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Measles Virus Neutralizing Antibody Response, Cell-Mediated Immunity, and Immunoglobulin G Antibody Avidity Before and After Receipt of a Third Dose of Measles, Mumps, and Rubella Vaccine in Young Adults

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Background. Two doses of measles, mumps, and rubella (MMR) vaccine are 97% effective against measles, but waning antibody immunity to measles and failure of the 2-dose vaccine occur. We administered a third MMR dose (MMR3) to young adults and assessed immunogenicity over 1 year.

Methods. Measles virus (MeV) neutralizing antibody concentrations, cell-mediated immunity (CMI), and immunoglobulin G (IgG) antibody avidity were assessed at baseline and 1 month and 1 year after MMR3 receipt.

Results. Of 662 subjects at baseline, 1 (0.2%) was seronegative for MeV-neutralizing antibodies (level, <8 mIU/mL), and 23 (3.5%) had low antibody levels (8–120 mIU/mL). One month after MMR3 receipt, 1 subject (0.2%) was seronegative, and 6 (0.9%) had low neutralizing antibodies, with only 21 of 662 (3.2%) showing a ≥4-fold rise in neutralizing antibodies. One year after MMR3 receipt, no subject was seronegative, and 10 of 617 (1.6%) had low neutralizing antibody levels. CMI analyses showed low levels of spot-forming cells after stimulation, suggesting the presence of T-cell memory, but the response was minimal after MMR3 receipt. MeV IgG avidity did not correlate with findings of neutralization analyses.

Conclusions. Most subjects were seropositive before MMR3 receipt, and very few had a secondary immune response after MMR3 receipt. Similarly, CMI and avidity analyses showed minimal qualitative improvements in immune response after MMR3 receipt. We did not find compelling data to support a routine third dose of MMR vaccine.

Keywords. measles; third dose of measles, mumps, rubella (MMR) vaccine; measles vaccine immunogenicity; vaccine-preventable disease; immunization; cell-mediated immunity; measles virus antibody avidity.

Measles is a contagious, viral rash illness; complications include pneumonia and encephalitis and can result in death [1]. High coverage with 2 doses of measles, mumps, and rubella (MMR) vaccine and improved measles control in the World Health Organization (WHO) Region of the Americas resulted in the declaration of measles elimination in the United States in 2000 [2].

Two doses of MMR vaccine are generally sufficient to provide long-lasting protection against measles [3]. Nonetheless, measles virus (MeV) is one of 3 viruses targeted by the MMR vaccine, and third doses have been administered during mumps outbreaks among highly vaccinated populations [4, 5] and in nonoutbreak settings among healthcare personnel, military recruits, international travelers, and college students who may have received 2 doses but lacked documentation [6–8].

The immunogenicity of the MeV component of a third MMR dose has not been studied. We assessed the magnitude and duration of an aggregate MeV neutralizing antibody response, cell-mediated immune response, and immunoglobulin G (IgG) antibody avidity before and after a third MMR dose (MMR3) in a healthy, young adult population.

METHODS

Setting

The study population comprised patients of the Marshfield Clinic, a private, multispecialty group practice with regional centers throughout central and northern Wisconsin. The clinic maintains an electronic vaccination registry (available at: http://www.recin.org) for immunizations administered by Marshfield Clinic providers, local public health agencies, and immunization providers. No measles cases were reported in the area during the study period.

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Subjects

Two cohorts comprising 685 subjects were enrolled during 2009–2010. Cohort 1 (113 subjects) participated in a 10-year longitudinal study at the Marshfield Clinic examining immunogenicity and adverse events following the second MMR vaccine dose (hereafter, "the longitudinal study") [9, 10]. To achieve adequate sample size, Marshfield's vaccination registry was used to recruit subjects from cohort 2 for whom receipt of 2 MMR doses was documented but who did not participate in the longitudinal study (572 subjects). Invitation letters were mailed to both cohorts, and follow-up telephone calls were made. Additionally, cohort 1 subjects who participated in the measles cell-mediated immunity (CMI) substudy during the longitudinal study were asked to participate in the current CMI substudy.

Although only 16 cohort 1 subjects (14.2%) had low or undetectable MeV antibody concentrations during the longitudinal study, 93 of 113 cohort 1 subjects had low or undetectable concentrations of antibody (defined previously [10–12]) to at least one of the viruses targeted by MMR during the longitudinal study, and all cohort 2 subjects were offered a third dose of MMR vaccine (M-M-R II; Merck). Serum was collected from all subjects immediately before (baseline) and 1 month and 1 year after MMR3 receipt.

Study Design

At each visit, subjects were questioned about measles occurrence, exposures, vaccinations, and other health events. MMR vaccine was administered during the initial visit. Informed consent was obtained from all subjects. Institutional review boards of the Marshfield Clinic Research Foundation and the Centers for Disease Control and Prevention approved the study. Sample size determination and exclusion criteria were previously described [13].

CMI Substudy

The 60 participants in the longitudinal measles CMI substudy or subjects with a low or undetectable MeV antibody concentration in ≥1 serum specimen collected during the longitudinal study were asked to participate in the current CMI substudy. However, only 34 subjects (56.7%) meeting these criteria were re-enrolled. A convenience sample from cohort 2 was used to reach the recruitment goal of 60 subjects.

Laboratory Methods

Laboratory testing was performed at the end of the study. Other than each subject's unique identifier code and serum collection dates, laboratories were blinded to study information.

