Antibody Induced by Immunization with the Jeryl Lynn Mumps Vaccine Strain Effectively Neutralizes a Heterologous Wild-Type Mumps Virus Associated with a Large Outbreak

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Recent mumps outbreaks in older vaccinated populations were caused primarily by genotype G viruses, which are phylogenetically distinct from the genotype A vaccine strains used in the countries affected by the outbreaks. This finding suggests that genotype A vaccine strains could have reduced efficacy against heterologous mumps viruses. The remote history of vaccination also suggests that waning immunity could have contributed to susceptibility. To examine these issues, we obtained consecutive serum samples from children at different intervals after vaccination and assayed the ability of these samples to neutralize the genotype A Jeryl Lynn mumps virus vaccine strain and a genotype G wild-type virus obtained during the mumps outbreak that occurred in the United States in 2006. Although the geometric mean neutralizing antibody titers against the genotype G virus were approximately one-half the titers measured against the vaccine strain, and although titers to both viruses decreased with time after vaccination, antibody induced by immunization with the Jeryl Lynn mumps vaccine strain effectively neutralized the outbreak-associated virus at all time points tested.

In the prevaccine era, mumps was a common viral disease, with >90% of the population demonstrating serologic evidence of infection by adolescence [1]. In the United States, mumps vaccine was first licensed in 1967, and it has been included in trivalent measles, mumps, and rubella (MMR) vaccines since 1971 [2]. The Advisory Committee on Immunization Practices (ACIP) first recommended the MMR vaccine in 1977, and they modified this recommendation to a 2-dose schedule in 1989 [3]. After implementation of these recommendations, the widespread use of mumps-containing vaccines in children led to a 97% decrease in the number of cases of mumps reported (from ~200,000 cases/year in the prevaccine era to 5270 cases/year by 1982) [4]. By 2003, the number of mumps cases in the United States decreased to an all-time low of 231 cases, and the number continued to remain low through 2005 [5], representing a 99.9% reduction in the number of reported cases since the prevaccine era. A similar dramatic reduction in the incidence of mumps has also been noted in other countries subsequent to the inclusion of a 2-dose MMR schedule in national immunization programs [6, 7].

The era of a low incidence of mumps in the United States came to an abrupt end in 2006, when 5783 cases were reported—the largest mumps outbreak in 20 years [8]. The majority of cases occurred in persons 18–24 years of age and, surprisingly, mostly among recipients of 2 doses of vaccine [8]. Dramatic increases in the num-
ber of mumps cases were also reported in this age group in the United Kingdom during 2004–2005 and in the Maritime region of Canada in 2007; however, these outbreaks differed from the outbreak in the United States in that most cases occurred among recipients of 1 dose of vaccine in Canada and among unvaccinated individuals in the United Kingdom [9, 10]. Of note, mumps vaccine was not routinely given before September 1987 in the United Kingdom, and a 2-dose MMR vaccine schedule was not implemented in the United Kingdom or Canada until 1996. Nonetheless, the high rate of attack among recipients of 1 or 2 doses of vaccine in the United States and Canada and, to a lesser extent, the United Kingdom has raised questions about vaccine efficacy. On one hand, all isolates obtained and sequenced from the outbreaks in the United States, the United Kingdom, and Canada were determined to be genotype G according to the small hydrophobic (SH) gene sequence classification system [10–13]; genotype G viruses are a phylogenetic group distinct from the genotype A (Jeryl Lynn) and B (Urabe-AM9) viruses contained in the vaccine used in these countries. This finding raised the controversial prospect that wild-type mumps viruses capable of escaping immune protection are circulating. Other mumps virus genotypes have also been associated with outbreaks among vaccine recipients elsewhere (e.g., genotypes C and H in Russia [14], genotypes D and H in Spain [15], and genotypes H and I in Korea [16, 17]). Although mumps virus is serologically monotypic, some investigators have implied that antigenic differences may offer an epidemiological advantage to viruses circulating in an immunized population, permitting breakthrough infections to occur [15, 18–21]. On the other hand, the overwhelming majority of mumps cases in these recent outbreaks occurred among individuals 18–24 years of age [8–10], a cohort immunized >10 years earlier; this finding suggests that waning immunity (i.e., secondary vaccine failure) may be an important factor contributing to susceptibility.

