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Measles infection in infants is associated with severe complications, and secondary infections are attributed to generalized immunosuppression. Measles binding to its monocyte receptor down-regulates IL-12 which is expected to diminish Th1-like cytokine responses, including IFN-γ. Whether young infants can be immunized effectively against measles is an important public health issue. We evaluated Ag-specific IL-12, IFN-γ, and T cell responses of infants at 6 (n = 60), 9 (n = 46), or 12 mo (n = 56) of age and 29 vaccinated adults. IL-12 and IFN-γ release by PBMC stimulated with measles Ag increased significantly after measles immunization in infants. IL-12 and IFN-γ concentrations were equivalent in younger and older infants, but IL-12 concentrations were significantly lower in infants than in adults (p = 0.04). IL-12 production by monocytes was down-regulated by measles; the addition of recombinant human IL-12 enhanced IFN-γ production by PBMC stimulated with measles Ag, but infant T cells released significantly less IFN-γ than adult T cells under this condition. Of particular interest, the presence of passive Abs to measles had no effect on the specific T cell proliferation or IFN-γ production after measles stimulation. Cellular immunity to measles infection and vaccination may be limited in infants compared with adults as a result of less effective IFN-γ and IL-12 production in response to measles Ags. These effects were not exaggerated in younger infants compared with effects in infants who were immunized at 12 mo. In summary, infant T cells were primed with measles Ag despite the presence of passive Abs, but their adaptive immune responses were limited compared with those of adults. The Journal of Immunology, 1999, 162: 5569–5575.

Measles is a leading cause of infant morbidity and mortality in many countries (1–3). In recent outbreaks in the United States, the incidence and mortality rates of measles were highest among children <12 mo old (4). Infants are at risk for measles pneumonia, suggesting an inadequate capacity to limit viral dissemination, and are susceptible to secondary infections, indicating that measles induces a generalized immunosuppression (1, 3, 5–7). Host responses assessed during and after measles infection, or immunization with live attenuated measles vaccines, exhibit immunologic patterns consistent with a diminished Th1 response or a polarization toward Th2 activation (5, 6, 8–12). Delayed type hypersensitivity reactions to other Ags, such as Mycobacterium tuberculosis, mitogen-stimulated T cell proliferation, and release of IL-2 and IFN-γ are diminished, and NK cell function is reduced (9–11, 13–19). In addition, nonspecific, spontaneous release of IL-4 by circulating PBMC and total IgE concentrations are increased (11, 20).

Measles virus infects monocytes through binding to the CD46 surface protein, which acts as a measles-specific entry mediator (21–23). Karp et al. (24) have demonstrated that measles binding to CD46 results in down-regulation of IL-12 production. Based on the Th1/Th2 model of CD4+ T cell responses, decreased IL-12 release would be expected to favor predominance of a Th2-like response, because IL-12 is a crucial early stimulus for Th1 clonal expansion. IL-12 stimulates resting and activated T cells, induces the production of IFN-γ by T cells, activates NK cells, and inhibits IL-4 production (25–31). Since IL-12 promotes the rapid induction of Ag-specific T cells in the naive host, decreased IL-12 production could impair the acquisition of adaptive immunity to measles and facilitate a generalized immunosuppression by failure to regulate IL-4 production. The purpose of this study was to compare virus-specific T cell responses in infants and adults with vaccine-induced measles immunity, using assays for IL-12 production, T cell proliferation, and IFN-γ release by PBMC stimulated with measles. Our hypothesis was that infants might have a limited Th1-like response to measles compared with adults.

