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Rubella virus-specific humoral immune responses and their interrelationships before and after a third dose of measles-mumps-rubella vaccine in women of childbearing age

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Abstract

In the U.S., measles, mumps, and rubella vaccination is recommended as two vaccine doses. A third dose of measles-mumps-rubella (MMR) vaccine is being administered in certain situations (e.g., identified seronegativity and during outbreaks).

We studied rubella-specific humoral immunity (neutralizing antibody, enzyme-linked immunosorbent assay/ELISA IgG titer and antibody avidity) and the frequencies of antigen-specific memory B cells before and after a third dose of MMR-II in 109 female participants of childbearing age (median age, 34.5 years old) from Olmsted County, MN, with two documented prior MMR vaccine doses. The participants were selected from a cohort of 1,117 individuals if they represented the high and the low ends of the rubella-specific antibody response spectrum. Of the 109 participants, we identified four individuals (3.67 % of all study participants; 7.14 % of the low-responder group) that were seronegative at Baseline (rubella-specific ELISA IgG titers < 10 IU/mL), suggesting a lack of protection against rubella before receipt of a third MMR vaccine dose. The peak geometric mean neutralizing antibody titer one month following the third dose of MMR vaccine for the cohort was 243 NT₅₀ (CI; 241, 245), which is expected for a cohort with two doses of MMR, and the peak geometric mean IgG titer was 150 IU/mL (CI; 148, 152) with no seronegative individuals at Day 28. One-third of all subjects (31.8% for the neutralizing antibody;

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Competing Interests

Dr. Poland is the chair of a Safety Evaluation Committee for novel investigational vaccine trials being conducted by Merck Research Laboratories. Dr. Poland offers consultative advice on vaccine development to Merck & Co. Inc., Avianax, Adjuvance, Valneva, Medicago, Sanofi Pasteur, GlaxoSmithKline, and Emergent Biosolutions. Drs. Poland and Ovsyannikova hold three patents related to measles and vaccinia peptide research. Dr. Kennedy holds a patent on vaccinia peptide research. Dr. Kennedy has received funding from Merck Research Laboratories to study waning immunity to measles and mumps after immunization with MMR-II®. All other authors declare no competing financial interests. 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.

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30.8% for the IgG titer) experienced a significant boost (4-fold) of antibody titers one month following vaccination. Antibody titers and other tested immune-response variables were significantly higher in the high-responder group compared to the low-responder group. The frequencies of rubella-specific memory B cells were modestly associated with the antibody titers. Our study suggests the importance of yet unknown inherent biologic and immune factors for the generation and maintenance of rubella-vaccine-induced humoral immune responses.

Keywords

Rubella; Rubella Vaccine; Measles-Mumps-Rubella Vaccine; Immunity, Humoral; Antibody; Viral

Introduction

Rubella is a mild, self-limited viral disease in children, but it is of public health importance given its devastating consequences when infection occurs during pregnancy (e.g., fetal death, miscarriage, stillbirth, and a wide range of fetal/infant birth defects, including those classified as congenital rubella syndrome [CRS]). Approximately 100,000 CRS cases occur worldwide every year [1]. Current rubella vaccines are considered effective and typically provide seroconversion rates above 95%—with vaccine effectiveness of approximately 95%—after a single dose of vaccine [1-3].

Nevertheless, sporadic rubella cases and outbreaks throughout Europe, Japan, South America, and to some extent the United States still occur despite the immunization programs in place (although vaccination/immunity gaps have been noted for some of the listed regions). Rubella and CRS have been eliminated in the United States since 2004; however, the United States reported an average of 10 imported or import-related rubella cases per year between 2004 and 2011. A total of 77 rubella cases and 4 CRS cases with a median age of infected individuals of 29 years were reported [4, 5]. Of these cases, 12% were in vaccinated individuals [5]. While the genesis of most of these cases and outbreaks is importation of disease and failure to vaccinate, vaccine failure or waning immunity is increasingly recognized as an important issue for which little data are available [6-14]. Previous studies report that between 1.8% and 17% of immunized individuals have low rubella-specific antibody titers consistent with a lack of protection approximately 7–20 years after the administration of a second dose of rubella-containing vaccine [7, 14-16]. Although uncommon, viral transmission, fetal infection, and CRS have been documented after maternal vaccination [17-19]. This raises concerns about rubella-specific herd immunity [20], especially in a population with low rubella incidence in which boosting from circulating rubella virus is unlikely to occur.

