokine receptor genes and other immune genes) have been reported and suggest phenotypic differences and functional dissimilarities in the mounting and sustaining of antigen-specific immunity [42, 43]. Consistent with this, we have previously identified both consistent and race-specific genetic associations (for Caucasians and African-Americans) between polymorphisms in immune response genes (e.g., cytokine and cytokine receptor genes, antiviral effector genes, Toll-like receptors, HLA and other immune function-related genes) and humoral and/or cellular immune responses following smallpox, measles and anthrax vaccination [21, 22, 31, 32, 43-45]. Large and well-designed population-based association studies (adjusting for all confounding variables) in diverse racial and ethnic groups are warranted to further identify and/or confirm phenotypic differences and genetic factors that underlie post-immunization immune response differences.

Our study failed to demonstrate consistent associations between sex (and other demographic/clinical variables) and immune response following rubella immunization, as previously reported [46, 47], a fact that might be due to the relative under-representation of females in the replication cohort, and/or other factors (e.g., different assays for immune outcome measures). Sex-based disparities and immune response differences following immunization are likely and the underlying biological mechanisms for sex bias in immune response have been nicely summarized by Klein *et al.* [2]. Thus, sex-based immune response variations following rubella and/or other immunizations warrant further in-depth investigation in future studies. There are several strengths of our study. We have used rubella-specific neutralizing antibody titers (i.e., functional neutralizing antibodies that protect against disease) as our major immune response outcome. We measured the antibody titers using a state-of-the-art, high-throughput assay, and also standardized protocols and stringent QC metrics for all immune assays. However, since the lowest serum dilution in our assay was 1:25, the rubella neutralizing antibody titers in our study cannot be related to the recognized rubella-specific protective neutralization titer of 1/8 and/or to the international correlate of protection of 10 IU/mL [48]. Although it would be difficult to directly compare our results with those from other studies, and interpret our findings (in terms of possible protection from infection), similar to other reports, we observed a substantial proportion of

subjects with low neutralizing antibody titers even after two MMR vaccine doses [49] [50]. This is suggestive of waning antibody levels in an elimination environment (with no or rare booster from wild-type rubella virus encounters), although with no clinical evidence of diminished protection. While the current vaccine is acknowledged to be a good vaccine, we and others have demonstrated that the immune response to rubella vaccine varies and immune response wanes over time (with no wild-type rubella virus immune boosting) [49-51]. Our study provides consistent evidence for racial/ethnic differences in functional neutralizing antibodies following rubella vaccination that correlate best with protection. Based on the phenomenon noted by others that higher pre-vaccination titers (after first rubella vaccine dose) are associated with higher titers after subsequent vaccination, even years after vaccination, we can speculate that the higher neutralizing antibody levels observed for African-Americans (compared to Caucasians and/or Hispanics) in our study may potentially denote genetic and racial differences in the long-term immunity and protection following vaccination [49-51]. Understanding the demographic factors influencing vaccine-induced immunity and infection susceptibility (e.g., race/ethnicity) may potentially help predict herd immunity in vaccinated populations with diverse demographics and/or lead to more effective vaccination practices (e.g., different timing and/or number of doses, higher/lower dose etc., for different racial/ethnic groups). The importance of cellular immunity and Th1 (and other) cytokines for conferring rubella vaccine-induced protection and long-term immunity is unknown, but cellular immunity may play a role in protection against symptomatic and/or severe disease in seronegative vaccinated individuals and/or individuals with antibody level below the level of protection, as observed for measles [48]. Recent data provides some degree of evidence for a relationship (correlation) between functional neutralizing antibodies and IFN γ after rubella vaccination [24]. For this reason, we also measured and assessed rubella-specific Th1/proinflammatory cytokines (IFN α and IL-6), detectable in our cohorts, but no consistent associations were observed for these immune response outcomes.

