Deficiency of the Humoral Immune Response to Measles Vaccine in Infants Immunized at Age 6 Months

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Context.—Measles causes serious morbidity in infants, with the highest risk among those who are 6 to 12 months of age. In the United States, measles vaccine has been given at age 12 to 15 months to minimize interference by passive antibodies and to achieve the high seroprevalence required for herd immunity. Infants of mothers with vaccine-induced immunity may lose passively acquired antibodies before 12 months, leaving them susceptible to measles infection.

Objective.—To assess the immunogenicity of measles vaccine in infants younger than 12 months.

Design.—Cohort study conducted before and after measles immunization.

Setting.—Pediatric clinic in Palo Alto, Calif.

Participants.—Infants 6 (n = 27), 9 (n = 26), and 12 (n = 34) months of age were enrolled; 72 provided both initial and follow-up samples.

Main Outcome Measures.—Evaluation of immunogenicity before and 12 weeks after measles vaccination, including measles neutralizing antibody titers, measles-specific T-cell proliferation, and cytokine profiles.

Results.—Measles neutralizing antibodies were present before vaccination in 52% (12/23), 35% (7/20), and 0% (0/22) of 6-, 9-, and 12-month-old infants, respectively. In the absence of detectable passive antibodies, geometric mean titers after vaccination were significantly lower in 6-month-old infants compared with 9-month-old infants (27 vs 578, P = .01) and 12-month-old infants (27 vs 972, P = .001). The seroconversion rate, defined as a 4-fold rise in antibody titer, in these 6-month-old infants was only 67%, and only 36% of these infants achieved seroprotective neutralizing antibody titers of 120 or higher after vaccination compared with 100% of 9- and 12-month-old infants lacking detectable passive antibody prior to vaccination. T-cell proliferation and cytokine responses to measles did not differ with age.

Conclusions.—Humoral immunity was deficient in 6-month-old infants given measles vaccine, even in the absence of detectable passively acquired neutralizing antibodies. Comparison of their responses with those of 9- and 12-month-old infants indicates that a developmental maturation of the immune response to measles may occur during the first year of life, which affects the immunogenicity of measles vaccine.

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The susceptibility of infants to serious disease caused by viruses is recognized during the immediate neonatal period but it also extends through the first year of life, suggesting that immunocompetence of the host develops gradually over this time interval. In the case of measles, clinical experience demonstrates that a critical maturation of the host response occurs between 6 and 12 months, based on the subsequent decline in measles mortality. The high rate of infant morbidity and mortality observed in developing countries and during recent outbreaks in the United States has renewed interest in evaluating measles immunization of infants at the youngest possible age. Past studies showed a high failure rate of measles vaccination in infants younger than 12 months. Poor immunogenicity was associated with the persistence of antibodies acquired transplacentally from mothers whose measles immunity was induced by natural infection. The recommendation for vaccination at 12 to 15 months of age was made to ensure that all, or almost all, infants had lost passive antibody when immunized and that optimal herd immunity was achieved. In contrast with the first 3 decades after measles vaccine was introduced, most infants in the United States are now born to mothers who have vaccine-induced immunity to measles. These infants can be expected to lose maternal antibodies by 9 to 12 months of age. Our recent study showed only 29% of 9-month-old infants and 5% of 12-month-old infants had persistent passive antibodies. As a result, more infants younger than 12 months now lack both passive and active measles immunity, leaving them unprotected and in the highest-risk group for life-threatening complications. Maintaining high seroprevalence rates to achieve herd immunity protects young infants but local epidemics confirm past studies showing that a small population of susceptible individuals can sustain a measles outbreak.

Little is known about the maturation of virus-specific immune responses in healthy infants following infection or immunization. Newborn infants have deficiencies in primary antigen presentation by dendritic cells, limited T-cell proliferation, impaired B-cell function, and reduced production of cytokines by helper T cells of the type 1 subset (Th1), including interleukin 2 (IL-2) and interferon γ (IFN-γ). Whether these deficiencies, which could diminish the immunogenicity of measles vaccine, are still present in 6- to 9-month-old infants has not been determined. Since many infants now have early loss of passive antibodies, it is feasible to distinguish the relative impact of deficiencies attributed to the maturation of the immune re-
spontaneous from passive antibody inhibition of measles vaccine immunogenicity.