Plaque Reduction Neutralization (PRN) Testing

PRN testing was performed using low-passage Edmonston MeV on Vero cell monolayers, as previously described [14]. End points were determined for all serum samples tested, and 50% neutralizing doses were calculated using the Kärber method. Serial 4-fold dilutions of serum were tested in duplicate starting at 1:8 and ending at 1:8192 against virus diluted to give 25–35 plaques/well and run in parallel with the Second WHO International Standard Reference Serum (66/202). After incubating the virus-serum mixtures at 37°C with 5% CO_2, the mixtures were transferred onto corresponding 24-well tissue culture plates containing confluent Vero monolayers; after incubating for 1 hour at 37°C, the inoculum was removed, and cells were overlaid with medium containing carboxy methylcellulose and returned to the incubator for 5 days prior to staining with neutral red and plaque counting. Serum samples from individual subjects were tested in the same assay run. Titers were standardized against the WHO reference serum, with a titer of 1:8 corresponding to 8 mIU/mL in this assay.

CMI

Cryopreserved peripheral blood mononuclear cells (PBMCs) were thawed and cultured overnight in 5% CO_2 at 37°C with Roswell Park Memorial Institute (RPMI) medium supplemented with 4% human serum type AB (Lonza), 1% penicillin/streptomycin, and 1% 200 mM L-glutamine. Following the overnight culture, interferon γ (IFN-γ) production by T cells was assessed using enzyme-linked immunospot assays of PBMCs (5×10^5 cells/well), as previously described [15]. PBMCs were stimulated either with a mixture of MeV hemagglutinin, fusion, and nucleoprotein proteins as 20–amino acid peptides (11 amino acids overlapping) at 1 μg/mL or with a lysate from MeV-infected Vero cells (Advanced Biotechnologies) at 10 μg/mL for 40 hours. RPMI medium and concanavalin A (5 μg/mL) were used as negative and positive controls, respectively. After stimulation, the plates were incubated with biotin-conjugated antibodies to human IFN-γ and then developed and read, as previously described [15]. Low and positive T-cell responses were categorized as <20 and ≥20 spot-forming cells (SFCs)/million PBMCs, respectively.

MeV IgG Antibody Avidity

MeV IgG antibody avidity was evaluated to determine whether there was a correlation between neutralizing antibody concentrations and strength of antibody binding. After neutralization results were available, avidity testing was performed using the method described previously [16]. Serum samples from all 662 subjects were split into quartiles based on baseline PRN antibody concentration. Subjects with undetectable neutralizing antibody concentrations were negative for MeV IgG by the Captia Measles IgG enzyme immunoassay assay (Trinity Biotech, Jamestown, New York); thus, avidity could not be measured. All subjects with low MeV neutralizing antibody concentrations at baseline, 1 month after MMR3 receipt, or 1 year after MMR3 receipt were tested for MeV antibody avidity. A random number generator selected specimens from at least 10 subjects from each of the remaining 3 quartiles for avidity testing; specimens from 59 subjects were evaluated. Each specimen was classified as negative if, at a 1:21 dilution, the Captia assay did not detect

IgG and as having low avidity, if the end-titer avidity index (AI) was ≤30%; intermediate avidity, if the end-titer AI was >30%–<70% (intermediate results were retested); and high avidity, if the end-titer AI was ≥70%.

Data Analysis

Based on previous studies [17, 18], serum samples were categorized as negative for MeV neutralizing antibody (level, <8 mIU/mL), indicating susceptibility to infection and disease; (2) having a low MeV neutralizing antibody level (8–120 mIU/mL), indicating potential susceptibility to infection and disease; (3) having a medium MeV neutralizing antibody level (121–900 mIU/mL), indicating potential susceptibility to infection but not disease; and (4) having a high MeV neutralizing antibody level (>900 mIU/mL), indicating nonsusceptibility to infection or disease. Serum samples were also dichotomized as indicating potential susceptibility (MeV neutralizing antibody level, <121 mIU/mL) and nonsusceptibility (≥121 mIU/mL).

We combined cohorts 1 and 2 during analysis because there were no statistically significant differences between the cohorts by sex, race/ethnicity, or age. However, cohort 1 had significantly lower geometric mean concentrations (GMCs) of MeV neutralizing antibody at baseline ($P = .0289$), so we stratified the χ^2 risk factor analysis at 1 month and 1 year after MMR3 receipt by baseline MeV neutralizing antibody concentrations.

Mantel–Haenszel χ^2 and Fisher exact tests were performed to assess categorical variables. Wilcoxon rank sum tests were used for continuous variables. Potential risk factors for testing negative for or having a low level of MeV neutralizing antibody included sex, age at first MMR dose, time since second MMR dose (we used <15 years vs ≥15 years, based on the average age of subjects at enrollment minus the age when the second dose was recommended), and (for serum samples obtained after MMR3 receipt) the binary variable of whether the subject was negative for or had a low level of MeV neutralizing antibody at baseline. In multivariate logistic regression, a backward selection approach that used P values of <.4 for inclusion and P values of <.05 for retention identified factors independently associated with being negative for or having a low level of MeV neutralizing antibody at baseline and 1 month and 1 year after MMR3 receipt.

For the CMI analysis, the mean number of SFCs resulting from PBMC stimulation with MeV peptide and MeV lysate was determined at baseline and 1 month and 1 year after MMR3 receipt. The MeV-specific T-cell response was calculated by subtracting the mean spontaneous response (no stimulation) from the mean peptide or lysate response. MeV T-cell responses were correlated with MeV neutralizing antibody levels at baseline and 1 month and 1 year after MMR3 receipt. For the avidity analysis, end titer avidity index percentages were correlated with MeV neutralizing antibody levels at all 3 time points.

GMCs of MeV neutralizing antibody were calculated from base 2 log-transformed data. Statistical significance was assigned for P values of <.05. Data were analyzed with SAS 9.3 (Cary, North Carolina). Reverse cumulative distribution curves were created in Excel to compare the shift in curves from baseline and 1 month and 1 year after MMR3 receipt.

