Laboratory Characterization of Measles Virus Infection in Previously Vaccinated and Unvaccinated Individuals

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Waning immunity or secondary vaccine failure (SVF) has been anticipated by some as a challenge to global measles elimination efforts. Although such cases are infrequent, measles virus (MeV) infection can occur in vaccinated individuals following intense and/or prolonged exposure to an infected individual and may present as a modified illness that is unrecognizable as measles outside of the context of a measles outbreak. The immunoglobulin M response in previously vaccinated individuals may be nominal or fleeting, and viral replication may be limited. As global elimination proceeds, additional methods for confirming modified measles cases may be needed to understand whether SVF cases contribute to continued measles virus (MeV) transmission. In this report, we describe clinical symptoms and laboratory results for unvaccinated individuals with acute measles and individuals with SVF identified during MeV outbreaks. SVF cases were characterized by the serological parameters of high-avidity antibodies and distinctively high levels of neutralizing antibody. These parameters may represent useful biomarkers for classification of SVF cases that previously could not be confirmed as such using routine laboratory diagnostic techniques.

The incidence of measles has been dramatically reduced because of the availability of live attenuated vaccines, either as single-antigen vaccines or as combined vaccines, such as measles-mumps-rubella (MMR) vaccine. Measles is no longer endemic in the Americas because of high 2-dose vaccination coverage rates with MMR vaccine [1]. Consequently, vaccination strategies that include a 2-dose schedule for measles vaccination have been adopted by many countries, which has facilitated tremendous advancements towards the goal of reducing global measles-related morbidity and mortality [2].

Measles outbreaks continue to occur, however, even in highly vaccinated populations, largely as a result of the exposure of vaccine-exempt populations (eg, those with religious and philosophical objections to vaccination) to imported cases and, much less frequently, as a result of exposure of those with primary or secondary vaccine failure (SVF) [3–12]. Following the adoption of a 2-dose MMR schedule in the United States in 1989 [13], measles cases decreased to an average of 63 cases per year for the period 2000–2007 [14]. In 2008, however, US measles cases were at the highest level seen in more than a decade, with nearly half of those cases involving children whose parents had rejected vaccination [14]. Since 2008, Israel, Ireland, Switzerland, Austria, Italy, Australia, Germany, France, Britain, and Canada

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have reported substantial outbreaks of measles among populations that have refused vaccination, and importations from these and other countries have fueled US outbreaks [15–25]. It is worth noting that occasional spread from unvaccinated individuals with measles to 2-dose vaccine recipients has recently been observed [5, 10, 14, 26].

Immunity to wild-type measles is generally thought to be lifelong [27]; however, life spans have increased, and the length of protection afforded by natural infection or vaccination in the absence of circulating wild-type MeV and subclinical boosting is unclear. It has long been recognized that an intense force of infection and/or an extended duration of exposure can produce a range of symptoms from classic to mild/modified or asymptomatic in previously immune individuals [28]. Waning immunity would be most likely to occur in vaccine recipients, because vaccinated persons have lower levels of measles-specific antibody than do those with immunity derived from exposure to wild-type MeV [29–32]. In addition, the decrease in measles antibodies is more rapid in vaccinees than in those who have recovered from measles disease [4]. A prospective cohort study in the United States demonstrated that, although measles plaque reduction neutralization (PRN) antibody persisted in all vaccinees 10 years after a second dose, there was a progressive decrease in levels of measles antibody as time since vaccination increased [33].

Laboratory confirmation of acute measles infection in previously immune individuals presents a greater challenge to the diagnostic laboratory than does detection of acute disease in unvaccinated persons, because immunoglobulin (Ig) M responses may be absent or short-lived. Moreover, because of restricted viral replication, molecular detection using RT-PCR is also limited. The lack of IgM antibodies and an inability to detect MeV in conjunction with the presence of modulated symptoms could lead to an underestimation of measles disease among the previously vaccinated population and suggests that more-sensitive assays or alternative approaches to detect MeV infection may be needed. In this study, we evaluated paired serum samples and clinical information obtained from measles cases among vaccinated and unvaccinated individuals in the Republic of the Marshall Islands (RMI) and presumptive SVF measles cases identified in the United States. RMI is an isolated Pacific island nation with high 1-dose vaccine coverage that implemented a 2-dose requirement in 1998 and was free of reported measles cases for 14 years. In 2003, the RMI experienced a large measles outbreak [6, 7, 34]. During the subsequent investigation, it was noted that some individuals exhibited a milder disease course. Serum specimens from these individuals, as well as from individuals with more-classical presentations, were collected. Subsequently, paired serum samples from US measles outbreaks were also examined. Clinical symptoms and measles IgM, IgG, IgG avidity, and serum neutralizing antibody titers were compared to characterize responses to measles infection in previously vaccinated and unvaccinated individuals.

