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Characterization of humoral and cellular immunity to rubella vaccine in four distinct cohorts

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Abstract Although vaccination campaigns have significantly reduced the global burden of rubella disease, there are still regional outbreaks and cases of congenital rubella syndrome. Rubella vaccination elicits a strong humoral as well as cellular response. The relationship between these two measures in response to rubella vaccine is poorly understood. We have previously reported no correlation between rubella-virus-specific cytokine secretion and IgG antibody levels after rubella vaccination. In the current study, we extend our previous work to report correlations between secreted cytokines and functional neutralizing antibodies after rubella vaccination in four distinct cohorts. There was evidence of significant differences ($p < 0.05$) in rubella-virus-specific humoral and cellular responses between cohorts. When investigating relationships between rubella-vaccine-specific humoral and cellular immunity, we observed a significant correlation between neutralizing antibodies and IFN-γ ($r_s = 0.21$, $p = 0.0004$). We also observed correlations in subjects with extreme humoral immune phenotypes and IFN-γ levels in two of the four cohorts ($r_s = 0.32$, $p = 0.01$; $r_s = 0.36$, $p = 0.01$, respectively). These findings indicate that there is a high level of heterogeneity in rubella-specific immune responses between study populations. We believe that the novel correlation discovered between IFN-γ and neutralizing antibody titers will give future insight into the functional mechanisms of immunity induced by rubella virus and other live viral vaccines.

Keywords Polymorphism, single nucleotide · Cytokines · Receptors, cytokine · Antibodies · Measles–mumps–rubella vaccine · Immunity · Immunity, humoral · Immunity, cellular

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Introduction

The year 2012 witnessed the highest number of rubella cases in England and Wales since 1999 [1]. In the first half of 2013, Poland reported 21,283 rubella cases and Japan recorded 5,442 cases [2, 3]. With these few examples of the resurgence of rubella cases comes the threat of larger outbreaks and exposure to the most at-risk population—unvaccinated pregnant women. The World Health Organization has targeted rubella for elimination from Europe, the Americas, and the Western Pacific Regions [4]. Vaccine campaigns have eliminated some areas of endemic rubella. However, recent increases in sporadic nationwide epidemics and a concomitant increase in the cases of congenital rubella syndrome (CRS) emphasize the importance of these campaigns.

Neutralizing antibodies are considered the best correlate of protection against rubella [5]. However, there is evidence of false-positive results using antibody-based assays due to the presence of infectious mononucleosis, Rh factor, and parvovirus infection [6–8]. Also, individuals with sub-protective titers (≤15 IU) of serum IgG antibodies may still be protected against rubella, which is likely due to cellular immunity [9]. Thus, the measurement of rubella-specific

humoral immunity as a correlate may not be ideal under certain conditions. Cellular immunity measures to rubella virus vaccine include a lymphoproliferative response and the production of inflammatory cytokines, such as IFN-γ, IL-6, and TNF-α [10–13]. It is not well understood what the relationship is between cellular and humoral immune responses and how they correlate with protection.

In this study, we characterized rubella-virus-specific humoral and cellular immune responses in four separate cohorts that received the measles–mumps–rubella-II (MMR-II) vaccine. We also examined correlations between neutralizing antibodies and inflammatory cytokines (IL-6 and IFN-γ). The objective of this study was to identify differences and commonalities in immune responses between cohorts and to elucidate potential correlates of rubella-vaccine-specific immunity.

Methods

Study participants

The following methods are similar or identical to our previous published studies [13–20].

The study cohorts were comprised of healthy children, older adolescents, and adults (age at enrollment of 11–40 years) who resided in Rochester, MN, and San Diego, CA, with clinical and demographic characteristics previously reported [16, 21, 22] (see Table 1 for demographics).

