

A B S T R A C T

Measles, mumps, and rubella-specific IgG antibodies were evaluated in 134 healthy infants routinely immunized with trivalent live attenuated measles-mumps-rubella (MMR) vaccine at one year of age. Blood samples were collected just before, and at 1, 3, and 12 months after MMR. Specific IgG was measured by commercial enzyme immunoassays. Before vaccination, 98.5%, 99.2%, and 98.5% of the infants tested were seronegative for measles, mumps, and rubella, respectively. One year after MMR, 16.4% and 22.4% of vaccinees lacked demonstrable antibody to measles and mumps while none were found to be seronegative for rubella. Response profile analysis revealed primary failure rates of 12.1% (measles) and 8.6% (mumps) while 4% (measles) and 13.8% (mumps) of the infants responded initially but became seronegative within one year. These observations suggest that earlier administration (at age 18 months) of the second dose of MMR may be more desirable than revaccination at school entry.

A B R É G É

Les anticorps IgG spécifiques à la rougeole, les oreillons et la rubéole ont été évalués chez 134 enfants en bonne santé qui avaient été systématiquement immunisés avec un vaccin associé Rougeole-Oreillons-Rubéole (ROR), vivant et atténué, à l'âge d'un an. Des prélèvements sanguins ont été effectués juste avant l'administration du vaccin ROR, puis à des intervalles de 1, 3, et 12 mois par la suite. Le dosage immunoenzymatique de l'IgG spécifique a été effectué grâce à des préparations de commerce. Avant l'immunisation, les taux de séro-négativité étaient de 98,5 %, 99,2 %, et 98,5 % pour la rougeole, les oreillons, et la rubéole respectivement. Un an après l'immunisation ROR, la présence d'anticorps contre la rougeole et les oreillons ne pouvait être démontrée chez 16,4 % et 22,4 % des enfants vaccinés, alors qu'aucun ne s'avérait séro-négatif pour la rougeole. L'analyse des profils de réaction a révélé des taux d'échec primaire de 12,1 % pour la rougeole et 8,6 % pour les oreillons, alors que 4 % (rougeole) et 13,8 % (oreillons) des enfants sont devenus séro-négatifs durant l'année après avoir manifesté une réaction initiale. Ces observations suggèrent que l'administration précoce (à 18 mois) de la seconde dose de vaccin ROR pourrait être préférable à la revaccination au moment de l'entrée à l'école.

Serologic Responses to Measles, Mumps, and Rubella (MMR) Vaccine in Healthy Infants: Failure to Respond to Measles and Mumps Components May Influence Decisions on Timing of the Second Dose of MMR

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Despite over two decades of vaccination programs employing live attenuated strains of measles, mumps and rubella viruses in trivalent MMR vaccines, periodic outbreaks of these diseases still occur. Incomplete vaccine uptake as well as waning immunity have been implicated. The increasing frequency and size of measles outbreaks since 1989 and concern about waning immunity have resulted in the implementation of second dose, as well as catch-up, MMR vaccination programs in many European countries and U.S. states. These appear to have been highly effective in lowering the numbers of measles, mumps, and rubella susceptible individuals and have almost eliminated these diseases.¹⁻⁵

Concern about the status of measles immunity in Canadian children led to the recommendation, in 1993, by the National Advisory Committee on Immunization (NACI) that MMR catch-up and reimmunization programs be undertaken in Canada.^{6,7} In 1995, alone, over 2,100 Canadian cases (approx. 31/100,000 popula-

tion) were reported, accounting for 70% of measles cases in the Western hemisphere (D. Scheifele, Chair, NACI, pers. comm.). This incidence was at least 10-fold higher than in the United States² or Finland⁴ where MMR revaccination programs have been in place for several years. This alarming increase in measles prompted campaigns in British Columbia (BC) and other provinces in which monovalent measles or divalent measles-rubella (MR) vaccines (but not mumps vaccine) were administered to individuals aged 18 months to 19 years. Now, BC, Quebec, Newfoundland, New Brunswick, Saskatchewan, PEI, the Northwest Territories, and the Yukon have adopted a two-dose MMR immunization policy in which the first dose is given at 12 months, followed by a second dose at 18 months of age. The remaining four provinces have elected to offer the second dose of MMR at school entry (age 4-6 years).

We report here the results of an infant MMR immunization study which suggest that after the first dose, vaccine failure rates for the measles and mumps components may be undesirably high, leaving infants at risk for disease before they receive their second dose of MMR vaccine.

METHODS

Study subjects were 134 healthy, full-term, breastfed infants. Parental informed

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consent was obtained according to the requirements of the University of British Columbia Clinical Screening Committee for Research Involving Human Subjects. All infants were routinely immunized at age 12-14 months with a single lot of trivalent live attenuated measles-mumps-rubella vaccine (MMR II, Merck, Sharpe & Dohme, West Point, PA). Careful attention was given to maintenance of vaccine temperature before administration to ensure appropriate and uniform handling conditions. Blood samples were collected by fingerprick just before vaccination and at 1, and 3 months after MMR. An additional 5 mL blood sample was collected by venipuncture at 1 year after MMR vaccination. Sera were prepared by centrifugation of blood samples to remove cellular elements and stored at -20°C until tested.

