

Persistence of Rubella Antibodies after 2 Doses of Measles-Mumps-Rubella Vaccine

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Background. Since 1990, most schoolchildren in the United States have received a second dose of measles-mumps-rubella vaccine (MMR2) at kindergarten entry. Elimination of endemic rubella virus circulation in the United States was declared in 2004. The objective of the current study was to evaluate the short- and long-term rubella immunogenicity of MMR2.

Methods. At enrollment in 1994–1995, children ($n = 307$) in a rural Wisconsin health maintenance organization received MMR2 at age 4–6 years. A comparison group of older children ($n = 306$) was vaccinated at age 9–11 years. Serum specimens were collected during a 12-year period. Rubella antibody levels were evaluated by plaque-reduction neutralization (lowest detectable titer, 1:10).

Results. Before administration of MMR2 in the kindergarten group, 9% of subjects were seronegative, 60% had the lowest detectable titer, and the geometric mean titer (GMT) was 1:13. One month after administration of MMR2, 1% were seronegative, 6% had the lowest detectable titer, and the GMT was 1:42. Four-fold boosts occurred in 62% of subjects, but only 0.3% were immunoglobulin M positive. Twelve years after MMR2 administration, 10% were seronegative, 43% had the lowest detectable titer, and the GMT was 1:17. The middle-school group showed similar patterns.

Conclusions. Rubella antibody response to MMR2 was vigorous, but titers decreased to pre-MMR2 levels after 12 years. Because rubella is a highly epidemic disease, vigilance will be required to assure continued elimination.

Rubella is an infectious viral disease, typically causing a mild fever, rash, and lymphadenopathy [1]. Infection of a pregnant woman, however, can have devastating effects on the fetus, including cataracts, hearing impairment, heart defects, and other disorders termed congenital rubella syndrome (CRS) [1]. Before the introduction of vaccine in the United States, CRS oc-

curred in ~1 of 2000 live births [2], consistent with rates in other developed nations [3]. However, the incidence of rubella was highly episodic [4], and during the 1963–1964 epidemic, as many as 1 in 100 births may have been affected [5].

Rubella vaccine was licensed in 1969 and then combined with measles and mumps vaccines in 1971, in a universal childhood vaccination program [6]. By 1989, reported cases of rubella and CRS had been reduced by 97%. In that year, the Advisory Committee on Immunization Practices (ACIP) recommended a 2-dose measles-mumps-rubella (MMR) vaccine schedule for improved measles control [7]. During the next decade, increased rubella vaccination efforts within the United States [8] and elsewhere in the hemisphere [9] resulted in historically low incidences of rubella and CRS [10]. In 2004, an expert panel concluded that ongoing rubella virus transmission had been eliminated from the United States [6].

Because more than two-thirds the world's children live in countries without rubella vaccination programs

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Table 1. Study Design and Subject Retention

Serum specimen collection	Period	Kindergarten group (<i>n</i> = 312)		Middle-school group (<i>n</i> = 309)	
		Subject age, years	No. (%) of subjects ^a	Subject age, years	No. (%) of subjects ^a
Before MMR2 administration	1994–1995	5	307 (98)	10	306 (99)
After MMR2 administration					
1 month	1994–1995	5	301 (96)	10	301 (97)
6 months	1994–1995	5	297 (95)	10	302 (98)
2 years	1996–1997	7	242 (78)	12	265 (86)
7 years	2001–2002	12	160 (51)	17	189 (61)
10 years	2004–2005	15	154 (49) ^b
12 years	2006–2007	17	144 (46) ^b

^a Percentages indicate the percentage of the originally enrolled cohort.

^b Serum specimens were not obtained after the study end point (age, 17 years).

[11], the risk of rubella importation remains. However, few studies have examined the long-term persistence of antibodies after administration of 2 doses of rubella vaccine in the absence of endemic wild-type virus circulation [12, 13]. In 1994, as reported elsewhere [14–16], the Centers for Disease Control and Prevention (CDC) began a longitudinal study of the immunogenicity of the second MMR vaccine dose (MMR2). We now report our rubella findings.

METHODS

Context

A second dose of measles vaccine is required for school attendance in all states in the United States, at kindergarten entry in most states and at middle-school entry in some [17]. Almost all measles vaccine is administered as MMR vaccine in the United States [8]. In 1990, Wisconsin passed a law requiring MMR2 for both kindergarten and middle-school entry, providing a setting to examine the immunogenicity of 2 frequently used schedules.

Setting and Subjects

As described elsewhere [14–16], the study population was drawn from patients of Marshfield Clinic, a comprehensive health maintenance organization that is the principal health care provider for rural central Wisconsin. In 1994, the clinic's computerized files were reviewed to identify 2 groups of children for whom MMR2 was required: kindergartners (age, 4–6 years) and middle schoolers (age, 10–12 years). Candidate study subjects were excluded if they (1) had received the first dose of MMR vaccine (MMR1) other than at 12–24 months of age; (2) had previously had measles, mumps, or rubella disease; (3) had lived in the same household with anyone who had had any of these diseases during the subject's lifetime; or (4) had any contraindication to MMR vaccination or any condition likely to impair immune response to MMR vaccine, according to ACIP recommendations [18]. Parents of study subjects

were provided with informed permission materials, and middle schoolers were also provided with informed assent materials. The study was approved by the human subjects protection offices of both the Marshfield Clinic and the CDC.

Design

Prevaccination serum specimens were obtained, and MMR2 (M-M-R_{II}; Merck) was administered <72 h thereafter by study nurses, along with any other vaccinations for which the child was eligible. Adverse events were evaluated, as reported elsewhere [14]. Serum specimens were collected according to a schedule (Table 1) that permitted antibody levels for the 2 groups to be compared between subjects at similar ages, with a study end point of age 17 years. At each collection, families were questioned concerning rubella disease, exposures, vaccinations, and other health events. Clinic and CDC data concerning rubella disease activity were also reviewed.

