Immune Responses to Measles and Mumps Vaccination of Infants at 6, 9, and 12 Months

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Immunizing infants against measles at the youngest age possible has the potential to reduce morbidity and mortality. The ability of infants at 6, 9, or 12 months to respond to measles and mumps vaccines was evaluated by measuring T cell proliferation, interferon-γ production, and neutralizing antibody titers before and after vaccination. Infants in all age groups had equivalent cellular immune responses to measles or mumps viruses, with or without passive antibodies when immunized. In contrast, 6-month-old infants without passive antibodies had low geometric mean titers of antibody to measles or mumps viruses and low seroconversion rates. Geometric mean titers of antibody to measles virus increased if infants were revaccinated at 12 months. Six-month-old infants had limited humoral responses to paramyxovirus vaccines, whereas cellular immunity was equivalent to that of older infants. T cell responses can be established by immunization with these live attenuated virus vaccines during the first year, despite the presence of passive antibodies.

The global eradication of measles will have a major impact on child health, because measles still causes 800,000 deaths annually in developing countries [1–3]. To achieve this goal, a better understanding of measles immunity in infants will help to optimize the use of live attenuated measles virus vaccines and to design novel vaccines with enhanced efficacy [4–6]. Measles morbidity and mortality are highest among the youngest infants in developed, as well as developing, countries [7–10]. Measles vaccine immunogenicity in younger infants is now a concern in the United States, because most women of childbearing age have vaccine-induced measles immunity. The associated early decline of transplacentally acquired antibodies means that the interval of susceptibility between the loss of passive antibodies and immunization at 12–15 months may be ⩾6 months [11, 12].

Potential obstacles to the immunization of younger infants against measles and other viral pathogens include immaturity of the immune system [13, 14], as well as interference by passive antibodies [15]. Although humoral immunity has been evaluated extensively, little is known about the ontogeny of cell-mediated responses to viruses during the first year of life. Our first objective was to investigate measles virus–specific cellular immunity in relation to age and to the presence of passive antibodies at the time of immunization, in large cohorts of infants who were vaccinated at 6, 9, or 12 months. Measles and, to a lesser extent, measles vaccination have been associated with transient immunosuppression that could create particular barriers to immunogenicity in younger infants [16–21]. Therefore, our second goal was to compare age-related patterns of cellular immunity elicited by measles vaccine to responses by mumps vaccine, another paramyxovirus. In a previous study [14], we showed that infants vaccinated against measles at 6 months had diminished neutralizing antibody titers. Responses to mumps vaccination were evaluated to determine whether this observation was unique to measles or was a general characteristic of the immunologic capacity of 6-month-old infants to respond to live attenuated paramyxovirus vaccines. To identify possible immunologic effects of early exposure to viral antigens, our third objective was to assess humoral and cell-mediated immunity in a subset of infants who were given a second dose of measles as measles-mumps-rubella (MMR) vaccine at 12 months of age after initial measles immunization given at 6 or 9 months.

Materials and Methods

Populations. The measles cohort included 248 infants vaccinated at 6 months ($n = 93$), 9 months ($n = 77$), or 12 months ($n = 78$); of these, 210 infants had prevaccine and 12-week blood

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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 and adults.

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samples, 36 had prevaccine specimens only (6 months, 12; 9 months, 7; 12 months, 17), and 2 had none. The mumps cohort was 139 infants immunized at 6 months ($n = 53$), 9 months ($n = 45$), or 12 months ($n = 41$); 97 infants had paired samples, 41 had prevaccine specimens only (6 months, 13; 9 months, 15; 12 months, 13), and 1 was not tested. Thirty-one infants (6 months, $n = 12$; 9 months, $n = 19$) had a third blood sample taken at 18 months of age, after a second dose of measles antigen, which was given as MMR vaccine at 12 months of age. Because of limitations in sample size and in the numbers of peripheral blood mononuclear cells (PBMC) recovered, not all T and B cell assays were done for each sample. Exclusion criteria were gestation of <36 weeks, birth weight of <2500 g, and acute or chronic illness. Adults (born after 1963), with no known exposure to measles and ⩾1 documented vaccination with MMR vaccine, were tested for immunity to measles ($n = 48$) or mumps ($n = 25$). No measles or mumps cases were identified in our area during the study.

Vaccines. Six- and 9-month-old infants were immunized with live measles virus vaccine (Attenuvax; Merck), 1000 median $TCID_{50}$, or live mumps virus vaccine (Mumpsvax; Merck) containing >20,000 $TCID_{50}$; these infants received MMR-II vaccine (Merck) at 12 months. Twelve-month-old infants received 1 dose of MMR-II vaccine. Adults were immunized >5 years before evaluation.

Antibody assays. Serum samples were tested for antibodies to measles virus in parallel with the World Health Organization measles reference immunoglobulin II by means of a modified plaque reduction neutralization (PRN) assay [22], which is more sensitive for low titers than is a commercial EIA for measles virus antibodies [23]. Fourfold serial dilutions of serum (1:4–1:4096) were mixed with low-passage Edmonston measles virus (25–35 pfu). The PRN titer was the dilution that reduced plaques by ⩾50%; <1:4 was considered to be negative.

Mumps virus–neutralizing titers were determined by a plaque neutralization assay [24]. Twofold serum dilutions (1:10–1:320) were mixed with mumps virus (wild-type Barnes; 80–100 plaques/well), and plaques were detected by EIA with monoclonal antibody to mumps virus (Chemicon International), horseradish peroxidase–labeled rabbit anti–mouse IgG conjugate (Accurate Chemical and Scientific), and aminoethylcarbazol/H_2O_2. The neutralizing titer was the dilution showing 50% plaque reduction; ⩽1:10 was considered to be negative. Cord blood specimens ($n = 55$) were tested to confirm detection of antibodies to mumps virus at high titers. Serum samples were also tested by ELISA for IgG to mumps virus (Wampole Laboratories).