A third dose of **measles-mumps-rubella** (MMR) vaccine is now being administered in certain situations, such as during mumps or measles outbreaks. In these cases, and in the case of rubella-specific seronegativity in young women and pregnant women, vaccine is administered even after two or more previously documented vaccine doses. For pregnant women, vaccine is administered postpartum. Further knowledge regarding rubella vaccine immunogenicity in such instances, as well as identifying the factors associated with high or

low antibody response, is necessary to achieve and maintain protection. Rubella virus neutralizing antibody responses were recently reported in a group of 679 young adults (18–31 years old) after a third dose of MMR vaccine [15]. A small proportion of study participants (1.8%) had neutralization titers < 10 U/mL before the third MMR administration and more than 50% experienced a 4-fold rise in neutralizing antibody titer one month following the third MMR vaccine [15]. We extended the findings of this study to a comprehensive assessment of rubella virus-specific humoral immune response outcomes, including neutralizing antibody response, antibody avidity, and memory B cell response, before and after a third dose of MMR in 109 women of childbearing age from Olmsted county, MN, and surrounding areas. Neutralizing antibody titers reflect the functional antibody ability to rapidly neutralize the virus, while the frequencies of antigen-specific memory B cells reflect the specific B cell ability to respond to subsequent antigen encounters with enhanced immune response kinetics and efficacy. We specifically designed the cohort to include individuals from the local community with the highest or the lowest antibody titers in order to evaluate the importance of inherent biology in vaccine response to a 3rd dose of MMR vaccine.

The hypothesis of this study was that pre-existing immunity and inherent biology factors, along with other demographic/clinical variables (e.g., age at enrollment, age at 1st and 2nd rubella vaccination, and time from 2nd rubella vaccination to enrollment), might have an impact on the response to a third dose of MMR vaccine. Baseline immune responses to rubella vaccination (median 22.9 years after second rubella vaccination in our cohort) served as a starting point for comparative analysis of humoral immune response outcomes years after the last vaccination and before the receipt of a 3rd dose of MMR vaccine. Our study cohort received a 3rd dose of MMR vaccine within the framework of the study, allowing us to examine the recall responses driven by memory lymphocytes (e.g., memory B cells) and to make comparisons between responder groups at different timepoints following vaccination. We selected the Day 8 timepoint based on the literature demonstrating a window of plasmablast activity during immune responses to multiple vaccines/infections, while Day 28 was representative of the peak humoral immune response, including antibody titers and the generation antigen-specific memory B cells following vaccination [21–23].

Methods

Study cohort

The study sample consisted of 109 healthy female subjects (20–45 years old) from Olmsted County, MN, and surrounding areas enrolled at Mayo Clinic, Rochester, MN, with two documented doses of MMR vaccine. Subjects were selected for this study if they were in the highest 30% (high antibody responder group) or lowest 30% (low antibody responder group) of the rubella-specific IgG antibody-titer spectrum based on enzyme-linked immunosorbent assay (ELISA) screening of 1,117 available serum samples from the local community (obtained from the Mayo Clinic Biobank), as published [24]. Subjects from the high or the low antibody responder groups (after two doses of MMR vaccine) received a 3rd dose of MMR vaccine within the framework of the current study. Serum and peripheral blood mononuclear cells (PBMCs) were collected prior to vaccination (Baseline) and at 8 and 28

days after the receipt of a third dose of MMR vaccine. Demographic and clinical variables for the study subjects were extracted from their medical records, including vaccination history, dates of previous MMR vaccinations, and height and weight (body mass index/BMI). If no MMR-vaccination history was available in the medical record, subjects were enrolled in the study only after providing (at the time of enrollment) written documentation of administration of two previous doses of MMR vaccine with dates of administration from their vaccination records. All study participants provided written informed consent, and all study procedures, including the Mayo Clinic Biobank protocol, were approved by the Mayo Clinic Institutional Review Board.

All samples were assigned a random study ID and all personal identifiers were removed before the samples were sent to the CDC for testing. Thus, the measurements of antibody titers and avidity were performed on de-identified samples at the CDC without personal identifiable information of the study subjects, and CDC human subjects review was not required.

Rubella-specific IgG antibody and antibody avidity assays

Rubella-specific IgG antibody titers were measured using the Zeus Rubella IgG ELISA Test System (Zeus Scientific Inc.; Branchburg, NJ), according to the manufacturer's instructions. The OD values were transformed into rubella-specific IgG antibody titer in International Units per milliliter (IU/mL), per the manufacturer's instructions. Sera with OD ratios of >2.2 were further diluted, and rubella-specific IgG antibody concentrations were determined from the diluted serum. Using the Zeus Rubella IgM ELISA Test System (Zeus Scientific Inc.; Branchburg, NJ) and the Diamedix Rubella IgM Capture EIA system (Erba Diagnostics Inc., Miami, FL), we measured rubella-specific IgM antibodies for a subset of subjects/sera that had the following IgG titers: IgG titers < 10 IU/mL and low rubella IgG titers with a 4-fold rise of IgG antibody titers (Day 28 after a third MMR vaccine dose compared to Baseline). The coefficient of variation (CV) for the ELISA assays based on four repeated measurements was 2.6%, which demonstrates a high degree of reproducibility.