The present study examines the ability of antibody induced by immunization with the Jeryl Lynn mumps vaccine virus strain to neutralize outbreak-associated virus and the possible contribution of waning immunity to recent mumps outbreaks. We evaluated the ability of antisera obtained from children before administration of a second dose of vaccine and at both 1 month and 10 years after administration of the second dose of vaccine to neutralize the homologous genotype A Jeryl Lynn vaccine strain and a genotype G wild-type mumps isolate (USA06-Iowa-G) obtained during the outbreak that occurred in the United States in 2006.

**SUBJECTS, MATERIAL, AND METHODS**

**Study subjects and serum samples.** Coded human serum samples were obtained from a related vaccine study wherein antibody persistence after MMR immunization was measured in patients attending Marshfield Clinic, a health care clinic in Marshfield, Wisconsin [22]. Children who received their first dose of MMR vaccine between 12 and 24 months of age were enrolled in the study and were given a second dose of MMR vaccine (manufactured by Merck) when they were between 4 and 6 years of age. Subjects were excluded from the study if they had a history of mumps, lived in the same household with anyone who had a mumps virus infection, had a contraindication to MMR vaccination, or had any condition that would affect vaccine uptake, as advised by the ACIP [23]. Parents of study subjects provided written informed consent, and the study was approved by the Human Subjects Protection Office of the Centers for Disease Control and Prevention, whereas serologic testing of the deidentified serum samples was approved by the Research Involving Human Subjects Committee of the US Food and Drug Administration (FDA).

The first serum samples were obtained from children who were 4–6 years of age at enrollment and who had received a single dose of MMR vaccine (MMR1) at 12–24 months of age. Within 3 days of serum sample collection, a second dose of vaccine (MMR2) was administered. Serum samples were collected periodically, as previously described, and the time points for sample collection were 1 month after administration of MMR2 and 10 years after administration of MMR2. All serum samples were collected between 1994 and 2005 and were stored frozen at −20°C until use. At the time of collection of each serum sample, families were questioned concerning mumps exposures, other vaccinations received, and other health events.

For the present study, serum samples collected from 88 individuals at 3 time points (at 2–5 years after MMR1 administration, 1 month after MMR2 administration, and 10 years after MMR2 administration [i.e., at 14–16 years of age]) were available. These 264 serum samples were provided in a blinded fashion for serologic testing. Samples were unblinded only after all testing was completed.

**Viruses.** Serum samples were tested for neutralizing activity against the Jeryl Lynn mumps virus vaccine strain and against USA06-Iowa-G, a wild-type virus obtained from a throat swab specimen from an individual with a clinical case of mumps associated with the mumps epidemic that occurred in the United States in 2006. The Jeryl Lynn mumps virus vaccine strain was obtained from Merck as a monovalent bulk lot [24]. The USA06-Iowa-G virus was isolated on primary monkey kidney cells and expanded by 2 passages in Vero cell culture. The infectivity titer of both virus stocks was determined by plaque assay, as described elsewhere [25]. According to the mumps virus genotype classification system for the SH gene sequence [26], Jeryl Lynn virus is a genotype A virus and USA06-Iowa-G virus is a genotype G virus.

