Age-Dependent Differences in IgG Isotype and Avidity Induced by Measles Vaccine Received during the First Year of Life

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Background. Measles remains an important cause of death worldwide, and vaccinating individuals at an earlier age could lead to better control of the disease. However, persistence of maternal antibody and young age affect the quantity of vaccine-induced neutralizing antibody and may also affect antibody quality.

Methods. Enzyme immunoassay was used to analyze measles virus–specific IgG levels, avidity maturation, and isotype changes, using serum samples from infants who received measles vaccine at 6 months of age and measles-mumps-rubella (MMR)–II at 12 months of age (n = 26), measles vaccine at 9 months of age and measles-mumps-rubella (MMR)–II at 12 months of age (n = 48), or only MMR-II at 12 months of age (n = 27).

Results. The median IgG level was lower among infants with maternal antibody than among those without maternal antibody. Compared with median avidity indices for infants aged 12 months, median values were lower for 6-month-old infants with maternal antibody (P < .0001), 6-month-old infants without maternal antibody (P = .001), 9-month-old infants with maternal antibody (P = .03), and 9-month-old infants without maternal antibody (P = .006). The median IgG3 level was highest at 6 months of age. IgG1 was predominantly at 12 months. Low avidity responses at 6 or 9 months of age did not hinder higher avidity responses or the switch to IgG1 after secondary vaccination. The 2-dose regimen did not augment the response, compared with the response in infants who received 1 dose at 12 months of age.

Conclusions. Avidity and isotype maturation of measles vaccine–induced antibody are affected by age, providing insight into the ontogeny of the immune response to measles vaccine.

There were ∼30 million cases of measles and 454,000 measles-related deaths in 2004. Measles is a leading cause of vaccine-preventable disease in infants [1]. Most deaths associated with measles occur in infants <12 months of age and are due to measles-induced increased susceptibility to secondary infections. The majority of measles virus (MV) transmission occurs in Asia and sub-Saharan Africa, but outbreaks of MV infection continue to occur in areas of the Americas and Europe with low vaccination rates and continued MV importation [2].

The current live-attenuated measles vaccine is administered to infants aged 9–15 months and is highly efficacious. At younger ages, infants’ response to vaccine is often reduced because of the interference of maternal antibody and the immaturity of the immune response [3, 4]. For infants, levels of transplacentally acquired MV-specific antibodies are determined by the amount of maternal antibody transferred and the rate of maternal antibody decay, resulting in a variable period of susceptibility to MV infection [5, 6]. This window of susceptibility before vaccination and after the amount of passively acquired antibody has waned to a nonprotective level accounts for a substantial portion of the deaths due to measles. Attempts to immunize younger infants with a high-titer measles vaccine resulted in unexpected excess mortality among females,
precluding use of the vaccine in this age group [7–10]. A 2-dose regimen has been suggested as a potential strategy to augment vaccine uptake by infants. However, to fully validate this or other novel approaches, it is essential to better characterize immune responses to measles vaccine in infants <12 months of age in the presence and absence of preexistent antibody.

Humoral immunity is a critical determinant of vaccine efficacy, and much work has focused on measuring the induction of neutralizing antibody in response to measles vaccination in infants [4, 11–14]. Preexistent antibody constitutes a primary obstacle to vaccine-induced humoral responses in infants aged 9 months, but age-dependent deficiencies in antibody responses to the vaccine also contribute to reduced responses in 6-month-old infants [4]. Quantitative aspects of humoral immunity have been well characterized in response to measles vaccine in infants, but there is little information on qualities of the antibody, such as isotype and avidity, which are likely to affect vaccine efficacy.

Class switch recombination, defined as the selection of heavy chain constant region exons through deletion-recombination events, determines the isotype and function of the antibody. The 4 IgG subtypes are encoded by separate heavy chain constant region genes. Recombination to a particular subtype is a T cell–dependent process that results in Fc regions with different effector functions.

Antibody avidity, defined as the total noncovalent interaction between antibody and antigen, is a product of somatic hypermutation and affinity-based selection of MV-specific B cells in the germinal centers of secondary lymphoid tissues. The process of affinity maturation results in antibody-producing cells that secrete antibodies with progressively higher affinities and faster on-rates [15]. Failure of affinity maturation may play a role in immune complex formation, delayed clearance of virus, poor neutralization of virus, and disease pathogenesis [16–20].