The rubella-specific IgG avidity was measured using the Zeus Rubella IgG ELISA Test System (Zeus Scientific Inc.; Branchburg, NJ) described above, with the following modifications [25]. All serum samples at or above 10 IU/mL were tested. Where needed, normalization/dilution of sera was performed to fit the range of values between 10 and 70 IU/mL. Serum samples were tested in pairs; one following the standard manufacturer's protocol and the other with a 35 mM diethylamine (DEA) wash step after the incubation with antigen. Avidity was calculated as the percentage of the absorbance value with and without DEA. Serum samples of high and low rubella IgG avidity were included as internal controls in each experiment. Avidity indices > 30% were considered indicative of the presence of high avidity antibodies.

Rubella neutralizing antibody assay

A soluble, immuno-colorimetric-based neutralization method (sICNA), optimized to a high-throughput micro-format, was used as previously described [15, 26-29]. The neutralization titer represented the highest dilution at which the input virus signal was reduced by at least

50% within the dilution series (NT₅₀) [27]. The NT₅₀ was determined using Karber's method. Serum-virus mixtures (samples) with less than 50% of the input infectivity at the last dilution were retested starting from a higher dilution. Serum-virus mixtures with more than 50% of input infectivity at the first dilution were retested starting from a lower dilution, with the lowest starting dilution being 1:10 [15]. For quality control purposes and to confirm assay reproducibility, 129 serum samples were retested. The reported intra-class correlation coefficient (ICC) based on log-transformed estimates from repeated NT₅₀ measurements for this assay was 0.89, which demonstrates a high degree of reproducibility [29].

Memory B cell ELISPOT assay

Rubella virus-specific memory-like IgG B cells were quantified pre- (Day 0/Baseline) and post-vaccination (Day 28) in subjects' PBMCs using the Mabtech ELISpot^{PLUS} kit for human IgG (Mabtech Inc.; Cincinnati, OH) according to the manufacturer's specifications and as previously described [30-32]. Antigen-specific memory B cell frequencies were measured/presented in spot-forming units (SFUs) per 2×10^5 cells as subjects' medians (median of rubella virus-specific SFUs response, measured in quadruplicate). The reproducibility of this assay (assessed as intraclass correlation coefficients between four replicate measurements) was high (average 0.88) [30].

Statistical Analysis

All analyses used log₂ scale and were done in R version 3.4.2. Univariate linear regression and Fisher's Exact test was used to identify differences between the high and low antibody responder groups for the demographic/clinical covariates. Spearman's correlations were used to evaluate differences between the demographic/clinical covariates and immune responses at each time point (Baseline, Day 8, Day 28, Day 28 – Baseline). The differences between high and low antibody responder groups for each humoral immune response outcome at each timepoint were evaluated using Wilcoxon rank sum tests, and the changes in immune response outcomes between timepoints were tested using Wilcoxon signed rank tests. The associations between humoral immune response outcomes were evaluated using Spearman correlations.

The CV for the ELISA assay performance is calculated as the ratio of the standard deviation to the mean of four repeated measurements. Intra-class correlation (ICC) was determined between four measurements using the Shrout and Fleiss's method [33].

Results

Demographic and clinical variables of the study subjects

All demographic and clinical variables of study participants are summarized in Table 1. Fifty-six females were enrolled in the low-antibody group and 53 females were enrolled in the high-antibody group based on their rubella-specific IgG antibody titer as measured by ELISA prior to enrollment in the study. As noted in Table 1, the majority of the study participants were White/Non-Hispanic or Latino and were between 30 and 40 years of age. The median body mass index (BMI) for the cohort was 27.5 (IQR, 23.9, 33.1). No

statistically significant differences in vaccine history or demographic/clinical variables were noted between the low-antibody group and the high-antibody group participants (Table 1).

As expected, we observed associations between the screening rubella-specific antibody titer (i.e., the assignment of subjects to the high- or the low-antibody titer group) and the pre- (Baseline) and post-vaccination immune response outcomes (Supplemental Table 1). The Baseline neutralizing antibody response was strongly associated with the screening rubella-specific antibody titer with a correlation of 0.753 ($p=3.76E^{-21}$).

