

# Measles and mumps vaccination as a model to investigate the developing immune system: passive and active immunity during the first year of life

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## Abstract

Evaluations of neutralizing antibody responses in 6-, 9- and 12-month-old infants given measles or mumps vaccine indicated that 6-month-old infants had diminished humoral immune responses associated with passive antibody effects, but also had an intrinsic deficiency in antiviral antibody production, which was independent of passive antibody effects. In contrast, lower neutralizing antibody titers in 9-month-olds were related only to passive antibody effects. Measles and mumps-specific T-cell proliferation and interferon-gamma (IFN $\gamma$ ) production were induced by vaccination at 6, 9 or 12 months, regardless of passive neutralizing antibodies or age. These observations suggest a need to refine concepts about passive antibody interference and primary vaccine failure, taking into account the sensitization of antiviral T-cells, which occurs in the presence of passive antibodies and is observed in infants who do not develop active humoral immunity. A second dose of measles vaccine given at 12–15 months enhanced antiviral T-cell responses to measles in infants who were vaccinated at 6 or 9 months, and produced higher seroconversion rates. Since T-cell immunity is elicited under the cover of passive antibodies, the youngest infants benefit from the synergistic protection mediated by maternal antibodies and their own capacity to develop sensitized antiviral T-cells, which prime for subsequent exposures to the viral antigens. Conceptually, maternal immunization approaches with vaccines that can be given to women of child-bearing age before pregnancy, or that are safe for administration during pregnancy, should enhance passive antibody protection. Rather than being detrimental to infant adaptive immune responses, maternal vaccination can be coupled effectively with vaccine regimens that elicit priming of antiviral immune responses in infants during the first year of life.

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## 1. Introduction

Measles virus is a highly contagious human pathogen, which is spread by respiratory transmission [1,2]. Infection with measles virus is usually associated with a systemic febrile illness, characterized by viremia and rash, which can be complicated by encephalitis or pneumonia. Before the development of the live attenuated measles vaccines, measles caused an estimated 7 million deaths each year in previously healthy children [1–3]. Measles vaccine campaigns have been quite effective in controlling the annual epidemics in most developed countries, but inadequate de-

livery of measles vaccine to the global population means that an estimated 800,000 children die from measles annually [3]. The morbidity and mortality due to measles is highest among infants who become infected during the first year of life. This pattern of disease burden is observed in developed as well as developing countries [4–8].

When live attenuated measles vaccines were introduced in the 1960s, protective efficacy was found to be significantly lower in children vaccinated at less than 12 months of age [1]. These youngest children can be protected from most exposures to measles in communities that maintain high levels of measles immunity by vaccinating older children. However, one important consequence of the efficiency with which measles virus is transmitted from person to person in respiratory secretions is that exceptionally high levels

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of ‘herd immunity’ must be preserved by immunization of each new birth cohort at the appropriate age. Measles control has been achieved through intensive mass and routine measles vaccination and re-vaccination programs in the western hemisphere [8], but infant deaths from measles are common when resources are inadequate to sustain the public health effort, as is the case in certain areas of Africa and southern Asia [3,9]. Periodic re-introduction of measles virus into well-vaccinated communities also results in severe disease and deaths among infants who have not yet received their routine measles immunization, which is given at 12–15 months. Measles re-introductions are of growing concern in developed countries because many women are protected from measles by childhood immunization. Since measles antibody titers are lower in vaccinated women than in those who had natural infection, their infants receive lower titers of transplacentally transferred, passive antibodies against measles than infants whose mothers had measles infection. As we and others have observed, many infants who are born to vaccinated mothers have lost detectable measles passive antibodies by 6–9 months of age, resulting in a longer period of susceptibility between loss of passive immunity and administration of the primary measles vaccine dose at 12–15 months of age [9–12].