RESULTS

Enrollment

We contacted 194 of 200 persons from the longitudinal study; 113 (58.2%) were enrolled, 45 (23.2%) refused, and 36 (18.5%) were ineligible (15 had previously received MMR3, and 21 had

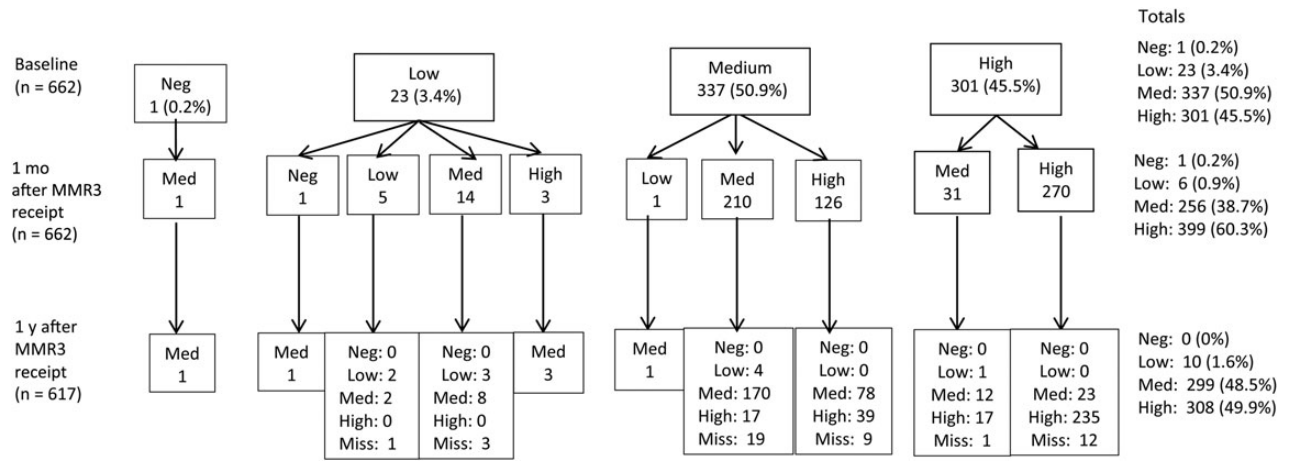

Figure 1. Flow chart of measles virus (MeV) neutralizing antibody concentrations at baseline and 1 month and 1 year following receipt of a third dose of measles, mumps, and rubella (MMR3) vaccine. Subjects were classified as negative for MeV neutralizing antibody (level, <8 mIU/mL; Neg), having a low MeV neutralizing antibody level (8–120 mIU/mL), having a medium MeV neutralizing antibody level (121–900 mIU/mL; Med), or having a high MeV neutralizing antibody level (>900 mIU/mL). Data are no. or no. (%) of subjects. Abbreviation: Miss, missing data.

other reasons). To achieve adequate sample size, we contacted 1379 of 1795 additional persons (76.8%). Of those, 572 (41.4%) were enrolled, 664 (48.2%) refused, and 143 (10.4%) were ineligible (4 had previously received MMR3, and 139 had other reasons; Supplementary Figure 1).

Baseline serum samples were obtained from 685 enrolled subjects. We excluded 20 cohort 1 subjects (2.9%) who had medium or high concentrations of antibody for all 3 antigens throughout the longitudinal study and were, therefore, not given MMR3. An additional 3 (0.4%) were excluded because they only had baseline samples. There were 662 subjects (96.6%) who received MMR3 and provided a serum specimen at 1 month; 617 (92.6%) provided a specimen at 1 year. Subjects were aged 18–28 years (mean age [±SD], 20.8 ± 2.1 years); 294 (44.4%) were male, and 649 (98.0%) were self-declared as being non-Hispanic and white. The mean interval between the second and third MMR doses was 15.8 years (range, 6.7–20.4 years).

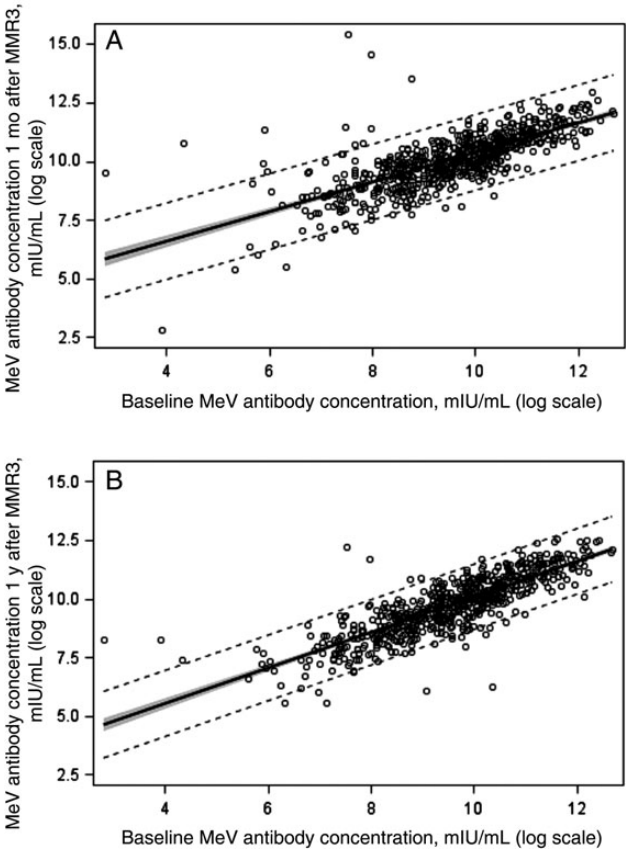

Figure 2. *A*, Comparison of individual measles virus (MeV) antibody concentration levels at baseline and 1 month following receipt of a third dose of measles, mumps, and rubella (MMR3) vaccine ($R^2 = 0.54$, $P < .0001$). *B*, Comparison of individual MeV antibody concentration levels at baseline and 1 year following MMR3 receipt ($R^2 = 0.68$, $P < .0001$). For both figures, data points are represented by circles and show the comparison result for each subject. The dark solid line represents the best fit of the comparison. The light shading around the line represents the 95% confidence limits. The dotted lines represent 95% prediction limits.