Dynamics of humoral immune response outcomes after a third MMR vaccine dose

In all subjects, vaccination with a third dose of MMR vaccine elicited a significant rise in rubella-specific neutralizing antibody titer from Baseline to Day 28 post-vaccination (Tables 2 and 3, Fig. 1). We also observed statistically significant differences in neutralizing antibody titers measured at all timepoints between the high- and low-antibody groups, with the high responders always having higher post-third dose neutralizing antibody titers compared to the low responders (Table 2). The rise in the neutralizing antibody titer from Baseline to Day 28 was higher for the low-antibody-responder group (4.73) compared to the high-antibody-responder group (1.88), ($p\text{-value}=7.7E^{-10}$, Table 2). The results for the total rubella-specific IgG antibody titers measured by ELISA and antibody avidity indices closely mirrored the neutralizing antibody titers (Table 2 and Fig. 1), although the methodology used to measure antibody avidity may introduce a bias toward lower avidity in low-antibody samples (Supplemental Fig. 1). The boost in these two humoral immune response outcomes (Day 28- Baseline) was significantly higher in the low-antibody-responder group compared to the high-antibody-responder group (Table 2). It is worth noting that all avidity values, measured at all time points for all subjects, were above 30% and thus indicate the presence of high avidity rubella-specific antibodies even at Baseline. Of the 109 study subjects, we identified four individuals (3.67 % of all study participants and 7.14 % of the low-responder group) that were seronegative at Baseline (i.e., had rubella-specific ELISA IgG titers < 10 IU/mL). Note that for three of the four seronegative individuals, avidity assays at Baseline/Day 8 were not applicable, as rubella-specific IgG was not detected, but the Day 28 serum samples contained rubella-specific antibodies with high avidity. In order to rule out a primary humoral immune response, we tested for rubella-specific IgM antibodies in subjects with IgG titers < 10 IU/mL, as well as subjects with low rubella IgG titers with a significant (4-fold or more) rise of IgG antibody titers after vaccination. None of these samples/subjects were positive for rubella-specific IgM antibodies.

Our study also assessed the dynamics of rubella-specific memory B cell response after a third dose of MMR vaccine, while specific plasmablast response was not measured due to the availability of cryopreserved cells for the ELISPOT assay. We observed a significant rise in rubella-specific memory B cell ELISPOT response from Baseline to Day 28 post-vaccination in all subjects (see Table 3 and Fig. 1). Although the high responders had higher frequencies of rubella-specific memory B cells, this difference was statistically significant only at Baseline ($p\text{-value}=0.0019$, Table 2). The difference did not reach statistical significance at Day 28 ($p\text{-value}=0.103$, Table 2).

Associations between demographic/clinical variables and humoral immune response outcomes after a third MMR vaccine dose

The correlation between the screening rubella-specific antibody titer and the peak neutralizing antibody titer at Day 28 after third MMR vaccine dose was $r=0.244$ ($p=0.0114$); and the correlation between the screening antibody titer and the boost in neutralizing antibody response (Day 28 – Baseline) was $r=-0.629$ ($p=4.13E^{-13}$). Similar results were observed also for Day 8 neutralizing antibody response as well as for rubella-specific IgG antibody titers measured pre- and post-vaccination. Likewise, we noted a correlation between the screening rubella-specific ELISA antibody titer and rubella-specific memory B cell frequencies, although this correlation was significant only at Baseline ($r=0.324$, $p\text{-value}=0.0021$). Other demographic/clinical variables (Supplemental Table 1) did not have significant and/or consistent associations with rubella-specific immune response outcomes. We observed a weak negative correlation ($r=-0.212$, $p\text{-value}=0.032$) between the subjects' BMI and the antibody avidity index rise/boost (Day 28 – Baseline, Supplemental Table 1).

We also observed a high correlation between neutralizing antibody response measurements and ELISA IgG antibody response measurements (Baseline and Day 8 $r=0.86$; Day 28 $r=0.70$; $p\text{-values} < 2.2E^{-16}$; Fig.2A). Correlations between antibody measures and the frequencies of rubella-specific memory B cells were relatively weak (see Fig.2B and C). The Baseline rubella-specific memory B cell ELISPOT response was weakly positively correlated with the Baseline rubella-specific neutralizing antibody titer ($r=0.33$, $p\text{-value}=0.0015$) and the Day 8 neutralizing antibody titer ($r=0.35$, $p\text{-value}=0.00076$). The increase of rubella-specific memory B cells after a third MMR vaccine dose (Day 28 – Baseline) was positively correlated with the boost (Day 28 – Baseline) in neutralizing antibody titer ($r=0.25$, $p\text{-value}=0.022$). Similar associations were observed between rubella-specific IgG titers and rubella-specific memory B cells (see Fig.2C).

Discussion

Although rubella vaccine is considered safe and highly effective in preventing clinical rubella, some studies have reported sub-optimal long-term vaccine-induced immunity and even seronegativity (antibody titer <10 IU/mL) in immunized individuals, including women of childbearing age, after two doses of rubella vaccine [14-16].