Given these considerations, understanding the obstacles to inducing antiviral immunity when infants are vaccinated at less than 12 months old is important for eliminating the threat of measles infection in infancy in developed as well as developing countries. We have undertaken a series of investigations to address this issue, through comparative analyses of the host response in infants given measles vaccine at 6, 9 and 12 months of age [9,13–15]. Work is in progress to evaluate the response to a second dose at 12 months given to infants whose first dose was administered at 6 or 9 months. While the immunogenicity of measles vaccine has been evaluated traditionally using serologic assays, we have examined the acquisition of measles-specific cellular immunity in parallel with measles neutralizing antibody responses. Comparisons with the responses of infants given live attenuated mumps vaccines have provided a means to determine whether observations about measles immunity are unique to measles, or are generally characteristic of the response to live viral vaccines given during the first year of life. In other work in progress, we are using new methodologies to assess the functional capacities of infant CD4 T-cell responses to measles antigens, including assays for cytokine production, quantitation of measles-specific responder CD4 T-cells, and up-regulation of surface molecules that are important in helper T-cell function.

The purpose of this report is to review our studies of adaptive antiviral immunity in which measles immunization is used as a model to examine the ontogeny of the host response during the first year of life. A fundamental goal of this work is to define the effects of passively acquired maternal antibodies on acquisition of adaptive immunity, both humoral and cellular, and to differentiate these effects,

as much as possible, from those that may be attributed to functional immaturities of the immune system during the first year of life.

## 2. Methods for evaluation of antiviral immunity to measles and mumps

The measles vaccines used in these studies were Measles Virus Vaccine Live (Attenuvax; Merck, Inc., West Point, PA), 1000 median tissue culture infective doses [TCID<sub>50</sub>], or Mumps Virus Vaccine Live (Mumpsavax; Merck, Inc., West Point, PA), >20,000 TCID<sub>50</sub>, and MMR-II vaccine (Merck, Inc., West Point, PA). Measles and mumps antibodies were measured using plaque reduction neutralization (PRN) methods [15]. Seroconversion was defined as a four-fold rise in antibody titer after pre-vaccine levels were corrected for decay over three half lives; measles seroprotection has been considered to be associated with PRN titer >1:120 mIU. Measles and mumps-specific T-cell responses were measured by proliferation of peripheral blood mononuclear cells (PBMC) incubated with measles or mumps antigen compared to an uninfected cell control [10,13–15]. T-cell proliferation was measured after 5 days by <sup>3</sup>[H]-thymidine uptake. The stimulation index (SI) was the ratio of mean counts per minute (cpm) in antigen and uninfected cell control wells. An SI >3.0 was defined as the cutoff for a positive response because it represents the mean SI + 1 S.D. above the mean SI as measured in the pre-vaccination specimens. Interferon-gamma (IFN $\gamma$ ), interleukin-12, and other cytokines were measured in supernatants from PBMC stimulated with measles or mumps antigen by ELISA methods [14]. Statistical analyses were done with Student paired or unpaired *t*-test,  $\chi^2$  and Fischer exact tests; GMTs were reported with 95th confidence intervals, and SI and IFN $\gamma$  with standard errors.

## 3. Humoral and cell-mediated immune responses to measles and mumps vaccines given to infants at 6, 9 or 12 months of age

In this study, we evaluated the ability of infants at 6, 9, or 12 months to respond to measles and mumps vaccines by measuring neutralizing antibody titers, T-cell proliferation and IFN $\gamma$  production before and after immunization [15]. Infants were vaccinated at 6 months (*n* = 93), 9 months (*n* = 77), or 12 months (*n* = 78) of age; pre-vaccine and 12 weeks blood samples were obtained from 210 infants. Mumps vaccine was given to 6 months (*n* = 53), 9 months (*n* = 45) or 12 months (*n* = 41) infants; 97 infants were tested both before and after vaccination. Eligibility requirements were gestation >36 weeks, birth weight >2500 g, and no acute or chronic illness. Vaccinated adults born after 1963 were tested for immunity to measles (*n* = 48) or mumps (*n* = 25).

The first significant observation from these investigations was that infants in all age groups had equivalent cellular immune responses to measles or mumps, which was independent of the presence of passive antibodies when immunized. The second important finding was that 6-month-old infants had low measles or mumps geometric mean titers (GMTs) and seroconversion rates, when immunized in the presence of passive antibodies, but also had lower neutralizing antibody responses in the absence of detectable maternally-derived measles or mumps antibodies at the time of vaccination.