Seroconversion was defined as a 4-fold increase in antibody titer after prevaccine levels were corrected for decay over 3 half-lives; measles seroprotection was defined as titer of ⩾120 mIU [25].

T cell proliferation assay. PBMC were recovered by ficoll-hypaque gradient purification and added to 96-well microtiter plates at 3.0×10^5/well in RPMI 1640 (Gibco) and 10% heat-inactivated normal human serum (Sigma); all PBMC cultures were prepared with fresh cells. Measles virus or mumps virus antigen was prepared from lysates of Vero cells inoculated with Attenuvax measles vaccine (more attenuated Enders' strain; Merck), or Mumpsvax (Jeryl Lynn strain; Merck). Vero cell lysates made in parallel from flasks that had been seeded with the same concentration of cells as the antigen flasks served as an uninfected cell control. Briefly, measles or mumps virus–infected cells were harvested at peak cytopathic effect (~90%), were sonicated, were centrifuged, were freeze/thawed 3 times, and were stored at −70°C until used; undiluted antigen resulted in <1 pfu/well. The antigen preparation, containing 320 μg/mL cell and viral protein, was equivalent to total protein in uninfected cell lysate. Preliminary studies were done with multiple dilutions of measles and mumps virus antigen (range, 1:16–1:512); dilutions of 1:16 and 1:32 were used because these dilutions stimulated T cells from 30 immune adult donors. Measles and mumps virus antigen and control cells were added at dilutions of 1:16 and 1:32 to triplicate wells for testing infant PBMC and in quadruplicate wells for adults. Adult PBMC were also incubated with antigen and control at a 1:64 dilution. The measles virus antigen used in these studies was not inactivated because pilot experiments documented no differences when T cells were stimulated with live or inactivated measles virus antigen. T cell proliferation was measured after 5 days by ^{3}[H]thymidine uptake. The stimulation index (SI) was the ratio of mean counts per minute in antigen and uninfected cell control wells; an SI >3.0 was established as a positive response because a ratio ⩾3.0 was observed when the mean counts per minute in triplicate antigen-stimulated wells was ⩾2 SD above the mean counts per minute in wells stimulated with uninfected cell control. The highest SI resulting from either antigen/control concentration was used for statistical analysis because subjects respond to antigen concentrations differently. All assays included uninfected cell control wells, which were determined to have no significant proliferation (<3.0) relative to media-only controls; mean counts per minute in control wells was consistently <2000. Phytohemagglutinin (PHA), 0.1 mg/mL (Difco), a nonspecific mitogen, was used to assure the capacity of the PBMC in each specimen to proliferate and to assess potential immunosuppressive effects of measles and mumps immunization on proliferation responses at 3 months after vaccination.

Interferon (IFN)–γ production. Supernatants from PBMC stimulated with measles or mumps antigen were collected on days 4–8, were stored at −70°C, and were tested for IFN-γ by ELISA (Endogen). No spontaneous release of IFN-γ was detected in supernatants of PBMC stimulated with control uninfected cell lysate in infant or adult specimens. The peak IFN-γ concentration was used for data analysis.

Statistics. Responses were compared by Student's paired or unpaired t test, χ^2, and Fisher's exact tests. Paired t test was used to compare pre- and postvaccination responses in individual infants to document significant increases in antigen-specific responses following immunization. Geometric mean titers (GMTs) are reported with 95% confidence intervals (CIs) and SIs and IFN-γ levels with SEs. The minimum size of the study populations needed for comparisons by use of the immunologic assays was based on a statistical projection in which differences in host response rates of 40% in 1 cohort, as opposed to 5% in another, would be detectable ($\alpha = .05$; $n = 20$); fewer subjects ($n = 13$) were projected to be sufficient to show a statistically significant difference if response rates were 70% versus 20% ($\alpha = .05$). Despite dropouts, the cohorts used for final data analyses met these projections.

Results

Cell-mediated immunity after measles vaccination. T cell proliferation to measles virus increased significantly after vac-

cination. The mean SIs were equivalent in all age groups (figure 1*A*). SIs (±SE) before and after vaccination were 2.1 ± 0.2 versus 7.5 ± 1.0 at 6 months (n = 74), 1.6 ± 0.1 versus 7.7 ± 1.3 at 9 months (n = 58), and 1.4 ± 0.1 versus 5.3 ± 0.6 at 12 months (n = 47). Measles SIs were ⩾3.0 in 72%, 69%, and 65% of 6-, 9-, and 12-month-old infants, respectively. Measles virus–specific T cell proliferation was elicited irrespective of measles virus antibody titers. Measles virus antibodies were detectable in 64% of infants at 6 months, 39% at 9 months, and 2% at 12 months in assays of serum samples obtained on the day of immunization immediately before the vaccine was given. SIs after immunization of the infants who had PRN titers ⩾1:4 mIU, compared with those without passive antibodies when vaccinated were 7.3 ± 0.9 versus 6.9 ± 2.2 in 6-month-old infants and 5.6 ± 0.8 versus 7.6 ± 1.6 in 9-month-old infants (figure 2*A*). In a further analysis based on stratification by how high the GMTs were at vaccination, mean SIs were found to be 8.2 ± 1.9 in 16 infants who had passive antibody titers >50 mIU and 7.9 ± 0.9 among 31 infants with titers of 4–50 mIU (not significant); SIs were equivalent in infants with titers of 4–25 mIU versus >25 mIU or 4–80 mIU versus >80 mIU.

IFN-γ production increased significantly after immunization at 6 months (n = 24), 9 months (n = 20), or 12 months (n = 13). IFN-γ concentrations (mean ± SE) were 184 ± 52, 349 ± 95, and 115 ± 26 pg/mL in 6-, 9-, and 12-months-old infants, respectively (not significant). IFN-γ concentrations were equivalent regardless of passive antibody status at the time that vaccine was administered. At 6 months, IFN-γ concentrations in infants with and without passive antibodies were 172 ± 38 and 273 ± 83 pg/mL, respectively (P = .22); at 9 months, responses were 188 ± 58 and 326 ± 86 pg/mL (P = .31).