In this study, we characterize qualitative as well as quantitative aspects of the antibody response to measles vaccine in infants by monitoring the dynamics of avidity maturation and isotype changes in infants aged 6 months or 9 months who received an early 2-dose vaccine regimen in the presence or absence of passively acquired antibodies and in infants aged 12 months who received a single measles vaccination.

**SUBJECTS AND METHODS**

**Population.** Serum samples were collected as part of a measles vaccine cohort study of healthy full-term infants at the Palo Alto Medical Foundation (Palo Alto, CA) [13]. A total of 101 infants who received primary vaccination against measles at 6 months of age (n = 26), 9 months of age (n = 48), or 12 months of age (n = 27) were evaluated for the current study. Because of the small volume of blood obtained from participants, all assays were not performed on all specimens, resulting in the possibility of a type II statistical error for some comparisons. A total of 1000 median tissue-culture infective doses of live MV vaccine (Attenuvax; Merck) were used to immunize infants aged 6 or 9 months, and blood samples were obtained before and 12 weeks after immunization. Measles-mumps-rubella vaccine (MMR-II; Merck) was administered at 12 months of age, and blood specimens were obtained 24 weeks after this second dose of MV vaccine. Infants who entered the study at the age of 12 months received a single dose of MMR-II, and blood samples were obtained before and 24 weeks after vaccination. No cases of measles were identified in the Palo Alto area during the study period. The study was approved by the Stanford University Committee for the Protection of Human Subjects and the institutional review board of the Palo Alto Medical Foundation. Written informed consent was obtained from parents or guardians.

**MV-specific antibody levels, avidities, and isotypes.** Samples were previously tested for MV neutralizing antibody by use of a modified plaque reduction neutralization (PRN) assay to determine whether maternal antibody was present at the time of vaccination [13, 14]. MV-specific IgG levels were measured using an EIA. An extract of Vero cells infected with the Edmonston strain of MV (Advanced Biotechnologies) was diluted in NaHCO₃ (pH 9.3), and 1 μg/well was used to coat flat-bottomed, 96-well plates (Nunc Maxisorp Immunoplates; Nalgene Nunc International), which were incubated overnight at 4°C. Plates were washed with PBS (pH 7.2) containing 0.05% Tween-20, blocked with 2% skim milk in PBS for 2 h at 37°C, and washed. Serum samples were diluted at a ratio of 1:100 in blocking buffer, plated in triplicate, and incubated for 1 h at 37°C. Plates were washed and incubated for 1 h at 37°C with alkaline phosphatase–conjugated rabbit antibody against human IgG (Accurate Chemical and Scientific). Following 3 washes, plates were incubated in the dark for 30 min with 50 μL of p-nitrophenyl phosphate (Sigma). Absorbance at 405 nm was read (Emax precision reader; Molecular Devices), and the MV-specific IgG level was expressed as optical density (OD), using Softmax Pro 3.1.1 software (Molecular Devices).

Antibody avidity was determined on the basis of MV-specific IgG dissociation due to the chaotropic agent ammonium thiocyanate (NH₄SCN) [21–23]. After incubation with serum as described above, plates were washed and incubated with 50 μL of 0–3 mol/L NH₄SCN in 0.5-mol/L increments at room temperature for 15 min. An avidity index (AI) was calculated as the concentration of NH₄SCN required to reduce MV-specific IgG binding by 50% [21–23]. Samples with absorbance readings of <0.3 in the absence of NH₄SCN were within 2 SDs of the background value and were therefore excluded from avidity analysis.

For isotype determination, plates were incubated with serum as described above, and bound IgG was detected with horse-
Age-Dependent Measles IgG Isotype and Avidity

Figure 1. Measles virus–specific IgG responses determined by enzyme immunoassay analysis (absorbance, 405 nm) after primary (shaded boxes) and secondary (unshaded boxes) vaccination among infants aged 6, 9, or 12 month with (MatAb) or without (No MatAb) maternal antibody. Age groups denote the age at which primary vaccination was received. Boxes, upper and lower quartiles; dots, outliers; lines inside boxes, median values; whisker bars, smallest and largest sample values.