MeV Neutralizing Antibody Concentrations Before and After MMR3 Receipt

Of 662 subjects at baseline, 1 (0.2%) was seronegative, 23 (3.5%) had low MeV neutralizing antibody concentrations, 337 (50.9%) had medium concentrations, and 301 (45.5%) had high concentrations (Figure 1). The seronegative subject was a female aged 20 years who received her last MMR dose 18 years prior. At 1 month and 1 year after MMR3 receipt, she had medium MeV neutralizing antibody concentrations. Of 23 subjects with low baseline antibody concentrations, 1 was seronegative, 5 had low concentrations, 14 had medium concentrations, and 3 had high concentrations 1 month after MMR3 receipt. One year after MMR3 receipt, 19 of 23 had serum specimens collected; 5 had low, 14 had medium, and 0 had high MeV neutralizing antibody concentrations.

Overall, at 1 month after MMR3 receipt, 1 of 662 subjects (0.2%) had no detectable MeV neutralizing antibodies, 6 (0.9%) had low MeV neutralizing antibody concentrations, 256 (38.7%) had medium neutralizing antibody concentrations, and 399 (60.3%) had high MeV neutralizing antibody concentrations. One year after MMR3 receipt, all 617 subjects who returned were positive for MeV neutralizing antibodies: 10 (1.6%) had low concentrations, 299 (48.5%) had medium concentrations, and 308 (49.9%) had high concentrations.

When MeV neutralizing antibody concentration was assessed as a continuous variable, subjects who were seronegative or had low baseline concentrations were more likely to be seronegative or to have low concentrations 1 month and 1 year after MMR3 receipt, whereas subjects with high baseline concentrations were more likely to have high neutralizing antibody concentrations at 1 month ($R^2 = 0.54$; $P < .0001$) and 1 year ($R^2 = 0.68$; $P < .0001$; Figure 2).

GMCs were significantly different between baseline and 1 month after MMR3 receipt (727 vs 1060 mIU/mL; $P < .0001$) and between baseline and 1 year after MMR3 receipt (727 vs

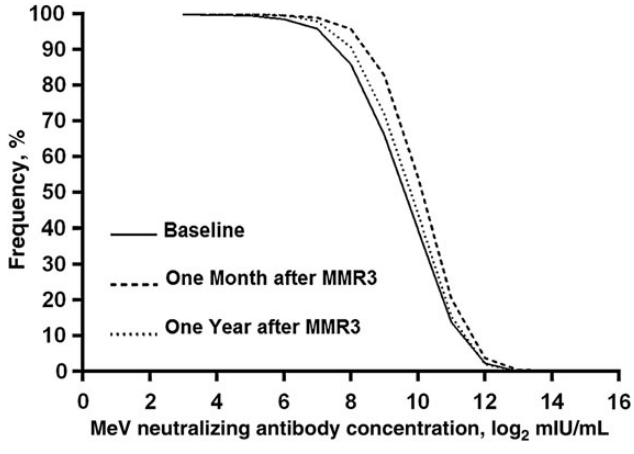

Figure 3. Reverse cumulative distribution curve by percentage of subjects who had measles virus (MeV) neutralizing antibody concentrations at baseline and 1 month and 1 year following receipt of a third dose of measles, mumps, and rubella (MMR3) vaccine.

843 mIU/mL; $P < .05$). However, the reverse cumulative distribution curves show that the shift in MeV antibody concentrations from baseline to 1 month and 1 year after MMR3 receipt was minimal (Figure 3).

4-fold Increases

Twenty-one (3.2%) of 662 subjects had ≥4-fold rises from baseline to 1 month after MMR3 receipt, of whom 1 was seronegative at baseline, 8 had low antibody concentrations, and 12 had medium MeV neutralizing antibody concentrations at baseline. Eight of 617 subjects (1.3%) had ≥4-fold rises from baseline to 1 year after vaccination, of whom 1 was seronegative at baseline, 4 had low concentrations, and 3 had medium MeV neutralizing antibody concentrations at baseline.

Risk Factors for Negative or Low MeV Neutralizing Antibody Concentrations Before and After MMR3 Receipt

The unadjusted odds ratios (ORs) showed that those who had their first MMR dose at age 12 to <15 months (OR, 3.47; 95% confidence interval [CI], 1.24–9.72; $P = .01$) had a higher odds of being seronegative or having a lower antibody concentration at baseline, compared with those who had their first dose at age ≥15 months, and those who had their second MMR dose <15 years earlier had a lower odds of being seronegative or having a low MeV neutralizing antibody concentration, compared with those who had their second dose ≥15 years prior (OR, 0.22; 95% CI, .05–.93; $P = .03$; Table 1).

Of 50 subjects (7.6%) who received their first dose at age 12 to <15 months, 5 (10.0%) were seronegative or had a low MeV antibody concentration at baseline, compared with 19 of 612 (3.1%) who were vaccinated with their first dose at age ≥15 months. Of 190 subjects (28.7%) who received their second dose <15 years prior, 2 (1.1%) were seronegative or had low MeV antibody concentrations at baseline, compared with 22 of 472 (4.7%) who received their second dose ≥15 years earlier. In multivariate analysis, having the first MMR dose at 12 to <15 months of age remained a significant risk factor at baseline (OR, 3.94; 95% CI, 1.37–11.30; $P = .01$), and those who had their second MMR dose <15 years prior continued to have a lower odds of being seronegative or having low MeV antibody concentrations (OR, 0.18; 95% CI, .04–.80; $P = .02$).

One month after MMR3 receipt, there were no significant risk factors for being seronegative or having a low MeV antibody concentration, after adjustment of χ^2 analysis by controlling for baseline GMCs. In multivariate analysis, a significant risk factor for seronegativity or having low MeV antibody concentrations 1 month after MMR3 receipt was whether a subject was seronegative or had low MeV antibody concentrations at baseline (OR, 195.8; 95% CI, 21.8 to >999.9; $P < .0001$).