The Rochester cohorts (Rochester 1, 2, and 3) consisted of individuals from three independent cohorts of healthy schoolchildren and young adults from all socioeconomic strata in Rochester, MN. Specifically, between December 2001 and August 2002, we enrolled 346 healthy children (age 12–19 years, Rochester 1). A detailed description of Rochester 1 has been published elsewhere [23, 24]. Between December 2006 and August 2007, we enrolled 440 healthy children (age 11–18 years, Rochester 2), as previously published [14, 16]. In November 2008–September 2009, we enrolled 388 healthy children, enriched with African American youth (age 11–22 years, Rochester 3) [25, 26]. All participants had written records of receiving two doses of age-appropriate measles–mumps–rubella (MMR II, Merck) vaccine.

In July 2005–September 2006, we enrolled an additional 1,076 healthy older adolescents and healthy young adults (age 18–40 years, San Diego cohort) from armed forces personnel in San Diego, CA. Subject enrollment for this study has been previously described in detail [21, 22]. We recruited these individuals because they were active military personnel who were recently vaccinated with smallpox vaccine. As members of the U.S. military, they represent a cross section of the U.S. population. The Institutional Review Boards of the Mayo Clinic and Naval Health Research Center approved this study, and written informed consent was obtained from each subject or from the parents of all children who participated in the study; written assent was obtained from age-appropriate participants.

Rubella-virus-specific cytokine secretion

The levels of secreted cytokines following stimulation of isolated PBMCs with live rubella virus were measured, as previously described by our group [13, 27]. Briefly, 2×10^5/mL PBMCs were stimulated with the W-Therien strain of rubella virus (a gift from Dr. Teryl Frey, Georgia State University, Atlanta, GA) with optimized multiplicity of infection (MOI) and incubation times depending on the specific cytokine measured. For the measurement of IL-6 and IFN-γ, PBMCs were stimulated with an MOI of 5. The supernatants were removed post-stimulation at 24 h for IL-6 and 48 h for IFN-γ. All samples were stored at −80 °C until assayed. Cytokine levels were quantified using BD Opt-EIA™ Human ELISA kits, and absorbance levels were measured using a Molecular Devices SpectraMax 340PC^{384}.

Rubella-specific neutralizing antibodies

The levels of neutralizing antibodies against live rubella virus were measured using a soluble immunocolorimetric assay (sICA) that was adapted for high throughput [28]. Briefly, serial dilutions of subject sera were incubated with the rubella vaccine virus strain HPV77. After incubation, the virus/sera mixture was added to Vero cells cultured in a flat-bottomed 96-well plate and incubated for 72 h at 37 °C, 5 % CO_2. Then, cells were fixed in cold methanol for 10 min and blocked with PBS supplemented with 5 % skim milk (Difco; BD, NJ, USA) and 0.1 % Tween 20 for 30 min and then washed three times with PBS supplemented with 0.05 % Tween 20 (PBS-T). Fixed cells were incubated with anti-E1 glycoprotein (CDC, GA, USA) for 30 min and washed three times with PBS-T. The secondary goat anti-mouse HRP-conjugated antibody (Invitrogen, CA, USA) was added for 30 min. Plates were washed again, and antibody conjugate was visualized by adding NeA-Blue TMB substrate (Clinical Science Products, MA, USA) for 10 min. The optical density (OD) values were read at 450/630 nm on an Eon microplate spectrophotometer (BioTek, VT, USA). The Loess method of statistical interpolation was used to estimate neutralization titers (NT_{50}) from observed values [29].

Statistical analysis

The differences between the median values of the stimulated observations and the median values of the