Measles-specific T-cell proliferation increased significantly after vaccination and mean SIs were equivalent regardless of age. Measles SI was  $>3.0$  in 72, 69, and 65 of 6-, 9-, and 12-month-old infants. Measles SIs after immunization of infants who had measles neutralizing antibodies when vaccinated compared to those who did not, were  $7.3 \pm 0.9$  versus  $6.9 \pm 2.2$  in 6-month-old infants and  $5.6 \pm 0.8$  versus  $7.6 \pm 1.6$  in 9-month-old infants (Fig. 1A). Measles T-cell proliferative responses were independent of the amounts of measles passive antibodies; SIs were  $8.2 \pm 1.9$  in 16 infants who had passive antibody titers  $>50$  mIU and  $7.9 \pm 0.9$  among those with titers of 4–50 mIU. Mumps-specific T-cell proliferation was elicited in infants vaccinated at 6, 9 or 12 months. Again, cellular immune responses were not affected by passive antibodies; mean SIs in infants with and without passive antibodies were  $8.0 \pm 2.4$  versus  $8.7 \pm 1.6$  in 6-month-olds and  $6.1 \pm 1.5$  versus  $7.1 \pm 1.1$  in 9-month-olds (NS) (Fig. 1B).

Infants given measles vaccine had IFN $\gamma$  production by PBMC obtained after immunization at 6 months ( $n = 24$ ), 9 months ( $n = 20$ ), or 12 months ( $n = 13$ ). IFN $\gamma$  concentrations were equivalent in all age cohorts; responses were  $184 \pm 52$ ,  $349 \pm 95$ , and  $115 \pm 26$  pg/ml, in 6-, 9-, and 12-month-olds. Passive antibodies did not affect IFN $\gamma$  production. IFN $\gamma$  concentrations in infants with and without passive antibodies when immunized at 6 months of age, were  $172 \pm 38$  and  $273 \pm 83$  pg/ml ( $P = 0.22$ ); at 9 months, responses were  $188 \pm 58$  and  $326 \pm 86$  pg/ml ( $P = 0.31$ ). IFN $\gamma$  production was also detected after mumps vaccination at 6, 9 or 12 months, with mean concentrations of  $239 \pm 77$ ,  $156 \pm 54$ , and  $327 \pm 96$  pg/ml, respectively. Passive antibodies had no effect on mumps-specific IFN $\gamma$  production.

Age-related differences in measles and mumps neutralizing antibody titers after vaccination were detected. Measles GMTs were 120 mIU (71–200) and 146 mIU (44–490), in 6-month-olds with ( $n = 47$ ) or without ( $n = 26$ ) passive measles antibodies (Fig. 2A). In contrast, passive antibodies at the time of vaccination was the only factor affecting responses of 9-month-olds; GMTs were 180 mIU (68–473) in those who had passive antibodies when immunized compared to 744 mIU (467–1183) in those without detectable passive antibodies ( $P = 0.01$ ). Measles GMT in 9-month-olds without passive antibodies were equivalent to the GMT of 1210 mIU (774–1893) in 12-month-olds. Measles GMT was 120 mIU (71–200) in 6-month-olds without passive antibodies which was significantly lower than GMTs in 9-month-old ( $P = 0.01$ ) or 12-month-old infants who had no passive antibodies ( $P = 0.0002$ ) (Fig. 2A).