Infants had lower measles virus–specific T cell proliferation than did adults (figure 1*A*). The mean SI (±SE) was 6.9 ± 0.6, compared with 11.0 ± 1.8 in adults (P = .009). However, the percentages of infants and adults who had measles SIs >3.0 were equivalent (69% of infants vs. 83% of adults; P = .11). Mean IFN-γ concentrations (±SE) were lower in infants (226 ± 42 pg/mL) than adults (449 ± 107 pg/mL; n = 20; P = .02; figure 3*A*).

Cell-mediated immunity after mumps vaccination. SIs to mumps virus (±SE) measured before and after vaccination, respectively, were 1.6 ± 0.1 and 8.0 ± 1.2 for 6-month-old infants (n = 39), 1.7 ± 0.2 and 7.5 ± 1.1 for 9-month-old infants (n = 26), and 1.5 ± 0.2 and 8.7 ± 1.8 for 12-month-old infants (n = 20; figure 1*B*). SIs elicited by mumps vaccine did not differ among infants vaccinated at 6, 9, or 12 months. SIs did not differ between infants with and without passive antibodies; the mean SIs were 8.0 ± 2.4 versus 8.7 ± 1.6 in 6-month-old infants and 6.1 ± 1.5 versus 7.1 ± 1.1 in 9-month-old infants (not significant; figure 2*B*). The mean SIs in infants (8.1 ± .80) and adults (9.7 ± 1.9; n = 25) did not differ (figure 1*B*); the percentages in each cohort for subjects with SIs ⩾3.0 were 72%, 73%, and 72% in 6-, 9-, and 12-month-old infants, respectively, and 80% in adults.

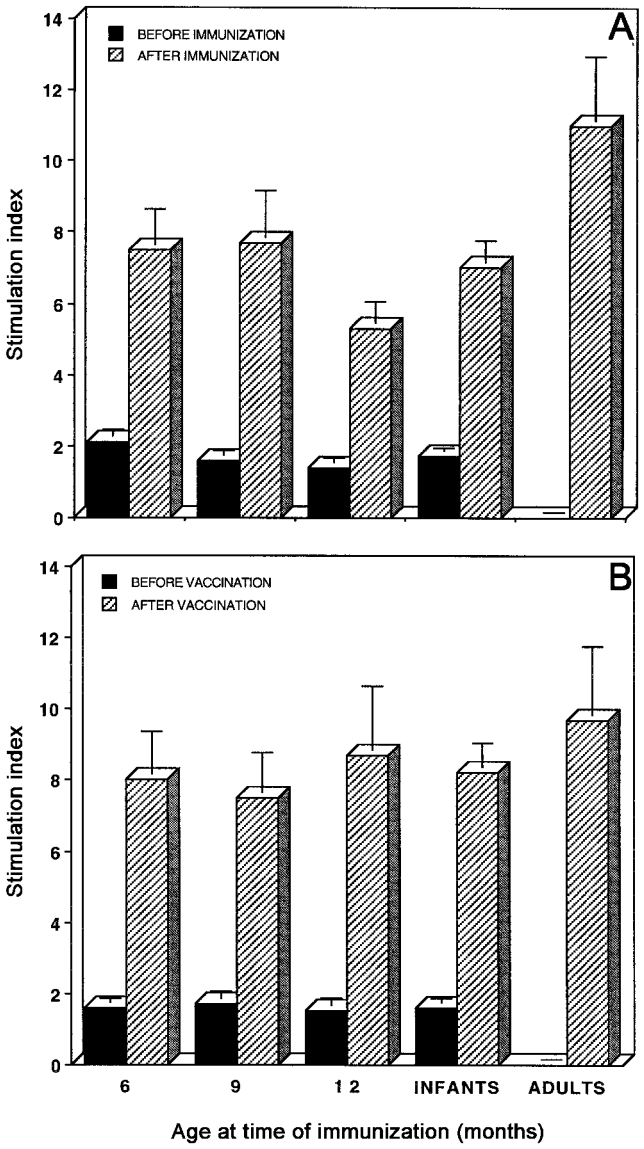

Figure 1. Antigen-specific T cell proliferation before and after immunization with measles *(A)* or mumps *(B)* vaccines. Stimulation index was calculated as ratio of mean counts per minute in antigen and uninfected cell control wells. Error bars indicate SEs. Stimulation index + SE is shown for infant groups by age at immunization (6, 9, or 12 months), all infants combined, and vaccinated adults. Positive stimulation index is defined as ⩾3.

After mumps vaccination, 6- (n = 17), 9- (n = 13), and 12-month-old infants (n = 9) had equivalent IFN-γ concentrations (mean ± SE) of 239 ± 77, 156 ± 54, and 327 ± 96 pg/mL, respectively. The mean (±SE) peak IFN-γ concentration in infants with passive antibodies (n = 6) was 402 ± 170 pg/mL, compared with 215 ± 43 pg/mL for those who had none (n = 31; not significant). However, mumps virus–specific IFN-

γ production was low in infants as a group, compared with a mean concentration of 692 ± 120 pg/mL in vaccinated adults ($P = .0001$; figure 3*B*).