At all study intervals, total specific levels of measles and mumps IgG were determined by commercial enzyme immunoassays (EIAs, Behring Enzygnost, Beringwerke AG, Marburg, Germany). EIA absorbance values ≥ 0.200 units were considered positive. Total levels of rubella-specific IgG were determined by two different commercial EIAs employing whole rubella virus: Behring Enzygnost (for pre-vaccine and 1-year post-vaccine samples) and BioWhittaker Rubestat (BioWhittaker, Inc., Walkersville, MD, for the 1- and 3-month post-vaccine samples). Absorbances ≥ 0.200 units (Behring) or an index of ≥ 1.00 (BioWhittaker) were considered positive. All EIA serologic evaluations were performed on contract by the Virology Laboratory at the BC Centre for Disease Control, Vancouver, BC, and were the methods in use at the time for viral antibody screening. Rubella virus-neutralizing antibody levels in pre-vaccine and 12-month post-vaccine samples only, were determined in our laboratory by bioassay as previously described.⁸ Titers $\geq 1:16$ were considered protective. Neutralization titers for measles and mumps antibodies were not evaluated because the methods were not available to our laboratory.

RESULTS

Results of tests performed with sera collected just before, and at 1, 3, and 12

TABLE I
Serologic Responses of Infants Immunized with MMR Vaccine at 12 Months of Age

Serologic Evaluation*	Number Seronegative/Number Tested (%)			
	Pre-Vaccine	Post-Vaccine		
		1 Month	3 Months	12 Months
Measles	132/134 (98.5)	24/126 (19.7)	15/133 (11.3)	19/116 (16.4)
Mumps	131/132 (99.2)	30/128 (23.4)	32/127 (25.2)	26/116 (22.4)
Rubella	130/132 (98.5)	8/124 (6.5)	0/128 (0)	0/110 (0)

* Measles- and mumps-specific IgG antibodies were determined by Behring Enzygnost EIAs at all study intervals. Rubella-specific IgG antibodies in the pre-vaccine and 12-month post-vaccine samples were determined by Behring Enzygnost EIA. Rubella-specific IgG levels in the 1- and 3-month post-vaccine samples were determined by the BioWhittaker Rubestat EIA.

TABLE II
Levels of Measles-, Mumps-, and Rubella-Specific Antibodies Following Primary Immunization of Infants at 12 Months of Age with MMR Vaccine

Serologic Evaluation*	Pre-Vaccine	Antibody Titer†		
		Post-Vaccine		
		1 Month	3 Months	12 Months
Measles	Mean	0.041	0.521	0.796
	Median	0.024	0.496	0.787
	Range	0.001-0.696	0.001-1.357	0.001-1.980
Mumps	Mean	0.016	0.461	0.413
	Median	0.001	0.448	0.394
	Range	0.001-1.007	0.001-1.221	0.001-1.227
Rubella	Mean	0.040	3.72	6.96
	Median	0.026	3.53	6.91
	Range	0.003-1.058	0.23-10.07	1.10-11.68

* Measles- and mumps-specific IgG antibodies were determined by Behring Enzygnost EIAs at all study intervals. Rubella-specific IgG antibodies in the pre-vaccine and 12-month post-vaccine samples were determined by Behring Enzygnost EIA. Rubella-specific IgG levels in the 1- and 3-month post-vaccine samples were determined by the BioWhittaker Rubestat EIA.

† Antibody titers are reported in EIA absorbance units, with the exception of rubella antibody determinations at 1 and 3 months post-vaccine which are reported in EIA indices as per the method employed for analysis of these samples (BioWhittaker Rubestat EIA, see Methods).

months after MMR vaccination are shown in Tables I and II. The majority of pre-vaccine sera tested were negative for measles, mumps, and rubella IgG when tested by the Behring Enzygnost EIAs. Those that were found to be seropositive (Table I) exhibited borderline reactivities and were not retested due to insufficient sample quantity. Six out of 56 pre-vaccine sera tested for rubella neutralizing antibody demonstrated borderline positive (titer = 1:16) levels (not shown) but were not retested due to insufficient quantity of serum. Of these, only one was found to be also positive for rubella IgG by the Behring EIA.