In this study, we examined the immune response of 109 young females of childbearing age (born between 1973 and 1998) to a third dose of rubella-containing vaccine and the demographic and clinical factors associated with this response. Our cohort contained individuals from the local community who had pre-vaccination antibody titers in the bottom or top 30th percentile. None of the demographic and clinical factors were significantly associated with Baseline humoral immune response measures. Only a few factors (age at first and second rubella vaccination, BMI) exhibited weak or suggestive associations with differences in levels of humoral immunity measures after the third MMR vaccination (see Supplemental Table 1). Higher weight and BMI have been typically associated with lower vaccine-induced antibody titers, as previously reviewed in the literature [34]; however, our study found minor effects of BMI on humoral immune response to a third dose of MMR vaccine. The assignment of subjects to the high- or the low-antibody group was the main

factor associated with the humoral immune response measured after the third MMR vaccination.

We identified four subjects who were seronegative approximately 23 years (median for the cohort) after the receipt of the second MMR vaccine dose [35, 36]. The majority of the subjects from the low-responder group had low-positive Baseline IgG titers between 10 IU/mL and < 40 IU/mL, while the majority of the subjects from the high-responder groups presented with medium-positive Baseline titers of 40 IU/mL to < 120 IU/mL (n=31) or with high-positive titers ≥ 120 IU/mL (n=19) [15]. Although, by study design, our results are not comparable to rubella seroprevalence studies across the general U.S. population, it is worth noting that the proportion of seronegative individuals in this study is consistent with other reports evaluating the persistence of rubella antibodies after vaccination. For example, McLean et al. found 1.8% out of 679 young adults had rubella neutralizing antibodies under 10 U/mL approximately 15 years after the second rubella vaccination [15], and Seagle et al. reported ~ 3.7% out of 296 study subjects followed up to 12 years after second MMR vaccination [6] had rubella neutralizing antibodies < 10 (reciprocal of serum dilution). Importantly, among 1,393 recently tested serum samples obtained from individuals residing in Olmsted County, MN, and the surrounding areas, we identified 30 (2.2%) individuals with rubella-specific IgG <10 IU/mL [24]. There are several reports of decreasing titers years after vaccination and higher rubella seronegativity rates in the United States and other countries. A longitudinal study carried out by the Centers for Disease Control and Prevention reported 9.7% rubella seronegativity in 144 subjects followed for 12 years after their second MMR immunization at 4–6 years of age [7]. A study in Finnish children followed up to 15 years after their second vaccine dose and reported 17 % rubella seronegativity rate (< 10 IU/mL) [16]. These data raise concerns regarding waning vaccine-induced rubella immunity and possible disease susceptibility upon rubella virus exposure with risks for CRS in pregnant women [1, 17-19]. None of the tested serum samples for rubella-specific IgM antibodies was positive. This may be indicative of a secondary or anamnestic immune response phenotype in study subjects. The individuals who were seronegative or had low baseline titers also had an anamnestic response to the third MMR vaccine dose, suggesting that secondary vaccine failure or waning of antibody response occurred after the previous vaccinations [15]. In addition, the avidity indices for all subjects' pre- and post-third MMR immunization were > 30%, which demonstrates the maintenance of high-avidity rubella-specific antibodies even in subjects with low titers. The seronegative individuals also had avidity indices > 30% at Day 28 post-third MMR immunization, consistent with results published by LeBaron *et al.* [7], which found rubella antibodies with a mean avidity of 53.3 in 98.9% of 188 study subjects tested one month after second rubella vaccination. The antibody avidity increased with the third MMR vaccination in our study; 60.6% of the subjects presented with a ≥ 10% increase in Day 28 antibody avidity compared to Baseline.

In our study, response to a third dose of MMR vaccine was strongly dependent on high- or low-immune-response group. At Day 28 post-vaccination, there were no seronegative individuals, and only three individuals remained with low-positive rubella IgG titers. One third of all subjects experienced a significant boost (≥ 4-fold) of antibody titers one month following immunization. Seventy subjects (64.2%; 28 low responders and 42 high

responders) had positive Day 28 IgG titers ≥ 120 IU/mL. The antibody titers were significantly and consistently higher in the high responders compared to the low responders, although the antibody increase/boost was higher in the low responders compared to high responders. For example, 54.5% of the low responders experienced a 4-fold increase in neutralizing antibody titer compared to only 7.7% of the high responders; similar percentages were observed for rubella IgG titers: 56.4% for the low responders vs. 3.8% for the high responders; $p < 0.001$ for all comparisons. The negative effect of preexisting antibodies on the secondary immune response is known and likely due to viral neutralization (i.e., the formation of antigen-antibody complexes lowering the viral load) and/or negative feedback mechanisms affecting B cells [37]. Other studies report overall higher percentages of individuals exhibiting a four-fold increase in rubella-specific antibody titer approximately one month after receipt of a second MMR vaccine dose [7] or third MMR vaccine dose [15]. Due to the enrichment of high/low antibody responders in our study, it may not be accurate to compare our study to those done by others. For example, the lower percentage of subjects with a four-fold increase in rubella-specific antibody titer in our study may reflect the enrichment of subjects with high ceiling antibody titers that are less likely to experience a significant boost in their antibody titer.