One year after MMR3 receipt, females had a lower odds of seronegativity or having a low MeV antibody concentration (OR, 0.34; 95% CI, .06–1.80; $P = .04$), compared with males, after adjustment of χ^2 analysis by controlling for baseline

Table 1. Risk Factors for Negative or Low Measles Virus (MeV) Neutralizing Antibody Concentrations at Baseline and 1 Month and 1 Year After Receiving a Third Dose of Measles, Mumps, and Rubella (MMR3) Vaccine

Factor	Baseline (n = 662)				1 mo After MMR3 Receipt (n = 662)				1 y After MMR3 Receipt (n = 617)			
	Unadjusted OR (95% CI)	*P* Value	Multivariate OR (95% CI)	Multivariate *P* Value	aOR[a] (95% CI)	*P* Value	Multivariate OR (95% CI)	Multivariate *P* Value	aOR[a] (95% CI)	*P* Value	Multivariate OR (95% CI)	Multivariate *P* Value
Sex												
Female	0.56 (.24–1.28)	.16	0.53 (.23–1.23)	.14	0.22 (.03–1.45)	.08	0.16 (.02–1.48)	.11	0.34 (.06–1.80)	.04	0.19 (.04–.99)	.049
Male	Reference		Reference		Reference		Reference		Reference		Reference	
Age at first MMR dose, mo												
12 to <15	3.47 (1.24–9.72)	.01	3.94 (1.37–11.30)	.01	0.83 (.09–7.57)	.15	. . .		1.37 (.19–9.74)	.90	. . .	
≥15	Reference		Reference		Reference		. . .		Reference		. . .	
Time since second MMR dose, y												
<15	0.22 (.05–.93)	.03	0.18 (.04–.80)	.02	1.75 (.20–15.29)	.79	. . .		2.38 (.49–11.61)	.26	2.64 (.50–14.04)	.25
≥15	Reference		Reference		Reference		. . .		Reference		Reference	
Baseline antibody concentration, mIU/mL[b]												
<121	NA		NA		NA		195.8 (21.8–>999.9)	<.0001	NA		54.95 (10.90–277.14)	<.0001
≥121	NA		NA		NA		Reference		NA		Reference	

A *P* value of <.05 was considered statistically significant.

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; NA, not applicable; OR, odds ratio.

[a] Adjusted by controlling for baseline measles virus neutralizing antibody concentration.

[b] By default, baseline MeV neutralizing antibody concentrations could not be a risk factor at baseline. We were also unable to assess the risk associated with baseline neutralizing antibody concentrations at 1 month or 1 year after MMR3 receipt during univariate analysis because this was the variable we adjusted for to account for the statistical differences between cohort 1 and cohort 2. This adjustment allowed us to combine the cohorts during the analysis to increase our power.

GMCs. In multivariate analysis at 1 year after MMR3 receipt, being female remained protective (OR, 0.19; 95% CI, .04–.99; $P = .049$) and seronegativity or having low MeV neutralizing antibody concentrations at baseline were risk factors (OR, 54.95; 95% CI, 10.90–277.14; $P < .0001$).

CMI

Of 60 subjects in the CMI substudy, 7 were excluded (6 did not receive MMR3, and 1 had insufficient blood collected); 1 of 53 subjects (1.9%) did not provide a serum specimen at 1 month, and 6 (11.3%) did not provide a specimen at 1 year. MeV lysate stimulation results were missing for an additional 2 subjects at baseline and 1 subject at 1 month. Positive controls were positive for all CMI subjects, indicating viable cells capable of spot formation. The mean number (±SD) of unstimulated spot-forming T cells/million PBMCs was 0.1 ± 0.1 at baseline, 0.1 ± 0.1 at 1 month, and 0.2 ± 0.2 1 year after MMR3 receipt.

Of 53 subjects in the CMI substudy, none were seronegative for MeV neutralizing antibody at baseline, and 5 (9.4%) had low baseline concentrations, of whom 1 had a positive baseline CMI response (≥20 SFCs/million PBMCs) to peptide stimulation, and none had a positive baseline response to lysate stimulation. Only 13 of 48 subjects (27.1%) with medium or high levels of baseline MeV neutralizing antibodies had a positive baseline CMI result by peptide stimulation, and 7 of 46 (15.2%) had a positive baseline CMI result by lysate stimulation.

The number of SFCs/million PBMCs was generally higher with peptide stimulation, compared with lysate stimulation.