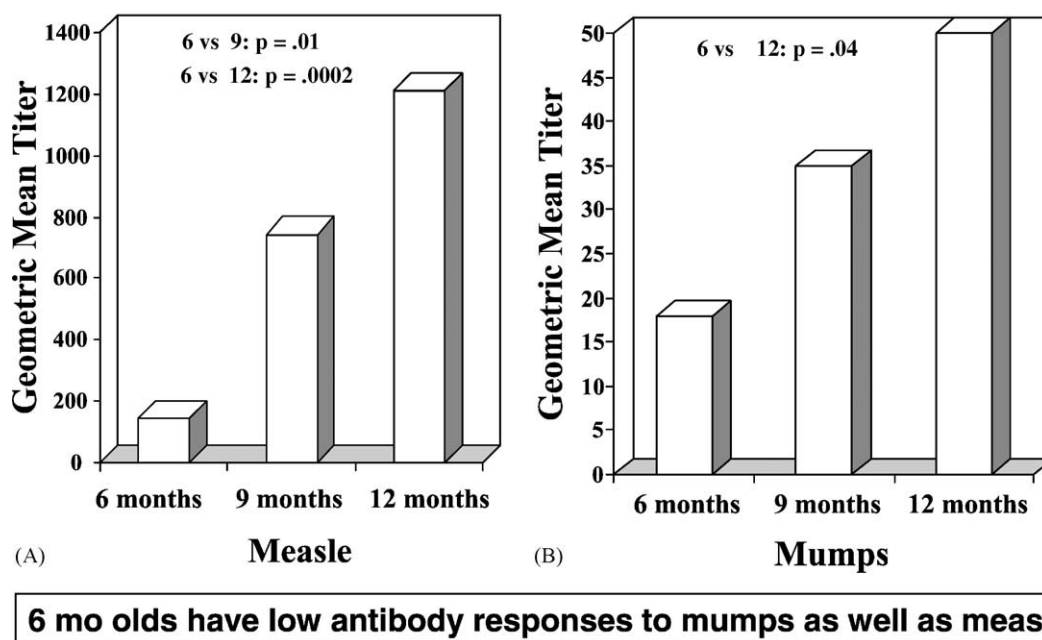


Fig. 1. Antigen-specific T-cell proliferation following measles or mumps immunization of infants with and without passive antibodies. The bars indicate the mean stimulation index (SI) 12 weeks after measles (panel A) or mumps (panel B) immunization in the presence (dark bars) and absence (striped bars) of passive antibodies. As shown on the horizontal axis, infants were 6- or 9-months-old at immunization. Error bars indicate standard error. A positive SI is defined as 3. Reprinted with permission from [15].

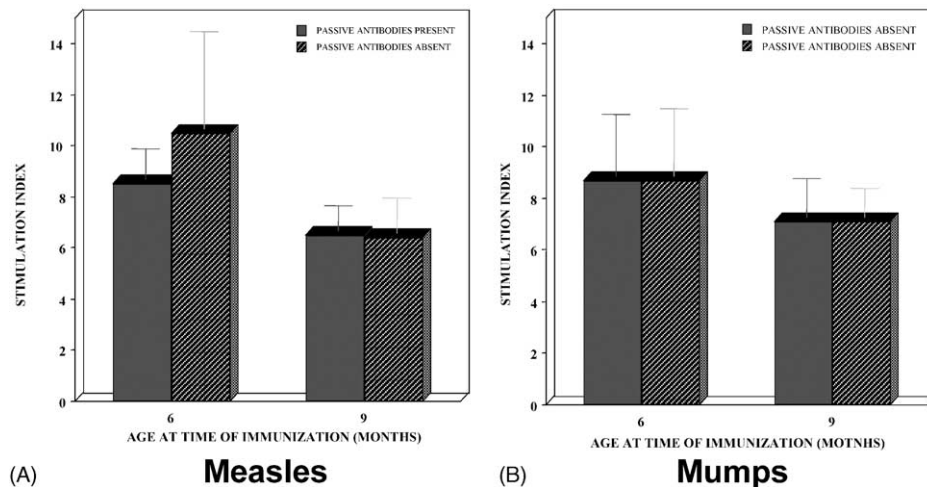


Fig. 2. Neutralizing antibody responses of infants after measles or mumps immunization in the absence of passive antibodies. Geometric mean titers (GMTs) were determined by plaque reduction neutralization assay of sera obtained 12 weeks after measles (panel A) or mumps immunization (panel B). Infants were 6, 9, or 12 months at time of immunization. The 95% confidence intervals for each GMT are given in the text. A measles neutralizing antibody titer of 120 mIU has been defined as protective. Reprinted with permission from [15].