At one year after MMR vaccination, 83.6%, 77.6%, and 100% of the study subjects followed were seropositive (by

Behring Enzygnost tests) for measles-, mumps-, and rubella-specific IgG, respectively, while 97.3% of these samples had rubella neutralizing antibody titers $\geq 1:16$. Thus, at 12 months after vaccination, 16.4% and 22.4% of the children studied lacked demonstrable antibody to measles and mumps, respectively. Of the 19 children who were measles seronegative one year after MMR vaccination, 14 (12.1% of the total group) and 5 (4% of the total group) appeared to be primary and secondary vaccine failures, respectively. In addition, 8 of the remaining 97 infants who were seropositive at this time (or 6.9% of the total group) had very low levels of measles-specific IgG (EIA absorbance > 0.200 but < 0.300 units). Of the 26 children found to be mumps seronegative at

one year after MMR vaccination, 10 (8.6% of the total group) were primary vaccine failures while 16 (13.8% of the total group) had become mumps seronegative over the course of follow-up. Nine of the 90 infants who were mumps seropositive one year after MMR (or 7.8% of the total group) had very low levels of IgG (EIA absorbance > 0.200 but < 0.300 units). In marked contrast to the outcome of measles and mumps vaccination, none of the children followed were seronegative for rubella at one year after MMR, as determined by EIA. However, 3/110 tested (2.7%) lacked rubella virus neutralizing antibody (neutralization titers < 1:16). None of the infants followed were seronegative for all three viruses at 12 months after MMR vaccine.

DISCUSSION

The results of our study indicate higher vaccine failure rates (16.4% [measles] and 22.4% [mumps]) than those (2-12% [measles] and 3-19% [mumps]) reported in earlier studies involving various follow-up intervals after administration of a single dose of MMR vaccine.⁹⁻¹² Ratnam et al.¹³ in a recent study of measles neutralizing antibody levels in 580 one-year-olds receiving their first dose of MMR vaccine, observed that at 4-6 weeks after vaccination, 15 (2.6%) lacked measles antibody while a further 80 (13.8%) had subprotective levels. Thus, their results using a functional antibody test (measles plaque reduction) are comparable to our observations in the present study where 24/126 (19.7%) of the infants tested were observed to be measles-seronegative by EIA at one month after MMR vaccination. Similarly, Davidkin et al.¹⁴ in a study of children undergoing a two-dose MMR immunization schedule, observed that, despite an initial seroconversion rate of 86% in 14- to 18-month-old vaccinees, anti-mumps antibody titers fell rapidly during the first year such that 27% of the vaccinees were seronegative. Hence, they reported a decay in titers similar to that observed in our study. Our observation of a low failure rate with respect to the rubella component in this small cohort is comparable to that recently observed by Ratnam et al.¹³ and is

also compatible with the previously predicted rubella seronegativity rate of 1-6% after a single dose of MMR in infancy.^{9,12,15}

The results of our study also suggest that primary vaccine failure rates may be somewhat higher than expected, being in the order of 12.1% and 8.6% respectively for measles and mumps. Although primary MMR vaccine failure has been attributed to improper storage,¹⁶ batch variability and different vaccine sources,¹⁰ we feel that these factors did not impact on the results of our study as a single lot of MMR vaccine was used and the cold chain was strictly maintained. Methodologic variations in serologic determinations could also contribute to the different estimations of seropositivity coming from various studies. This would be particularly evident in comparisons between studies where antibodies are measured by a total quantitative assay for virus-specific IgG (EIA) in one, and by a functional antibody test (neutralization) in the other. Thus, primary antigen binding assay methods such as EIA may "overestimate" by measuring not only biologically relevant (e.g., neutralizing) antibodies but also irrelevant antibodies. Although some differences in antibody levels measured by different EIA methods are expected to occur, the assumption is that assay cutoffs should be approximately equivalent in that the assay validation involves the same international reference standard(s). Thus, our conclusions are based on the numbers of subjects shown to be either seropositive or seronegative by the method employed.

The observed secondary failure rates for measles (4.3%) and mumps (13.8%), taken together with the numbers of seropositive individuals with low levels of measles (6.9%) and mumps (7.8%) specific IgG, also introduce considerable concern about waning immunity during the interval between the first and second doses of MMR vaccine, particularly in immunization strategies where the second dose is administered at school entry age. Canadian epidemiologic studies suggest that infants receiving their first dose of MMR before 15 months of age may be at higher risk for measles in an outbreak situation.¹⁷ Moreover, there may be concerns about mumps immunity in communities where

catch-up and reimmunization programs have employed only monovalent measles or divalent measles-rubella vaccines.

In conclusion, the results of this study suggest that primary and secondary failure rates for the measles and mumps components (but not the rubella component) of MMR following the first dose of the vaccine may be undesirably high. The observation of low titers of measles- and mumps-specific IgG (which could possibly be insufficient to protect against disease) in 7-8% of infants vaccinated with MMR one year earlier, is also worrisome considering the potential for further decay of these antibody levels. These observations strongly support the need to revaccinate children earlier (i.e., at 18 months of age) rather than later (i.e., at school entry age), and indicate that further studies (including epidemiologic) on retention of immunity to these viruses should be considered.

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