MATERIALS AND METHODS

RMI Clinical Case Definition

A suspected measles case patient in RMI was defined as a patient with fever, rash, and either cough, coryza, or conjunctivitis who resided in the RMI during the period 13 July–7 November 2003. A laboratory-confirmed case patient was defined as a patient with serological (defined as positive measles IgM enzyme immunoassay [EIA] results or a 4-fold increase in measles PRN antibody titer) or virological (defined as measles virus RNA detected in blood or secretions by reverse-transcription polymerase chain reaction [RT-PCR]) evidence of acute measles infection. For the purposes of this study, acute primary measles infection was defined as a laboratory-confirmed case in an unimmunized individual who had not received a dose of measles vaccine during the outbreak and whose initial serum sample had low-avidity measles antibodies. Individuals meeting the clinical case definition (CCD) who had documented previous measles vaccination and high-avidity antibodies were considered potential SVF cases. Because such individuals could not be distinguished from measles-vaccinated individuals with a rash illness other than measles, only those with laboratory-confirmed measles disease were used for comparison of clinical symptoms among those with acute measles and SVF.

RMI Case Investigation Form

A standardized case investigation form, developed and distributed to all RMI health care providers, collected detailed demographic information, self-reported clinical features, vaccination history, and illness outcome for all suspected measles cases. Vaccination history was obtained from parental/patient recall, personal and medical records, and immunization logs maintained by the local health department. Patients were classified as vaccinated if recall or documentation provided the number and/or dates of vaccinations; as having no history of vaccination if they reported no receipt of previous measles vaccine and had no documentation of vaccination; as having unknown vaccination status if their vaccination status was uncertain or lacked documentation.

Collection of Clinical Samples

Serum specimens were collected for serological testing and nasopharyngeal swab samples were collected for virus isolation and genetic characterization from a subset of RMI case patients with suspected measles. Commonly, specimen 1 was collected during the first medical contact and specimen 2 was collected approximately 1 month later. Patients were selected by convenience, and those patients who were examined do not represent a systematically selected, representative sample of the outbreak

population. Anecdotal reports from co-investigators suggested that patients with milder symptoms may have been oversampled. RT-PCR assays to detect MeV RNA were performed on all nasopharyngeal samples that were collected, as previously described [34]. Specimens from patients with suspected measles disease who were involved in US outbreaks were referred to the Centers for Disease Control and Prevention (CDC) for initial or confirmatory testing.

Measles IgM Testing

Serum specimens were tested for measles-specific IgM antibodies using an IgM EIA with a capture format, as previously described [35]. ImmunoWELL Measles Recombinant IgM Test (GenBio) was used for the quantitative detection of IgM to MeV according to the manufacturer's directions.

Measles IgG Testing

The ImmunoWELL Measles Recombinant IgG Test was used for the qualitative detection of measles IgG. This test utilizes an EIA microtiter plate technique in an indirect format for the detection of measles antibodies. Serum is added to microtiter plates coated with baculovirus expressed recombinant measles nucleoprotein (N) and allowed to react. After removal of unbound antibodies, goat anti-human IgG antibody conjugated with horseradish peroxidase is allowed to react with bound antibodies. After a series of washes, colorless chromogenic substrate (3, 3', 5, 5'-Tetramethylbenzidine-H2O2) is added, and bound peroxidase reacts developing a color change. The substrate-peroxidase reaction is stopped by adding 2M phosphoric acid, and the resulting OD is read with a spectrophotometer.

Avidity Testing

Avidity testing of RMI specimens was accomplished using an ImmunoWELL rubeola assay (GenBio) using purified recombinant N protein. Specimens were tested at 4 dilutions (1:100, 1:1000, 1:5000, and 1:25,000). After incubation with the antigen, samples were washed with either the manufacturer's wash buffer or with avidity reagent. An avidity index was calculated by dividing the optical density value of the well washed with the avidity reagent by the optical density value of the well washed with the manufacturer's wash buffer. The linear range was below 1.5 absorbance units. Only the first ratio within the linear range was interpreted. Receiver operating characteristic (ROC) analysis was performed on blood donors with known measles exposure histories and on unvaccinated laboratory-confirmed cases to determine an avidity cutoff value. Avidity ratios $<45\%$ were considered to be low, whereas ratios that $\geqslant 50\%$ were considered to be high. An avidity index between 45%–49% was considered to be an equivocal response.