Laboratory Methods

Neutralization tests. Antibody levels were evaluated by the plaque-reduction neutralization test, with immunoenzymatic staining to visualize plaques, as described elsewhere [16]. Testing was performed at the end of the study, and specimens from individual subjects were tested in the same run. Other than each subject's unique identifier and serum collection dates, the laboratory was blinded to all study information. The baby hamster kidney cell line (BHK-21; American Tissue Culture Collection) and 48-well plates (Nunc) were used in the virus neutralization tests. The rubella virus used was the Gilchrist strain, a wild-type strain first isolated in 1963 at the National Institutes of Health; it produces practically no cytopathic effect within 72 h after infection. For positive virus controls, 6 wells were infected with virus but no serum; for negative controls, 6 wells were not infected. Serum controls were high-positive, low-positive, and negative serum specimens, as evaluated by enzyme immunoassay (EIA). All test and control serum specimens were

2-fold serially diluted from 1:10 to 1:160 in minimal essential Eagle's and Earle's cell culture medium. Titers <1:10 were considered to indicate seronegativity.

For the virus neutralization step, a cell-free dilution of rubella virus pretitrated to ~90–100 plaques per well was added to each serum dilution and incubated at 37°C in a carbon dioxide (CO₂) incubator for 90 min. Then, 40,000 freshly trypsinized BHK cells were added, and the plates were transferred to the CO₂ incubator. After 2 h of incubation, the serum-virus mixture was removed from all wells and supplemented with freshly prepared medium containing 4% fetal bovine serum. After 72 h of incubation, the cell culture medium was aspirated from all wells and dried at room temperature, and the cell monolayer was fixed with 80% cold acetone. After 15-min fixation, the acetone was removed, and the cell monolayer was dried again at room temperature, when the plates were ready for immediate staining or storage at –70°C for future probing.

For the immunoenzymatic staining step, pretitrated pooled monoclonal antibody (Abcam) to rubella viral proteins (capsid, E1, and E2) was added and incubated at 37°C for 60 min. After unbound antibody was removed, the plates were washed 3 times with phosphate-buffered saline (0.05 mol/L; pH 7.4). Pretitrated horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin (Ig) G (Accurate Chemical & Scientific) was added, and the plates incubated at 37°C for 60 min and then washed again 3 times. HistoMark (Kirkegaard and Perry Laboratory) was added, the plates were incubated at 37°C for 30 min, and the substrate solution was removed and rinsed briefly with water to stop further color development.

All wells were inspected visually with a magnifying glass. A black-brown spot ≥1 mm in diameter was considered a plaque. Compared with the mean plaque count of the positive virus control wells, a 70% reduction in plaque count was considered indicative of neutralizing antibody.

IgM EIAs. Serum specimens obtained 1 month after MMR2 administration were evaluated for anti-rubella IgM by the California State Laboratory proprietary EIA, using methods described elsewhere [19]. Rheumatoid factor and excess IgG were removed by absorption with anti-human IgG (Gull SORB; Gull Laboratories). Concurrent testing was performed using the Captia Rubella-M enzyme-linked immunosorbent assay (Trinity Biotech), an IgM capture format assay found to be highly specific among commercial assays [20]. To help evaluate the single discrepant result, we used the Sorin ETI-RUBEK-M assay (Sorin Biomedica), which was also found to be highly specific among commercial assays [21, 22].

Avidity testing. Selected serum specimens obtained 1 month after MMR2 were also evaluated for anti-rubella IgG avidity (the strength with which antibody binds to antigen), using a washing step with urea to an indirect solid-phase enzyme-linked immunosorbent assay with alkaline phosphatase-conjugated anti-human IgG (Labsystems), as described elsewhere [23–25]. Avidity was classified as low (<15%), consistent with a naive or primary immune response, or high (>25%), consistent with an anamnestic or secondary immune response. Intermediate avidity (15%–25%) was considered to be indeterminate.

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Analytic Methods

Serum specimens with reciprocal titers of <10 or >160 were assigned values of 5 and 320, respectively, for estimation of geometric mean titers (GMTs). The distribution of titers, the proportion seronegative, and the proportion with the lowest detectable reciprocal titer (10) were examined as primary indicators of antibody level. These indicators were used to compare pre- and postvaccination antibody levels, and the 2 study groups were compared at the same serum collections and at the same ages. Risk factors examined for the antibody indicators were sex, age at MMR1, and mother's birth year. In addition, the interval since MMR1 was examined for pre-MMR2 antibody levels, and pre-MMR2 antibody levels were compared with post-MMR2 antibody levels. Data were insufficient to examine race or ethnicity (604 of 613 subjects were non-Hispanic white) or concurrent receipt of other vaccinations with MMR2 (305 of 306 middle schoolers received no additional vaccinations). Distribution of titers was treated as an ordinal variable, and the following tests were used: (1) Cochran-Mantel-Haenszel row mean scores for comparisons across levels of categorical variables; (2) Jonckheere-Terpstra test for comparisons of ordinal variables; (3) and Kruskal-Wallis and linear regression analysis for comparisons involving continuous variables. For association of categorical variables, Pearson χ^2 and Fisher exact tests were used.

RESULTS

Study Population

The kindergarten and middle-school groups did not differ significantly by sex, race/ethnicity, or age at administration of MMR1 (Table 2). The study subject retention rate was 96.5% (599 of 621 subjects) during the first 6 months and 53.6% (333 of 621 subjects) at the last serum specimen collection (Table 1). The 321 children who contributed serum specimens at every collection did not differ significantly from the 300 others for sex, race, receipt of other vaccinations, age at receipt of MMR2, or rates of seronegativity at the first collection. Study completers had received MMR1 ~1 week earlier than noncompleters (15.6 vs 15.9 months; $P < .001$) and had lower antibody levels at the first collection (GMT, 10.6 vs 12.6; $P < .001$).

