Frequency of Measles Virus-Specific CD4$^+$ and CD8$^+$ T Cells in Subjects Seronegative or Highly Seropositive for Measles Vaccine

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The protective effect of measles immunization is due to humoral and cell-mediated immune responses. Little is known about cell-mediated immunity (CMI) to measles vaccine virus, the relative contribution of CD4$^+$ and CD8$^+$ T cells to variability in such immune responses, and the immunologic longevity of the CMI after measles vaccination in humans. Our study characterizes cellular immune response in subjects seronegative or highly seropositive for measles vaccine immunoglobulin G-specific antibody, aged 15 to 25 years, previously immunized with two doses of measles-mumps-rubella II vaccine. We evaluated the ability of subjects to respond to measles vaccine virus by measuring measles virus-specific T-cell proliferation. We examined the frequencies of measles virus-specific memory Th1 and Th2 cells by an ELISPOT assay. Our results demonstrated that proliferation of T cells in seronegative subjects was significantly lower than that for highly seropositive subjects ($P = 0.003$). Gamma interferon (IFN-γ) secretion predominated over interleukin 4 (IL-4) secretion in response to measles virus in both groups. The median frequency of measles virus-reactive CD8$^+$ T cells secreting IFN-γ was 0.09% in seronegative subjects and 0.43% in highly seropositive subjects ($P = 0.04$). The median frequency of CD4$^+$ T cells secreting IL-4 in response to measles virus was 0.03% in seronegative subjects and 0.09% in highly seropositive subjects ($P = 0.005$). These data confirm the presence of measles virus-specific cellular immune responses post-measles vaccine immunization in humans. The detection of measles virus-induced IFN-γ and IL-4 production by ELISPOT can be used to identify measles virus-specific low-frequency memory T cells in subjects immunized with measles vaccine. These differences agree in directionality with the observed antibody response phenotype.

Measles vaccine coverage is widespread due to extensive immunization programs and has significantly reduced the mortality and morbidity caused by measles (20). However, measles outbreaks can occur even in highly vaccinated populations (27). Among persons in the United States who contracted measles in the 1989 to 1991 outbreaks, 20 to 40% had been previously vaccinated (27). The failure rate of the measles vaccine is between 2 and 10% (8, 29, 37), leading to a second-dose policy and to further investigation of the immunological mechanisms underlying vaccine response. For this reason, we sought to characterize the nature of the cell-mediated immunity (CMI) in individuals previously vaccinated with two doses of measles vaccine.

Measles vaccine virus immunization induces both humoral immunity and CMI responses. Humoral immunity to measles vaccine has been extensively studied, and it is believed that both primary vaccine failure (complete lack of antibody after immunization) and secondary vaccine failure (waning or insufficient antibody after immunization) may be responsible for the variable efficacy of measles vaccine. Investigators speculate that measles vaccine failure may result from the induction of a polarized cytokine profile, which induces an immune response that may not be sufficiently protective (35). Preferential activation of interleukin-4 (IL-4)-producing Th2 cells by measles vaccine has been reported previously in response to measles immunization, which may lead to a lack of long-lasting immunity post-measles vaccination (35). Although humoral immunity plays a pivotal role in protection against measles, several reports suggest that CMI is essential for recovery from measles and may be sufficient for long-term immunity (13, 18, 20, 32, 34). This is best illustrated in children with isolated agammaglobulinemia who recover from measles and develop lifelong immunity to measles (15). In contrast, individuals with T-cell deficiencies such as leukemia and human immunodeficiency virus infection experience a progressive illness with 50 to 100% mortality (19, 22). These findings clearly suggest that cellular immunity plays a central role in immunity to measles infection and in protection against reinfection.

Despite the importance of cellular responses in measles, relatively little is known about the cellular immune response induced by measles vaccination or the relative importance of CD4$^+$ and CD8$^+$ T cells in this response. This is partially due to the experimental difficulties of inducing stimulation, as the measles virus causes immunosuppression. Technical methods to provide precise quantification of measles virus-specific memory T cells have been limited as well. With the emergence of new technology, assays like ELISPOT provide an opportunity to quantify antigen-specific T lymphocytes at a single-cell level.
level (9, 24). The purpose of this pilot study is twofold: (i) to characterize the cellular response at a single-cell level in humans by measuring the frequency of measles virus-specific CD4+ and CD8+ memory T cells years after completion of the two-dose vaccine series and (ii) to determine if a correlation exists between seronegative and highly seropositive humoral responses following measles vaccination and the level of measles virus-specific lymphoproliferation.