Avidity testing for measles-specific IgG in US specimens was performed as described (S. Mercader, personal communication). Briefly, a commercially available EIA platform for measles IgG detection was modified to include 3 protein-denaturing washes to elute low-avidity antibodies. Serum was diluted in 2 dilution series: one series was washed with the manufacturer's wash buffer (WB), whereas the other was washed with diethylamine in WB (DEA). The titer value at optical density signal extinction for each dilution series was calculated, and the ratio of the 2 titers was obtained and expressed as a percentage; end-titer avidity index (etAI%) = (end-titer DEA curve/end-titer WB curve) $\times$ 100.

PRN Test

PRN tests were performed as described previously using low-passage Edmonston MeV on Vero cell monolayers [36], and end point titers were calculated using the Kärber method [37]. Serum specimens were run in parallel with the Second World Health Organization (WHO) International Standard Reference Serum (66/202), and samples with reciprocal titers of <8 were assigned a value of 4 for calculating conversion rates. In this assay, a titer of 1:8 corresponded to 8 mIU/mL.

Radioimmunoprecipitation

Immunoprecipitation using ^{35}S-methionine-labeled lysates of MeV-infected cells was performed as previously described, except that Vero/hSLAM cells were used instead of Vero cells [38]. H-protein specific monoclonal antibodies (MAbs) CV-2, CV-4, CV-5 and CV-11 have been described previously [38]. Mabs79VV17D (V17), 80IIIB2 (B2), and 81-I-366 (366) were obtained from the laboratory of Dr D. McFarlin (National Institutes of Health).

RESULTS

Laboratory Testing of Paired Serum Samples From RMI

Paired serum samples (63 pairs) were collected from case patients with suspected measles who met the CCD but did not receive a dose of vaccine during the outbreak response immunization campaign during 2003. Twenty five (40%) of the 63 specimens were IgM positive by the quantitative IgM EIA and were therefore designated as laboratory-confirmed measles cases. In contrast, 38 (60%) of the cases in this cohort were measles IgM negative. Within the IgM-negative group, 2 additional cases (for a total of 27 cases) could be identified because they were either RT-PCR positive for wild-type MeV or had a 4-fold increase in neutralization titer. Although the remaining 36 individuals met the CCD, all had negative measles IgM results and thus were not considered to have laboratory-confirmed measles; therefore, they were excluded from the analyses described below.

Avidity. Antibody avidity measurements were performed on serum specimens from the 27 laboratory-confirmed cases of measles (Figure 1). Of the 8 cases in patients with recorded documentation of vaccination, 2 (ages 1.4 years and 18 years) had low-avidity IgG antibody detected, which suggested that they had experienced primary vaccine failure, whereas 6 cases