The evaluation of adaptive immunity to measles vaccination in infants who are younger than 12 mo has important clinical relevance because it would be advantageous to achieve protective immunity as early as possible during the first year of life. Potential obstacles to immunization of younger infants against measles include neutralization of the live attenuated vaccine virus by measles Abs acquired transplacentally and immaturity of the immune system (32–39). We and others have shown that passive Abs to measles interfere with vaccine-induced humoral immunity, but little is known about the effects of maternally derived measles Abs on induction of virus-specific cell-mediated immunity (40–43). Infants whose mothers have measles vaccine-induced immunity lose passive Abs at a shorter interval after birth than those who receive higher concentrations of measles Abs because their mothers have had natural measles (37, 42–46). In a recent study, we showed that the humoral response to measles vaccine was deficient in 6-mo-old infants compared

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with 9 and 12-mo-old infants even when passively acquired Abs were not detectable (41). This further evaluation of measles-specific T cell responses in infants was undertaken to assess whether there are differences in proliferation, IL-12 or IFN-γ responses when younger infants are immunized with measles vaccine, and whether passive Abs affect these cell-mediated immune responses.

Materials and Methods

Study population

The study subjects were 162 healthy infants, without intercurrent illnesses, who were 6 mo (n = 60), 9 mo (n = 46), or 12 mo old (n = 56) and healthy adults (n = 29; age 20–40 yr). Infants were enrolled if they were 6 mo old (range, +3 wk), 9 mo old (range, +3 wk), or 12 mo old (range, +3 wk). Children born before 36 wk gestation, whose birth weight was <2500 g, or who had chronic underlying illnesses were excluded. Among the infants who were enrolled, 2 infants were withdrawn as participants before blood samples were obtained and 26 infants were evaluated only before measles immunization, including 6 who were 6 mo old, 4 who were 9 mo old, and 15 who were 12 mo old. Prevaccine specimens as well as samples taken 12 + 3 wk after measles vaccination were available from 134 infants; because of limitations in the numbers of PBMC recovered, not all assays were performed in all infants. 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 adult participants. No cases of measles were identified in our geographic area during the study period.

Infants 6 and 9 mo old were immunized with measles virus vaccine live (Attenuvax (Merck, West Point, PA)) containing 1000 median tissue culture-infective doses (TCID50) of the U.S. reference measles virus strain. Twelve-month-old infants were immunized with M-M-R virus vaccine live (Merck; containing measles virus vaccine live, 1000 TCID50). Adults had received at least one measles vaccination years before evaluation.

T cell proliferation assay

PBMC were separated from whole blood by Ficoll-Hypaque gradient and added to 96-well microtiter plates at concentrations of 3.0 × 105/well in RPMI 1640 (Life Technologies, Gaithersburg, MD), and 10% normal human sera (Sigma, St. Louis, MO). Measles Ag, prepared from lysates of Vero cells inoculated with Attenuvax measles vaccine (more attenuated Edmonston strain, Merck) or an uninfected cell control 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 Ag and control at a 1:64 dilution. Preliminary studies were performed with multiple measles Ag dilutions (range, 1:16 to 1:512); dilutions of 1:16 and 1:32 were assigned a value of 1 for statistical analysis. Seroconversion was defined as the plaque number by 50%; titers >1:4 were considered positive and were assigned a value of 1 for statistical analysis. Seroconversion was defined as a fourfold rise in Ab titer after levels before vaccination were corrected for decay over three half-lives.

Cytokine production

Supernatants from PBMC stimulated with measles Ag or uninfected cell control were collected from wells on days 4–8, stored at −70°C. Supernatants were tested for the p40 and p70 subunit of human IL-12 using the ELISA ultrasensitive assay (Biosource, Camarillo, CA) and for IFN-γ using the ELISA method from Endogen (Cambridge, MA). Supernatants from PBMC stimulated with measles Ag or uninfected cell control, in the presence or absence of rhIL-12 (added at 50 or 100 U/ml or 2.9 and 5.8 ng/ml, respectively), were collected on days 4–8, stored at −70°C, and tested for IFN-γ. The optimal concentrations of rhIL-12 were established in preliminary assays and used for all subsequent tests; peak values from either concentration were used for statistical analysis. Sensitivities of cytokine detection were defined by reference standards in each assay.