An important concept of our study design is the replication of results in an independent validation cohort to prevent and/or minimize the risk of false positive observations. In addition, we used genomic data and a principal components approach, described previously [6, 21-23], for precise race/ethnicity categorization of study participants and analysis methodology that corrects for potential known confounding variables. Limitations include the influence of additional confounding factors on the immune measures and analysis results (e.g., potential wild-type rubella virus exposure in deployed military personnel from the San Diego cohort, type of rubella-containing vaccine/vaccines received in the past [MMR, MMRV, MMR+V], or other vaccines that subjects might have received concurrently with the last MMR vaccination for the San Diego cohort, as well as pre-vaccination titers). For example, it is known that yellow fever vaccine given concurrently with MMR reduces immune responses to some of the MMR components (including rubella), and MMRV (measles-mumps-rubella-varicella vaccine) or MMR + V given concurrently, induces significantly different rubella-specific post-immunization titers compared to MMR administered alone [52, 53]. These factors were well controlled and uniform for the Rochester cohort (U.S. born children/adolescents from Olmsted county, MN, with two and only two documented doses of MMR vaccine, and no wild-type rubella virus circulating in the geographical location etc.). As members of the U.S. military, the San Diego cohort represents a cross section of the U.S. population with proven vaccine-induced immunity to MMR and a documented receipt of MMR vaccine; however, the number of vaccine doses and timing of vaccinations are unknown for most of the subjects (unlike the Rochester cohort), as it is difficult to get exact vaccination record data from adults. The potential confounding effects of these unknown variables (for the San Diego cohort) on the study results are alleviated by the use of two independent (with varying age, sex distribution, racial distribution, geographical location, vaccination history etc.) cohorts to confirm the findings

(i.e., the discovery and replication study design). Furthermore, Davidkin *et al.* [51] present data on MMR vaccine-induced immunity throughout a 15-year follow-up, demonstrating that the second MMR booster vaccine had little effect other than a short-term (< one year) boost in rubella-specific antibody titer, followed by a decline over time that paralleled the pre-boost decline in antibody titer with only a slight upward adjustment of the average titer.

Despite the availability of rubella vaccine for more than four decades, rubella and congenital rubella syndrome/CRS continue to cause considerable morbidity, particularly in the developing countries, with recent resurgence of rubella cases in some developed countries. Our rubella vaccine study provides evidence for racial/ethnic differences in the magnitude of humoral immune response (neutralizing antibodies) following rubella vaccination. Further studies will aim at explaining and deciphering the genetic factors, biological processes and pathways involved in forming and maintaining the humoral and cellular immune responses following vaccination in different racial/ethnic groups. Such knowledge may be used to design and develop better vaccines for individuals and/or racial/ethnic groups through increasing vaccine efficacy and reducing vaccine adverse events.

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Highlights

- !! We studied demographic influences on rubella vaccine-induced immunity
- !! The discovery findings were verified through replication in an independent cohort
- !! African-Americans had higher neutralizing antibody titers compared to Caucasians
- !! Rubella vaccine-specific humoral immunity significantly varies by race

Figure 1.

Plots of genetic similarity according to PCA-based axes of genetic variation: A and B for the San Diego cohort, C and D for the Rochester cohort. Ultimate genetic groupings are shown by different symbols/colors and illustrate the consistent clustering of racial and ethnic groups. African-American and admixed African-American clusters were combined in analyses, but are shown in different symbols/colors for the Rochester cohort to highlight the differences between the admixed African-American and Somali groups.

Figure 1(A and B) reprinted with permission from Human Genetics [21].

Table 1

Demographic and immunological characteristics of the study subjects

	<i>Rochester (Discovery) (N=1052)</i>	<i>San Diego (Replication) (N=942)</i>
Age at Enrollment		
Median, IQR (Years)	15 (13, 17)	24 (22, 27)
Age at Last Vaccination (Years)		
Median, IQR ^a	9 (5, 12)	19 (18, 22)
Time from Last Vaccination to Enrollment		
Median, IQR (Years)	6.4 (4.6, 8.6)	3.0 (2.2, 4.0)
Sex (N, %)		
Male	578 (54.9%)	683 (72.5%)
Female	474 (45.1%)	259 (27.5%)
Race, genetically-determined (N, %)^a		
African American	62 (5.9%)	190 (20.2%)
Caucasian	897 (85.3%)	506 (53.7%)
Hispanic	0 (0.0%)	198 (21.0%)
Other	58 (5.5%)	48 (5.1%)
Somali	35 (3.3%)	0 (0.0%)
Ethnicity, genetically-determined (N, %)^a		
Not Hispanic or Latino	1014 (96.4%)	686 (73.9%)
Hispanic or Latino	0 (0.0%)	198 (21.0%)
Unknown	38 (3.6%)	48 (5.1%)
Neutralizing Antibodies (NT₅₀)		
Median, IQR	57 (35, 96)	66 (44, 113)
IL-6 (pg/mL)		
Median, IQR	3596 (3032, 4008)	4122 (3529, 4791)
IFN-γ (pg/mL)		
Median, IQR	6 (2, 20)	-1 (-6, 3)

^a Race/ethnicity determined by principal component analysis, as described in the Methods section. PCA-defined groupings for the Rochester cohort: "African-American" (combining "African-American" and "African-American Admixed"), Caucasians, Somali and "Other" group. PCA-defined groupings for the San Diego cohort: "African-American", Caucasians, Hispanics and "Other" group.