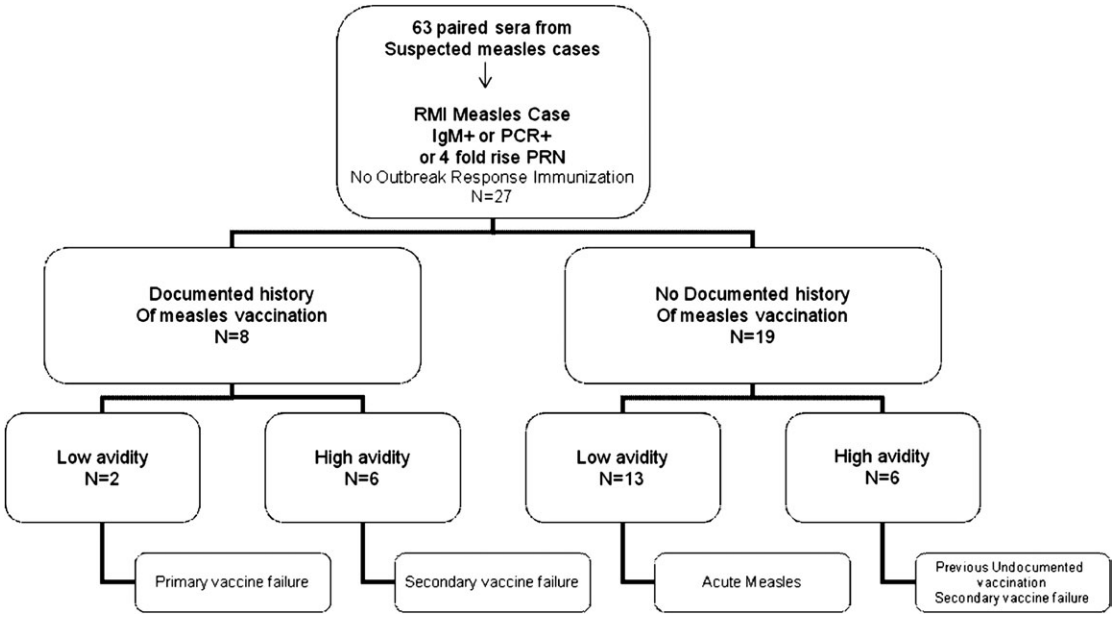

Figure 1. Avidity results and vaccination status for laboratory confirmed measles cases in the Republic of the Marshall Islands (RMI). Laboratory-confirmed case patients included case patients who did not receive outbreak response immunization and were immunoglobulin M (IgM) positive or had a 4-fold increase in measles plaque reduction neutralization (PRN) titer or had reverse-transcription polymerase chain reaction (RT-PCR) results that were positive for wild-type measles virus. Vaccination history was obtained from parental or patient recall, personal and medical records, and immunization logs maintained by the local health department. Patients were classified as vaccinated if recall or documentation provided the number and/or dates of vaccinations or were classified as having no history of vaccine if they reported no previous measles vaccination and had no documentation of vaccination.

(ages 10, 10, 11, 14, 15, and 19 years) had high-avidity antibody, which demonstrated that they likely had SVF. Of the cases with no reported measles vaccine history, the majority (13 of 19) had low-avidity antibody indicative of a primary response to infection. In contrast, 6 of the 19 cases among the group with no reported receipt of vaccine were found to have high- or equivocal-avidity antibody (1–13 days after rash onset), which indicated that they likely had received an undocumented dose of measles vaccine or, less likely, that they had previously experienced measles infection.

Age Distribution of IgM-Positive Patients

When the age distribution of IgM-positive RMI patients in this study was plotted against the results of antibody avidity testing (Figure 2), it was striking that almost all samples containing low-avidity antibody were from acute cases that occurred in children aged <1 year and in adults aged >20 years. Children aged <1 year would not yet have been eligible for vaccination, and adults aged >20 years would have been too old to have received routine vaccination when it began in 1982 and must have missed any supplemental immunization activity (SIA) "catch-up" vaccination efforts. In contrast, the majority of patients with high-avidity antibody were between the ages of 10 and 20 years and had received vaccination 10–15 years earlier. The high-avidity IgG response observed in these individuals indicates that they represent SVFs, because they had been primed by at least 1 dose of vaccine in the past, met the CCD, and were measles IgM positive.

Disease Severity in Acute vs Secondary Vaccine Failure Cases. Disease symptoms for individuals who met the definition for SVF (Table 1) were compared with symptoms for patients who had acute primary measles infection (Table 2). There were fewer complications in those with presumed SVF than in those with acute primary infection. There were 4 hospitalizations, 3 cases of pneumonia, and 6 other reported complications in the 10 patients with acute illness, whereas there were no hospitalizations, no cases of pneumonia and only 2 complications in the 6 patients with SVF. In this subset of cases with paired measles serological testing, all cases of acute primary measles infection that required hospitalization occurred in infants <1 year of age.

Neutralizing Antibody Responses in RMI. The PRN test is an accepted serological measure of protection because it measures functional neutralizing antibody, which is believed to confer immunity. Five of 6 identified SVF cases had paired serum samples for testing by the PRN test (Table 1). Interestingly, the PRN titers in each of the initial samples, obtained 0–28 days after rash onset, were observed to be exceptionally high; they were 6–60 times the mean PRN titer seen after routine measles vaccination (1 or 2 dose) and were markedly higher than the titers observed after acute measles infection in unvaccinated individuals (Table 3). These PRN titers were also compared with

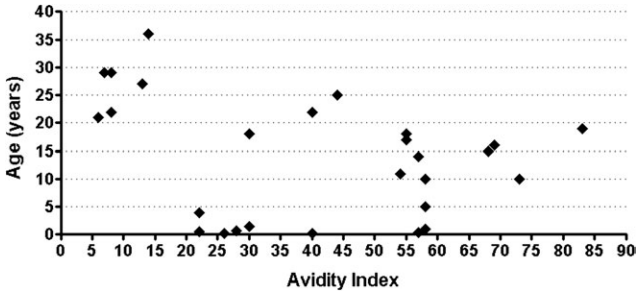

Figure 2. Age distribution and avidity index of laboratory-confirmed cases. Avidity testing was performed using an ImmunoWELL Rubeola assay (GenBio) using purified recombinant N protein. Specimens were tested at 4 dilutions (1:100, 1:1000, 1:5000, and 1:25,000). After incubation with the antigen, samples were washed with either regular assay wash buffer or with avidity reagent. An avidity index was calculated by dividing the optical density value of the well washed with the avidity reagent by the optical density value of the well washed with the kit's regular wash buffer. The linear range was below 1.5 absorbance units. Only the first ratio within the linear range was interpreted. Receiver operating characteristic analysis was performed for blood donors with known measles exposure histories to determine a cutoff value. If the avidity ratio is less than 45% the specimen is likely to contain low-avidity measles antibodies. If the avidity ratio is >50%, the specimen is likely to contain high-avidity measles antibodies. If the avidity index is between 45%–49%, it is considered to be an equivocal response.