Monocyte isolation and stimulation

Monocytes were isolated by density gradient centrifugation from whole blood from adult subjects and incubated with measles at a multiplicity of infection of 5, or with an uninfected Vero cell control, under nonadherent conditions. Monocytes were enriched by adherence to culture flasks and added to 96-well plates at concentrations of 2 × 105/well in RPMI 1640 (Life Technologies), and 10% normal human sera (Sigma). Staphylococcus aureus Cowan strain 1, 0.0075% (Calbiochem, La Jolla, CA), was added to monocyte cultures after 60 h; after 24 h, supernatants were collected and tested for the p40 and p70 subunit of human IL-12 using the ELISA ultrasensitive assay (Biosource). All cell reagents were LPS free to the limits of detection of the Limulus amebocyte lysate pyrogen kit (1–2.3 pg/ml) (BioWhittaker, Walkersville, MD).

Measles Ab assays

Sera were obtained from infants participating on the day that vaccine was given to determine whether passive Abs were present at the time of immunization. Sera were stored at −80°C and tested for measles-neutralizing Ab using a modified plaque reduction neutralization (PRN) assay (47). The PRN assay was used because of its superiority in detection of low titers of measles Abs compared with commercial ELISA methods (48). Briefly, serial fourfold dilutions of heat-inactivated serum (1/4–1/4096) were mixed with an equal volume of a low passage strain of Edmonston measles virus containing 25–35 PFU. Each serum dilution was incubated in duplicate in 24-well plates with Vero cell monolayers for 1 h and 45 min at 36°C in 5% CO2. The PRN titer was defined as the serum dilution that reduced the number of plaques by 50%. Titers <1:4 were considered negative and were assigned a value of 1 for statistical analysis. Seroconversion was defined as a fourfold rise in Ab titer after levels before vaccination were corrected for decay over three half-lives.

Statistical analysis

Comparison of T cell and cytokine responses in the same patient were evaluated by Student’s paired t test, whereas comparisons between patients were analyzed using a Student’s unpaired t test. ANOVA was performed to discern differences in the means between more than two groups. The χ2 and Fisher exact tests were used to compare the number of vaccinees in each cohort with proliferation responses. Values of p ≤ 0.05 were considered significant.

Results

Measles-specific T cell proliferation responses in relation to age at immunization

T cell proliferation after stimulation of PBMC with measles Ag was evaluated in 119 infants who were 6 mo (n = 49), 9 mo (n = 36), or 12 mo (n = 34) old and in 29 adults (Fig. 1A). T cell responses to measles were detected after immunization in all of the infant age groups; the mean SI ± SE increased from 2.0 ± 0.2 to 6.1 ± 0.8 in 6-mo-old (p = 0.001), from 2.1 ± 0.3 to 6.6 ± 1.6 in 9-mo-old (p = 0.01), and from 1.4 ± 0.1 to 5.6 ± 0.9 in 12-mo-old (p = 0.0002) infants. The mean SI after vaccination did not differ by age cohort. In addition, there were no age-related differences among the infants when the percentage who had detectable T cell proliferation to measles, defined as SI > 3.0, was compared. Responses were detected in 71% of 6-mo-old, 69% of 9-mo-old, and 62% of 12-mo-old infants. The percentage of adults with SI > 3.0 was 86%, which was not significantly higher than the 68% in infants. However, the mean SI in vaccinated infants.

Abbreviations used in this paper: SI, stimulation index; rhIL-12, recombinant human IL-12; PRN, plaque reduction neutralization.
was 6.1 ± 0.66, which was significantly lower than the mean SI of 11.3 ± 1.9 in adults (p = 0.002) (Fig. 1A).