Table 2

Associations between race/ethnicity and rubella vaccine-induced immune responses

Rochester (Discovery)				
Immune Outcome	Race/ Ethnicity	N	Median (IQR) ^a	Adjusted p-value ^b
Neutralizing antibodies	AA	61	77.30 (49.17, 112.23)	0.0007
	Cauc	889	55.43 (34.43, 91.30)	
	Other	58	54.08 (33.67, 96.13)	
	Somali	34	118.64 (71.70, 276.00)	
Secreted IL-6	AA	56	2958.04 (2335.48, 4095.06)	0.0604
	Cauc	856	3629.28 (3095.35, 4003.81)	
	Other	57	3439.32 (3114.28, 4006.29)	
	Somali	32	2807.82 (2111.63, 4072.40)	
Secreted IFN γ	AA	56	3.17 (−0.77, 13.23)	0.8365
	Cauc	839	6.37 (1.66, 19.70)	
	Other	56	4.48 (0.59, 14.41)	
	Somali	31	22.81 (4.29, 73.87)	
San Diego (Replication)				
Neutralizing antibodies	AA	189	86.20 (56.20, 130.60)	<.0001
	Cauc	506	61.85 (41.50, 105.60)	
	Hisp	198	61.17 (39.23, 100.90)	
	Other	48	76.03 (56.60, 163.83)	
Secreted IL-6	AA	182	4195.05 (3479.27, 4753.44)	0.9547
	Cauc	481	4126.64 (3508.78, 4812.69)	
	Hisp	194	4027.82 (3580.33, 4684.85)	
	Other	47	4441.94 (3612.45, 5024.20)	
Secreted IFN γ	AA	178	−0.69 (−6.36, 3.44)	0.3330
	Cauc	470	−1.75 (−6.37, 2.99)	
	Hisp	190	−1.15 (−6.40, 3.60)	
	Other	45	−0.43 (−3.59, 5.37)	

^aIQR, inter-quartile range with 25% and 75% quartiles^b Calculated using linear (for antibodies) and linear mixed models (secreted cytokines) while adjusting for the potentially confounding variables of sex, age at enrollment, vaccination history (age at most recent immunization and time since last immunization to blood draw), batch/mn number of immune assay used to measure immune outcome. Analysis for neutralizing antibody titers were performed using log2 transformed NT50 measurements.

Table 3

Associations between sex and rubella vaccine-induced immune responses

Rochester (Discovery)				
Immune Outcome	Sex	N	Median (IQR) ^a	Adjusted p-value ^b
Neutralizing antibodies	Females	469	59.03 (35.20, 94.90)	0.5673
	Males	573	56.20 (34.43, 96.60)	
Secreted IL-6	Females	452	3616.51 (3061.40, 3987.92)	0.0321
	Males	549	3590.37 (3015.27, 4024.21)	
Secreted IFN γ	Females	440	6.99 (1.69, 20.10)	0.0862
	Males	542	5.11 (1.34, 20.46)	
San Diego (Replication)				
Neutralizing antibodies	Females	258	63.72 (39.90, 102.07)	0.0994
	Males	683	67.33 (44.77, 116.33)	
Secreted IL-6	Females	237	3963.90 (3441.15, 4571.73)	0.0043
	Males	667	4164.30 (3540.72, 4891.39)	
Secreted IFN γ	Females	234	−1.61 (−7.84, 3.18)	0.2975
	Males	649	−1.25 (−5.76, 3.14)	

^aIQR, inter-quartile range with 25% and 75% quartiles^bCalculated using linear (for antibodies) and linear mixed models (secreted cytokines) while adjusting for the potentially confounding variables of race, age at enrollment, vaccination history (age at most recent immunization and time since last immunization to blood draw), batch/run number of immune assay used to measure immune outcome. Analysis for neutralizing antibody titers were performed using log2 transformed NT₅₀ measurements.