Seroconversion rates were 77% in these 6-month-olds versus 97% in 9-month-olds ( $P = 0.02$ ) and 96% in 12-month-olds ( $P = 0.01$ ), and measles titers  $>1:120$  were detected in 59% of 6-month-olds compared to 97 and 94% of 9- and 12-month-olds who had no passive antibodies (6 months versus 9 months,  $P < 0.0001$ ; 6 months versus 12 months,  $P = 0.0001$ ). Passive antibody interference alone accounted for the difference in GMT between 9- and 12-month-olds ( $P = 0.003$ ), as shown by the equivalent GMTs when 9-month-olds without passive antibodies were compared with 12-month-olds. Mumps GMTs were also lower in 6-month-olds immunized in the absence of passively acquired mumps neutralizing antibodies (Fig. 2B); GMTs were 18 (9–37), 35 (20–63), and 51 (28–89) at 6, 9, and 12 months (6 months versus 12 months,  $P = 0.04$ ).

In summary, 6-month-old infants had an intrinsic limitation in humoral responses to both of these live attenuated paramyxovirus vaccines which was independent of the inhibitory effects of passive antibodies, whereas their antiviral cellular immune responses, measured as measles or mumps-specific T-cell proliferation and  $\text{IFN}\gamma$  production, were equivalent to older infants. Furthermore, these studies demonstrated that T-cell responses are established by immunization with live attenuated viral vaccines during the first year, despite the presence of passive antibodies. These observations indicate that assessing the induction of adaptive antiviral immunity using serologic methods only provides an incomplete picture of the host response in infants given live attenuated viral vaccines. A corollary is that defining primary vaccine failure as a failure to develop antiviral humoral immunity does not take into account the priming of antiviral T-cell responses in infants immunized during the first year of life. These analyses also suggest that the traditional concept that passive antibodies block the infant immune response by preventing the vaccine virus replication is oversimplified.

Measles and mumps vaccines contain low concentrations of viral antigens, which would not be expected to elicit antiviral T-cell responses without amplification of the antigenic signal by replication. Other mechanisms, specific for the induction of B-cell responses, are more likely to affect the production of measles or mumps antibodies in the presence of passive antibodies, independently of induction of antiviral cellular immunity. Examples of these mechanisms include formation of antigen-antibody complexes which undergo phagocytosis, facilitating presentation of viral peptides to naïve T-cells but inhibiting B-cell sensitization, and masking of B-cell epitopes by pre-existing antibodies to these regions of viral protein antigens [16].

#### 4. Interleukin-12, $\text{IFN}\gamma$ and T-cell proliferation responses to measles in immunized infants

The morbidity and mortality caused by measles in infants results in part from an enhanced susceptibility to secondary bacterial infections [2]. These secondary infections can be explained by the generalized immunosuppression that persists for a prolonged interval after the resolution of clinical signs of acute measles. In addition, some of the pathogenic mechanisms associated with measles virus infection are predicted to interfere with the induction of helper T-cells, classified as Th1-like, because of their capacity to produce IL2,  $\text{IFN}\gamma$ , related cytokines. Specifically, Karp et al. found that the binding of measles virus to CD46, which is the receptor that it uses to infect monocytes, results in the down-regulation of IL12 production [17]. IL12 is critically important for enhancing the production of the Th1-like cytokine responses, including  $\text{IFN}\gamma$ . Since IL12 promotes the rapid induction of antigen-specific T-cells in the naive host, decreased IL12 production could impair the acquisition

of adaptive immunity to measles, and has the potential to drive the CD4 T-cell response towards the production of Th-2 like cytokines, which may have immunosuppressive effects. Although we found no age-related differences in measles-specific T-cell proliferation or IFN $\gamma$  production in infants vaccinated at 6, 9 or 12 months of age, these responses were lower in infants compared to vaccinated adults. Therefore, we examined measles-specific T-cell responses in infants and adults who had vaccine induced measles immunity, using assays for IL12 production, T-cell proliferation and IFN $\gamma$  release by PBMC stimulated with measles [14].

Our hypothesis was that infants might have a limited Th1-like response to measles, which could contribute to a diminished measles-specific cellular immune response. Release of IL12 and IFN $\gamma$  by PBMC stimulated with measles antigen was measured along with measles-specific CD4 T proliferative responses of infants before and after measles vaccination. Infants were vaccinated at 6 months ( $n = 52$ ), 9 months ( $n = 42$ ), or 12 months ( $n = 40$ ); their responses were compared with those of 29 vaccinated adults. In these experiments, both IL12 and IFN $\gamma$  release by PBMC that were obtained before and after measles immunization of infants and stimulated with measles antigen in vitro, increased significantly following immunization. Whereas there were no differences in production of IL12 and IFN $\gamma$  in younger infants, evaluated after immunization at 6 months of age, and older infants vaccinated at 9 or 12 months, IL12 release by PBMC stimulated with measles antigen was significantly lower in infants than adults ( $P = 0.04$ ). In order to determine whether this deficiency in IFN $\gamma$  release was due only to the limited production of IL12 by infant monocytes, exogenous recombinant human IL12 was added to PBMC cultures during the period of stimulation with measles antigen.