Results

**MV-specific IgG OD.** EIA analysis of specimens from 6-month-old infants immunized with monovalent measles vaccine showed that the median MV-specific IgG OD increased from 0.2 before to 1.6 twelve weeks after the first vaccination for 19 infants (P = .0001) and to 2.8 twenty-four weeks after the second vaccination for 13 infants (P = .002) (figure 1). The median OD increased from 0.1 before to 2.1 after the first vaccination for 19 infants aged 9 months (P = .0002) and to 2.6 after the second vaccination for 16 (P = .1). A median OD of 2.7 was observed after MMR immunization in 25 infants aged 12 months. The median OD for 6-month-old infants after a single dose of vaccine was lower than that for 9-month-old infants (P = .02) and 12-month-old infants (P = .001). The median ODs for 6-month-old infants and 9-month-old infants after a second dose of vaccine were comparable to that for 12-month-old infants after a single dose.

Of the 74 children <12 months of age included in this study, PRN analysis revealed that 21 (16 aged 6 months and 5 aged 9 months) had an MV-specific neutralizing antibody level of >1 mIU at the time of vaccination [13, 14]. EIA analysis of samples obtained before vaccination revealed that infants aged 6 or 9 months with maternal antibody detected by PRN analysis had a median IgG OD of 0.2, compared with a median OD of 0.1 for those with no maternal antibody detected (P = .04 for 6-month-old infants, and P = .3 for 9-month-old infants) (figure 1).

The median IgG OD after the first dose of measles vaccine increased to 1.4 for 13 infants aged 6 months with maternal antibody (P = .002) and to 2.1 for 6 without maternal antibody (P = .03; P = .003 for those with vs. those without maternal antibody) (figure 1). The median IgG OD after the first dose of vaccine increased to 1.2 for 4 infants aged 9 months with maternal antibody (P = .1) and to 2.6 for 15 without maternal antibody (P = .001; P = .069 for those with vs. those without maternal antibody). There were no differences in the median IgG ODs after a single dose of vaccine between infants aged 6 or 9 months without maternal antibody and infants aged 12 months. For 10 infants aged 6 months without maternal antibody, there was no correlation between the median IgG OD...
after a single dose of vaccine and previously determined PRN findings ($\rho = 0.19; P = .6$), whereas there was a positive correlation between these variables for 45 infants aged 9 or 12 months without maternal antibody ($\rho = 0.69; P < .0001$).

After the second dose of vaccine, the median IgG OD increased to 2.6 for 7 infants aged 6 months with maternal antibody ($P = .02$) and to 3.0 for 6 without maternal antibody ($P = .03$). The median IgG OD after the second dose of vaccine was 2.1 for 4 infants aged 9 months with maternal antibody ($P = .1$) and 2.7 for 12 without maternal antibody ($P = .4$).

**MV-specific IgG avidity.** MV-specific IgG AIs were calculated (figure 2). After the first vaccination, the median AI was 0.9 for 6-month-old infants, 1.0 for 9-month-old infants, and 1.8 for 12-month-old infants. Statistical analyses revealed significant differences between infants aged 6 months and those aged either 9 months ($P = .0016$) or 12 months ($P = .0001$) and between infants aged 9 months and those aged 12 months ($P = .001$).

Among infants aged 6 months, median AIs after the first dose of vaccine were not significantly different between 16 with and 9 without maternal antibody (0.8 vs. 0.9); among infants aged 9 months, median AIs after the first dose of vaccine were not significantly different between 5 with and 16 without maternal antibody (1.0 vs. 1.1) (figure 2). The median AI was lower for 6-month-old infants without maternal antibody, compared with 9-month-old infants without maternal antibody ($P = .05$); median AIs for 12-month-old infants were higher than those for 6-month-old infants with maternal antibody ($P = .0001$), 6-month-old infants without maternal antibody ($P = .001$), 9-month-old infants with maternal antibody ($P = .03$), and 9-month-old infants without maternal antibody ($P = .006$). For 10 infants aged 6 months without maternal antibody, the median AI after a single dose of vaccine was not correlated with previously determined PRN values ($\rho = -0.3; P = .4$), whereas there was a positive correlation between these variables for 45 infants aged 9 or 12 months without maternal antibody ($\rho = 0.49; P = .0006$).

After the second dose of measles vaccine, the median AI increased to 1.1 for 7 infants aged 6 months with maternal antibody ($P = .02$) and to 2.0 for 6 without maternal antibody ($P = .03$); median AIs were not significantly different between the groups (figure 2). The median AI after the second vaccination was 0.5 for 11 infants aged 9 months with maternal antibody ($P = .1$) and 1.5 for 12 without maternal antibody ($P = .04$) ($P = .03$ for those with vs. those without maternal antibody). For all infants except those aged 9 months who had maternal antibody, the median AI after the second vaccination was similar to that for infants aged 12 months after a single vaccination.