**IL-12 production to measles in relation to age at immunization**

IL-12 production elicited by measles Ag was evaluated in 67 infants, including 37 who were 6 mo old, 16 who were 9 mo, and 14 who were 12 mo old, and in 13 adults. Mean IL-12 concentrations (±SE) before vaccination were 6.2 ± 2.4, 3.0 ± 1.1, and 7.0 ± 3.8 pg/ml compared with levels after vaccination of 21.3 ± 7.5, 17.9 ± 7.2, and 13.2 ± 5.9 pg/ml, in the 6-, 9-, and 12-mo-old infants, respectively (Fig. 1B). When the infants were evaluated as one group, there was a significant increase in IL-12 levels after compared with before measles vaccine (p = 0.01). No significant differences were seen when IL-12 responses were compared between the infant groups but the mean IL-12 concentration after vaccination of infants was 19.6 ± 4.4, which was statistically lower than the mean IL-12 concentration of 41.4 ± 11.7 pg/ml measured in adults (p = 0.04) (Fig. 1B).

**IFN-γ production to measles in relation to age at immunization**

IFN-γ concentrations were measured in 65 infants who were 6 mo (n = 30), 9 mo (n = 23), or 12 mo (n = 12) old and in 13 adults. An Ag-specific response was observed in each infant age group (Fig. 1C). IFN-γ concentrations (±SE) before and after vaccination were 21.1 ± 5.5 pg/ml vs 197.7 ± 59.7 pg/ml (p = 0.002), 38.9 ± 16 pg/ml vs 304.3 ± 100.7 pg/ml (p = 0.01), and 42.6 ± 13.7 pg/ml vs 110.5 ± 27.8 pg/ml (p = 0.04) in the 6-, 9-, and 12-mo-old infants, respectively. No significant differences were found when IFN-γ concentrations were compared among the infant groups or when the mean of 213.2 ± 43.4 pg/ml for all infants was compared with the mean IFN-γ concentration of 404.7 ± 124.7 pg/ml measured in adults (Fig. 1C).

**Effect of passive Abs to measles on measles-specific T cell proliferation and cytokine responses in infants**

The presence of passive Abs did not affect the frequency with which T cell proliferation to measles was elicited after vaccination of infants (Fig. 2A). Twenty-six of 42 infants who were 6 mo old had passive Abs, compared with 15 of 35 infants 9 mo old and 0 of 36 infants 12 mo old. No significant differences were detected when the mean SI of infants with passive Abs within each age cohort was compared with the mean SI in infants with no detectable Abs. When data for all age groups were combined, the mean SI of infants who had passive Abs was 5.7 ± 0.92 compared with 5.8 ± 1.1 in those who had none (p = 1.0). Cohorting 6- and 9-mo-old infants according to titer of passive Ab present before vaccination, at both 1:25 and 1:80, did not correlate with decreasing T cell responses, as has been described with humoral immune responses in infants (49, 50).

Passive Abs had no adverse effect on measles-induced IL-12 release by PBMC after vaccination of infants (Fig. 2B). The mean IL-12 concentration was 29.2 ± 9.5 in 17 infants who had passive Abs compared with 9.2 ± 1.9 among 22 infants who had none (p = 0.03).

Passive Abs had no significant effect on IFN-γ production in response to measles Ag after immunization (Fig. 2C). Of 16 infants who had passive Abs, the mean IFN-γ concentration was 105.9 ± 36.5 compared with 235.1 ± 62.6 among 32 infants who had none (p = 0.2).
Effect of measles immunization on mitogen-induced T cell proliferation in relation to age

Infants were tested for T cell proliferation to PHA responses just before and 3 mo after vaccination. The mean cpm $\pm SE$ in PHA-stimulated cultures of PBMC were not statistically different among the infant groups or when measured before immunization and 3 mo later (data not shown). The mean cpm for all infants tested after immunization was $43,500 \pm 2,900$, which was significantly lower than the mean cpm of $58,000 \pm 6,700$ observed in adults ($p = 0.03$) (Fig. 3).