Table 1 Study cohorts demographics

Study demographics					
	Rochester 1	Rochester 2	Rochester 3	San Diego	Total
Age at enrollment					
*N**	318	380	341	986	2,025
Mean (SD)	15.6 (2.0)	14.5 (2.3)	15.2 (2.3)	25.1 (4.5)	20.0 (6.2)
Median	16	15	15	24	19
Q1, Q3	14.0, 17.0	13.0, 17.0	13.0, 17.0	22.0, 27.0	15.0, 24.0
Range	(12.0–19.0)	(11.0–18.0)	(11.0–22.0)	(19.0–40.0)	(11.0–40.0)
Age at most recent vaccination					
*N**	318	380	341	698	1,737
Mean (SD)	10.9 (2.3)	7.8 (3.4)	6.8 (3.2)	20.6 (3.4)	13.3 (6.9)
Median	12	8	5	19	12
Q1, Q3	11.0, 12.0	5.0, 11.0	4.0, 10.0	18.0, 22.0	7.0, 19.0
Range	(2.0–17.0)	(1.0–14.0)	(1.0–15.0)	(17.0–39.0)	(1.0–39.0)
Time since most recent vaccination to enrollment					
*N**	318	380	341	698	1,737
Mean (SD)	4.7 (2.4)	6.7 (2.4)	8.4 (2.7)	3.4 (1.7)	5.3 (3.0)
Median	4.8	6.7	8.5	3	4.9
Q1, Q3	2.8, 6.1	4.9, 8.2	6.4, 10.3	2.2, 4.0	2.9, 7.4
Range	(0.6–12.3)	(0.4–15.4)	(0.6–16.8)	(0.1–8.6)	(0.1–16.8)
Gender					
Male	170 (53.5 %)	209 (55.0 %)	195 (57.2 %)	723 (73.3 %)	1,297 (64.0 %)
Female	148 (46.5 %)	171 (45.0 %)	146 (42.8 %)	263 (26.7 %)	728 (36.0 %)
Self-declared race					
American Indian or Alaska Native	2 (0.6 %)	2 (0.5 %)	0 (0.0 %)	19 (1.9 %)	23 (1.1 %)
Asian, Hawaiian or Pacific Islander	7 (2.2 %)	15 (3.9 %)	5 (1.5 %)	48 (4.9 %)	75 (3.7 %)
Black or African American	4 (1.3 %)	4 (1.1 %)	76 (22.3 %)	162 (16.4 %)	246 (12.1 %)
White	299 (94.0 %)	337 (88.7 %)	247 (72.4 %)	530 (53.8 %)	1,413 (69.8 %)
Multiple	6 (1.9 %)	14 (3.7 %)	8 (2.3 %)	82 (8.3 %)	110 (5.4 %)
Other	0 (0.0 %)	2 (0.5 %)	5 (1.5 %)	126 (12.8 %)	133 (6.6 %)
Unknown	0 (0.0 %)	6 (1.6 %)	0 (0.0 %)	19 (1.9 %)	25 (1.2 %)

* The total number of subjects included in this study is determined by the availability of demographic data and immune measures

unstimulated observations were determined for IL-6 and IFN-γ, and these measures were used in analysis. Distributions of outcomes and demographics were described with counts and percentages for categorical variables, with measures of central tendency (means and medians), and with variability (range, interquartile ranges, and standard deviation) for continuous variables. Wilcoxon rank sum tests were utilized to ascertain whether differences in cytokine and neutralizing antibody levels existed between cohorts. Spearman correlations were employed to investigate associations between neutralizing antibody titers and levels of both IL-6 and IFN-γ, with and without controlling for age; age at most recent recorded vaccination; years from most recent recorded vaccination to enrollment age; gender; self-declared race and ethnicity. Correlations were then repeated within a subset of 224 "extreme responders," defined as individuals who exhibited no meaningful neutralizing antibody response (NT_{50} estimated as <25; 18 subjects from Rochester 1, 34 from Rochester 2, and 30 each from Rochester 3 and San Diego), and an equal number of individuals from within each cohort with the highest NT_{50} values.

Results

Humoral and cellular responses to rubella vaccine across cohorts

The median neutralizing antibody titer (NT_{50}) was 64.1 (IQR; 38.4, 107.2) for Rochester 1; 54.2 (IQR; 33.9, 88.0) for Rochester 2; 57.2 (IQR; 34.0, 93.9) for Rochester 3;