**Virus neutralizing antibody assay.** A plaque reduction neutralization assay was used to determine anti–Jeryl Lynn and anti–USA06-Iowa-G mumps virus antibody titers for each se-
rum sample. In brief, serum samples were thawed at room temperature, heated at 56°C for 45 min, and then serially diluted 2-fold (from 1:2 to 1:2048) in minimal essential medium (MEM) to a final volume of 120 μL in a 96-well plate; they were then mixed with an equal volume of MEM containing ~80 pfu of either the Jeryl Lynn virus or the USA06-Iowa-G mumps virus, for a final dilution range of 1:4–1:4096. To serve as virus controls, 120 μL of each virus preparation was added to wells in a 96-well plate containing 120 μL of MEM (in the absence of serum). The virus/serum and virus/mock-serum mixtures were incubated for 1 h at 37°C in an atmosphere of 5% CO₂, and then 100 μL was transferred in duplicate from each well of the 96-well plates to wells in 24-well plates containing Vero cell monolayers. After incubation for 1 h at 37°C in an atmosphere of 5% CO₂, the inocula were removed, and 1.0 mL of MEM containing 2% carboxymethylcellulose (MP Biomedicals) supplemented with 10% fetal bovine serum and antibiotics was added. After incubation for 5 days at 37°C in an atmosphere of 5% CO₂, the inocula were replaced, and 1.0 mL of MEM containing 2% carboxymethylcellulose (MP Biomedicals) supplemented with 10% fetal bovine serum and antibiotics was added. After incubation for 6 days, the overlay medium was aspirated, cell monolayers were fixed in a 3.7% solution of formaldehyde in water, and plaques were counted. The mean plaque number was determined for duplicate wells at each serum dilution and for virus control wells.

Mumps reference immune serum, Lot 3, a lyophilized preparation of human mumps immune serum prepared by the Division of Biologics Standards, National Institutes of Health (now the Center for Biologics Evaluation and Research, FDA) was reconstituted using sterile water and served as the positive serum control in each assay run. For a valid assay, the titer of mumps reference immune serum, Lot 3, was required to be within 2 SDs of its cumulative historical mean value of 1:330 when tested against the Jeryl Lynn strain and 1:277 when tested against the USA06-Iowa-G strain. In addition, each valid assay met the following criteria: the mean plaque number in the virus control wells was 30 ± 15, cell control wells had no visible plaques, and the cell monolayers were intact and free of visible contamination. Samples obtained from the same child were tested on the same day; assays not meeting the validation requirements were retested.

The neutralizing antibody titer for each serum sample was defined as the highest dilution of serum capable of reducing the number of virus plaques by $\geq 50\%$, compared with the virus control, as determined by the Kärber formula [27]. Seropositivity was defined as a neutralizing antibody titer of $\geq 1:4$ [28, 29]. To calculate geometric mean titers (GMTs) of neutralizing antibody, pooling of data across assay runs was required. Neutralizing antibody titers from successive assays were pooled if the titer for mumps reference immune serum, Lot 3, was within 2 SDs of its cumulative historic mean value.

Statistical analyses. The reciprocal neutralizing antibody titer measured for each sample was log transformed, group means were calculated, and GMTs and 95% confidence intervals (CIs) were reported after antilogarithm transformation. Assessments of statistical significance between group GMTs were conducted using Student’s $t$ test and the Mann-Whitney rank-sum test. Statistical significance was denoted by $P < .05$.

RESULTS

Serum samples were obtained from 88 study subjects at each of 3 time points: 2–5 years after administration of MMR1, 1 month after administration of MMR2, and 10 years after administration of MMR2. Valid test results were obtained for all but 1 of the subjects; overuse of one serum sample resulted in insufficient volume for additional testing. Of the 87 subjects for whom valid test results were obtained, the post-MMR1 serum sample of 1 subject was of insufficient volume to allow retesting against the USA06-Iowa-G virus, because of a prior invalid assay run. In total, of the original 264 serum samples (the total number of samples obtained from 88 subjects at 3 time points), valid neutralizing antibody titers against the Jeryl Lynn mumps virus vaccine strain and against the USA06-Iowa-G mumps wild-type virus were obtained for 261 and 260 serum samples, respectively.