The purpose of our study was to assess whether there are intrinsic immunological barriers to immunization of infants younger than 12 months with measles vaccine, or whether neutralization of the vaccine virus by passive antibodies constitutes the only significant obstacle to early vaccination. In the past, potential interference by passive antibodies prevented the analysis of developmental changes in the host response to measles.

METHODS

Study Population

Subjects included healthy infants, without documented intercurrent illnesses, who were 6 (n = 27), 9 (n = 26), or 12 (n = 34) months of age and were seen for their well-child visit at the Palo Alto Medical Foundation, Palo Alto, Calif. A total of 87 infants were enrolled in the study; 2 infants never participated after consent was given because their mothers decided against phlebotomy (6-month-olds: n = 1, 12-month-olds: n = 1), 13 infants provided only the initial blood sample (6-month-olds: n = 1, 9-month-olds: n = 2, 12-month-olds: n = 10), and 72 infants provided both the initial and follow-up samples (6-month-olds: n = 23, 9-month-olds: n = 24, 12-month-olds: n = 23), but not all T-cell and B-cell assays were performed for each sample. 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 consent was obtained from parents or guardians. Children born before 36 weeks’ gestation, whose birth weight was less than 2500 g, or who had chronic underlying illnesses were excluded. Mothers were grouped by birth weight at concentrations of 3.0 \times 10^6 per well in RPMI 1640 (Gibco, Gaithersburg, Md), 10% normal human sera (Sigma, St Louis, Mo). Measles antigen, prepared from infected Vero cell lysates, or an uninfected cell control was added at dilutions of 1:16 and 1:32 to triplicate wells. T-cell proliferation was measured by adding tritiated thymidine (2.5 µCi per well) after 5 days for 6 to 18 hours. The stimulation index (SI) was calculated as the mean counts per minute (cpm) in measles antigen–stimulated wells divided by the mean cpm in control wells.

A positive SI to measles was 3.0 or greater, based on the mean and SD of responses in infants before vaccination. Phyothenagglutinin (Difco, Detroit, Mich) was used as a positive control.

Assays for Cytokine Production

Supernatants from PBMCs stimulated with measles antigen prepared from infected Vero cell lysates or uninfected cell controls were collected from duplicate wells for 8 consecutive days, stored at –70°C, and tested using ELISA assays, with sensitivities of detection defined by reference standards in each assay. The supernatants collected on days 1 through 8 after initial incubation of T cells with measles antigen were tested in parallel to determine the peak cytokine response; this peak concentration was used as the value for statistical analysis. Interleukin 4 and IL-10 production were measured using assays from Genzyme Inc (Cambridge, Mass). Specimens were also tested for IL-4 using Cytoscreen ultrasensitive assay (Biosource Inc, Camarillo, Calif). The ELISA method from Endogen Inc (Cambridge, Mass) was used to measure IL-2 and IFN-γ.

Statistical Analysis

The reciprocals of the measles PRN titers were transformed, and geometric mean titers (GMTs) were calculated. Differences in antibody titers among groups were evaluated by the Mann-Whitney U test. Stimulation indexes and cytokine responses in individual patients before and after vaccination were compared using the paired Student t test; the unpaired t test was used to compare study populations, but only on paired data points. Analysis of variance was performed to evaluate differences among the means of all 3 groups. The χ² and Fisher exact tests were used to compare the number of vaccinees in each cohort who had antibody or proliferation responses. The Spearman rank coefficient was used to evaluate correlations between SI and GMTs or cytokine responses. Statistical significance was defined at P < .05 for all analyses performed.

RESULTS

Humoral Immune Responses

Measles neutralizing antibody titers were determined in 65 infants before vaccination. Twelve (42%) of 29 6-month-old infants had detectable passive antibodies compared with 7 (9%) of 20 9-month-old infants and 0 (0%) of 22 12-month-old infants (6 months vs 12 months, P = 0.03; 9 months vs 12 months, P = .01) (Table). There were no
statistical differences in measles GMTs before vaccination when comparing 6- and 9-month-old infants born to the oldest mothers, who were born before 1957, and the youngest mothers, who were born after 1963.