and 67.0 (IQR; 44.0, 114.5) for the San Diego cohort (Fig. 1). There were statistically significant ($p \leq 0.05$) differences in median NT_{50} for all comparisons except Rochester 2 and Rochester 3 ($p = 0.3$). Secreted cytokine levels were calculated as the difference between the median of rubella-virus-stimulated replicates minus the median of the unstimulated replicate values. The median of the differences for IL-6 production across study cohorts was 3,910.3 pg/mL (IQR; 3,622.3, 4,220.1) for Rochester 1; 3,378.9 pg/mL (IQR; 2,874.3, 3,817.7) for Rochester 2; 3,328.0 pg/mL (IQR; 2,771.4, 3,829.8) for Rochester 3; and 4,117.4 pg/mL (IQR; 3,521.8, 4,787.9) for the San Diego cohort. The median of the differences for IFN-γ production for Rochester 1 was 7.9 pg/mL (IQR; 3.2, 23.4), Rochester 2 was 9.3 pg/mL (IQR; 2.3, 23.5), Rochester 3 was 1.9 pg/mL (IQR; −0.9, 9.3), and the San Diego cohort was −1.4 pg/mL (IQR; −6.4, 3.1). There were also statistically significant differences in rubella-virus-induced secreted cytokine levels (IL-6 & IFN-γ) between study cohorts. IL-6 levels were significantly different ($p \leq 0.0001$) between all cohorts, except in the Rochester 2 and Rochester 3 cohorts ($p = 0.7$). IFN-γ levels were also remarkably different between all cohorts ($p \leq 0.0001$), except for Rochester 1 and Rochester 2 ($p = 0.4$).

Correlation between neutralizing antibodies and secreted cytokines

Correlation analyses revealed a correlation between serum neutralizing antibodies and IFN-γ secretion after rubella vaccination in Rochester 3 ($r_s = 0.2084$, $p = 0.0004$) and a trend toward significance after adjustment in Rochester 2 ($r_s = 0.0979$, $p = 0.06$). There was also a correlation between IL-6 and NT_{50} in Rochester 1 ($r_s = 0.1045$, $p = 0.08$) that approached significance (Table 2). The observed correlation between IFN-γ and NT_{50} in two cohorts suggests that this cellular immune marker may be further investigated as a co-correlate of protection. The low-level correlation between IL-6 and NT_{50} was only observed in one cohort, making it difficult to speculate what the biological significance is for the correlations between this inflammatory cytokine and NT_{50}.

Next, we investigated correlations between the extremes of NT_{50} with rubella-virus-specific secreted cytokine levels