The neutralizing antibody titer of mumps reference immune serum, Lot 3, against the Jeryl Lynn (407; 95% CI, 156–1043) and USA06-Iowa-G (251; 95% CI, 118–523) viruses in each valid assay run was within 2 SDs of its cumulative mean value across all assays, as is shown in figure 1. Test results were pooled across 15 assays using Jeryl Lynn virus and 13 assays using USA06-Iowa-G virus.

Jeryl Lynn and USA06-Iowa-G mumps neutralizing antibody titers. With the exception of one individual, all children were seropositive (neutralizing antibody titer, $\geq 1:4$) against both Jeryl Lynn and USA06-Iowa-G virus at each time point tested. The individual who was the exception had a neutralizing antibody titer of $<1:4$ against the Jeryl Lynn vaccine strain 1 month after administration of MMR2. Because all subjects possessed neutralizing antibody to mumps after immunization with MMR1, there were no cases of primary vaccine failure in the study population. Therefore, we cannot account for seroconversion resulting from subclinical wild-type mumps virus infection that may have occurred between administration of MMR1 and MMR2.

The GMTs of neutralizing antibody to the Jeryl Lynn and USA06-Iowa-G viruses are shown in table 1. The GMTs to USA06-Iowa-G virus at the 3 time points assessed are approximately one-half the GMTs to the Jeryl Lynn virus (for all, $P < .001$, by the Mann-Whitney rank-sum test).

The cumulative distribution of fold differences in neutralizing antibody titers against the 2 viruses was also analyzed, and these data are shown in figure 2. Most serum samples neutralized the Jeryl Lynn and USA06-Iowa-G viruses with equal efficiency at the 3 time points tested, with 49%–60% having a $<2$-fold dif-
ference in antibody titer when the neutralizing end point titers against each virus were compared on an individual basis. In contrast, 14%–22% of vaccinated children had a >4-fold difference in titer, depending on the time point tested. In nearly all cases, titers to USA06-Iowa-G virus were lower than titers to Jeryl Lynn virus. In 11 (12.5%) of 88 individuals, 10- to 28-fold differences in neutralizing antibody titers against the 2 viruses were noted.

An overall decrease in neutralizing antibody activity against USA06-Iowa-G mumps virus, compared with the activity noted against the homologous vaccine strain, was also seen when we compared titers by use of reverse cumulative distribution curves at each time point (figure 3).

Waning immunity. Although there was no evidence indicating a complete loss of neutralizing antibody activity after vaccination, antibody titers clearly waned over time. Although the GMT of neutralizing antibody to the Jeryl Lynn and USA06-Iowa-G viruses increased by ~2.6-fold and 2.0-fold, respectively, in response to a second dose of vaccine (for all, \(P < .001\), by \(t\) test), by 10 years after administration of MMR2, the measured neutralizing antibody titers to both viruses decreased to levels noted before MMR2 administration (table 1). This decrease is also demonstrated in the reverse cumulative distribution curves (figures 3 and 4).

Clinical significance of observed mumps neutralizing antibody levels. There is no established mumps neutralizing antibody titer predictive of protection against infection or disease. Therefore, the clinical significance of the observed differences in virus-specific neutralization and the implications of declining

<table>
<thead>
<tr>
<th>Virus</th>
<th>2–5 years after MMR1</th>
<th>1 month after MMR2</th>
<th>10 years after MMR2</th>
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<tbody>
<tr>
<td>Jeryl Lynn</td>
<td>107.9 (83.8–139.2)</td>
<td>280.5 (212.1–371.0)</td>
<td>94.8 (73.2–122.9)</td>
</tr>
<tr>
<td>USA06-Iowa-G</td>
<td>56.2 (46.8–67.6)</td>
<td>110.4 (90.3–135.0)</td>
<td>56.6 (47.3–67.5)</td>
</tr>
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</table>