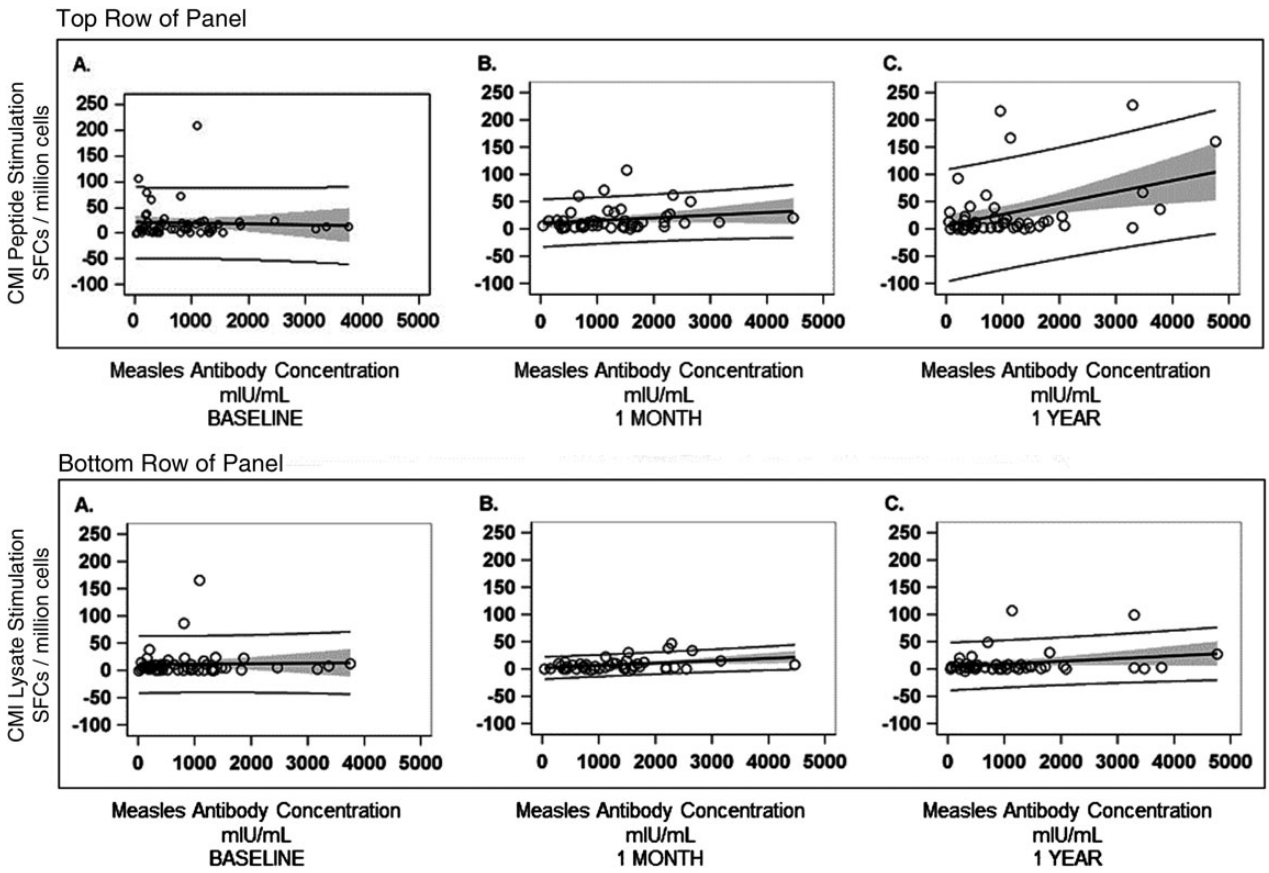

Figure 4. Top Row of Panel. *A*, Comparison of baseline measles virus neutralizing antibody concentration levels (mIU/mL) and baseline measles virus T-cell response to measles virus peptide stimulation (spot-forming cells [SFCs]/million cells; $R^2 = 0.002$, $P = .73$ [n = 53]). *B*, Comparison of measles virus neutralizing antibody concentration levels (mIU/mL) and measles virus T-cell response to measles virus peptide stimulation (SFCs/million cells) 1 month after receiving a third dose of measles, mumps, and rubella (MMR) vaccine ($R^2 = 0.05$ and $P = .13$ after removal of 2 outliers [n = 50]; $R^2 = 0.30$ and $P < .0001$ after inclusion of 2 outliers [n = 52], and the *x*-axis on the graph extended beyond 40 000 mIU/mL). *C*, Comparison of measles virus neutralizing antibody concentration levels (mIU/mL) and measles virus T-cell response to measles virus peptide stimulation (SFCs/million cells) 1 year after receiving a third dose of MMR vaccine ($R^2 = 0.17$, $P = .004$ [n = 47]). Bottom Row of Panel. *A*, Comparison of baseline measles virus neutralizing antibody concentration levels (mIU/mL) and baseline measles virus T-cell response to measles virus lysate stimulation (SFC/million cells), $n = 51$. $R^2 = 0.0008$, $P = .85$. *B*, Comparison of measles virus neutralizing antibody concentration levels (mIU/mL) and measles virus T-cell response to measles virus lysate stimulation (SFC/million cells) 1 month after receiving a third dose of MMR vaccine, $n = 49$. $R^2 = 0.14$, $P = .007$ (Note that 1 outlier was removed from the figure; the other outlier was already missing because of insufficient blood drawn to analyze the measles virus lysate response). When the outlier was included, the results were: $n = 50$. $R^2 = 0.001$, $P = .80$, and the x-axis on the graph extended beyond 40 000 mIU/mL. *C*, Comparison of measles virus neutralizing antibody concentration levels (mIU/mL) and measles virus T-cell response to measles virus lysate stimulation (SFC/million cells) 1 year after receiving a third dose of MMR vaccine, $n = 47$. $R^2 = 0.06$, $P = .09$. For all figures, data points are represented by circles and show the comparison result for each subject. The dark solid line represents the best fit of the comparison. The light shading around the line represents the 95% confidence limits. The dotted lines represent 95% prediction limits. Abbreviation: CMI, cell-mediated immunity.

Table 2. Correlation Between Measles Virus (MeV) Neutralizing Antibody (NAb) Geometric Mean Concentrations, as Measured by Plaque Reduction Neutralization, and MeV Antibody Avidity Levels, According to End-Titer Avidity Indexes, at Baseline and 1 Month and 1 Year After Receiving a Third Dose of Measles, Mumps, and Rubella (MMR3) Vaccine

		Baseline						1 mo After MMR3 Receipt						1 y After MMR3 Receipt[b]					
			Avidity Index						Avidity Index						Avidity Index				
Quartile[a]	Subjects, No.	MeV NAb Concentration GMC, mIU/mL	Mean[c]	Neg[d]	Low	Int	High	MeV NAb Concentration GMC, mIU/mL	Mean[c]	Neg[d]	Low	Int	High	MeV NAb Concentration GMC, mIU/mL	Mean[c]	Neg[d]	Low	Int	High	Miss
1	27	69	71	8 (29.6)	0	10 (37.0)	9 (33.3)	249	75	2 (7.4)	0	5 (18.5)	20 (74.1)	143	73	1 (3.7)	0	6 (22.2)	16 (59.3)	4 (14.8)
2	11	556	78	0	0	0	11 (100)	606	81	0	0	0	11 (100)	466	80	0	0	0	10 (90.9)	1 (9.1)
3	11	990	79	0	0	1 (9.1)	10 (90.9)	1222	78	0	0	0	11 (100)	750	79	0	0	1 (9.1)	10 (90.9)	0
4	10	2130	78	0	0	2 (20)	8 (80)	2435	78	0	0	2 (20)	8 (80)	2225	81	0	0	3 (30)	7 (70)	0
Total	59	299	75	8	0	13	38	582	77	2	0	7	50	415	77	1	0	10	43	5

Data are no. or no. (%) of subjects, unless otherwise indicated.