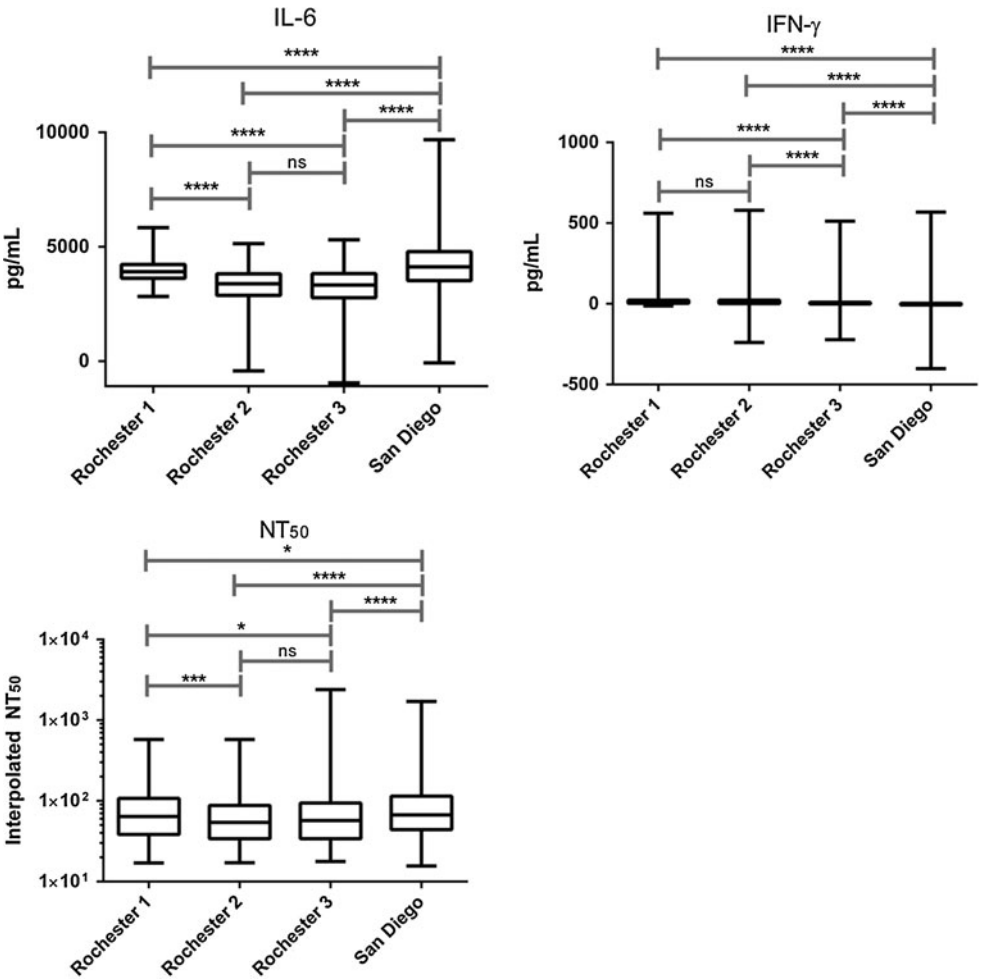

Fig. 1 Distribution of humoral and cellular immune responses across cohorts. *Box plots* comparing secreted cytokine levels and NT_{50} across cohorts. Rubella-specific IL-6 (Rochester 1 $n = 297$, Rochester 2 $n = 380$, Rochester 3 $n = 311$, San Diego $n = 946$) and IFN-γ (Rochester 1, $n = 297$, Rochester 2, $n = 380$, Rochester 3 $n = 292$, San Diego $n = 925$) levels were measured by ELISA and reported as the difference between rubella-virus-stimulated and unstimulated values. NT_{50} levels (Rochester 1, $n = 318$; Rochester 2, $n = 373$; Rochester 3, $n = 338$; San Diego, $n = 985$) were calculated using interpolated data from a high-throughput soluble immunochemical assay. There were significant differences in immune measures across cohorts. However, Rochester 2 and Rochester 3 displayed strikingly similar median values of NT_{50} and IL-6 (*ns* not significant; $^*p \leq 0.05$; $^{***}p \leq 0.001$; $^{****}p \leq 0.0001$)

Table 2 Summary of correlations between rubella-specific cellular immune measures and neutralizing antibodies

Cohort	Cytokine (pg/mL)	Simple correlation (95 % CI)	Adjusted correlation (95 % CI)
Rochester 1			
	IFN-γ	0.0809 (−0.0334, 0.1928)	0.07 (−0.0454, 0.1834)
	IL-6	0.1162 (0.0023, 0.2269)	0.1045 (−0.0108, 0.2167)
Rochester 2			
	IFN-γ	0.1148 (0.0132, 0.2137)	0.0979 (−0.0046, 0.1982)
	IL-6	0.0181 (−0.0836, 0.1194)	0.0187 (−0.0839, 0.1208)
Rochester 3			
	IFN-γ	**0.2637 (0.1525, 0.3675)**	**0.2084 (0.0937, 0.317)**
	IL-6	−0.0918 (−0.2013, 0.0203)	−0.0629 (−0.1781, 0.0542)
San Diego			
	IFN-γ	0.0259 (−0.0386, 0.0903)	0.0218 (−0.0554, 0.0988)
	IL-6	−0.0516 (−0.115, 0.0122)	−0.0538 (−0.1304, 0.0234)

Correlations between in vitro secreted cytokine levels and serum NT_{50} using Spearman correlation of coefficients. Simple correlations are unadjusted for confounding factors. The Adjusted correlations account for age, age at most recent recorded vaccination, years from most recent recorded vaccination to enrollment, gender, self-declared race, and ethnicity. Bolded text represents a significant correlation between IFN-γ and NT_{50} (p value = 0.0004)