Antibody responses to measles virus. GMTs to measles virus, measured by PRN, increased significantly after vaccination at 6 months ($n = 73$), 9 months ($n = 61$), or 12 months ($n = 53$). However, postvaccination GMTs were low in 6-month-old infants with and without passive antibodies when they were immunized. The GMTs were 120 mIU (95% CI, 71–200) and 146 mIU (95% CI, 44–490) in 6-month-old infants with ($n = 47$) or without ($n = 26$) passive measles antibodies, respectively. In contrast, the postvaccination GMT was significantly lower, 180 mIU (95% CI, 68–473), among 9-month-old infants who had passive antibodies ($n = 24$) when immunized, compared with 744 mIU (95% CI, 467–1183) in those without detectable passive antibodies ($n = 37$; $P = .01$). The higher GMT to measles virus observed in 9-month-old infants who had no passive antibodies was equivalent to the response of 12-month-old infants, who had a GMT of 1210 mIU (95% CI, 774–1893). The GMT of 120 mIU (95% CI, 71–200) in 6-month-old infants with no passive antibodies when immunized was lower than the GMT in 9- ($P = .01$) or 12-month-old infants without passive antibodies ($P = .0002$; figure 4*A*). The seroconversion rate was 77% in 6-month-old infants who had no passive antibodies, compared with 97% in 9-month-old infants ($P = .02$) and 96% in 12-month-old infants ($P = .01$). Only 59% of these 6-month-old infants developed titers ⩾120 mIU, compared with 97% and 94%, respectively, of 9- and 12-month-old infants who had no passive antibodies when given measles vaccine (6 vs. 9 months, $P < .0001$; 6 vs. 12 months, $P = .0001$).

Although postvaccination GMTs were lower in the cohort of infants who had passive antibodies, there was no correlation between the highest PRN titers at the time of vaccination and the lowest postvaccination titers. GMTs were 121 mIU (95% CI, 72–204) in 49 infants with titers >25 mIU and 164 mIU (95% CI, 59–457) in 21 infants with titers of 4–25 mIU when immunized (not significant); no correlation was observed with stratification by 4–80 mIU versus >80 mIU prevaccine titers.

Overall, when infants with and without passive antibodies were evaluated together, the GMT was 128 mIU (95% CI, 74–215) in 6-month-old infants, which was below the GMTs of 426 mIU (95% CI, 241–657) and 1209 mIU (95% CI, 774–1893) in 9- and 12-month-old infants, respectively (6 vs. 9 months, $P = .002$; 6 vs. 12 months, $P < .0001$). Passive antibody interference alone accounted for the difference in GMTs between 9- and 12-month-old infants ($P = .003$), as shown by the equivalent GMTs when 9-month-old infants without passive antibodies were compared with 12-month-old infants, as described above. Among the 6-month-old infants who had passive antibodies, interference could not be differentiated from the age-related impairment of the humoral immune response, which was documented in the analysis of the cohort of 6-month-old infants who lacked passive antibodies when immunized.

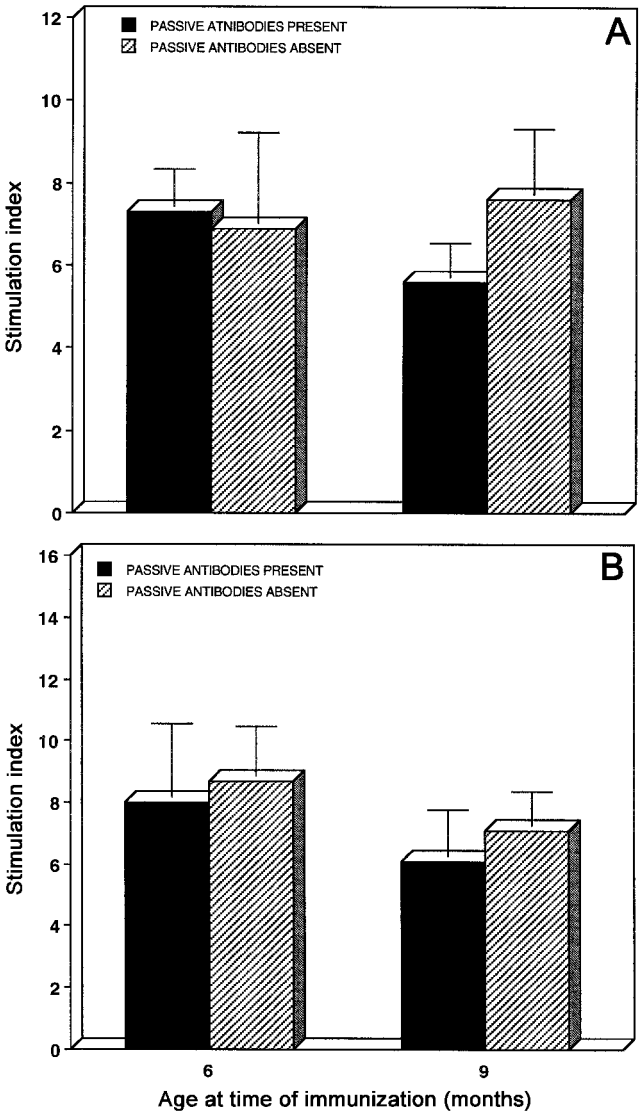

Figure 2. Antigen-specific T cell proliferation 12 weeks after immunization with measles *(A)* or mumps *(B)* vaccine in infants with and without passive antibodies. Stimulation index was calculated as ratio of mean counts per minute in antigen and uninfected cell control wells. As shown on horizontal axis, infants were 6 or 9 months old at immunization. Error bars indicate SEs. Positive stimulation index is defined as ⩾3.

The percentages of infants with titers ⩾120 mIU were 60%, 89%, and 94% at 6, 9, and 12 months, respectively (6 vs. 9 months, $P = .0003$; 6 vs. 12 months, $P < .0001$).