Pre-MMR2 Antibody Levels

Compared with the middle-school group (10 years after MMR1 administration), the kindergarten group (4 years after MMR1

Table 2. Study Population Characteristics

Characteristic	Kindergarten group (n = 307)	Middle-school group (n = 306)	P
Age group related			
Age at MMR2, median years (range)	5.1 (4.2–6.1)	11.2 (10.1–12.5)	<.001
Mother's birth year median (range) ^a	1961 (1946–1972)	1957 (1940–1967)	<.001
Other vaccinations with MMR2, no. (%)	217 (71)	1 (0.3)	<.001
Other			
No. (%) of female subjects	151 (49)	150 (49)	NS
No. (%) of white subjects	300 (98)	304 (99)	NS
Age at MMR1, median months (range)	15.6 (12.8–24.7)	15.7 (14.1–24.5)	NS

NOTE. MMR1, first dose of measles-mumps-rubella (MMR) vaccine; MMR2, second dose of MMR vaccine; NS, not significant.

^a Mother's birth year was not available for 13 subjects (3 in the kindergarten group, 10 in the middle-school group).

administration) had higher overall antibody levels before MMR2 (GMT, 12.5 vs 10.5 for kindergarten vs middle-school group; $P < .001$), and fewer children with negative titers (9.4% [29 of 307 subjects] vs 24.5% [75 of 306 subjects]; $P < .001$) (Figure 1). The majority of subjects in each group had the lowest detectable titer (59.6% [183 of 307 subjects] in the kindergarten group vs 52.3% [160 of 306 subjects] in the middle-school group; $P = .073$).

Initial MMR2 Response

Overall. For each group, titers increased ~3-fold 1 month after MMR2 and then decreased at 6 months but were still significantly higher than pre-MMR2 titers (Figure 1). At 6 months, the proportion in each group with negative titers was reduced to near zero (kindergarten group, 0.3% [1 of 297 subjects]; middle-school group, 0.99% [3 of 302 subjects]), and the proportion with the lowest detectable titer was significantly diminished (kindergarten group, from 59.6% [183 of 307 subjects] to 14.1% [42 of 297 subjects]; middle-school group, from 52.3% [160 of 306 subjects] to 21.5% [65 of 302 subjects]; $P < .001$). Subjects in the kindergarten group continued to have higher antibody levels than those in the middle-school group (GMT, 31.8 vs 27.1; $P = .006$).

Four-fold rises. Almost two-thirds of each group exhibited a 4-fold increase (kindergarten group, 62.2% [194 of 312 subjects]; middle-school group, 66.3% [205 of 309 subjects]; $P = .315$). Four-fold increases were more likely in subjects with lower pre-MMR2 titers; they were seen in 87.5% [91 of 104 subjects] with negative pre-MMR2 titers (ie, <10), 75.2% [258 of 343 subjects] with low pre-MMR2 titers (ie, 10), 31.8% [50 of 157 subjects] with medium pre-MMR2 titers (ie, 20–40), and 0% [0 of 9 subjects] with high pre-MMR2 titers (ie, ≥ 80 ; $P < .001$).

IgM assay results. Of the 612 specimens tested 1 month after MMR2 administration, 3 (0.5%) were positive for IgM,

each with confirmation by a second assay; these specimens included 1 (0.3%) of 304 from the kindergarten group and 2 (0.6%) of 308 from the middle-school group. Each of the 3 subjects had negative pre-MMR2 titers and 4-fold increases in response to MMR2.

Avidity. Of 188 specimens tested 1 month after MMR2 administration, 2 (1.1%) had low avidity, none had intermediate avidity, and 186 (98.9%) had high avidity. The mean avidity was 53.3 (range, 7.0–83.5). Both subjects with low-avidity specimens had negative pre-MMR2 titers, 4-fold increases in response to MMR2, and specimens positive for IgM.

Persistence of Antibodies

Comparison with pre-MMR2 titers. By age 17 years (12 years after MMR2 administration), titers for subjects in the kindergarten group (GMT, 16.9) were significantly lower ($P < .001$) than the postvaccination peak (Figure 2) but still higher than pre-MMR2 titers (GMT, 12.5; $P < .001$). However, the proportion with negative titers (9.7% [14 of 144 subjects]) was now the same as before MMR2 administration (9.4% [29 of 307 subjects]; $P = .926$) (Figure 3). The pattern for the middle-school group (at 7 years after MMR2 administration) was the same: overall titers were higher (GMT, 12.1) than before MMR2 administration (GMT, 10.5; $P = .014$), but the proportions with negative titers were similar (24.5% [75 of 306 subjects] before MMR2 administration vs 20.6% [39 of 189 subjects] after 7 years; $P = .320$).

Comparison between groups. At each serum specimen collection, subjects in the kindergarten group had significantly higher titers than did those in the middle-school group, and a significantly smaller proportion with negative or the lowest detectable titer (Figure 4). At the study end point (subject age, 17 years), the kindergarten group had significantly higher titers than did the middle-school group (GMT, 16.9 vs 12.1; $P < .001$), a smaller proportion with negative titers (9.7% [14 of