We and others have consistently found antibody response dynamics and patterns following rubella vaccination that depended on rubella-specific pre-immunization titer and the propensity of the individual to respond to vaccination [7, 15, 16, 38]. The same holds true for the measles component of the MMR vaccine, where seronegativity and low antibody titer following previous vaccination were demonstrated to be a significant risk factor for persistent low antibody titer and/or seronegativity after a subsequent dose of MMR [39-42]. No statistically significant differences were noted in the demographics and vaccine history between the two studied groups in our current study, suggesting that host factors may be involved in the variability of the immune response after rubella vaccination and implies that subsequent MMR immunizations would elicit similar subject-specific immune responses [1, 43-46].

Studies assessing relationships between antigen-specific memory B cell frequencies and antibody response after vaccination present conflicting results but generally agree that both memory B cells and long-lived plasma cells play roles in maintaining antibody responses and mediating the anamnestic response upon exposure/vaccination [47-49]. Our results point to modest correlations between the frequencies of circulating rubella-specific memory B cells and rubella-specific antibody titers/boost in antibody titers. Antigen-specific B cell responses and antibody titers are regulated autonomously and/or may have different kinetics or localization [47-49]. Serum antibodies may be maintained by long-lived plasma cells in the bone marrow rather than in circulation [47]. Other factors, such as antigen-specific long-lived plasma cells/progenitors and/or follicular T-helper/Tfh cells, play a significant role in mediating and facilitating humoral immune response to vaccination [50-54].

The limitations of our study include the constraints of our study design, the sample size, and the geographic area of enrollment. Due to the enrollment of high and low antibody responders and the enrollment of females from specific geographic areas, our study is not representative of—and the results may not be generable to—the entire U.S. population. On

the other hand, the assessment of subjects representing the extremes of the antibody response may enhance the identification of factors and differences that are biologically relevant and fundamental for MMR booster vaccine-induced immunity. Our statistical analyses did not apply correction for multiple comparisons; therefore, the results/p-values should be interpreted with caution (particularly for the weak associations and differences). It should be noted that correction for multiple testing, assuming that all tests are independent of each other, is overly conservative. We have presented actual p-values and confidence intervals to allow the reader to assess the magnitude of the effect. Another limitation of the study is that the methodology used to measure antibody avidity may introduce a bias toward lower avidity in samples with very low antibody titer. High titer sera are diluted to reach the 10–70 IU/ml for avidity testing (2–24 fold), and the samples are evenly distributed between 10 and 70 IU/ml for the avidity testing. However, many low titer sera are already within the 10–70 IU/ml window (e.g., baseline-low responder group has a mean IU/ml of 22.2) and thus the titer of sera used for avidity testing for this group is skewed toward the lower end of the window (Supplemental Fig. 1). Therefore, the difference in avidity between the low and high antibody titer groups should be interpreted with caution. Furthermore, as noted previously, the measured avidity indices for all study samples at all time points were > 30%, which demonstrates the presence of high-avidity rubella-specific antibodies in all subjects. The clinical significance of the reported differences in avidity indices between high and low responders is not clear and will require further investigation. We did not follow study subjects for a longer period of time after the third MMR immunization and did not assess cellular immunity parameters other than memory B cell ELISPOT frequencies. Long-term follow-up to evaluate the durability of rubella-specific immune responses to the third MMR vaccine dose (and the possibility of a third dose vaccine failure/immune response waning in the low responder group) and a parallel study focused on parameters of cellular immunity are currently underway. Such studies, along with the findings of the current study, could identify the key factors necessary to achieve and maintain protection from disease and potentially inform vaccine policies under certain situations (e.g., administration of a third dose of MMR vaccine to women at the lower end of the antibody response spectrum before planned conception/pregnancy) to benefit public health.