Antibody responses to mumps vaccine. Only 16% of 6-month-old infants ($n = 38$), 14% of 9-month-old infants ($n = 32$), and 12% of 12-month-old infants ($n = 26$) had passive antibodies to mumps virus. To be certain that transplacentally acquired antibodies could be detected by neutralization, 55 cord blood samples were tested and showed a GMT of 231 (95% CI, 169–315). PRN titers of antibody to mumps

virus increased after vaccination in all age groups, but the GMT was lower in 6-month-old infants than 12-month-old infants ($P = .04$). As was observed in 6-month-old infants given measles vaccine, this poor response was observed even if the infants who had passive antibodies when immunized with mumps vaccine at 6 months were excluded. In infants without passive antibody present before immunization, the GMTs after immunization were 18 (95% CI, 9–37), 35 (95% CI, 20–63), and 51 (95% CI, 28–89) at 6, 9, and 12 months, respectively (6 vs. 12 months, $P = .04$; figure 4*B*). Considering only the infants without passive antibodies at vaccination, seroconversion rates were 74% in 6-month-old infants, compared with 91% and 93%, respectively, in 9- and 12-month-old infants. The difference in humoral responses of 6- and 12-month-old infants was confirmed by EIA, showing means of 2.43 versus 2.91 U ($P = .02$). The presence of passive antibodies did not result in lower GMTs of antibody to mumps virus after immunization.

Induction of adaptive immunity by live virus vaccines. When cell-mediated as well as humoral immune responses were considered, only 6% of infants lacked measles virus–specific immunity (figure 5*A*). Ten percent of 6-month-old infants, 2% of 9-month-old infants, and 2% of 12-month-old infants given measles vaccine had neither T cell proliferation nor seroconversion. Six percent of infants failed to develop T cell proliferation or neutralizing antibodies to mumps virus (figure 5*B*). Twelve percent of 6-month-old infants, 4% of 9-month-old infants, and none of the 12-month-old infants had SIs to mumps virus <3.0 and titers of antibody to mumps virus <1:10. Although more infants immunized at 6 months failed to develop either humoral or cellular immunity, the numbers of infants who failed to respond in each age cohort were not significantly different for measles or mumps vaccine cohorts.

Measles revaccination. Among the subset of infants who were immunized at 6 months and revaccinated with MMR vaccine ($n = 19$), SIs (mean ± SE) were 8.9 ± 3.1 after the initial monovalent measles vaccine and 11.6 ± 2.4 after MMR vaccine (figure 6*A*). The SIs in 9-month-old infants ($n = 12$) were 8.2 ± 2.7 after monovalent measles vaccine and 9.3 ± 2.8 after MMR vaccine. Seventy-five percent of the 6-month-old infants and 30% of 9-month-old infants in these cohorts were in the subgroups of infants who had passive antibodies when first vaccinated. The mean SIs after MMR vaccine in infants given monovalent measles vaccine at 6 or 9 months were higher than the mean SI of 5.3 ± 0.6 in 12-month-old infants who had received MMR vaccine only (6 vs. 12 months, $P = .0008$; 9 vs. 12 months, $P = .04$). PRN titers after measles vaccine were 267 mIU (95% CI, 67–1064) in 6-month-old infants ($n = 8$), compared with 776 mIU (95% CI, 300–2012) in 9-month-old infants ($n = 10$; figure 6*B*). GMTs after MMR vaccine rose to 1487 mIU (95% CI, 884–2497) in the 6-months cohort and 1994 mIU (95% CI, 809–4920) in the 9-months cohort (figure 6*B*). Among the 5 infants who had passive antibodies when given measles vaccine at 6 months, the GMT increased from 85 mIU (95% CI, 22–3160) to 1300 mIU (95% CI, 588–2877). All infants developed titers ⩾120 mIU after receipt of MMR vaccine.

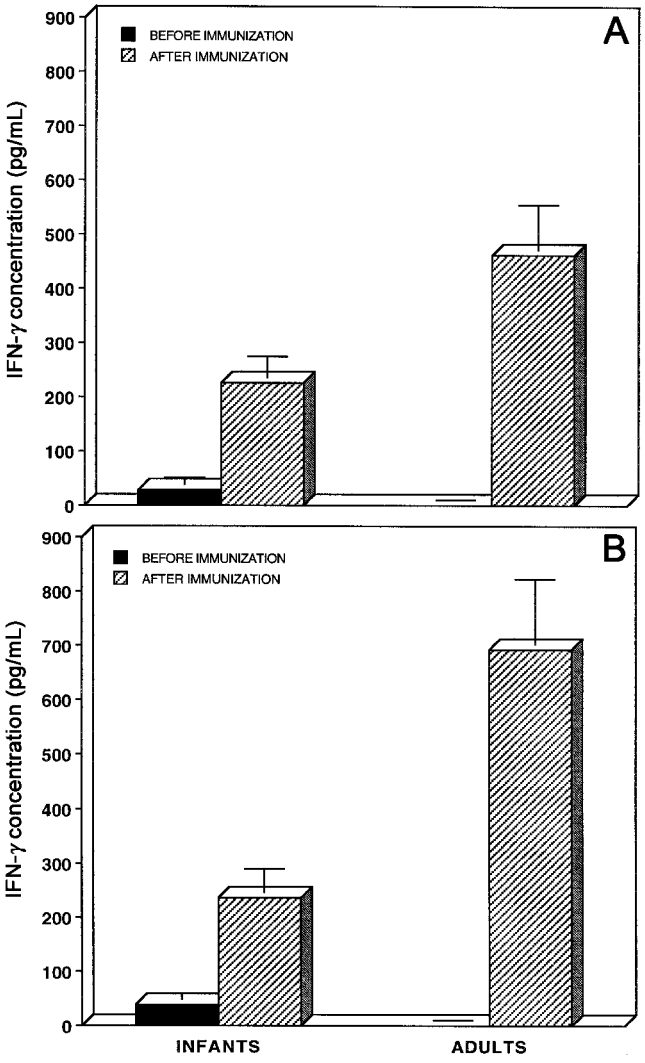

Figure 3. Interferon (IFN)–γ production before and after measles *(A)* and mumps *(B)* immunization in infants taken as one group vaccinated at 6, 9, or 12 months and vaccinated adults. Error bars indicate SEs.