Abbreviations: GMC, geometric mean concentration; IgG, immunoglobulin G; Int, intermediate; Miss, missing.

[a] Quartiles were established based on baseline plaque reduction neutralization measles antibody concentration. Subjects with the lowest baseline measles neutralizing antibody concentrations were placed in quartile 1 and subjects with the highest baseline measles neutralizing antibody concentrations were placed in quartile 4. The number of subjects selected from quartile 1 is more than from the other 3 quartiles, because we tested the avidity on every subject who had a negative or low measles neutralizing antibody concentration during at least 1 time point. Of note, 24 of 27 subjects in quartile 1 had a negative or low baseline measles antibody concentration; the remaining 3 subjects in quartile 1 had a medium neutralizing antibody concentration at baseline (but were still in the lowest quartile).

[b] Five subjects were missing data at 1 year.

[c] The mean avidity index excludes the specimens that tested negative by Captia Measles IgG enzyme immunoassay, since avidity could not be performed on those specimens.

[d] Negative (Neg) means that at 1:21 dilution, the specimen had undetectable IgG by the Captia Measles IgG enzyme immunoassay.

At baseline, the mean number of MeV peptide–stimulated SFCs (±SD) was 19.6 ± 9.3 SFCs/million PBMCs, compared with 11.9 ± 7.2 SFCs/million PBMCs by lysate stimulation. One month after MMR3 receipt, the mean number of MeV peptide–stimulated SFCs (±SD) was 18.5 ± 7.6 SFCs/million PBMCs, with 13 of 52 specimens (25.0%) positive by peptide stimulation, compared with 7.3 ± 2.9 SFCs/million PBMCs, with 5 of 51 (9.8%) positive by lysate stimulation. At 1 year after MMR3 receipt, the mean number of SFCs (±SD) was 29.7 ± 15.9 SFCs/million PBMCs, with 14 of 47 (29.8%) positive by peptide stimulation, compared with 10.3 ± 6.4 SFCs/million PBMCs, with 7 of 47 (14.9%) positive by lysate stimulation.

Baseline MeV antibody concentrations did not correlate with baseline MeV-specific T-cell responses to peptide stimulation ($R^2 = 0.002$, $P = .73$) or lysate stimulation ($R^2 = 0.0008$, $P = .85$; Figure 4). MeV neutralizing antibody concentrations at 1 month after MMR3 receipt correlated with MeV T-cell responses at 1 month by peptide stimulation ($R^2 = 0.30$, $P < .0001$), but the correlation did not remain after removing the 2 outliers ($R^2 = 0.05$, $P = .13$). There was no correlation between MeV antibody concentrations and lysate stimulation at 1 month ($R^2 = 0.001$, $P = .80$), but after removing the outlier, there was a correlation ($R^2 = 0.14$, $P = .007$). At 1 year after MMR3 receipt, there was a significant correlation between MeV antibody concentrations and MeV T-cell responses by peptide stimulation ($R^2 = 0.17$, $P = .004$), but no correlation by lysate stimulation ($R^2 = 0.06$, $P = .09$).

MeV IgG Antibody Avidity

Overall, 38 of 59 subjects (64.4%) evaluated had MeV antibodies with high avidity at baseline (Table 2), including 7 of 24 subjects (29.2%) with low MeV antibody concentrations at baseline. The avidity results did not correlate with MeV antibody concentrations at baseline ($R^2 = 0.07$, $P = .07$) or 1 month ($R^2 = 0.01$, $P = .50$) or 1 year ($R^2 = 0.02$, $P = .31$) after MMR3 receipt (Figure 5).

DISCUSSION

A modest but significant boost in MeV geometric mean neutralizing antibody concentrations occurred 1 month and 1 year after MMR3 receipt, compared with baseline. However, almost all subjects were MeV seropositive prior to receiving MMR3, and subjects' antibody levels returned to near-baseline levels 1 year after vaccination. Nonetheless, for the 24 subjects (3.6%) with low or negative baseline MeV antibody concentrations, 18 (75%) moved into medium or high categories at 1 month, of whom 12 (67%) remained medium or high at 1 year. Among the subsets tested for CMI and avidity, we did not find compelling qualitative data to support a routine third dose of MMR vaccine.

The second MMR vaccine dose was recommended to provide measles immunity to individuals who did not respond to the first dose [19]; 2 doses are 97% effective at preventing measles [20, 21]. Although 95% of vaccinated persons have detectable MeV antibodies 10–15 years after the second MMR dose