NOTE. Data are the GMT (95% confidence interval). Titers were measured before administration of 2 doses of the measles, mumps, and rubella (MMR) vaccine (MMR2) (given 2–5 years after administration of 1 dose of the MMR vaccine [MMR1]) and at 1 month and 10 years thereafter.
titers over time cannot be definitively ascertained. However, in an investigation of a small outbreak of mumps in the prevaccine era, preexposure neutralizing antibody titers of $\geq 1:8$ were associated with protection against mumps [30], a finding that suggests that this neutralizing antibody level is a possible correlate of protection. All but 2 of 87 individuals in the present study possessed a neutralizing antibody titer of $\geq 1:8$ against both viruses at all 3 time points assessed. The 2 exceptions included one individual whose titer to the vaccine strain was 1:11 before MMR2 administration, decreased to 1:3 one month after MMR2 administration, and was 1:6 ten years after MMR2 administration, as well as another individual whose titer to the vaccine strain was 1:43 one month after MMR2 administration and decreased to 1:6 ten years after MMR2 administration. Interestingly, the corresponding anti-USA06-Iowa-G antibody titers for these 2 individuals at 10 years after MMR2 administration were 1:26 and 1:19, respectively. If the minimum neutralizing antibody titer required for protection is 1:8, our data suggest that protective titers of mumps neutralizing antibody persist for 10 years after MMR2 administration in 98%–100% of vaccine recipients, depending on the target virus used.

**DISCUSSION**

Several mumps outbreaks have occurred in populations vaccinated with $\geq 1$ dose of vaccine containing mumps, including the recent outbreaks in the United Kingdom, the United States, and Canada [8–11]. The SH gene of viruses isolated from these outbreaks was sequenced and identified as genotype G, 1 of 13 phylogenetic groups described to date [31, 32]. Because the mumps vaccine viruses currently used in these countries are genotype A, the question was raised as to whether additional differences in the known protective antigens permitted genotype G mumps viruses to evade vaccine immunity. Previous studies using monoclonal antibodies or polyclonal antimumps serum suggested the possible existence of virus strain–specific neutralization epitopes that, in theory, might contribute to immune escape [21, 33–36]. On one hand, it was reassuring to note that all 261 serum samples obtained from the 88 vaccinated subjects tested in the present study, including those obtained 10 years after vaccination, could effectively neutralize the outbreak genotype G mumps virus.
isolate; likewise, all serum samples possessed a neutralizing antibody titer of $1:8$, a titer previously associated with protection against mumps virus infection [30]. On the other hand, field studies have not confirmed a minimum protective level of antibody, and a titer $1:8$ might be required for protection. We saw a $4$-fold reduction in the ability of the serum samples obtained from $22\%$ of the vaccine recipients to neutralize the outbreak virus relative to the ability to neutralize the homologous vaccine strain. Although we did not compare neutralization epitopes of the hemagglutinin and fusion envelope glycoproteins of Jeryl Lynn virus versus USA06-Iowa-G virus as a part of this study, our results lend some support to the idea that antigenic differences exist and may influence individual immunity.

Antibody titers against both the Jeryl Lynn and USA06-Iowa-G viruses were augmented after receipt of MMR2, indicating that vaccine recipients clearly benefited from the second dose; however, this advantage was only transient and was followed by a decrease in neutralizing antibody titers with increasing time after vaccination, so that GMTs measured $10$ years after MMR2 administration approximated levels noted before administration of dose $2$. Importantly, we do not know whether such a decline in antibody concentration correlates with a loss of immunity, because the minimal level of neutralizing antibody required for protection against mumps has not been firmly established. On the basis of the data reported in the present study, if the serum concentration of neutralizing antibody required for protection is much greater than $1:8$ [30], then the observed decreases in antibody titer with time after vaccination may indeed play a role in mumps outbreaks in older, vaccinated populations.