Measles-neutralizing antibody titers after vaccination, determined in the same 65 infants and expressed as GMTs, were 35 (95% confidence interval [CI], 13-95), 201 (95% CI, 69-685), and 972 (95% CI, 669-1415) in 6-, 9-, and 12-month-old infants, respectively (6 months vs 9 months, \( P < .003 \); 6 months vs 12 months, \( P < .001 \); 9 months vs 12 months, \( P = .01 \)) (Figure 1). The seroconversion rate (4-fold rise in antibody titer) in 6-month-old infants was only 65% (15/23) compared with 90% (18/20) of 9-month-old infants and all of the 22 infants who were 12 months old (6 infants vs 12 months, \( P = .01 \)). Six-month-old infants were also less likely than 9- and 12-month-old infants to develop seroprotective neutralizing antibody titers of 120 or more. Only 10 (43%) of 23 6-month-old infants had seroprotective titers after vaccination compared with 17 (85%) of 20 9-month-old infants and 21 (95%) of 22 12-month-old infants (6 months vs 9 months, \( P = .01 \); 6 months vs 12 months, \( P = .001 \)).

In the presence of passive antibodies, determined by PRN, there was no statistical difference in the seroconversion rates and GMTs among all infants, regardless of age. Sixty-three percent of 6-month-old infants who had passive antibody before vaccination seroconverted, with a postvaccination GMT of only 45. This rate of response was not statistically different from the 40% of 9-month-old infants who seroconverted in the presence of passive antibodies and who had a GMT of 85 after vaccination.

In contrast, age-related differences were observed among infants who had no detectable passive antibodies by PRN prior to vaccination. Among these infants, the measles GMTs after vaccination were lower in 6-month-old infants compared with 9-month-old infants (27 vs 578; \( P = .01 \)) and 12-month-old infants (27 vs 972; \( P = .001 \)) (Figure 2). Analysis of variance showed \( P = .001 \). The seroconversion rate in these 6-month-old infants was 67%, and only 36% of these infants reached seroprotective GMTs after vaccination, compared with 100% of the 9- and 12-month-old infants lacking detectable passive antibody prior to vaccination. The GMTs, seroconversion, and seroprotective rates of the 9-month-old infants who lacked detectable passive antibodies showed no statistical difference compared with those of 12-month-old infants, all of whom lacked detectable passive antibodies prior to vaccination.

Six infants who had primary measles vaccine failure at 6 months (\( n = 5 \)) or 9 months (\( n = 1 \)) were evaluated for neutralizing antibodies up to 2 years after revaccination with M-M-R. Their GMTs increased significantly, from 4.7 to 403 (\( P = .02 \)).

No correlations were found between measles GMT and cytokine responses to measles in any of the infant groups.

### T-cell Proliferation

T-cell proliferation to measles antigen was measured in 67 infants and 17 vaccinated adults. Infants from all age groups but the mean IL-2 concentration among infants after vaccination were 41.4 (10.93), 49.8 (19.69), and 42.5 (13.69) pg/mL in 6-, 9-, and 12-month-old infants, respectively (Table). A significant rise in IL-2 production was detected after vaccination of only 6- and 12-month-old infants, to 148.6 (25.77) and 99.8 (24.32) pg/mL in the 9-month-old children (6 months, \( P = .003 \); 12 months, \( P = .005 \)).

No age-related differences were detected among infant groups but the mean IL-2 concentration in vaccinated adults was 306.3 (60.84) pg/mL, which was higher than levels among all infants (\( P = .002 \)).

The production of IFN-\( \gamma \) by T cells stimulated with measles antigen was measured in 42 infants and 8 adults. Before vaccination, the mean (SE) IL-2 concentrations were 46.2 (18.0), 65.3 (16.39), and 32.5 (9.83) pg/mL in 6-, 9-, and 12-month-old infants, respectively (Table). A significant rise in IL-2 production was detected after vaccination of only 6- and 12-month-old infants, to 148.6 (25.77) and 99.8 (24.32) pg/mL, respectively (6 months, \( P = .003 \); 12 months, \( P = .005 \)). No age-related differences were detected among infant groups but the mean IL-2 concentration in vaccinated adults was 306.3 (60.84) pg/mL, which was higher than levels among all adults (\( P = .002 \)).

Five infants who had serologically defined primary vaccine failure had SIa of 3 or greater after measles immunization. Among infants who had passive antibodies prior to vaccination, 8% (1/12) of 6-month-old infants and 14% (1/7) of 9-month-old infants had neither humoral nor T-cell proliferation to measles vaccine. All infants who lacked passive antibodies had either humoral or cell-mediated immunity or both after immunization.