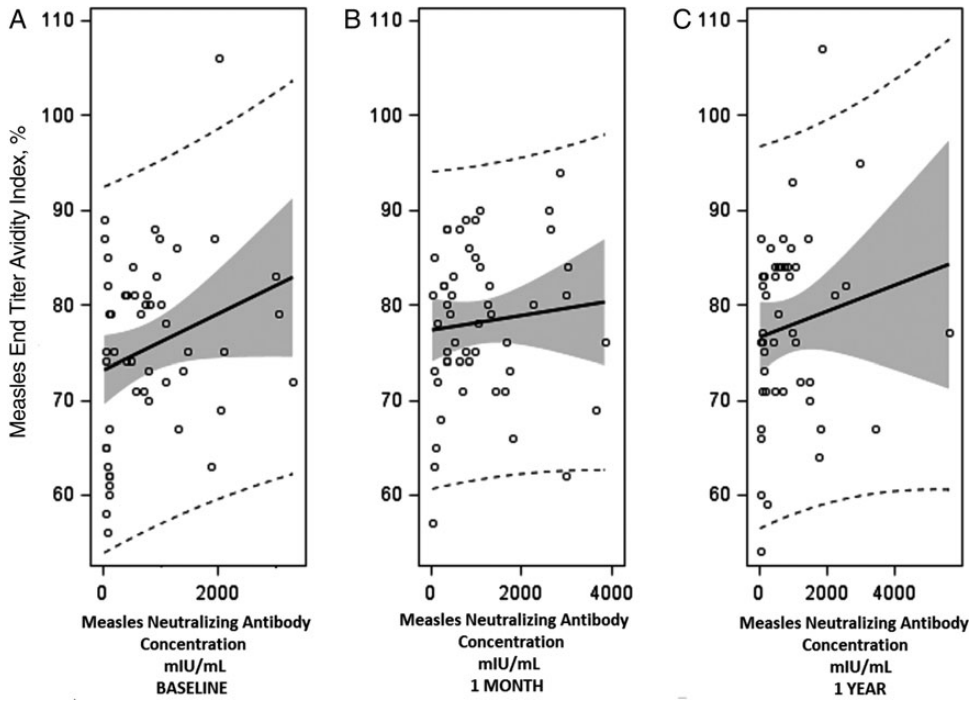

Figure 5. *A*, Comparison of baseline measles virus neutralizing antibody concentration levels (mIU/mL) and baseline measles virus antibody avidity levels (end-titer avidity index [AI]; $R^2 = 0.07$, $P = .07$ [n = 51]). *B*, Comparison of measles virus neutralizing antibody concentration levels (mIU/mL) and measles virus antibody avidity levels (end-titer AI) 1 month after receiving a third dose of measles, mumps, and rubella (MMR) vaccine ($R^2 = 0.01$, $P = .50$ [n = 51]). *C*, Comparison of measles virus neutralizing antibody concentration levels (mIU/mL) and measles virus antibody avidity levels (end-titer AI) 1 year after receiving a third dose of MMR vaccine ($R^2 = 0.02$, $P = .31$ [n = 47]). For all figures, data points are represented by circles, and they show the comparison result for each subject. The dark solid line represents the best fit of the comparison. The light shading around the line represents the 95% confidence limits. The dotted lines represent 95% prediction limits.

[10, 22], waning immunity occurs after 2 doses [10, 23], and 2-dose failures have been documented [24].

Having a low or negative baseline MeV antibody concentration was the biggest risk factor for low or negative antibody concentrations 1 month and 1 year after MMR3 receipt, suggesting that inherent biology may be partially responsible for a person's MeV antibody levels [10, 25]. Although our results concurred with those in other reports that the timing of administration of the first and second MMR doses significantly affected MeV antibody levels later in life [26, 27], our findings represented only a small proportion of the study population (only 50 subjects [7.6%] received their first dose at age 12 to <15 months).

Most subjects did not have a positive CMI result at baseline, despite the majority of subjects having medium or high baseline MeV antibody concentrations. Nonetheless, low levels of SFCs generally occurred for most specimens after stimulation, suggesting T-cell memory. However, this was not greatly boosted by MMR3. After removing outliers, we found mixed results 1 month after MMR3 receipt, with no correlation between MeV antibody response and MeV T-cell response by peptide stimulation but a significant correlation by lysate stimulation. Although we did find a significant correlation between CMI response by peptide stimulation and MeV antibody concentration at 1 year after MMR3 receipt, less than one third of subjects had positive cell-mediated responses by peptide stimulation, and even fewer had positive responses by lysate stimulation at 1 year. These findings could have been because transient increases in circulating MeV-specific T-cells were missed due to specimen collection timing (antigen-stimulated T-cell responses typically peak 2 weeks after vaccination [28], whereas samples were taken 1 month and 1 year after MMR3 receipt). Other studies assessing antibody and T-cell responses after a second MMR dose showed no correlation [29, 30]. Another possibility is that numbers of T cells producing IFN-γ in response to MeV did not increase after MMR3 receipt, owing to lack of infection by vaccine virus in the presence of neutralizing antibodies.

The MeV IgG avidity results did not correlate with neutralization results. Most subjects reached an IgG avidity plateau. Typically, IgG avidity maturation for MeV shifts from low to high 4 months following immunization or infection [16], which might negate additional increases in antibody avidity with subsequent doses of MeV-containing vaccine. Nonetheless, only 29% of subjects with low baseline MeV neutralizing antibody concentrations had high avidity results at baseline. It could be interpreted that subjects with poor antibody response and intermediate avidity results were potentially susceptible prior to revaccination. However, the avidity results are an average of the MeV-specific IgG and should be interpreted cautiously, since whole MeV is used as the

target antigen in the avidity assay, whereas the neutralization assay measures antibodies that bind MeV surface glycoproteins [31].

Our study had additional limitations. Subjects were not representative of the US population. Selection bias may have occurred in cohort 1, because MMR3 was only offered to those who had a low or negative MeV, mumps virus, or rubella virus antibody concentration during the longitudinal study.

Overall, MeV neutralizing antibody concentrations initially increased after MMR3 but declined to near-baseline levels 1 year later. Although our findings showed that MMR3 increased antibody levels for the small percentage of subjects with low MeV neutralizing antibody concentration levels who were on the cusp of protection, the CMI and avidity results in the subset tested showed that MMR3 did not result in substantial improvements in the quality of the immune response. While a third MMR dose may successfully immunize the rare individual who did not respond after 2 doses, MMR3 is unlikely to solve the problem of waning immunity in the United States. A better strategy for maintaining US measles elimination would be to improve vaccination coverage in pockets of unvaccinated individuals and maintain high 2-dose coverage nationally with the current 2-dose MMR recommendation.

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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