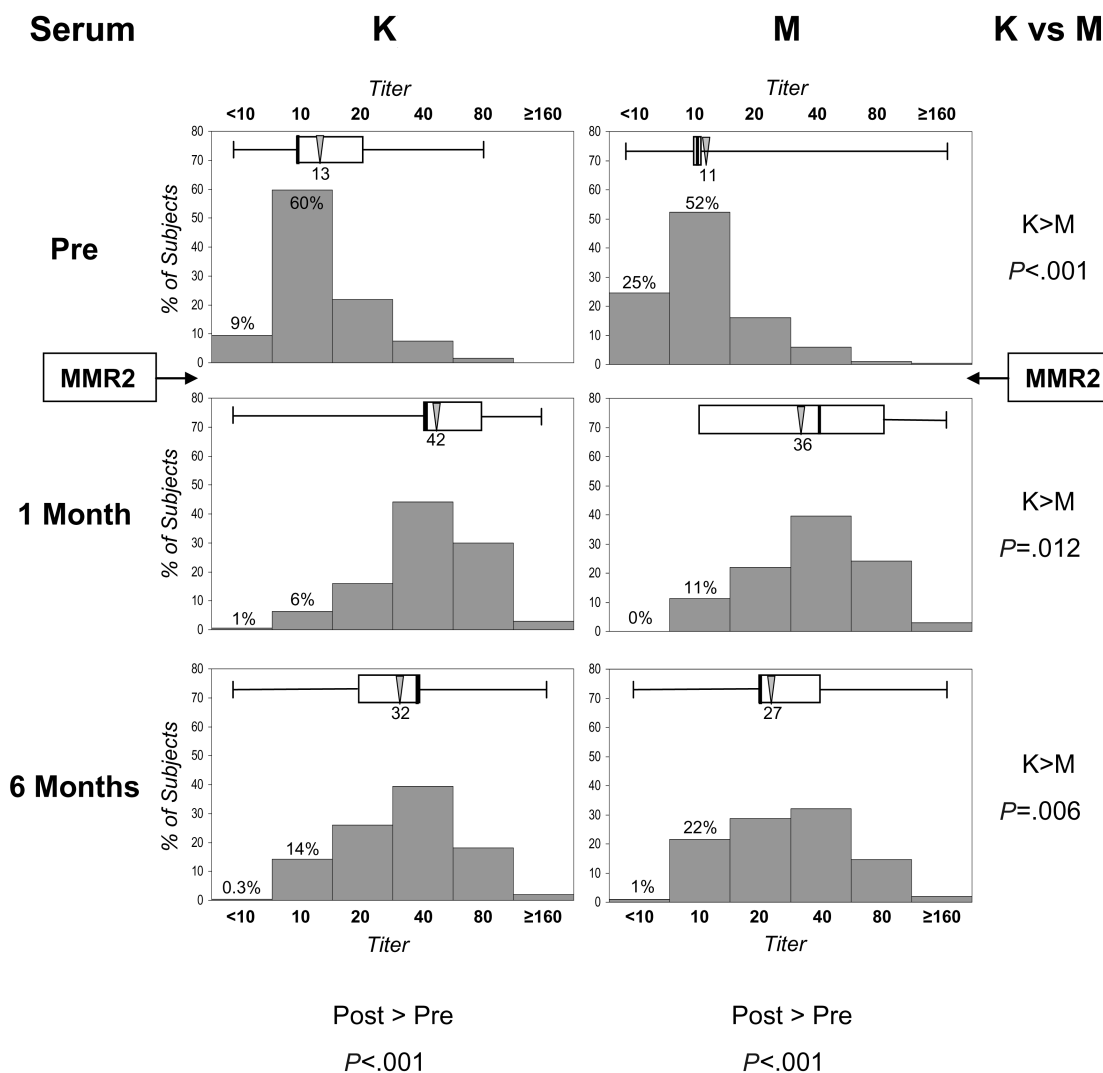


Figure 1. Initial rubella antibody response to the second dose of measles-mumps-rubella vaccine (MMR2). Each graph represents a serum collection, and bars represent proportions of the population with specified titers from the rubella plaque-reduction neutralization test. Box-and-whiskers plots represent ranges and 25th and 75th percentiles, dark vertical lines represent median titers, and triangles represent geometric mean titers. Left-hand graphs represent the group that received MMR2 at kindergarten (K) entry; right-hand graphs represent the group that received MMR2 at middle-school (M) entry. P values refer to differences between titer distributions (Jonckheere-Terpstra test). Pre, before MMR2; Post, after MMR2.

144 subjects] vs 20.6% [39 of 189 subjects]; $P = .007$), and a similar proportion with the lowest detectable titer (43.1% [62 of 144 subjects] vs 43.9% [83 of 189 subjects]; $P = .964$).

Evidence of Non-MMR2 Boosting

Of the 1144 specimens tested beyond the period of likely MMR2 vaccination effect (≥ 2 years after MMR2 administration), 87 (7.6%) demonstrated a 4-fold titer boost (mean, 5.9-fold) in parallel testing. Subsequent neutralization results were available for 33 of these subjects, with the 4-fold increase sustained at the next collection in 12 (36.4%). Four-fold boosts were seen at each serum collection in both groups, with no significant

trend in rates observed. The likelihood of a 4-fold boost was significantly associated ($P < .001$) with a lower preceding titer; such boosts were seen in 30.1% (28 of 93 subjects) with negative (ie, <10), 9.6% (32 of 334 subjects) with low (ie, 10), 4.4% (27 of 609 subjects) with medium (ie, 20–40), and 0% (0 of 108 subjects) with high (ie, >80) titers at the preceding collection. Of the 333 children with neutralization results from the final collection, 60 (18.0%) overall had experienced a non-MMR2 boost at some point, significantly more in the kindergarten group (29.2% [42 of 144 subjects]; 4 collections over 10 years) than in the middle-school group (9.5% [18 of 189 subjects]; 2 collections over 5 years; $P < .001$).