Table 3 Correlation between IFN-γ levels and NT_{50} extremes

Cohort	*N*	Cytokine (pg/mL)	Simple correlation (95 % CI)	Adjusted correlation (95 % CI)
Rochester 1				
	36	IFN-γ	0.1666 (−0.1959, 0.485)	0.07 (−0.2172, 0.5346)
		IL-6	0.1954 (−0.1676, 0.5071)	0.1136 (−0.2884, 0.4781)
Rochester 2				
	68	IFN-γ	**0.3392 (0.1072, 0.5326)**	**0.3197 (0.0733, 0.5254)**
		IL-6	0.0072 (−0.2317, 0.2452)	−0.015 (−0.2636, 0.2358)
Rochester 3				
	60	IFN-γ	**0.4999 (0.2661, 0.673)**	**0.3606 (0.0877, 0.5782)**
		IL-6	−0.2452 (−0.4756, 0.0211)	−0.1512 (−0.4109, 0.1343)
San Diego				
	60	IFN-γ	0.2192 (−0.0484, 0.4542)	0.2207 (−0.1865, 0.5572)
		IL-6	−0.1462 (−0.3929, 0.1226)	0.0572 (−0.3386, 0.434)

Correlations between in vitro rubella-specific secreted cytokine levels and serum NT_{50} extremes using Spearman correlation of coefficients. Simple correlations are unadjusted for confounding factors. The Adjusted correlations account for age, age at most recent recorded vaccination, years from most recent recorded vaccination to enrollment, gender, self-declared race, and ethnicity. Bolded text highlights a moderate, positive relationship between NT_{50} extremes and IFN-γ levels (p value <0.0001 for Rochester 2 and Rochester 3)

(Table 3). The extremes were defined as the observations for those people who had no meaningful neutralizing antibody response (NT_{50} <25) and an equal number of people with the highest neutralizing antibody response in each cohort ($N = 224$). The median NT_{50} in the highest response group was 272.0, compared with 22.5 in the lowest responders ($p \leq 0.0001$). There were also significant differences in IFN-γ levels, with a median of 9.8 pg/mL in the high group and 2.3 pg/mL in the low group ($p \leq 0.0001$). When investigating correlations between IFN-γ levels and NT_{50} in the separate cohorts, we discovered a moderate positive association in Rochester 2 ($r_s = 0.3197$, $p = 0.01$) and Rochester 3 ($r_s = 0.3606$, $p = 0.01$).

Discussion

When measuring humoral and cellular responses to rubella vaccine, we observed statistically significant differences in NT_{50}, IL-6, and IFN-γ between cohorts. However, this was not true for all comparisons. Specifically, the Rochester 2 and Rochester 3 cohorts exhibited very comparable rubella-specific NT_{50} and IL-6 levels; Rochester 1 and Rochester 2 cohorts displayed similar levels of IFN-γ. A significant correlation between NT_{50} and IFN-γ levels was observed for Rochester 3 with a trend in the same direction for Rochester 2. This association was strengthened when our focus shifted to the extreme responders in these cohorts, indicating a relationship between a strong IFN-γ response and a strong humoral response to rubella virus.

The immune response to vaccination is influenced by numerous environmental and host factors [30–33]. Therefore, it is not surprising that we observed differences in immune responses across cohorts. Although all individuals received the same rubella-containing MMR-II vaccine, there were other confounding attributes that could potentially influence immunity. Three of the Rochester cohorts were recruited as residents of Olmsted County, MN, while the fourth (San Diego) represents military personnel from all over the United States (see "Study participants"). There were differences in age at vaccine, age at enrollment, and time since last recorded vaccine event. We have previously

reported a high level of heritability (46 %) in rubella-specific antibody titers [32] and discovered numerous host genetic factors associated with differences in immunity to rubella vaccination [14–17, 34–36]. The differences and similarities observed between these cohorts highlight that other factors not associated with host genetic composition may influence immunity after rubella vaccination. In fact, a model aimed at defining all contributing factors to inter-individual differences in rubella-specific immunity will incorporate host, viral, and environmental factors. The ideal population-based study will target recruitment efforts to avoid disparities in these variables or use statistical methodology to adjust, such as those used in this study.