### Cytokine Production

Interleukin 2 production in response to measles antigen was measured in 41 infants and 8 adults. Before vaccination, the mean (SE) IL-2 concentrations were 46.2 (18.0), 65.3 (16.39), and 32.5 (9.83) pg/mL in 6-, 9-, and 12-month-old infants, respectively (Table). A significant rise in IL-2 production was detected after vaccination of only 6- and 12-month-old infants, to 148.6 (25.77) and 99.8 (24.32) pg/mL, respectively (6 months, \( P = .003 \); 12 months, \( P = .005 \)).

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Figure 1.—Neutralizing antibody responses of infants before and after measles immunization. Shown are the geometric mean titers (GMTs) as measured by plaque reduction neutralization assay before and after measles vaccination in infants who were 6, 9, or 12 months of age at the time of measles immunization. The Table provides the 95% confidence intervals. A neutralizing antibody titer of 120 or greater, defined as protective, is indicated by the dashed line.

Figure 2.—Neutralizing antibody responses of infants to measles immunization in the presence and absence of passive antibodies. Shown are the geometric mean titers (GMTs) as measured by plaque reduction neutralization assay after measles vaccination of infants in the presence and absence of passive antibodies. Infants were 6, 9, or 12 months of age at the time of measles immunization. The Table provides the 95% confidence intervals. A neutralizing antibody titer of 120 or greater, defined as protective, is indicated by the dashed line.

Figure 3.—T-cell proliferative responses of infants before and after measles immunization. Shown is the stimulation index to measles antigen before and after measles vaccination in infants who were 6, 9, or 12 months of age at the time of immunization. Error bars indicate SEs. A positive stimulation index is defined as 3 or greater.

Vaccination of 6-, 9-, and 12-month-old infants, respectively (Table). However, when responses of individual infants were evaluated by paired t test, significant increases were detected after vaccination of 9- and 12-month-old infants but not 6-month-old infants (6 months, \( P = .06 \); 9 months, \( P = .03 \); 12 months, \( P = .04 \)). The mean (SE) INF-\( \gamma \) concentration was 300.6 (147.66) pg/mL in vaccinated adults, which was not significantly different from the infants.

Interleukin 4 release was not detected after stimulation of T cells from infants or adults with measles antigen. Interleukin 10 production by PBMC stimulated with measles antigen was evaluated in 38 infants and 8 vaccinated adults. The mean (SE) IL-10 concentrations before and after vaccination were not statistically different in all age groups; the responses were 81.9 (15.54) vs 73.9 (26.44) pg/mL in 6-month-old infants, 84.5 (17.87) vs 77.4 (25.80) pg/mL in 9-month-old infants, and 59.9 (11.98) vs 50.4 (10.49) pg/mL in 12-month-old infants. Vaccinated adults had higher IL-10 concentrations than infants with a mean (SE) of 152.4 (32.03) pg/mL (\( P = .02 \)).

No age-related differences in the kinetics of cytokine production, defined as the interval to detection of the peak concentration, were detected. There was no correlation between SI and cytokine responses in individual patients.

COMMENT

Interference due to passively acquired antibodies among infants younger than 12 months has been observed since the live attenuated measles vaccine was introduced in the 1960s. Since 48% of 6-month-old infants in our population had undetectable levels of passive antibodies by the most sensitive PRN assay, it was possible to evaluate the capacity of the developing immune system to respond to measles vaccine without the confounding variable of interference by neutralizing antibodies acquired from the mother. Vaccination of 6-month-old infants who had no detectable passive antibodies elicited seroconversion in only 67% and seroprotective titers in only 36% of these infants. In contrast, 100% of 9-month-old infants lacking passive antibodies seroconverted and achieved titers considered seroprotective; their responses were not statistically different from those of 12-month-old infants.