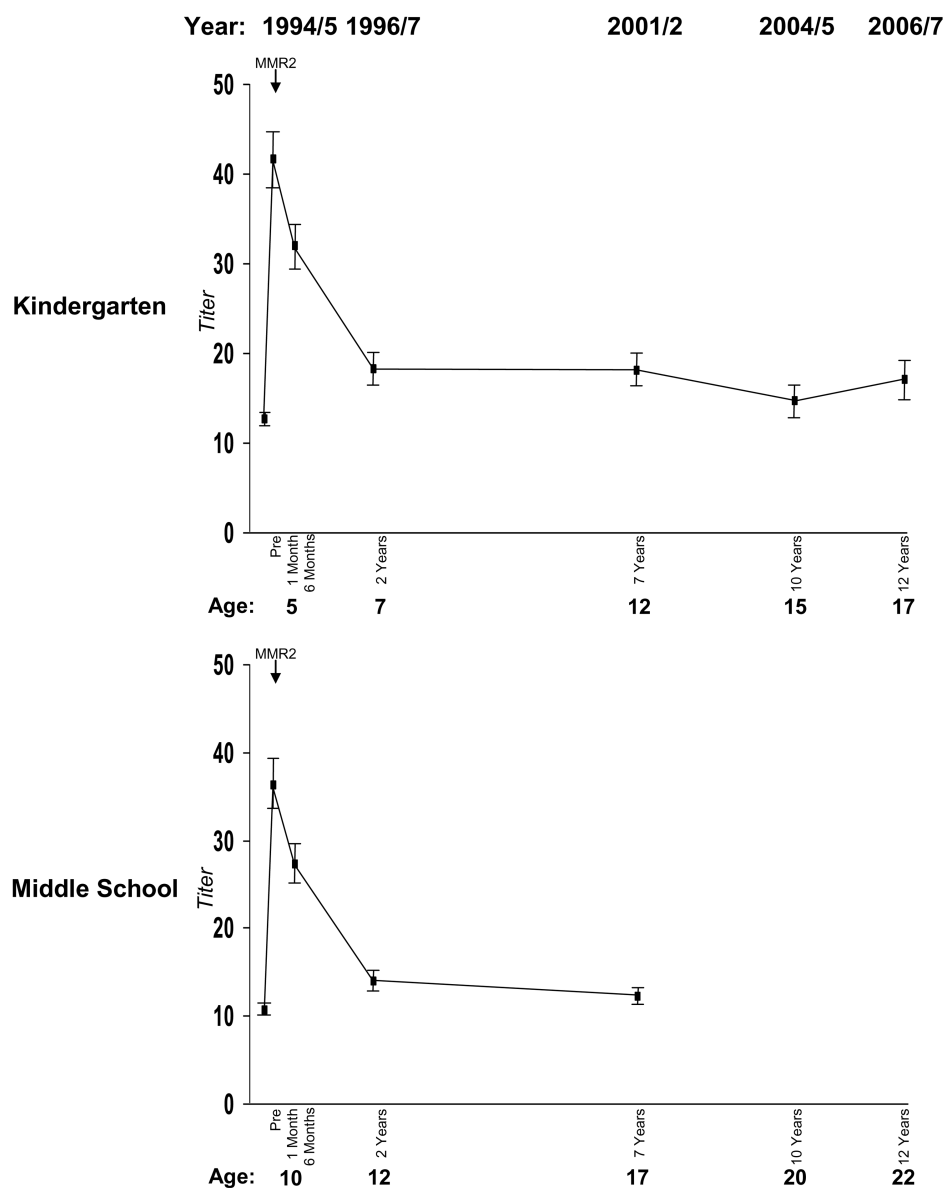


Figure 2. Changes in rubella antibody geometric mean titers (GMTs) after second dose of measles-mumps-rubella vaccine (MMR2). Graphs represent changes over time in rubella GMTs (*solid lines*) as demonstrated by plaque-reduction neutralization tests, with 95% confidence intervals (*whiskers*). Top graph represents the group that received MMR2 at kindergarten entry; bottom graph represents the group that received MMR2 at middle-school entry.

Rubella Disease Reports

During the study subjects' lifetimes before the study period (1981–1993), 11,110 US rubella cases were reported to the CDC, including 214 from Wisconsin and 1 from the 7 counties surrounding Marshfield Clinic. During the study period (1994–2007), 1673 US rubella cases were reported to the CDC, including 4 from Wisconsin and none from the 7 counties surrounding Marshfield Clinic.

Risk Factors for Antibody Levels and Seronegativity

Pre-MMR2 risk factors. The shorter the interval since the child had received MMR1, the higher the titer ($P < .001$), but the effect was small in aggregate ($R^2 = .021$) and disappeared when data were examined by group. Seronegativity showed a similar pattern. Male subjects had higher rates of seronegativity than female subjects (20.2% [63 of 312 subjects] vs 13.6% [41 of 301 subjects]; $P = .032$), an effect seen in each group but