Measuring humoral immunity is considered the gold standard as a correlate of protection against rubella virus, and protective humoral immunity is defined as any measurable antibody titer $\geq$10 IU/mL [37]. Vaccination with two doses of the RA27/3 strain of rubella virus induces a protective level of immunity that lasts >20 years [38, 39]. The estimated half-life for rubella-vaccine-specific antibodies is $\sim$114 years [40]. However, there is evidence of waning immunity over time. Rubella-specific neutralizing antibody titers 12-year post-vaccination can be one-half the original values [41]. Our current study revealed lower median values for NT_{50} in Rochester 2 (NT_{50} 54.2) and Rochester 3 (NT_{50} 57.2). The demographic data are strikingly similar between these two cohorts (Table 1). The median age at enrollment was identical between the two cohorts (15.0 years), with similar median age ranges at most recent vaccination event, and time elapsed since last recorded vaccination event. Although waning immunity may contribute to the lower NT_{50} in these cohorts, it is difficult to assess the exact influence in this study because: (1) there are no complete data available representing antibody titers shortly after vaccination in our cohorts; (2) unlike other rubella-specific antibody assays, our interpolated NT_{50} values were not calibrated against a standardized sample, such as the WHO 2nd International Standard Preparation for Anti-Rubella Serum. To address the second concern, we are currently testing for correlations between NT_{50} and those values obtained with Beckman Coulter's Access® Rubella IgG Assay [42]. This commercial assay is calibrated against a standardized serum sample. Initial analyses reveal a strong correlation between total IgG and NT_{50} titers (unpublished data). It is uncertain what the mechanism is behind the lower levels of secreted IL-6 in the Rochester 2 and 3 cohorts. This may, in part, be due to a combination of the multiple similarities noted between these two cohorts and not necessarily attributable to waning cellular immunity.

To our knowledge, this is the first study to investigate correlations between functional neutralizing antibodies against rubella virus and in vitro secreted cytokine levels. We previously observed no correlation between rubella-specific IgG EIA and secreted cytokine levels in vaccinated subjects [43]. The difference between this study and previous findings is that we found positive correlations between rubella-specific IFN-γ and NT_{50} in one cohort, which was then amplified when analyzing antibody response extremes. We conclude that this correlation may be lost when investigating across a large cohort, but the relationship is revealed when the study focus is directed toward those subjects that were characterized as having an extreme humoral response to rubella vaccination.

It is difficult to interpret the statistically significant findings into a more biologically relevant context. We have previously characterized the cellular immune response to rubella as "skewed" toward a proinflammatory response with high levels of IL-6, GM-CSF, and TNF-α [13] and moderate levels of IL-2 and IFN-γ. Intuitively, a robust Th1/proinflammatory response may lead to attenuation of the humoral response through such mechanisms as suppression of IL-4 production [44]. However, studies have revealed a synergistic relationship between IFN-γ and humoral immunity. In mice, IFN-γ enhances the secretion of IgG2A in vitro and in vivo [45, 46]. There is also evidence that IFN-γ can induce a viral-specific memory T cell to adopt a Th1/Th2 combined phenotype that could potentially contribute to a robust humoral and cellular response [47]. The contributing role of IFN-γ in humans on antibody production remains unclear. The importance of IFN-γ production in response to live viral vaccination is highlighted by the discovery that measles infection in infants with a lack of humoral response after vaccination is attenuated, and this is due to IFN-γ production by $CD4^+$ T cells. The measurement of IFN-γ is considered a correlate of protection in tuberculosis and malaria vaccine models [48–50, 51]. We propose that IFN-γ may be used, alongside humoral measures, as a co-correlate of protection in rubella vaccine studies. Moreover, the amplification of the correlation between IFN-γ and NT_{50} when analyzing extreme immune outcomes suggests that vaccination in high responders induces a robust humoral and cellular response.

In summary, we detected significant differences in humoral and cellular immune response between cohorts, but a correlation between IFN-γ and neutralizing antibodies. This is the first step toward validating that IFN-γ may be used as a co-correlate of protection against rubella virus. These data may give insight into future mechanisms behind inter-individual differences in response to vaccination against rubella and other live viral vaccines. The next generation of rubella vaccine candidates may be constructed to elicit a strong IFN-γ and antibody response regardless of genetic and other environmental influences.

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Conflict of interest 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. 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.

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