those published for individuals who experienced natural infection or were given 1 or 2 doses of measles-containing vaccine. The geometric mean titer (GMT) of 30 unvaccinated children from Venezuela infected with measles was 4764, which is similar to the GMT of 4798 reported previously for 122 women born before 1957 (Table 3). As noted previously and shown in Table 3, PRN titers in vaccinated persons (1 or 2 doses) are considerably lower than those seen in naturally infected individuals.

PRN titers in the second paired serum sample from those with SVF cases were similar to or lower than those seen in the first samples when the pairs were compared, and no 4-fold or greater increases in antibody titer were detected in this group (Table 1). In these laboratory-confirmed cases of measles SVF, very high PRN titers were apparent immediately after rash onset and demonstrate that these individuals, although still susceptible to infection at exposure, were nonetheless capable of rapidly producing an impressive quantity of high-avidity antibody that likely mitigated their symptoms and limited the severity of their disease.

Measles PRN titers in 5 paired serum samples from individuals with acute primary measles infection were also measured (Table 2). As expected, in young infants with acute measles infection, case patient 1 (Table 2), an 8-month-old child, had a low level of measles antibody in the acute sample that was obtained 3 days after rash onset and had a modest increase in measles PRN antibody ~30 days later. If one takes into account the loss of passively acquired maternal antibody over time and compares the observed versus the expected titer in the convalescent sample, case 1 meets the criteria for a 4-fold increase in antibody titer that is consistent with acute measles infection. In contrast, cases 5, 6, and 8, who were 3–6 months of age at the time of infection, had measles antibody titers of 5496, 6640, and 7656 mIU/mL, respectively, in the acute samples obtained within 4 days of rash onset, which was consistent with the presence of passively acquired antibody from mothers previously infected with wild-type measles. Comparisons of measles titers for cases 5, 6, and 8 indicate that seroconversion did not occur even when the natural loss of maternal antibody overtime was taken into account.

Neutralizing Antibody Responses of Secondary Vaccine Failure Cases in the United States. To determine whether the unusually high plaque neutralization results observed for SVF cases in RMI were unique to this population, paired serum samples were obtained from SVF case patients in the United States (Table 4). US case patients had documented receipt of MMR, were laboratory confirmed by IgM and/or RT-PCR, and

Table 1. Self-Reported Disease Severity of Case Patients With Secondary Vaccine Failure

Case patient	Age, years	Rash duration[a]	Cough	Coryza	Conj	Complications	Prev vac	Timing spec 1, days	Timing spec 2, days	PRNT spec 1	PRNT spec 2	Avidity spec 1	Avidity spec 2
1	15	2	Yes	Yes	Yes	Diarrhea	2	28	61	297,070	13,929	68	65
2	14	7	No	No	Yes	None	2	7	N/A	119,228	95,704	57	56
3	11	4	Yes	No	No	Diarrhea	2	0	46	55,715	37,134	54	62
4	10	5	Yes	Yes	No	None	3	2	41	34,124	34,124	73	79
5	19	3	Yes	Yes	No	None	2	4	48	82,196	80,818	65	56
6	10	6	Yes	Yes	Yes	None	1[b]	3	47	N/A	N/A	58	N/A

NOTE. All patients had rash and fever. All patients had documented previous vaccination (case patients 2–5 had recorded dates of vaccination in their immunization logs, whereas donor 1 had a history of receiving 2 vaccine doses but no dates of vaccination), did not receive an outbreak response vaccination, had high-avidity antibodies, and were immunoglobulin M positive or indeterminate. Conj, Conjuctivities; Conv, convalescent; N/A, not available; Prev Vac, number of previous measles-mumps-rubella vaccinations; PRNT, plaque reduction neutralization test; Timing spec 1, days after rash onset that specimen 1 was collected; Timing spec 2, days after rash onset that specimen 2 was collected.

[a] Mean rash duration is 4.56 days (range, 2–7 days).

[b] Patient 6 received 1 dose at age 17 months and a second dose 2 days prior to rash onset.