By comparison with PBMC incubated with measles antigen only, rhIL12 enhanced IFN $\gamma$  production but even when IL12 was provided, infant T-cells released significantly less IFN $\gamma$  than adult T-cells in response to measles antigen (Fig. 3). The data was further analyzed by classifying the vaccinated infants according to whether they had passively acquired neutralizing antibodies to measles at the time of immunization. No differences were observed in measles-specific T-cell proliferation or in the concentrations of IFN $\gamma$  produced by PBMC stimulated with measles antigen.

In summary, cellular immunity to measles infection and vaccination may be limited in infants compared to adults as a result of less effective IFN $\gamma$  and IL12 production in response to measles antigens. The quantities of IL12 made may not be sufficient to induce concentrations of IFN $\gamma$  high enough to promote the maximal expansion of infant T-cells that recognize measles antigens. Nevertheless, these effects were not exaggerated in younger infants compared to infants who were immunized at 12 months and the presence of passive antibodies had no contributing effect. The increased susceptibility of infants to severe measles is likely to be multi-factorial, and may be mediated in part by lower IL12 responses compared to adults, in combination with an intrinsic limitation in the capacity of infants to produce IFN $\gamma$  [15].

## 5. Further analysis of the limitations of the infant CD4 T-cell response to measles

In studies that are in progress, we are examining CD4 T-cell immunity in infants given measles vaccine at 6, 9 or 12 months, using flow cytometry-based intracellular cytokine assays [18]. This approach is being used to enumerate

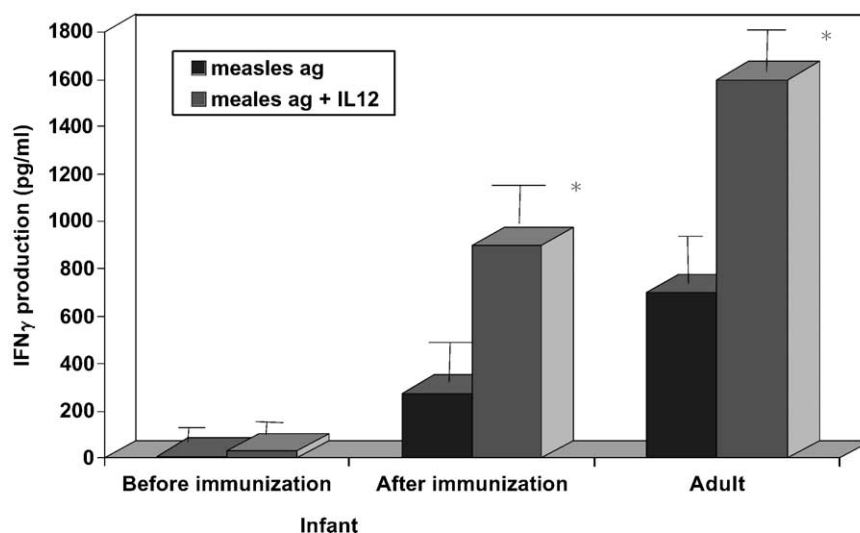


Fig. 3. Limited effect of recombinant IL12 on IFN $\gamma$  release by infant PBMC stimulated with measles antigen. Interferon- $\gamma$  production in infants before and 12 weeks after measles vaccination in the presence and absence of interleukin-12. Shown is the interferon- $\gamma$  concentration (pg/ml) in infants before (black bars) and 12 weeks after (striped bars) measles immunization in the presence and absence of interleukin-12 (50 or 100 U/ml). Infants were 6, 9, or 12 months of age at time of immunization. Error bars represent standard errors. Reprinted with permission from [14].

responder cell frequencies, to characterize the production of cytokines and to examine up-regulation of CD4 T-cell activation markers, CD69 and CD40L-ligand (CD40L) (Gans et al., CD4+ T-cell activation with limited interferon- $\gamma$  production to measles in vaccinated infants—IDSA 2002). Responder cell frequencies of CD4 T-cells, expressing CD69 or CD40L, are measured by flow cytometry after in vitro stimulation with measles antigen or *Staphylococcus* enterotoxin B (SEB). The proportion of activated cells detected with these cell surface markers is evaluated with respect to the percentage of these activated CD4 T-cells that also produced IFN $\gamma$ .