In summary, our study findings expand the current understanding of immune response and long-term humoral immunity to live rubella vaccination in female subjects of childbearing age, including immune response to a third MMR vaccine dose, and suggest the importance of yet unknown intrinsic biology and/or genetic influences for the generation and maintenance of vaccine-induced humoral immune responses. Longitudinal and/or systems biology studies providing comprehensive assessment of multiple vaccine-induced immunity measures in targeted populations are needed to decipher the genetic, environmental, and other factors underlying inter-individual differences in immune response to rubella vaccination and the factors affecting antibody persistence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Dynamics of rubella-specific humoral immune response in healthy women after a third dose of MMR vaccine

Immune response dynamics by neutralization assay (A), B-cell ELISPOT (B) IgG ELISA (C) and IgG avidity (D) over three time points are displayed using boxplots for all subjects (“Overall”, grey box), the high responders (“High”; black box) and the low responders (“Low”, white box). The neutralizing antibodies, the number of B cells, and rubella IgG titers are plotted in log2 scale. Each box was plotted using the 25% to 75% interquartile range and the median was represented by the bold line in the box. The “whiskers” extend up to 1.5 times the interquartile range above or below the 75th or 25th percentiles respectively.

Fig. 2. Correlation between the rubella-specific immune response measured pre- and post-vaccination with a third dose of MMR vaccine

A) Correlation between rubella-specific total IgG antibody titer and neutralizing antibody titer at each time point. B) Correlation between rubella neutralizing antibody titer and rubella-specific B cell ELISPOT response at the indicated time points. C) Correlation between rubella IgG antibody titer and rubella-specific B cell ELISPOT response at the indicated time points. Each graph contains the Spearman correlation coefficients (r) and p -values for the immune outcomes and time points contained in that graph. Different symbols are depicting the low (o) and the high (+) antibody responder groups. The scales of the axis reflect the untransformed values. The rise in the immune outcome from Baseline to Day 28 is presented as (Day 28 – Baseline).

Table 1.

Demographic and clinical information for the study subjects

	Low antibody group (n=56)	High antibody group (n=53)	Total (n=109)	P-value
Age at Enrollment (years)				0.282 ¹
Median (Q1, Q3)	36.0 (31.4, 40.3)	33.1 (29.5, 39.9)	34.5 (30.4, 40.3)	
Age at 1st Rubella Vaccination (months)				0.672 ¹
Median (Q1, Q3)	15.8 (15.1, 18.3)	15.4 (14.9, 16.0)	15.7 (15.0, 17.0)	
Age at 2nd Rubella Vaccination (years)				0.930 ¹
Median (Q1, Q3)	12.3 (9.1, 16.7)	12.2 (11.0, 15.4)	12.2 (9.8, 15.9)	
Time from 2nd Rubella Vaccination to Enrollment (years)				0.348 ¹
Median (Q1, Q3)	23.4 (19.9, 25.0)	21.3 (17.6, 25.6)	22.9 (18.4, 25.3)	
Race				0.486 ²
Asian	0 (0.0%)	1 (1.9%)	1 (0.9%)	
White	56 (100.0%)	52 (98.1%)	108 (99.1%)	
Ethnicity				1.00 ²
Non-Hispanic or Latino	54 (96.4%)	52 (98.1%)	106 (97.2%)	
Hispanic or Latino	2 (3.6%)	1 (1.9%)	3 (2.8%)	
BMI				0.800 ¹
Median (Q1, Q3)	27.5 (22.3, 34.5)	28.1 (24.5, 32.3)	27.5 (23.9, 33.1)	
Screening Rubella IgG Ab Titer (ELISA OD value)				<0.001 ³
Median (Q1, Q3)	0.24 (0.15, 0.31)	1.25 (1.03, 1.62)	0.35 (0.24, 1.23)	

¹Linear Model ANOVA²Fisher's Exact Test for Count Data³Not applicable

Table 2.

Humoral immune responses to rubella in healthy women before and after a third dose of MMR vaccine