Table 2. Self-Reported or Parent-reported Disease Severity of Case Patients With Acute Measles

Case patient	Age, years	Rash duration[a]	Cough	Coryza	Conj	Complications	Prev vac	Timing spec 1, days	Timing spec 2, days	PRNT spec 1	PRNT spec 2	Avidity spec 1	Avidity spec 2
1	0.7	5	Yes	Yes	Yes	D, V, H	0	3	45	84	168	28	25
2	63	14	Yes	Yes	Yes	None	0	21	N/A	N/A	N/A	12	N/A
3	21	4	Yes	Yes	Yes	DE, V	0	24	N/A	N/A	N/A	6	N/A
4	29	7	Yes	Yes	Yes	None	0	28	N/A	N/A	N/A	8	N/A
5	0.25	4	Yes	No	No	P, H	0	4	43	5,496	4,362	40	31
6	0.56	5	Yes	Yes	Yes	P, D, H	0	4	43	6,640	7,345	22	22
7	22	3	Yes	Yes	Yes	None	0	8	N/A	N/A	N/A	8	N/A
8	0.25	3	Yes	No	No	P, H	0	4	39	7,556	8,192	26	34
9	27	3	Yes	Yes	Yes	D	0	2	N/A	N/A	N/A	17	N/A
10	4	N/A	Yes	Yes	Yes	None	0	1	34	427	810	22	22

NOTE. All patients had rash and fever. All patients had no previous vaccination, did not receive an outbreak response vaccination, had low-avidity antibodies, and were immunoglobulin M positive. Data for patients with acute cases who had missing clinical information are not shown. Conj, Conjuctivities; Conv, convalescent; D, diarrhea; DE, dehydration; H, hospitalized; N/A, not available; P, pneumonia; Prev Vac, number of previous measles-mumps-rubella vaccinations; PRNT, plaque reduction neutralization test; Timing spec 1, days after rash onset that specimen 1 was collected; Timing spec 2, days after rash onset that specimen 2 was collected; V, vomiting.

[a] Mean rash duration is 4.8 days (range, 3–14 days).

had high-avidity measles antibodies. Cases 1–3 were part of a well-documented multistate measles outbreak associated with an international youth sporting event [10]. As seen with SVF case patients in the RMI, US laboratory-confirmed SVF case patients also had extremely high PRN titers in their paired serum specimens, which suggested that this high PRN response may be representative of immune responses to MeV in those individuals who were previously primed by measles vaccination.

Immunoprecipitation

To further characterize PRN responses observed following natural measles infection in 2-dose vaccinees, serum specimens were evaluated using immunoprecipitation. As expected, the antibody response was primarily directed against measles H protein; even when diluted 1 to 10,000, anti-hemagglutinin activity was still detected (data not shown).

DISCUSSION

The importance of waning immunity as an impediment to measles elimination remains an open question. Concern exists because measles seroprevalence rates appear to decrease as time since vaccination increases, and the proportion of the population possessing only vaccine-induced immunity continues to grow. Likewise, opportunities for boosting caused by wild-type measles exposure are becoming increasingly rare, and waning antibody titers could, over time, result in an accumulation of measles-susceptible individuals in the population. Recent outbreaks of measles in highly vaccinated populations and among individuals with 2 age-appropriate MMR vaccinations in the US and elsewhere contribute to these concerns [8, 11].

Waning of measles antibody, however, does not necessarily equate to waning immunity because cell-mediated responses are known to play an important role in protection. In addition, the incidence of measles in adults has not increased and measles attack rates, even 10 or more years after vaccination, remain low and consistent with primary vaccine failure rates [41–43]. Furthermore, among US residents during the period 2001–2008, the highest incidences of measles disease were in children ages 6–11 months and 12–15 months, and 65% of the total cases reported were considered to be preventable (eligible for vaccination but unvaccinated), which suggests that failure to vaccinate still plays a greater role in current US measles cases than does vaccine failure. More importantly, secondary spread from measles-infected 2-dose vaccinees to other susceptible individuals has not been documented [8, 10, 26]. No evidence of viral shedding was seen in a laboratory study of asymptomatic or mildly ill vaccinated contacts of persons with measles [44]; however, additional studies using existing, more sensitive laboratory techniques are needed. Disease in those with SVF is frequently muted, and the presence of virus, viral RNA and measles specific IgM are often difficult to detect; therefore, it is likely that transmission is limited and that spread of virus to other susceptible individuals occurs rarely, if at all. Such cases are not likely to be epidemiologically important with respect to transmission. Careful surveillance of vaccinated adolescents and adults, as well as a more thorough understanding of the clinical and laboratory presentation of measles disease in SVF, are needed to better identify cases and to investigate measles transmission capacity.