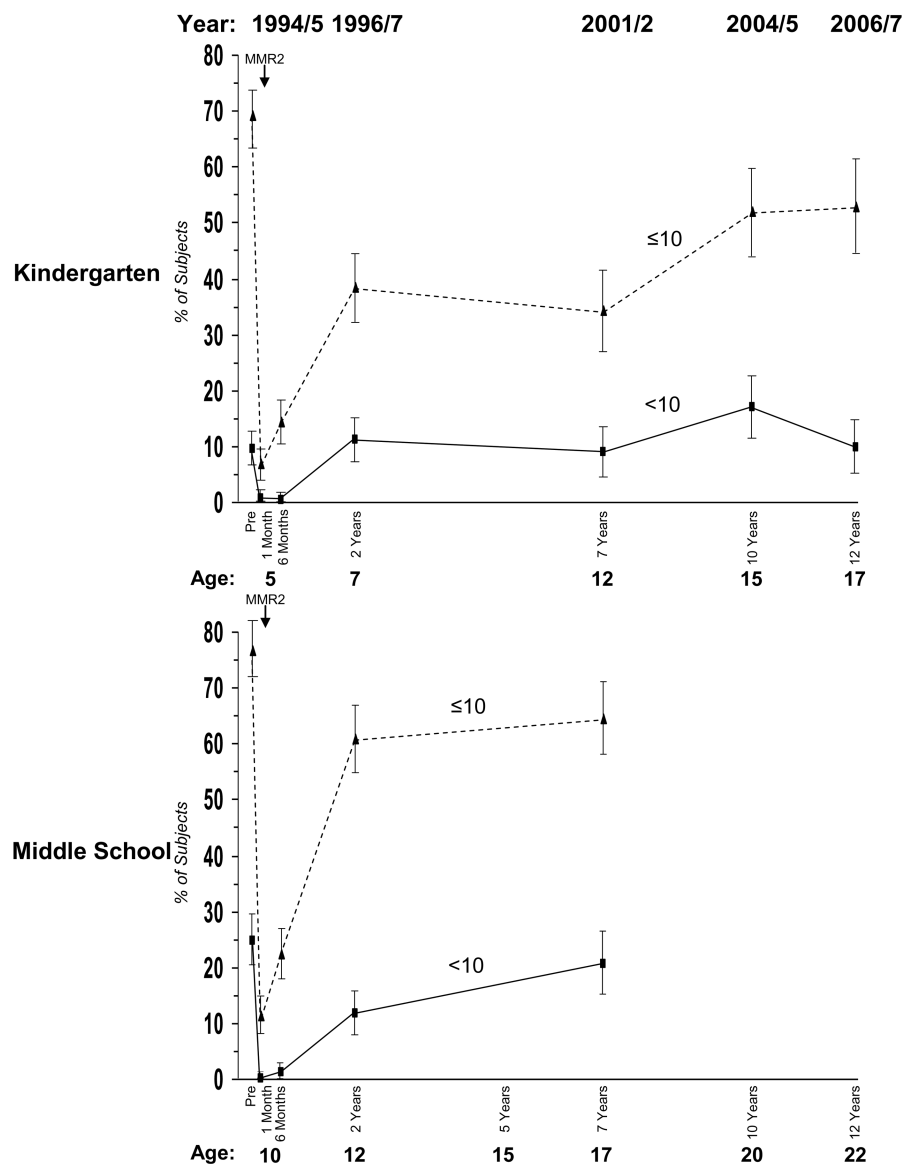


Figure 3. Change in proportions of subjects with negative or low rubella antibody titers as demonstrated by plaque-reduction neutralization tests, with 95% confidence intervals (*whiskers*). Top graph represents the group that received the second dose of measles-mumps-rubella vaccine (MMR2) at kindergarten entry; bottom graph represents the group that received MMR2 at middle-school entry.

significant only for the kindergarten group. Male subjects tended to have slightly lower titers, but this was not significant in aggregate (GMT, 11.4 vs 11.6 in male vs female subjects; $P = .132$) or within groups.

Post-MMR2 risk factors. The higher the titer before MMR2, the more likely ($P < .001$) a high titer after MMR2 (Figure 5). Of the 53 subjects who were seronegative at the final collection, 49 (92.5%) had negative (<10) or low (10) titers before receiving MMR2 ($P < .001$), an effect seen in both groups. At the final collection, male subjects had significantly higher rates of seronegativity than female subjects (20.2% [33 of 163 subjects] vs 11.8% [20 of 170 subjects; $P = .037$), an

effect seen in both groups. Male subjects also had significantly lower titers (GMT, 12.6 vs 15.4; $P = .015$), an effect seen in both groups but significant only for the kindergarten group.

DISCUSSION

In summary, we found that, before receiving MMR2, 9% of subjects in the kindergarten group and 25% in the middle-school group had negative neutralization titers, and most of the others had the lowest detectable titer. In response to the second dose, 62% in the kindergarten and 66% in the middle-school group exhibited 4-fold titer rises, with the incidence of