Measles virus-induced suppression of IL-12 production by monocytes

The experiment described by Karp et al. was repeated to confirm that the measles virus used to prepare measles Ag for T cell proliferation and cytokine assays had the capacity to suppress IL-12 production by monocytes to concentrations equivalent to those measured in control wells. Furthermore, addition of *S. aureus* Cowan strain 1 stimulated IL-12 production in control Vero cell cultures but had no effect on human monocytes cultured with measles Ag (data not shown).

Effects of rhIL-12 on measles-specific T cell proliferation and IFN-γ production

T cell proliferation was measured in 11 vaccinated adults after stimulation with measles Ag alone and with the addition of rhIL-12. All the adults had a positive SI to measles (SI > 3), with a mean SI $\pm SE$ of $14.1 \pm 2.9$. No difference in measles-induced T cell proliferation was demonstrated when rhIL-12 was added. The mean SI was $7.4 \pm 1.5$ ($p = 0.06$).

IFN-γ release by T cells from 6 adults was measured at Days 1, 3, 5, and 7 after incubation with measles Ag alone, measles and rhIL-12, or rhIL-12 alone. IFN-γ concentrations were higher when PBMC were stimulated with measles in the presence of rhIL-12 compared with measles Ag alone ($p = 0.02$) or rhIL-12 alone ($p = 0.0003$) (Fig. 4). The peak difference was observed on Day 7, with mean IFN-γ concentrations of $1634.12 \pm 196.0$ in the measles-stimulated wells with rhIL-12 added.
lacked detectable passive Abs (data not shown).

Measles-neutralizing Ab titers before vaccination in the presence and absence of IL-12. Shown is the IFN-γ concentration (picograms/ml) in infants before (◼) and 12 wk after (□) measles immunization in the presence and absence of IL-12 (50 or 100 U/ml). Infants were 6, 9, or 12 mo of age at time of immunization. Error bars represent SEs.

When 15 vaccinated infants were tested, the addition of rhIL-12 to PBMC stimulated with measles Ag also resulted in a significant increase in IFN-γ production by T cells (Fig. 5). The mean IFN-γ concentrations in measles-stimulated wells were 151.8 ± 55.6 pg/ml compared with 747.3 ± 180.2 in the presence of rhIL-12 and measles Ag (p = 0.01) (Fig. 5). The peak IFN-γ concentration after rhIL-12 and measles stimulation in children was 747.3 ± 180.2 which was significantly lower than the peak of 1634.2 ± 196 from adult PBMC stimulated with measles and rhIL-12 (p = 0.01).

Measles-specific humoral immune responses

Measles-neutralizing Ab titers before vaccination were 13 (95% confidence interval, 7–26), 4 (95% confidence interval, 2–8), and 1 (95% confidence interval, 1–1) in the 6-, 9-, and 12-mo-old infants, respectively. Twelve weeks after measles vaccination, neutralizing Ab titers rose to 76 (95% confidence interval, 37–156), 353 (95% confidence interval, 164–756), and 1023 (95% confidence interval, 756–1704) in the 6-, 9-, and 12-mo-old infants, respectively. (6 vs 9 mo, p = 0.0002; 6 vs 12 mo, p = 0.0001; 9 vs 12 mo, p = 0.01). As previously reported, in the absence of detectable passive Abs, seroconversion and neutralizing Ab titers of 6-mo-old infants were statistically lower than those of 9- or 12-mo-old infants. There was no statistical difference in the humoral immune responses between 9- and 12-mo-old infants who lacked detectable passive Abs (data not shown).