**MV-specific IgG isotype distribution.** Median MV-specific

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**Figure 2.** Avidity of measles virus–specific IgG after primary (shaded boxes) and secondary (unshaded boxes) vaccination among infants aged 6, 9, or 12 months with (MatAb) or without (No MatAb) maternal antibody. Age groups denote the age at which primary vaccination was received. Boxes, upper and lower quartiles; dots, outliers; lines inside boxes, median values; whisker bars, smallest and largest sample values.
IgG1 ODs after the first vaccination were 0.5 for 6-month-old infants, 1.7 for 9-month-old infants, and 1.4 for 12-month-old infants ($P = .003$ for infants aged 6 months vs. those aged 9 months; $P = .003$ for infants aged 6 months vs. those aged 12 months) (figure 3A). After the second vaccination, the median IgG1 OD increased to 1.4 for 13 infants aged 6 months ($P = .002$) and to 1.9 for 16 aged 9 months ($P = .1$); these values were similar to that for 12-month-old infants after a single vaccination. Median MV-specific IgG3 ODs after the first vaccination were 0.6 for 6-month-old infants, 0.6 for 9-month-old infants, and 0.1 for 12-month-old infants ($P = .07$ for infants aged 6 months vs. those aged 12 months; $P = .09$ for infants aged 9 months vs. those aged 12 months) (figure 3B). After the second vaccination, the median IgG3 OD decreased to 0.3 for 12 infants aged 6 months ($P = .3$) and to 0.2 for 16 infants aged 9 months ($P = .001$).

After the first measles vaccination, the median IgG1 OD for 6-month-old infants was 0.2 for those with and 1.1 for those without maternal antibody ($P = .05$) (figure 3A). The median IgG1 OD for 9-month-old infants was 1.3 for those with and 1.7 for those without maternal antibody ($P = .3$). For infants with maternal antibody, the median IgG1 OD for 6-month-old infants was less than that for 9-month-old infants ($P = .003$); however, the IgG1 ODs for 6-month-old and 9-month-old infants without maternal antibody did not differ significantly ($P = .1$). After receipt of a single dose of measles vaccine, the median IgG1 OD for 12-month-old infants was higher than that for 6-month-old infants with maternal antibody ($P = .005$) but not for 6-month-old infants without maternal antibody ($P = .2$).

After the second dose of vaccine, the median IgG1 OD increased to 1.0 for 7 infants aged 6 months with maternal antibody ($P = .02$) and to 1.8 for 6 without maternal antibody ($P = .04$). For 9-month-old infants, the median IgG1 OD increased to 1.9 after the second dose of vaccine for 12 without maternal antibody ($P = .04$); there was no significant change in this value after the second dose of vaccine for 4 infants aged 9 months with maternal antibody (median IgG1 OD, 0.8; $P = .7$). There were no significant differences in the median IgG1 values between 6-month-old and 9-month-old infants with maternal antibody or 6-month-old and 9-month-old infants without maternal antibody after the second dose of vaccine.

After the first measles vaccination, the median IgG3 OD for 6-month-old infants was 0.5 for those with and 1.0 for those without maternal antibody ($P = .008$) (figure 3B). For 9-month-old infants, the median IgG3 OD was 0.1 for those with and 0.7 for those without maternal antibody ($P = .5$). A decrease in the median IgG3 titer for 12 infants aged 9 months without maternal antibody was observed after the second dose ($P = .005$). IgG2 and IgG4 were not detected in any infants.

**DISCUSSION**

Quantitative and qualitative features of the antibody response to an early 2-dose measles vaccine regimen were characterized. EIA analysis revealed age-dependent differences in the generation of MV-specific IgG; age-dependent differences in class switching and avidity maturation were also observed. However, a lower level of MV-specific IgG was seen only in infants who had residual maternal antibody at the time of vaccination, whereas the age-dependent defect in avidity maturation was independent of the presence of maternal antibody. The MV-specific IgG3 level was highest in 6-month-old infants, whereas older infants primarily produced IgG1. Lower avidity responses...
to initial measles vaccination did not hinder the generation of higher avidity responses to a second dose of vaccine or the switch to IgG1. These data show that the ability to induce a mature, high-avidity IgG1 antibody response after measles vaccination increases with development of the immune system between 6 and 12 months of age.