Discussion

Enhancing measles vaccine efficacy in infants <12 months old is important for global eradication [1, 26]. Several points about the immunogenicity of measles vaccine emerged from this parallel evaluation of cellular and humoral immunity in infants immunized at 6, 9, or 12 months and from the comparison with mumps vaccine. With respect to cell-mediated immunity, infants evaluated at 6, 9, or 12 months had no age-related differences in T cell proliferation, which primarily reflects the memory CD4 T cell response to viral antigen, or in IFN-γ production, which

demonstrates the functional capacity of antigen-specific T cells to make this critical cytokine. Measles vaccine elicited cellular immunity as effectively as did mumps vaccine, indicating that the vaccine virus did not have an immunosuppressive effect on antigen-specific T helper responses, and neither vaccine was associated with low PHA responses by 3 months after immunization. In addition, the induction of memory T cell responses was not influenced by transplacentally acquired passive antibodies to measles or mumps virus, regardless of the neutralizing antibody titer at the time of vaccination. This observation is of particular significance because of the attention that has been given to the capacity of novel vaccine strategies, such as DNA vaccines, to induce adaptive immunity in animal models in the presence of passive antibodies. Our studies indicate that live attenuated vaccines against paramyxoviruses also exhibit this characteristic.

The pattern of rapid decline of transplacentally acquired passive antibodies when mothers have vaccine-induced immunity and our approach of stratifying the infant cohorts by serological testing on the day of vaccination provided the opportunity to investigate the functional maturity of the host response to paramyxoviruses in infants at 6, 9, and 12 months while controlling for the variable of passive antibody interference. In contrast to cellular immunity, the capacity of the infant immune system to generate humoral responses to measles and mumps vaccines was diminished in the 6-month-old cohorts. PRN titers to measles and mumps viruses were lower in infants vaccinated at 6 months when those with detectable passive antibodies were excluded. This observation confirmed our previous findings about measles immunization [14] and demonstrated that this intrinsic limitation in antibody production was not unique to measles vaccine but extends to mumps, another virus in the paramyxovirus family. Although it might be argued that passively acquired measles virus antibodies were present at levels below detection, we consider this explanation unlikely because of the sensitivity of the measles virus PRN assay; in addition, although very few infants (16%) had antibodies to mumps virus by 6 months, the same phenomenon of poor antibody production was observed in 6-month-old infants given mumps vaccine. Furthermore, there was no interference observed even in the presence of passive mumps virus antibodies and, thus, even low, undetectable levels would not be expected to explain the 6-month-old infants' limited humoral response to mumps vaccine. Understanding the mechanism of this restricted B cell function will be important for devising novel vaccines strategies that will be effective in younger infants, even when passive antibody interference is not an obstacle. Interactions between infant T and B cells may be impaired by diminished CD40 ligand expression and IFN-γ production, reducing helper T cell activity [27–32]. Antigen presentation for class II–restricted CD4 T cell activation may also be limited [33, 34]. T cell–independent B cell activation may be less efficient in response

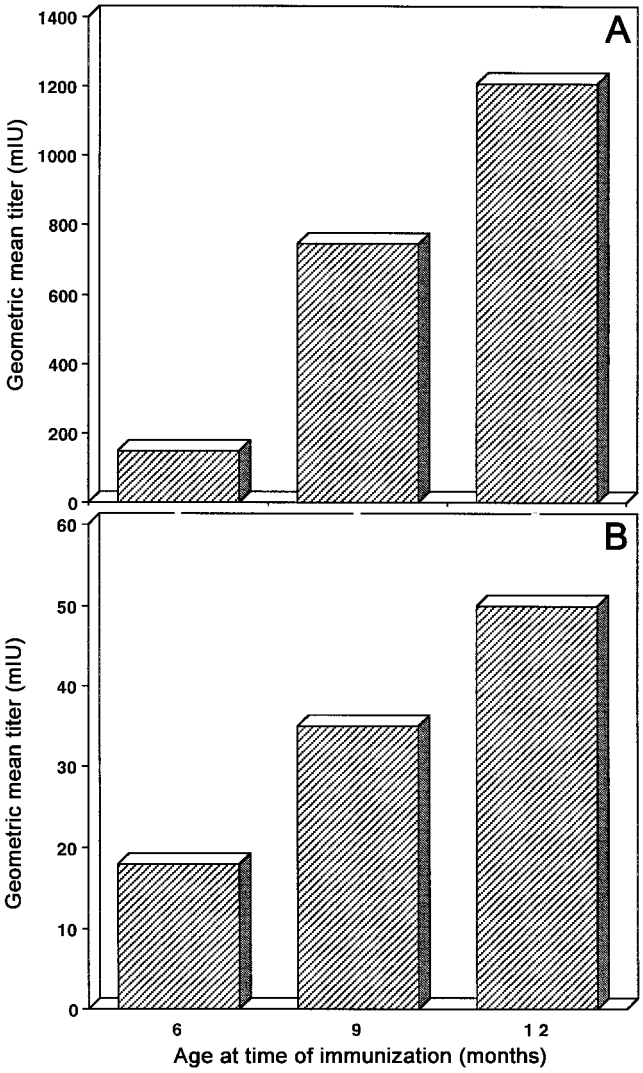

Figure 4. Neutralizing antibody responses of infants after measles *(A)* or mumps *(B)* immunization in absence of passive antibodies. Geometric mean titers were determined by plaque reduction neutralization assay of serum samples obtained 12 weeks after immunization. Infants were 6, 9, or 12 months at time of immunization. The 95% confidence intervals for each geometric mean titer are given in the text. Measles virus–neutralizing antibody titer of $\geqslant$120 mIU has been defined as protective.

to heavily glycosylated viruses [6], and younger infants could be more susceptible to this effect.

Evaluation of 9-month-old infants confirmed the well-documented effect of passive antibodies, even at low titers, on measles virus PRN titers induced by vaccination. However, passive antibodies to mumps virus did not predict lower titers after immunization. Passive antibodies to measles virus may have a particular capacity to interfere with antigen-specific B cell responses. Our analysis of antibody responses in 9-month-old infants with and without passive antibodies, compared with the

responses of 12-month-old infants, permits the conclusion that passive antibody effects constitute the primary obstacle to vaccine-induced humoral immunity in 9-month-old infants. When these infants were immunized in the absence of passive antibodies, their responses were equivalent to those of the 12-month-old cohort, indicating that 9-month-old infants have no intrinsic impairment of B cell function.