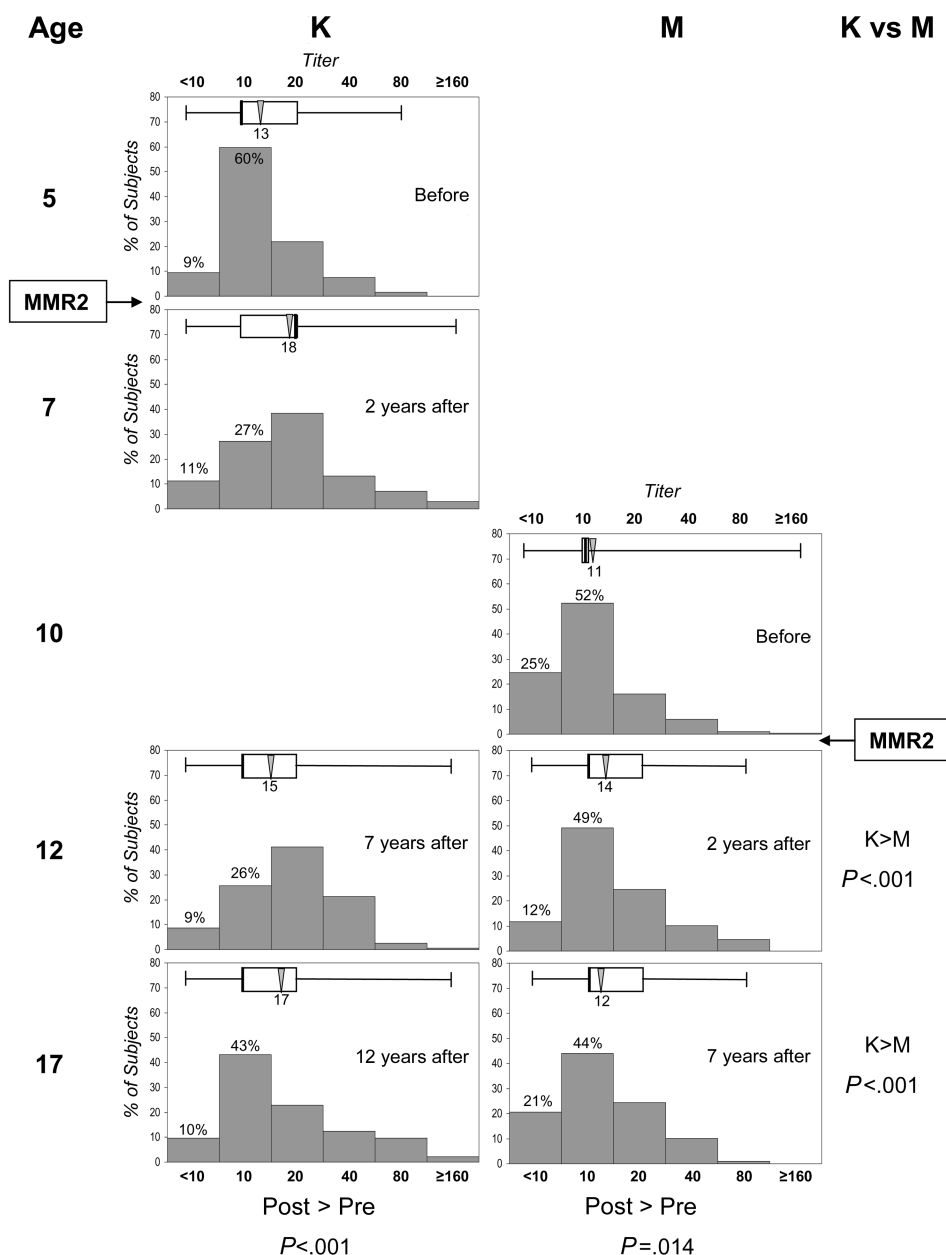


Figure 4. Age-specific rubella antibody levels before and after administration of the second dose of measles-mumps-rubella vaccine (MMR2). Each graph represents a serum collection, and bars represent proportions of the population with specified titers from the rubella plaque-reduction neutralization test. Box-and-whiskers plots represent ranges and 25th and 75th percentiles, dark vertical lines represent median titers, and triangles represent geometric mean titers. Left-hand graphs represent the group that received MMR2 at kindergarten (K) entry; right-hand graphs represent the group that received MMR2 at middle-school (M) entry. *P* values refer to differences between titer distributions (Jonckheere-Terpstra test). Post, after MMR2 administration; pre, before MMR2 administration.

seronegativity reduced to <1%. Less than 1% tested IgM positive, and 99% had high-avidity antibodies, suggesting an anamnestic response. During the 12-year study period, no rubella disease cases or exposures were reported among study subjects, but 4-fold boosts not attributable to vaccination were detected in 8% of specimens. By the age of 17 years, the kindergarten group's overall titers were less than one-half of those at the

postvaccination peak, and the proportion of seronegative subjects was similar to that before the second dose. The middle-school group showed similar patterns but had more seronegative subjects at the age of 17 years, the study end point. At each serum specimen collection and age, the kindergarten group had significantly higher antibody levels than the middle-school group, despite a considerably longer interval since the

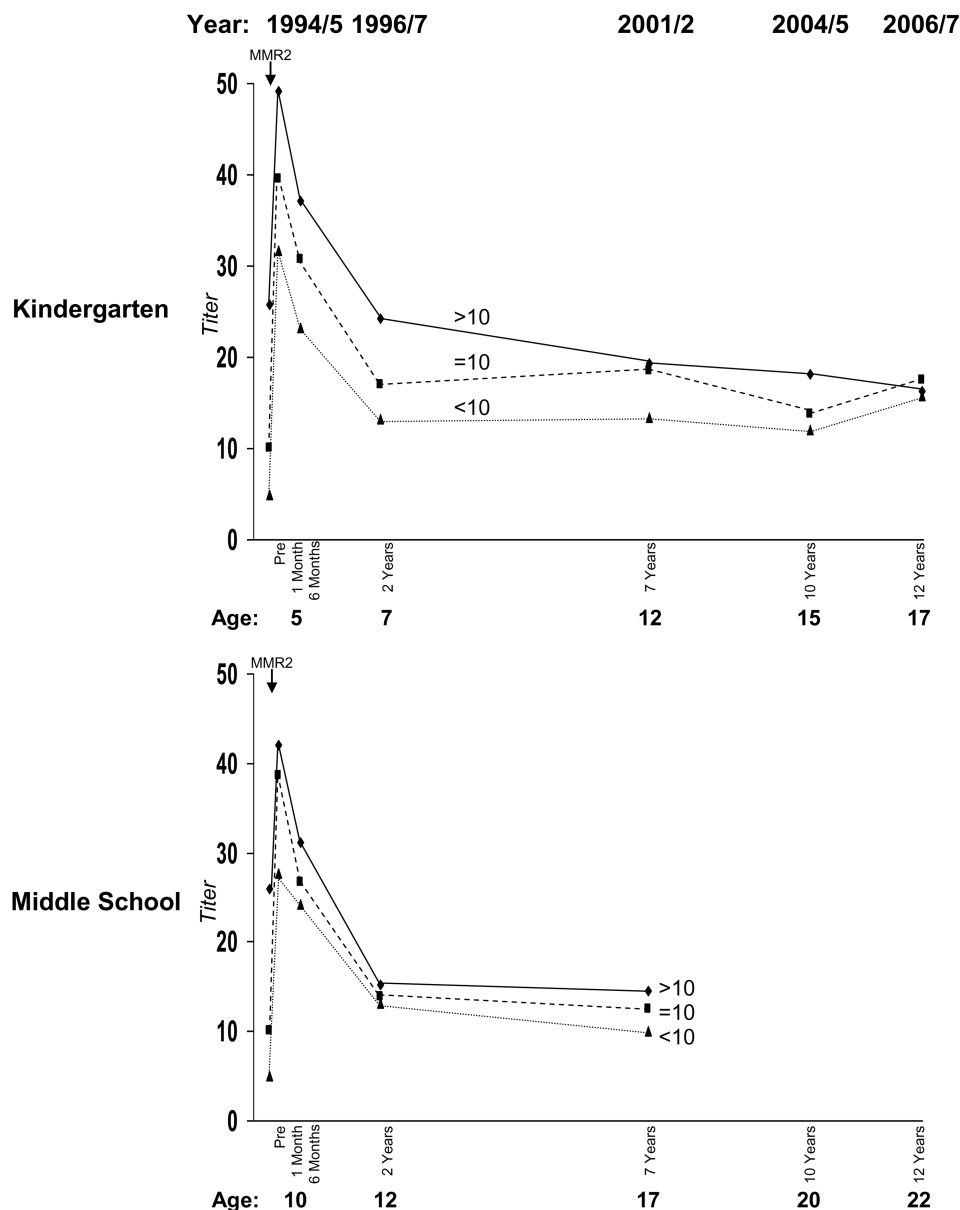


Figure 5. Rubella antibody geometric mean titers (GMTs) in cohorts grouped by titer before the second dose of measles-mumps-rubella vaccine (MMR2). The study population was divided into 3 cohorts on the basis of pre-MMR2 titer: seronegative (<10) (dotted line), lowest detectable titer (10) (dashed line), or medium-high titer (>10) (solid line). The GMT for each cohort was then calculated for each serum collection. Top graph represents the group that received MMR2 at kindergarten entry; bottom graph represents the group that received MMR2 at middle-school entry.

second dose. Antibody levels before and after the second dose were strongly correlated.