Discussion

The morbidity and mortality rates caused by infectious diseases, including measles, are highest among infants and young children, suggesting that a maturation of immune responses occurs during this developmental phase (1, 51–53). Although cell-mediated immunity is critical in controlling viral infections, information about the capacity of infants to respond to specific viruses or viral vaccines is limited. Deficiencies in Ag-induced T cell proliferation and cytokine production, particularly IFN-γ, have been documented in infants with herpes simplex infections during the first few weeks of life (52, 54). Descriptions of the age-dependent immunogenicity of certain vaccines suggests that the limited immune responses seen in neonates may extend beyond the first year, but when immune maturation takes place is not known and is likely to vary depending on the antigenic stimulus (33, 55, 56). Animal experiments indicate that newborn responses to Ags are associated with diminished levels of TNF-α and IFN-γ production and are shifted toward a Th2-type cytokine pattern (34, 35). Since measles infection and measles vaccine have been associated with spontaneous IL-4 release by circulating PBMC and other Th2-like responses, immunization of young infants could enhance their predominance relative to the induction of antiviral Th1-like responses (6, 9, 10, 12). This issue is of practical importance because protection of younger infants against serious or life-threatening measles would be beneficial in geographic areas where measles remains endemic (1, 2). Our experiments addressed these questions with a comparative analysis of cellular immunity elicited by measles immunization of infants at 6, 9, and 12 mo of age. We found that T cell recognition of measles Ag was elicited in 71, 69, and 62%, respectively, and no age-related decreases in IL-12 or IFN-γ production among younger infants were detected. However, measles-specific T cell proliferation and IL-12 responses of infants were significantly lower than those of adults with vaccine-induced immunity to measles.

IL-12 is critical for the induction of IFN-γ, a major Th1 T cell cytokine which is involved in the clonal expansion of Ag-specific T cells (25–31, 57, 58). Earlier studies have shown that measles-specific IFN-γ release and T cell-proliferative responses are lower after measles infection or immunization than those induced by other viruses (6, 59, 60). Diminished IL-12 production, associated with the direct binding of measles to its monocyte receptor, may account for these differences (24). Our hypothesis was that infants may be particularly susceptible to the effects of low IL-12 production to measles Ag because their T cells may be inherently less efficient at IFN-γ gene transcription (34, 54–56, 61). First, we confirmed the block of IL-12 production by monocytes exposed to high concentrations of measles. Second, we demonstrated that the addition of rhIL-12 to PBMC cultures along with measles Ag was associated with a dramatic increase in IFN-γ release by T cells from adults with vaccine-induced measles immunity. Finally, we...
showed that whereas IL-12 also enhanced IFN-γ production by infant T cells stimulated with measles Ag, the IFN-γ concentrations were significantly lower than those produced by T cells from immune adults under these conditions. Taken together, these observations suggest that despite some measles-specific induction of IL-12 release, the quantities of IL-12 made may not be sufficient to induce concentrations of IFN-γ high enough to promote the maximal expansion of infant T cells that recognize measles Ags. The increased susceptibility of infants to severe measles is likely to be multifactorial and may be mediated in part by lower IL-12 responses than with adults, associated with a more limited capacity to produce IFN-γ.

An increased susceptibility to secondary infections is an important reason for the high rates of infant morbidity and mortality associated with measles (2, 3). These complications are attributed to generalized immunosuppression caused by measles infection (6). Immunization with live attenuated measles vaccine has been followed by a transient decrease in mitogen-induced T cell proliferation for a few weeks after vaccination (16, 17). We found no suppression of proliferation to PHA when the responses of infants tested just before immunization were compared with those measured 3 mo later. There was no evidence that generalized immunosuppression persisted for this time interval regardless of the age at measles immunization; PHA responses of 6-mo-old infants were equivalent to those of infants who were 9 and 12 mo old.

Interference by passively acquired measles Abs with the immunogenicity of measles vaccine has been a deterrent to immunization of infants younger than 12 mo (4, 37, 42, 43, 62–64). In infants immunized at 6 mo who had no interference attributable to passively acquired Abs, but T cell responses appear to be intact even in younger infants. As has been reported for DNA vaccination (32), passive Abs did not influence whether measles-specific T cell proliferation or IFN-γ was induced by immunization of infants with live attenuated measles vaccine. Whether these virus-specific T cell responses result in protection is not known, but successful immunization of infants as young as 3 mo has been described during measles outbreaks or in endemic areas (65).

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References