By the current analysis, the overall numbers of CD4 T-cells that exhibited CD69 or CD40L expression after incubation with measles antigen in vitro, did not differ by infant age, when frequencies were compared in infants who were vaccinated at 6, 9 or 12 months, or when the infant responses were compared to those of the vaccinated adults. In the cohort of infants evaluated thus far, infants have had fewer CD40L+/IFN $\gamma$ + and CD69+/IFN $\gamma$ + CD4 T-cells after stimulation of PBMC with measles antigen when compared to the expression on activated PBMC from vaccinated adults. No age-related differences were noted in numbers of measles-specific activated, IFN $\gamma$ -producing CD4 T-cells, but both CD40L+/IFN $\gamma$ + and CD69+/IFN $\gamma$ + CD4 T-cell frequencies were lower after stimulation with SEB in the youngest cohort. CD40L+/IFN $\gamma$ + frequencies were 34 in 6-month-olds compared to 84 in 12-month-olds ( $P = 0.03$ ), and CD69+/IFN $\gamma$ + frequencies were 52 at 6 months and 192 at 12 months ( $P = 0.01$ ). These observations suggest that CD4 T-cells from immunized infants respond effectively to measles antigen by up-regulation of activation markers, CD69 and CD40L, but again indicate that infant CD4 T-cells are impaired for IFN $\gamma$  production. Nevertheless, the detection of measles-specific activation suggests that T-cells from immunized infants, while functionally limited in IFN $\gamma$  production, are primed to respond to an exposure to measles or to a second vaccination.

## 6. Effects of a two-dose measles vaccine regimen

Humoral and cellular immune responses have been examined in a subset of infants who were immunized at 6 months and re-vaccinated with MMR ( $n = 19$ ) [15]. Among these infants, the mean SIs were  $8.9 \pm 3.1$  after the initial monovalent measles and  $11.6 \pm 2.4$  after MMR. The SIs in 9-month-olds ( $n = 12$ ) were  $8.2 \pm 2.7$  after monovalent measles vaccine and  $9.3 \pm 2.8$  after MMR. The measles-specific T-cell proliferation responses measured after MMR was given to infants who had received monovalent measles vaccine at 6 or 9 months were higher than those of 12-month-olds who had received MMR only (6 months versus 12 months,  $P = 0.008$ ; 9 months versus 12 months,  $P = 0.04$ ). In addition, the measles neutraliz-

ing antibody responses increased after MMR to GMTs of 1487 mIU (884–2497) in the 6-month-olds and 1994 mIU (809–4920) in the 9-month-olds. All infants developed titers of 120 mIU after MMR. These studies showed that an early two-dose measles immunization regimen elicited measles-specific T-cell responses in infants as young as 6 months, which were associated with enhanced humoral responses after receiving MMR at 12 months of age. Further, the observations indicate that developmental immaturities of the immune response in young infants at the time of first measles vaccination do not suppress future responses, and that initial interference from passive antibodies was not associated with any limitation in seroconversion and seroprotective rates following the second measles vaccine dose.