The progressive increase in seronegativity in the years after the second dose raises concerns. Two other studies examined the persistence of rubella antibodies after the second dose. Kremer et al [12], using a commercial EIA, found no seronegative subjects aged 1–8 years after administration of the second dose in children in Luxembourg, although antibody levels decreased. Davidkin et al [13], using the same EIA, also identified no seronegative subjects 15 years after the second dose among a cohort

of Finnish children, despite decreasing titers. Seronegativity was based on the manufacturer's threshold of <4 rubella international units (RIU). The US standard threshold is <10 RIU [26]. When Davidkin and colleagues used this threshold, the rate of seronegativity increased to 17%, similar to our rate of 10%–21%; with the common European threshold of <15 RIU [25], the rate increased to 36%.

Unlike antibody thresholds for measles [27, 28] and mumps [29, 30], the antibody threshold that provides protection from rubella has not been evaluated by prospective disease attack

rate studies. In our study, 88% of seronegative subjects and 75% of those with the lowest detectable titer experienced 4-fold antibody boosts in response to the second dose, suggesting a risk of infection, despite the presence of high-avidity antibodies. However, O'Shea et al [31] in the United Kingdom, in a series of challenge experiments using vaccine virus, documented that at least some individuals without detectable neutralizing antibody may be protected against rubella infection and that 4-fold boosts in antibodies were only rarely associated with detectable viremia or shedding of vaccine virus [32, 33]. In contrast, Schiff et al [34] in the United States found that among vaccinees who had reverted to seronegativity, all shed virus, and one-half had detectable viremia after challenge with the Howell strain, a low-passage, wild-type strain similar to the Gilchrist strain used in our neutralization assay. A substantial number of case reports have documented the occurrence of rubella infection and CRS in infants born to women with apparent secondary vaccine failure [35–39]. Thus, the possibility of rubella susceptibility among those whose titers have waned cannot be ruled out.

Nonetheless, if protection in the doubly vaccinated young adult population were dropping close to or below the commonly accepted rubella herd immunity threshold of 85%–90% [40], one might expect to see some evidence in US disease rates. For the period 2001–2008, a total of 108 rubella cases (range, 7–23 annually) were reported; none occurred in subjects who had received 2 doses of rubella vaccine, and only 1 was a spread case (CDC; unpublished data). Six cases of CRS were reported; all patients were born to unvaccinated foreign-born mothers who had acquired infection abroad. These patterns are reassuring evidence that endemic rubella is not currently regaining a foothold in the United States. They are also consistent with findings of an ecological study in Massachusetts that suggested that high vaccination coverage may provide herd immunity, even if individual levels of rubella antibody decrease to less than commonly accepted thresholds of clinical protection [41].

However, surveillance for rubella may be difficult in a highly vaccinated population. Secondary vaccine failure tends to manifest as subclinical infection or illness without a rash [34, 42]. Among infants born to rubella-infected women whose antibody titers have apparently dropped below protective levels, rates of infection and congenital anomalies appear to be markedly reduced in frequency, although not prevented entirely [42]. In an elimination environment, rubella and CRS may be sufficiently rare not to be considered in a differential diagnosis [43], particularly if the presentation is atypical. In our study, we detected a number of 4-fold increases in antibody titers not attributable to vaccination, suggesting that exposures to wild

rubella may have occurred, despite the lack of reported rubella disease in the study population or its geographical area.

Furthermore, in the prevaccine era, rubella incidence was highly periodic, with low disease rates producing a gradual increase in susceptibility, followed by a major resurgence [1–5]. Thus, the relative rarity of disease reports in the United States, although reassuring, may not be conclusive proof of indefinite protection, particularly in an environment of markedly reduced risk of hemispheric importations [9, 44].

At every collection and every age, antibody levels were higher in the kindergarten group than in the middle-school group. Because we did not randomize the study population to different vaccination ages, the 2 study groups were not comparable in terms of subject age, maternal age, or risk of prior exposure to disease, so this finding must be regarded with caution. The difference in antibody response may be related to our finding that the higher the titer before vaccination, the higher the titer after vaccination, a phenomenon noted by other investigators for rubella [13, 45].

Our study has a number of limitations. The study population was not representative of the US population. At the study end point, 46% of the subjects had been lost to attrition; as a result, the number of specimens in the final serum collections was relatively small, reducing the power of the study to detect significant differences. We did not attempt virus isolation to confirm vaccine virus infection. We did not quantify neutralization titers beyond $>1:160$, so our GMTs may be an underestimation. Although 4-fold increases not attributable to vaccination were relatively rare, antibody levels in our study may be different from what would be obtained in a population free of exposures that boost rubella antibodies. Although neutralizing antibodies are thought to correlate best with protection [1], almost all clinical and research testing employs EIAs with results expressed in rubella international units [26]. Thus, it would be difficult to apply our results to clinical situations or to compare our findings with those of most studies. We did not evaluate cellular responses to vaccination. Even in the absence of detectable antibodies, memory cells might respond sufficiently rapidly to prevent infection.

Despite these limitations, we believe our study suggests that where wild-type virus boosting is rare, vaccine-induced rubella antibody levels may decrease, and seronegativity rates may rise. Rubella disease rates in the United States are historically low and vaccine failure very rare, but the epidemic nature of rubella demands continued vigilance to assure that elimination is maintained.

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