The induction of cellular immunity in infants given measles or mumps vaccine provides a new and reassuring perspective on the immunogenicity of live attenuated virus vaccines [14, 35–37]. Primary vaccine failure has been defined by lack of seroconversion, most common in younger infants [13, 14] and those with passive antibodies [15]. Nevertheless, measles and mumps vaccines elicited memory T cells in many of the youngest infants. Although it may be assumed that passive antibodies block immunogenicity by preventing replication of the attenuated virus, our observations suggest that it is necessary to consider a more complex mechanism. If viral replication were inhibited, T cell proliferation and IFN-γ production should be more robust in infants with no passive antibodies, but cellular responses to measles or mumps vaccines were equivalent in infants with and without passive antibodies. Although attenuated measles and mumps vaccines contain noninfectious defective interfering particles [38] and viral proteins that could sensitize T cells, the total antigen content in the 0.5-mL vaccine dose is low. Amplification of viral protein expression by vaccine virus replication is likely to be required to achieve adequate antigenic stimulation, unless proteins are combined with effective adjuvants; although transient antibody responses might be elicited, it is unlikely that the antigen component of these vaccines alone would be sufficient to elicit persistent virus-specific memory T cells. As has been described elsewhere [35, 37], some infants in our vaccine cohorts lacked T cell responses but developed antibodies to measles or mumps virus. Such observations suggest that helper T cells had been elicited but were below detection. Although a T cell–independent B cell response cannot be excluded, analyses of measles immunity do not support this possibility [19, 39].

In our study, measles and mumps vaccines induced memory T cell immunity in infants who had limited antibody responses. By use of related assays, Bautista-Lopez et al. [35] and Pabst et al. [37] also detected cellular immunity to measles in infants vaccinated at 6 or 12 months. In the absence of measles virus challenge, the contribution of virus-specific T cells to vaccine efficacy can only be presumed from their characteristic helper and cytotoxic functions. A measles virus PRN titer $\geq$120 mIU has been identified as a correlate of protection and was validated as a marker in the macaque model [4]. However, distinguishing between antiviral effects of humoral and cellular responses is difficult because immune individuals have both. That cellular immunity alone can protect is supported by the clinical observation that children with agammaglobulinemia were not susceptible to repeated measles virus infections [40]. A role for

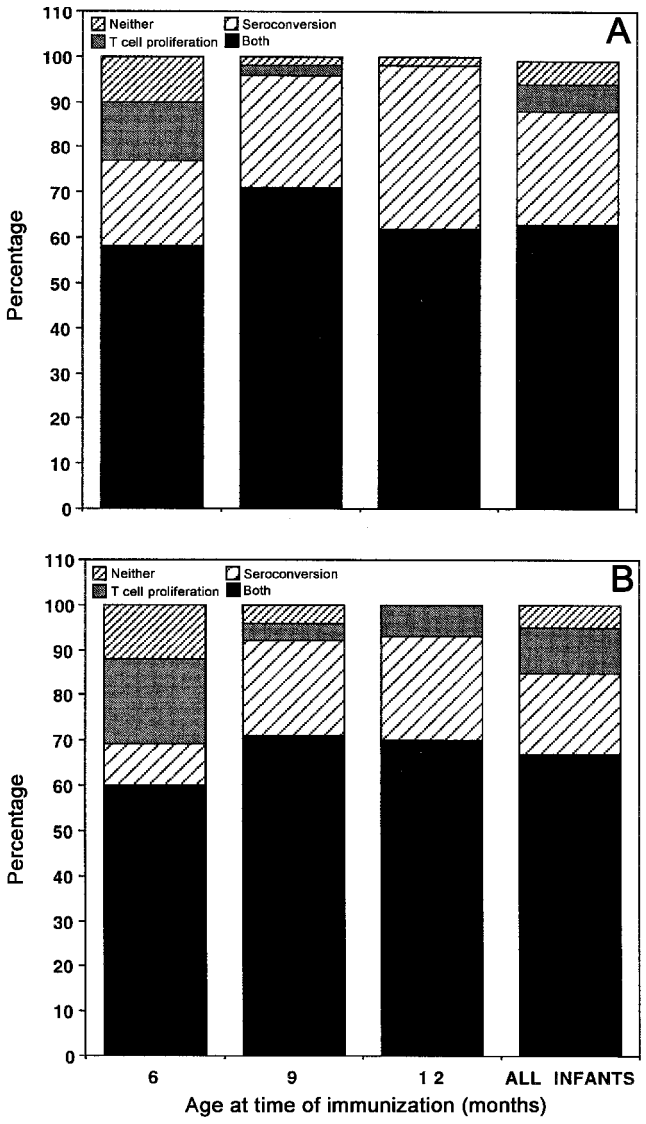

Figure 5. Percentage of infants with T cell proliferation and neutralizing antibody responses 12 weeks after measles *(A)* or mumps *(B)* immunization. Seroconversion, 4-fold increase in antibody titer; T cell proliferation, stimulation index $\geq$3. Infants were 6, 9, or 12 months old when immunized.

cellular immunity is also suggested by the experience of vaccinating infants in developing countries at ages when humoral responses would be subject to age-related deficiencies that we documented or to passive antibody interference. The clinical experience is that many of these infants had mild measles or developed symptoms only on exposure to a high inoculum by close contact [41, 42]. In macaques, animals with measles virus–specific T cell immunity were protected, despite deficient humoral responses following vaccination in the presence of passive antibodies [43]. These observations may be explained by priming of adaptive cellular immunity under the "cover" of

passive antibodies or at an age when humoral immune responses are impaired. Experiments in infant animals have demonstrated the capacity of the developing immune system to achieve a "prime-boost" response [44]. In our study, a subset of infants was reimmunized. Among these children, measles virus–specific T cell proliferation was higher after revaccination of those infants who had been immunized at 6 or 9 months than among infants who were given MMR vaccine only at 12 months. GMTs were also increased, although too few infants were evaluated to document a significant boost; nevertheless, the data indicate that the neutralizing antibody response to the second dose was not blunted. By use of both humoral or cellular immunity as measures of vaccine immunogenicity, we found that >90% of infants immunized at 6 or 9 months may have some protection from measles or at least from severe disease.