Our study demonstrates that the persistence of passive antibodies remains an obstacle to measles immunization, affecting responses in about one third of 9-month-old infants and half of 6-month-old infants. Yet, the deficiency of the humoral immune response to measles vaccine among 6-month-old infants without detectable passive antibodies in this study, compared with that among 9- or 12-month-old infants, indicates that some component of the immune response to measles antigens undergoes maturation late in the first year of life. Younger infants may have a functional defect in the Th2-cell response to measles antigen since INF-\( \gamma \) production did not increase significantly after vaccination of 6-month-old infants. Infants infected with herpes simplex virus and cytomegalovirus also have low or absent INF-\( \gamma \). T-cell recognition of measles antigens, as measured by in vitro proliferation, was detected among vaccinated infants and adults but the response rates were less than 75% among all cohorts, as has been described in previous studies of cell-mediated immunity to measles.
The limited proliferation of measles-specific memory T cells in in vitro assays may be related to the down-regulation of IL-12 production shown previously to be triggered by measles. Interleukin 12, which is secreted by monocytes, is important for optimal Th1 differentia-

tion and plays an integral role in the acquisition of the cell-mediated immune response.

If infant T cells have a diminished capacity to produce IFN-γ, decreased IL-12 production after measles infection or immunization would be expected to further impair the induction of measles-specific cellular immunity.

In vivo, natural measles infection and live attenuated measles vaccine induce transient, nonspecific immunosuppression, characterized by predominance of type 2 Th2 cell responses and associated with the increase in the spontaneous release of IL-4 and IL-10. Infants may have an age-related susceptibility to the generalized immunosuppression elicited by measles virus in vivo, including attenuated vaccine strains. In contrast with the increased spontaneous release of these cytokines, our evaluation of the measles-specific production of IL-4 and IL-10 did not demonstrate a shift toward a predominant Th2 cell response. Naïve T cells of neonates shift preferentially toward a Th1 cell response when stimulated by foreign antigens, but whether this pattern persists later in the first year of life has not been determined.

The decreased synthesis of measles neutralizing antibodies in younger infants may represent impaired T-cell and B-cell interactions. Specifically, a maturation deficiency in CD40 lipid expression by activated T cells could inhibit the development of T-cell-dependent B-cell immunity to measles. It is also possible that the limited immunogenicity of measles vaccine in 6-month-old infants reflects a defect in antigen presentation by dendritic cells. The switch from naïve CD45RA T cells to memory T cells expressing CD45RO is crucial for acquisition of an effective antigen-specific immune response and requires antigen processing by dendritic cells, which have been shown to be functionally immature in neonates. Finally, diminished humoral immunity to measles in 6-month-old infants could represent a primary B-cell deficiency, although impaired immunity to viral pathogens has usually been associated with altered T-cell-dependent responses. Infants have decreased T-cell–independent B-cell activation by poly saccharides and heavily glycosylated proteins made by bacteria and viruses.

Regardless of the underlying immunologic mechanism, our experience was that the maturational deficiency in the humoral response to measles vaccine was transient in individual infants. Re-vaccination at age 12 to 15 months resulted in a marked increase in measles neutralizing antibody titers among infants with serologically defined primary vaccine failure. These observations suggest that early immunization does not induce tolerance to subsequent doses of measles vaccine, which was an earlier concern in considering the immunization of infants younger than 12 months.

Our observations are consistent with recent reports that these infants seroconvert after a second dose of measles vaccine.

Most 6-month-old infants with poor humoral immune responses to measles had detectable T-cell proliferation to measles antigen after vaccination. Whether the presence of measles-specific T cells predicts protection, despite low or undetectable titers of neutralizing antibodies, is not known. The importance of cellular immunity against measles is suggested by clinical experience demonstrating that patients with cellular immunodeficiencies are susceptible to severe or fatal measles, whereas children with congenital agammaglobulinemia had no complications from measles and developed immune reinfection. Nevertheless, seroconversion after measles vaccination, with induction of measles neutralizing antibody titers greater than 120, correlates with protection against wild-type measles infection.

Our study demonstrates that host response of most infants immunized at age 6 months is insufficient to achieve this criterion of protective immunity.

Given the heterogeneity of the population of women of childbearing age in the United States, which includes women with natural as well as vaccine-induced immunity to measles, interference by passive antibodies will continue to affect the responses of many 6- and 9-month-old infants to measles vaccine. Our study indicates that postnatal maturation of the immune system is also likely to restrict the immunogenicity of measles vaccine in 6-month-old infants. This possibility warrants further investigation when considering lowering the recommended age of measles immunization for infants whose mothers have vaccine-induced immunity.

References


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