In this study, we identified and characterized measles SVF case patients using paired serum samples obtained from a large measles outbreak in the RMI (2003) and from recent outbreaks in the United States. We used only laboratory-confirmed cases and compared laboratory diagnostic results and reported clinical symptoms for acute primary measles cases versus vaccine failure

Table 3. Plaque Reduction Neutralization (PRN) Results Following Vaccination or Wild-type Infection Based on Exposure History

Study group	Vaccination history	Exposure to wild-type measles	Geometric mean titer plaque neutralization	95% CI	Reference	Comments
1	Unvaccinated	Yes	4,764 (n = 30)	3,467–1,547	Present study	Unvaccinated Venezualen children
2	Unvaccinated	Yes	4,798 (n = 122)	3,945–5,835	Markowitz et al, 1996 [39]	Women born before 1957
3	Unvaccinated	Yes	1,559 (n = 312)	…	LeBaron et al, 2007 [33]	Kindergarten children who received MMR between 12 and 24 months
4	Vaccinated with 1 dose	No	757 (n = 309)	…	LeBaron et al, 2007 [33]	Middle school children who received MMR between 12 and 24 months of age
5	Vaccinated with 1 dose	No	1,162 (n = 7)	…	Wong-Chew et al, 2003 [40]	Adult health care workers (age, 26–40 years) who received first dose of MMR as infants
6	Vaccinated with 2 doses	No	2,814 (n = 304)	…	LeBaron et al, 2007 [33]	One month after second MMR dose
7	Vaccinated with 2 doses	No	1,368 (n = 6)	…	Wong-Chew et al, 2003 [40]	Adult health care workers 4 weeks after receiving second MMR dose
8	Vaccinated with 1 dose	Yes	53,014 (n = 3)	12,313–219,721	Chen et al, 1990 [28]	Measles outbreak at Boston University; Preexposure PRN titers were <16, 80, and 86
9	Vaccinated with 2 doses	Yes	20,501 (n = 3)	3,837–540	Chen et al, 1990 [28]	Measles outbreak at Boston University; Preexposure PRN titers were 98, 118, and 120
10	Vaccinated with 2 doses	Yes	32,141,245,580	32,141–245,580	Present study	RMI measles outbreak, 2004

NOTE. PRN tests were performed using low-passage Edmonston MeV on Vero cell monolayers. CI, confidence interval; MMR, measles-mumps-rubella vaccine; RMI, Republic of the Marshall Islands.

cases. Additionally, laboratory results from SVF cases in the United States were compared with those observed in the RMI. Several interesting properties of the measles immune response emerged during this analysis that may help to characterize SVF cases.

First, the age distribution of IgM-positive laboratory confirmed cases reflected the history of MMR vaccination in the RMI. The RMI started routine single-dose measles vaccination in 1982, administering MMR vaccine routinely to individuals at 9 months of age. In 1998, a 2-dose MMR vaccine schedule was implemented (administered at 12 and 13 months of age), and 3 SIAs were conducted during the period 1994–2002 [6]. In the 2003 outbreak, IgM-positive cases clustered among children <5 years of age who would have received 0 or 1 dose of measles

Table 4. Case Patients With Secondary Vaccine Failure in the United States

Case	Age, years	Cough	Coryza	Conj	Prev vac	Timing spec 1, days	Timing spec 2, days	PRNT spec 1	PRNT spec 2	Avidity spec 1	Avidity spec 2	RT-PCR	IgM
1	33[a]	No	Yes	No	2	1	6	1573	207,954	N/A	N/A	+	+
2	19[b]	No	No	Yes	2	5	10	1858	119,287	High	High	+	−
3	19[b]	No	No	Yes	2	N/A	7	N/A	217,812	N/A	High	+	+
4	34	N/A	N/A	N/A	2	0	49	248,686	152,734	High	High	N/A	+
5	45	Yes	Yes	No	1	0	6	30,208	21,730	High	N/A	N/A	+

NOTE. All patients had rash and fever. Conj, conjuctivities; Conv, convalescent; Ig, immunoglobulin; N/A, not available; Prev vac, no. of previous measles-mumps-rubella vaccinations; PRNT, Plaque reduction neutralization test; RT-PCR, reverse-transcription polymerase chain reaction; Timing spec 1, days after rash onset that specimen 1 was collected; Timing spec 2, days after rash onset that specimen 2 was collected.

[a] Airport worker from Michigan involved in the outbreak reported in Guris et al [41].

[b] College students involved in the outbreak reported in Guris et al [41].

vaccine, representing the cohort of unvaccinated children and those with primary vaccine failure.