## 7. Conclusions

Evaluation of neutralizing antibody responses in 6-month-old infants given measles or mumps vaccine suggest an intrinsic deficiency in humoral responses in this age group which is independent of passive antibody effects. In contrast, no age-related differences were identified in measles or mumps-specific T-cell proliferation or IFN $\gamma$  production with vaccination at 6, 9 or 12 months (Fig. 4). Passive antibodies had no effect on T-cell proliferation or cytokine production. However, differences were observed in measles-specific T-cell responses between infants and adults, including lower measles-specific CD4 T-cell proliferation and IFN $\gamma$  and IL12 production, lower frequencies of CD69+/IFN $\gamma$ + CD4 T-cells and fewer CD40L+/IFN $\gamma$ + CD4 T-cells induced by measles stimulation. Thus, possible mechanisms for less robust humoral immune responses in younger infants may be related to limited IFN $\gamma$  production by helper T-cells, decreased measles-induced IL12 production affecting induction of CD4 T-cells and limited antigen-induced up-regulation of CD40-Ligand which could restrict interactions of helper T-cells and B-cells. With respect to concepts about antiviral immunity in the first year of life, these observations suggest a need to redefine passive antibody interference, since antiviral CD4 T-cells are elicited despite passive antibodies. The concept that maternal antibodies neutralize live viral vaccines is oversimplified. Inhibition of the infant antibody response by passively derived maternal antibodies will need to be investigated by identifying specific mechanisms that modulate antibody production independently of the induction of antiviral T-cells. An alternative paradigm is that inhibition of antibody production in the presence of passive antibodies is not 'interference', but modulation to avoid antibody excess and immune-mediated disease in early infancy. These studies also indicate a need to redefine the concept of primary vaccine failure. Priming of antigen-specific helper T-cells occurred in some infants without seroconversion, providing these infants with active,

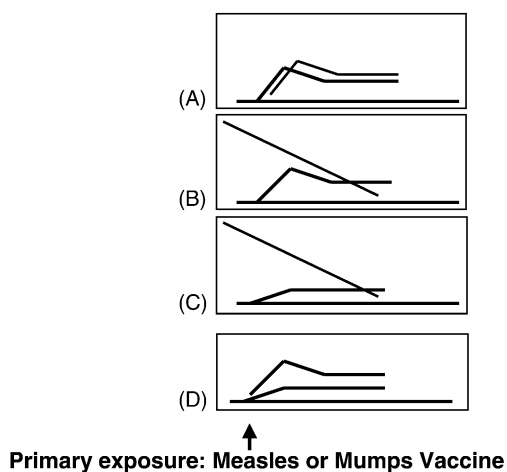


Fig. 4. Patterns of passive antibodies and acquisition of adaptive humoral and cellular immunity. The diagrams illustrate age-related differences in humoral immune responses and effects of passive antibodies on humoral immune responses. In contrast, T-cell responses are elicited regardless of age and are not affected by passive antibodies. Panel (A) demonstrates the antigen-specific T-cell and humoral responses in the absence of passive antibodies at the time of immunization of 9- and 12-month-old infants. Panel (B) illustrates the antigen-specific T-cell response of 6- or 9-month-old infants which are unaffected by the presence of passive antibodies. Panel (C) shows the inhibitory effect of passive antibodies on the humoral immune responses of 6- and 9-month-old infants. Panel (D) depicts the intrinsically lower humoral immune responses, with no age-related effect on T-cells in absence of passive antibodies at 6 months of age.

antiviral adaptive immunity. This finding is consistent with clinical experiences in which early immunization resulted in protection from severe measles disease and mortality even though breakthrough infections occurred [19]. Although measles-specific responder CD4 T-cell frequencies, T-cell proliferation, and IFN $\gamma$  and IL12 production were lower in infants than adults, a second dose of measles vaccine at 12–15 months increased antiviral T-cell responses to measles in infants who were vaccinated at 6 or 9 months, and resulted in equivalent seroconversion rates. Since T-cell immunity is elicited under the cover of passive antibodies, giving repeated doses of live viral vaccines represent a ‘prime’ and ‘boost’ strategy that mimics the pattern of exposure and re-exposure to pathogens during the first few years of life, which occurred universally before introduction of these vaccines. Therefore, vaccines that can be given to women of child-bearing age before pregnancy, or that are safe for administration during pregnancy, are predicted to be beneficial by prolonging the persistence of passive antibody protection during the first year of life. This strategy can be predicted to have no interfering effects on the induction of adaptive T-cell immunity, regardless of passive antibody titers. Maternal immunization approaches can be coupled with multiple dose vaccine regimens designed to compensate for lower initial antibody responses in the youngest infants, whether related to

down-modulation by passive antibodies or to immunologic immaturity.

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