Timepoint	Overall ¹	Low antibody group ¹	High antibody group ¹	P-Value ²
Neutralizing Antibody Titer (NT₅₀)				
Baseline	80.82 (78.3, 83.35)	41.81 (40.55, 43.07)	162.18 (160.22, 164.14)	6.087E-16
Day 8	96.87 (94.37, 99.38)	51.98 (50.43, 53.53)	187.03 (185.13, 188.93)	4.766E-15
Day 28	243.01 (241.09, 244.94)	194.14 (192.25, 196.03)	308.16 (306.43, 309.9)	5.542E-04
Day 8-Baseline ³	1.2 (0.33, 2.07)	1.24 (0.23, 2.26)	1.15 (0.49, 1.82)	4.028E-01
Day 28-Baseline ³	3.02 (0.8, 5.25)	4.73 (2.67, 6.78)	1.88 (0.38, 3.39)	7.654E-10
ELISA IgG Antibody Titer (IU/mL)				
Baseline	47.29 (44.48, 50.1)	22.21 (20.57, 23.85)	105.07 (103.21, 106.93)	1.205E-16
Day 8	49.24 (46.42, 52.06)	23.94 (22.14, 25.75)	105.47 (103.51, 107.43)	1.977E-15
Day 28	149.7 (147.66, 151.74)	125.84 (123.82, 127.85)	179.88 (177.94, 181.83)	1.506E-02
Day 8-Baseline ³	1.04 (0.24, 1.84)	1.08 (0.12, 2.03)	1 (0.43, 1.58)	4.130E-02
Day 28-Baseline ³	3.19 (0.51, 5.86)	5.72 (3.16, 8.28)	1.72 (0.35, 3.09)	1.716E-12
Avidity Index (%)				
Baseline	53.87 (53.33, 54.4)	49.66 (49.22, 50.11)	58.43 (57.9, 58.95)	3.834E-07
Day 8	54.01 (53.43, 54.58)	49.35 (48.88, 49.82)	59.1 (58.54, 59.66)	4.525E-07
Day 28	61.83 (61.4, 62.27)	60.21 (59.81, 60.61)	63.6 (63.14, 64.06)	1.942E-02
Day 8-Baseline ³	1 (0.75, 1.26)	0.99 (0.72, 1.27)	1.01 (0.77, 1.25)	1.542E-01
Day 28-Baseline ³	1.15 (0.66, 1.64)	1.22 (0.66, 1.78)	1.09 (0.74, 1.44)	3.454E-05
Memory B cell ELISPOT response (SFUs/2 x 10⁵ cells/PBMCs)				
Baseline	8.01 (5.72, 10.31)	6.17 (3.93, 8.4)	10.54 (8.42, 12.66)	0.0019
Day 28	27.69 (25.28, 30.09)	23.56 (21.04, 26.08)	32.78 (30.58, 34.99)	0.1027
Day 28-Baseline ³	3.48 (0.77, 6.19)	3.88 (0.85, 6.9)	3.11 (0.77, 5.45)	0.2642

¹Geometric Mean (95% Confidence Interval). Overall is defined as all the 109 subjects in this study.²Wilcoxon rank sum tests p-values comparing immune outcomes between high responders and low responders³Day 28 (or Day 8)-Baseline represents fold difference and is the geometric mean of (log₂(Day28)-log₂(Day0), i. e., 2^{^(ave(log₂(Day28)-log₂(Day0)))}).

Table 3.

Immune responses to a third dose of MMR in our study cohort.

Prior Antibody Status	Baseline Immune Response ¹	Day 8 Immune Response ¹	Day 28 Immune Response ¹	Day 8 vs Baseline p-value ²	Day 28 vs Baseline p-value ²
Neutralizing Antibody Titer (NT₅₀)					
Overall ³	80.82 (78.3, 83.35)	96.87 (94.37, 99.38)	243.01 (241.09, 244.94)	1.71e-10	7.82E-19
Low	41.81 (40.55, 43.07)	51.98 (50.43, 53.53)	194.14 (192.25, 196.03)	2.95e-07	1.14E-10
High	162.18 (160.22, 164.14)	187.03 (185.13, 188.93)	308.16 (306.43, 309.9)	9.46e-05	2.52E-09
ELISA IgG Antibody Titer (IU/mL)					
Overall ³	47.29 (44.48, 50.1)	49.24 (46.42, 52.06)	149.7 (147.66, 151.74)	0.584	5.59E-19
Low	22.21 (20.57, 23.85)	23.94 (22.14, 25.75)	125.84 (123.82, 127.85)	0.0702	1.14E-10
High	105.07 (103.21, 106.93)	105.47 (103.51, 107.43)	179.88 (177.94, 181.83)	0.224	1.52E-09
Avidity Index (%)					
Overall ³	53.87 (53.33, 54.4)	54.01 (53.43, 54.58)	61.83 (61.4, 62.27)	0.98	6E-12
Low	49.66 (49.22, 50.11)	49.35 (48.88, 49.82)	60.21 (59.81, 60.61)	0.296	4.02E-07
High	58.43 (57.9, 58.95)	59.1 (58.54, 59.66)	63.6 (63.14, 64.06)	0.3	1.62E-05
Memory B cell ELISPOT response (SFUs/2 x 10⁵ cells/PBMCs)					
Overall ³	8.01 (5.72, 10.31)	NA	27.69 (25.28, 30.09)	NA	9.87E-14
Low	6.17 (3.93, 8.4)	NA	23.56 (21.04, 26.08)	NA	3.17E-09
High	10.54 (8.42, 12.66)	NA	32.78 (30.58, 34.99)	NA	2.87E-10

¹ Geometric Mean (95% Confidence Interval)² Wilcoxon Signed Rank Test p-value for comparison between time points.³ Overall is defined as all the 109 subjects in this study.