Second, a plot of the age distribution of IgM-positive patients with laboratory-confirmed cases versus their avidity measurement also reflected the RMI MMR vaccine recommendations. The majority of low-avidity antibody (acute) cases were seen, as expected, in unimmunized children <1 years of age but also, somewhat surprisingly, in adults > age 20. The low-avidity antibody observed in adult cases suggested that these individuals had not been primed for an immune response to measles and likely missed opportunities to receive measles vaccine when the program was initiated in 1982, may have missed subsequent catch-up vaccination via SIA, or may have been primary vaccine failures. This finding illustrates that pockets of susceptible individuals can accumulate in populations because of changes in vaccination policy and can, in some circumstances, provide a sufficient base to maintain transmission, particularly in densely crowded populations [45]. Recent measles outbreaks among one-dose vaccine recipients and unvaccinated adults in Boston, Sao Paulo, Ukraine, and Australia further highlight this point and demonstrate that this scenario is not unique to RMI [25, 45, 46]

An interesting third characteristic noted was that the majority of patients who were identified with high-avidity antibody were between the ages of 10 and 20 years, which would mean that they had received MMR vaccination 10–15 years earlier. These vaccinated individuals would likely never have been exposed to wild-type virus, because RMI had been free of measles for the previous 14 years. These individuals were IgM-positive and met the CCD, yet they demonstrated high-avidity IgG responses, indicating that they had been primed by vaccine at least once in the past. Past priming appeared to afford protection from severe disease, because these patients reported milder symptoms and experienced fewer complications than did those with acute measles.

The fourth and most striking observation was the magnitude (PRN titers >30,000) of the neutralizing antibody response in SVF cases, which has not, as a rule, been observed following vaccination or primary acute measles. Although these individuals lacked sufficient protective neutralizing antibody to completely inhibit measles infection at the time of exposure, they rapidly mounted an impressive neutralizing response that likely mitigated extensive viral replication and resulted in mild measles with minimal symptoms and few complications. When these high-titered serum samples were examined using immunoprecipitation, it was apparent that, as expected, the PRN titers reflected a predominant anti-H antibody response. Additional study is needed to determine whether elevated PRN titers can be used as a biomarker for SVF in cases that cannot be confirmed using traditional laboratory methods.

Although we do not know the initial PRN titer or immune status of the RMI or US SVF case patients, it is significant that a few were RT-PCR positive for wild-type measles virus, which suggests that these cases have the potential to shed and spread virus to susceptible contacts. It is important to note, however, that there was no known or documented transmission from the SVF cases in RMI. Likewise, an investigation of 2 US SVF cases did not reveal secondary spread of measles to other students within their college community [10]. Similarly, measles in 2 fully immunized (2-dose recipient) siblings exposed during an airplane flight [26] was mild, and these children did not subsequently transmit measles even though a nasal swab sample from 1 of the children was positive for measles virus RNA by RT-PCR. Together, these data suggest that viral transmission from those with SVF cases to other susceptible individuals may be very limited or may not occur at all.

In conclusion, we have characterized measles SVF in 2 populations: in the RMI, which represents a fairly typical international setting as countries move from enhanced control and mortality reduction to regional elimination, and in a highly vaccinated US population with broad 2-dose MMR coverage. SVF in both situations was characterized by documentation of prior measles vaccination, the presence of high-avidity anti-measles IgG, and markedly elevated levels of PRN antibodies. Elevated PRN titers appear to represent a biomarker for SVF, and additional studies are needed to determine whether elevated titers persist and whether they can be used to identify SVF cases within a highly vaccinated society. The duration of MMR vaccine-induced immunity in the absence of circulating virus is not well understood and may be significantly impacted by age at first vaccination, as well as by the timing of the second dose. Consistent implementation of a 2-dose schedule is also needed to maintain high population immunity against measles, to minimize disease, and to prevent subsequent outbreaks. Because SVF cases are generally mild, they may be missed unless they are seen within an outbreak setting and linked to an acute, severe measles case. Until the transmission capacity of SVF cases has been fully established, the presence of measles disease in twice-vaccinated persons illustrates the need to (1) closely monitor levels of measles antibodies in adolescents and adults in the US population, (2) be vigilant of modified disease presentation during outbreaks, and (3) evaluate vaccinated close contacts when investigating sporadic unknown source cases that have no apparent link to importation. Based on accumulating evidence, and as reported by Rota et al [48] in this supplement, patients with SVF cases do not appear to efficiently transmit virus, and their occurrence will likely not impede measles eradication efforts [8, 10, 26, 44, 47].

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