Although no age-related differences were observed between infant groups, measles virus–specific T cell proliferation and IFN-γ concentrations were lower than responses observed in vaccinated adults. Measles reexposures cannot be excluded, but young adult age and continuous residence in the United States makes this explanation of higher responses less likely. A relative decrease in IFN-γ production was also observed in infants given mumps vaccine, suggesting a general limitation in infant adaptive T cell function. Measles down-regulates interleukin-12 production, which may inhibit the induction of cell-mediated immunity transiently [21, 45]. We found that infant and adult PBMC released interleukin-12, but concentrations were lower in infants, indicating that infant PBMC may be more susceptible to this effect; infant T cells also released less IFN-γ when supplemented with recombinant interleukin-12 [36]. Despite these relative differences, it is important to note that the overall percentages of infants and adults with detectable T cell responses to measles or mumps viruses were equivalent. In addition, the clinical experience shows that infants given MMR vaccine at 12 months are protected as well as adults, despite the lower cell-mediated immunity that we observed in this infant cohort. How or whether the lower cellular immune responses in infants relate to the acquisition of protective immunity is not clear. A less robust helper T cell response might have a more substantial impact on the capacity of the youngest infants to make measles– or mumps virus–specific antibodies, or, as noted above, diminished antibody responses in the youngest infants could reflect differences in primary B cell function. These observations indicate the need to further investigate the functional maturation of humoral as well as cellular components of adaptive immunity in human infants. Important information is likely to emerge when new methods are used to assess age-related differences in responses to various viral pathogens.

Our observations about the immunogenicity of measles vaccine during the first year of life have practical implications. Two-dose regimens, with the initial dose given at 6 months and a second dose at 12–15 months, may improve the protective

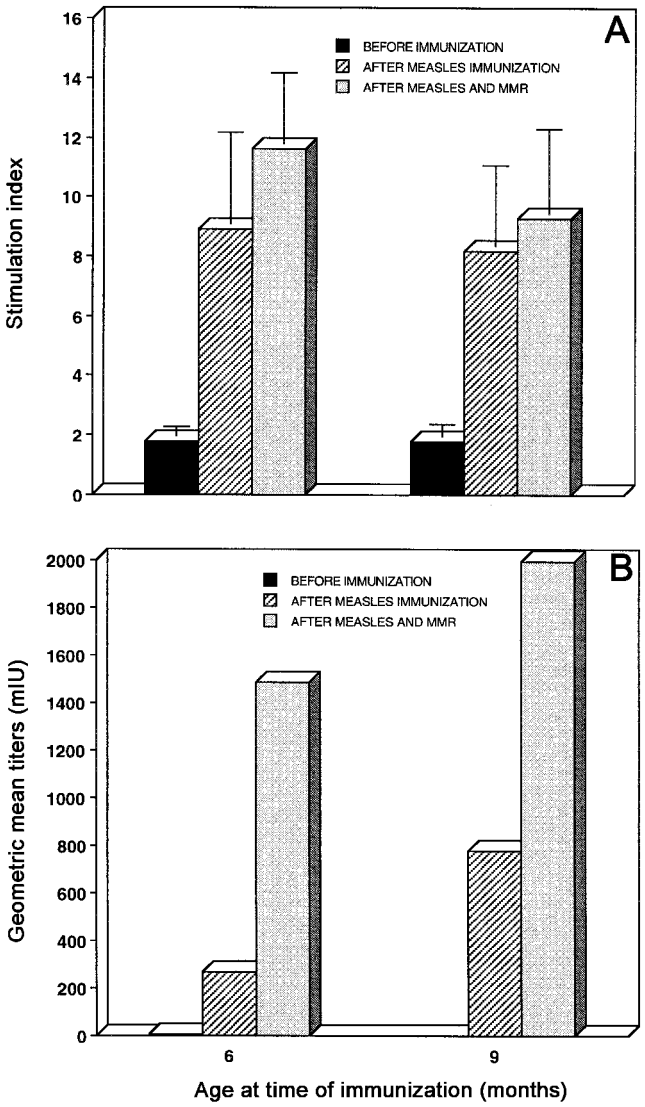

Figure 6. T cell proliferation and neutralizing antibody responses in infants after 1 and 2 doses of measles vaccine. *A,* Mean stimulation index, calculated as ratio of counts per minute in measles virus antigen–stimulated and control wells; *B,* geometric mean titers, measured by plaque neutralization assay, before and 12 weeks after measles immunization and at 6 months after measles-mumps-rubella (MMR) immunization. Infants were 6 or 9 months old at primary immunization with measles vaccine and 12 months old at revaccination with MMR vaccine. Error bars indicate SEs. Positive stimulation index is defined as ≥ 3. The 95% confidence intervals for each geometric mean titer are given in the text. Measles virus–neutralizing antibody titer ≥ 120 mIU has been defined as protective.

efficacy of live attenuated measles vaccine and could compensate for the early loss of passive antibodies in infants of vaccinated mothers. Even if neutralizing antibody titers are poor in the youngest infants and passive antibodies interfere with humoral immunity in some 9-month-old infants, the initial measles vaccine dose could prime the adaptive immunity by

sensitizing helper T cells. Over the longer term, new strategies, such as DNA vaccines that express viral proteins, along with costimulatory molecules or other adjuvants, may also be useful to compensate for limitations in antiviral responses of the developing infant immune system.

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