During recent mumps outbreaks, the highest age-specific rate of attack of mumps occurred among persons $18–24$ years of age, many of whom were last vaccinated $>10$ years earlier [8–10], a time frame in which mumps virus-specific immune responses have been shown to decrease [37–39]. The susceptibility of young vaccinated adults to mumps infection provides further evidence that secondary vaccine failure is a possible contributor to outbreaks in this population. In a logistic regression model presented by Vandermeulen et al. [40], the odds ratio for developing mumps was shown to increase by $27\%$ with each year since vaccination. In a recent study by Cohen et al. [41], the effectiveness of $1$ dose of vaccine against mumps was found to have decreased from $96\%$ at $2$ years of age to $66\%$ at $11–12$ years of age and, after $2$ doses of vaccine, from $99\%$ at $5–6$ years of age to $86\%$ at $11–12$ years of age. In an analysis of the 2006 mumps outbreak in the United States, the incidence of disease increased with age, but only up to $18–24$ years of age; in groups older than $18–24$ years of age, the incidence of mumps decreased [11]. Thus, the interval between vaccination and exposure was obviously important but was not the only factor influencing susceptibility during recent outbreaks of mumps. As herd immunity increases with higher rates of vaccine coverage, it can be expected that circulation of wild-type virus will be curtailed, dampening the frequency of exposures and mumps outbreaks. In the absence of opportunities for antigenic boosting, mumps antibody titers will likely continue to wane, and greater numbers of individuals who are susceptible to mumps may eventually accumulate, resulting in outbreaks that occur less frequently but with an amplitude larger than that noted in previous outbreaks.

The present study has several limitations that should be noted. First, we did not have access to serum samples obtained immediately after MMR1 administration; this access would have allowed us to be certain that neutralizing antibody responses that inhibited USA06-Iowa-G virus were induced by vaccination alone and did not result from subclinical infection with wild-type mumps virus during the interval between doses. Second, this retrospective study was small, with only $88$ children followed for $10$ years after immunization. We were not able to mimic the postvaccination interval of $14–20$ years, which may be a more accurate representation of the interval between immunization and development of mumps cases during recent outbreaks.

Figure 4. Reverse cumulative distribution curves of neutralizing antibody against Jeryl Lynn mumps vaccine strain (A) and USA06-Iowa-G mumps virus (B) $2–5$ years after administration of $1$ dose of measles, mumps, and rubella (MMR) vaccine (MMR1) (solid line), $1$ month after administration of $2$ doses of MMR vaccine (MMR2) (dotted line), and $10$ years after administration of MMR2 (dashed line).
outbreaks. Antibody titers measured 10 years after immunization may overestimate the level of protection 4–10 years hence. Third, in Canada and the United Kingdom, some individuals in the 18- to 24-year age cohort may have received Urabe-AM9 mumps vaccine, a genotype B strain [42, 43]. Our study failed to compare immunity after administration of a genotype B vaccine with that noted in association with an outbreak genotype G virus. Fourth, we did not perform cross-neutralization assays with Jeryl Lynn and USA06-Iowa-G viruses for Archetti-Horsfall analysis to determine antigenic relatedness [44–46]. In addition, this retrospective study and the use of archived serum samples did not allow us to evaluate cellular immunity to Jeryl Lynn and USA06-Iowa-G viruses. It is important to consider that individuals possessing low levels of antibody or even those lacking measurable antibody might not necessarily be susceptible to symptomatic mumps virus infection, because they might be fully protected by cell mediated immunity, which has been demonstrated in seronegative vaccine recipients, even 21 years after vaccination [47, 48].

In summary, this is the first study documenting serum neutralizing antibody titers against a contemporary mumps outbreak genotype G virus in recipients of 2 vaccine doses. Antibodies capable of neutralizing USA06-Iowa-G virus were detected after vaccination with the Jeryl Lynn mumps vaccine strain and persisted for ≥10 years after immunization, albeit at levels lower than those seen against the vaccine strain. Although mumps neutralizing antibody persisted, there was an observed decrease in serum levels of neutralizing antibody to both viruses with increasing time after vaccination. Additional studies are needed to more fully assess the role of virus genotype, antigenic differences, and persistence of T and B cell immunity to mumps in individuals with a remote history of immunization, to better understand the relative contributions of each to durable long-term protection against mumps infection.

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