The patterns in the development of MV-specific IgG differed from those for neutralizing antibody [13]. EIA analysis showed that 6-month-old infants without maternal antibody produced levels of MV-specific antibody similar to those in 12-month-old infants. Furthermore, for these infants there was no correlation between the titers measured by PRN and either the ODs or AIs measured by EIA. In contrast, for 9-month-old infants without maternal antibody, the IgG response measured by EIA correlated with PRN titers and was equivalent to the response for 12-month-old infants [11], and there was a positive correlation between PRN titers and AIs. The induction of MV-specific IgG, as measured by EIA, was affected by the presence of passive antibody, as was also observed for neutralizing antibody. PRN primarily measures levels of antibody against the hemagglutinin (H) protein, whereas EIA measures levels of antibody against all MV proteins. The H protein binds to cellular receptors for MV, and interference with this interaction is a prime target for neutralizing antibody. Lack of correlation between EIA findings and neutralizing-antibody titers suggests that 6-month-old infants may have a particular defect in their ability to produce antibody against the H protein, as well as a defect in affinity maturation. High-avidity antibody is important for neutralization of wild-type strains of MV that preferentially use CD150 as a receptor, whereas it is less important for neutralizing tissue culture-adapted strains of MV used for PRN assays that can use CD46 as well as CD150 as a receptor [19].

The generation of lower antibody avidity responses by infants aged 6 and 9 months, compared with those for infants aged 12 months, independent of the presence of preexisting antibody corroborates previous findings that 6-month-old infants have intrinsic deficiencies in humoral immune responses to measles vaccine [4, 12–14]. The mechanism determining age-dependent differences in the quality and quantity of the antibody responses has yet to be determined. Reduced CD4+ T cell help for B cells may impair the process of affinity maturation in germinal centers, resulting in antibody avidity in infants that is lower than that in adults. Previous work has shown that infants respond with lower rates of MV-specific CD4+ T cell proliferation and IFN-γ and IL-12 production, fewer CD69+/IFN-γ+ CD4+ T cells, and fewer CD40L+/IFN-γ+ CD4+ T cells, compared with adults. However, no age-related differences were observed in measles vaccine–induced T cell proliferation or IFN-γ production at 6, 9, or 12 months of age [24, 25]. The lower avidity response in young infants is likely related to limitations in somatic hypermutation in human B cells in early life [26–29].

Somatic mutations in the IgG VH6 region are rare in infants <6 months of age and increase, concomitant with evidence for positive selection of mutated B cell clones, during the first year of life [28].

After immunization, including administration of additional doses of vaccine, infant B cells receive an adequate T cell stimulus to successfully switch from IgM to IgG [26]. However, this switch is characterized by a strong predominance of IgG1 and IgG3, whereas the level of IgG2 antibody remains low [30]. The low ratio of IgG2 to IgG1 has been proposed to signify a Th2 skewing of the immune response in early life. When Th1 cytokines are supplemented in vitro, infant B cells can produce IgG2 [31]. Although increased levels of Th2 cytokines such as IL-4 and IL-10 were not observed in response to measles vaccination in infants [11], IFN-γ responses may be insufficient to induce a complete deployment of the Th1 response. The isotypes of MV-specific antibodies have been previously studied after immunization and natural infection [32–36]. These studies show that, in general, IgG3 is detected early and associated with decreasing levels over time, whereas levels of IgG1 continue to increase; IgG2 is rarely observed. Younger individuals often have higher levels of IgG3, compared with older individuals [34]. The decrease in IgG3 levels may reflect the short half-life of the antibody and an increased efficiency of switching to IgG1 with increased age. The g3 locus lies farthest upstream in the immunoglobulin heavy chain constant region germ-line sequence, and increased IgG3 synthesis by the youngest infants may reflect immaturities in infant B cell class switch recombination.

A 2-dose measles vaccine regimen for younger infants is being studied, the goal of which is to provide earlier protection against measles in infants <12 months of age, a susceptible population expected to increase in size as the proportion of mothers with vaccine-induced immunity increases. We assessed the qualitative and quantitative features of the antibody response generated by such a regimen. Our findings show that passive antibodies do not, but young age does, interfere with avidity maturation of IgG responses to measles vaccine, and they provide insight into the functionality and ontogeny of the humoral immune response during early life.

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References

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