**MATERIALS AND METHODS**

**Study subjects.** We investigated measles immunity in subjects between the ages of 15 and 25 years living in Rochester, Minn. We recruited subjects with low (seronegative) and high (seropositive) antibody responses after measles immunization for this study. The study subjects consisted of 15 healthy human subjects in Olmsted County, Minn., selected from a measles seroprevalence study conducted by our group (28). Subjects were identified and randomly sampled from two categories based on their measles virus-specific serum immunoglobulin G (IgG) antibody status: (i) IgG antibody-seronegative subjects (enzyme immunoassay [ELISA] index values of ≤0.8 following a first dose of measles vaccine; n = 10) and (ii) highly seropositive subjects (upper 10th percentile of IgG antibody levels; n = 5). For this study, we recruited individuals as members of two groups: seronegative if their ELISA result was ≤0.8 and seropositive if their ELISA result was ≥2.9. We recruited highly seropositive subjects because they represented a convenience sample of those seropositive individuals in the highest 10th percentile of antibody levels following the first dose of measles vaccine. Thus, our sample included the two ends of the biologic continuum of antibody responses. The subjects’ medical records documented that each subject had been previously immunized with two doses of measles-mumps-rubella (MMR-II) vaccine (Merck Research Laboratories, West Point, Pa.) containing the Edmonston strain of measles virus (≥1,000 50% tissue culture infective doses [TCID50]), the Jeryl Lynn B strain of mumps virus (≥20,000 TCID50), and the Wistar RA 27/3 strain of rubella virus (≥1,000 TCID50). The first dose was administered at 12 to 20 months of age, and the second was administered at 5 to 12 years of age. All subjects resided in a geographic area where no wild-type measles virus had circulated in the community during the subjects’ lifetimes. Therefore, the presence of measurable measles virus antibody level was attributed to vaccination. The Institutional Review Board of the Mayo Clinic granted approval for the study, and all obtained informed consent from each subject.

**Preparation of peripheral blood mononuclear cells (PBMC).** We obtained blood (30 ml) by venipuncture from all 15 subjects after obtaining informed consent. Venous blood samples were collected in serum separator tubes for serum separation and heparinized Vacutainer tubes for PBMC isolation and processed within 2 h of collection. Blood from heparinized tubes was diluted by twice the original volume with phosphate-buffered saline (pH 7.4), and peripheral blood mononuclear leukocytes were isolated by Ficoll-Hypaque (Amersham Pharmacia Biotech, Piscataway, N.J.) density gradient centrifugation. These freshly isolated PBMC were used for the lymphocyte proliferation assay as described below, and the remaining cells were counted and frozen in liquid nitrogen in aliquots of 106 cells/ml for ELISPOT assays. The viability of isolated cells ranged from 95 to 98%. We observed no changes in cellular viability between the PBMC samples before or after cryopreservation as determined by the trypan blue exclusion test.

**Measles virus antibody assay.** Circulating measles virus-specific IgG antibody titers for all serum specimens were determined by whole-virus-specific ELISA (Measles ELISA; BioWhittaker, Walkersville, Md.). Sera were separated by centrifugation at 900 × g for 15 min at room temperature, aliquoted, and stored at −70°C until used in the assay. Tests were done in duplicate, and the mean value was used for all analyses. According to the manufacturer, optical density readings of ≤0.8 index units are “negative,” readings of 0.8 to <1.0 are “equivocal,” and readings of ≥1.0 are “positive” (6). We defined optical density readings with levels of 2.9 or greater as highly seropositive, based on the upper 10th percentile of the entire distribution of subjects. The coefficient of variation for this assay in our laboratory was 6.6%.

**Lymphocyte proliferation assay.** We assessed in vitro lymphoproliferation by a [3H]thymidine incorporation assay as previously described with slight modifications (4, 34). Cells were washed twice in RPMI 1640 and resuspended at a concentration of 2 × 105/ml of complete RPMI 1640, supplemented with penicillin-streptomycin (100 U/ml), 5% fetal horse serum (Sigma, St. Louis, Mo.), 2% serum replacement medium (TCM; Celox, St. Paul, Minn.), and 2-mercaptoethanol and sodium pyruvate (Sigma). Cells were plated in triplicate in flat-bottomed 96-well microtiter tissue culture plates (Costar; Corning Inc.) and cultured either without stimulation or with stimulation with phytoshemagglutinin (PHA; 5 μg/ml; positive control) or live attenuated measles vaccine virus (more attenuated Enders strain; Attenuvac; Merck). Cells were incubated with live measles virus (50 PFU/well) for 20 months and then previously determined was optimal for proliferation with the use of 2 × 105 cells/well (data not shown). We measured spontaneous proliferative activity by culturing unstimulated PBMC. The cells were then pulsed with 1 μCi of tritiated thymidine/well for 18 h and harvested on fiberglass filters by using a semiautomated 96-well harvester (Skatron Instruments, Lier, Norway). The radioactivity associated with the labeled filters was counted with a liquid scintillation counter (Packard Instrument Company, Boston, Mass.), and the results were expressed as stimulation indices (SI), which is a ratio of mean counts per minute of antigen-stimulated cells to counts per minute of unstimulated cells. Stimulation indices of ≥3 were considered to be positive (4, 34).

**Enrichment of CD4+ and CD8+ T-cell subsets.** We thawed cells and separated CD4+ or CD8+ T cells from subjects’ PBMC by using immunomagnetic beads according to the manufacturer’s instructions (Dynabeads M-450; Dynal, Oslo, Norway). A suspension of 107 PBMC was incubated with 100 μl of anti-CD4 microbeads for 30 min at 4°C on a rotating shaker. Immunomagnetic beads were detached from isolated T cells by using Detachabeads (Dynal). The unbound cells were then incubated with anti-CD8 immunomagnetic beads for the selection of CD8+ T cells. Cells were washed and resuspended in RPMI 1640, supplemented with 10% human AB serum (1 mM l-glutamine and 1% human AB serum). A total of 107 PBMC routinely yielded 2 × 105 to 2.5 × 104 CD4+ and CD8+ T lymphocytes and 5 × 105 to 6 × 105 negatively depleted CD4+ or CD8+ T lymphocytes from a single blood sample. The purity of positively and negatively selected cells was confirmed by flow cytometry. Flow cytometric analysis for CD4-phycoerythrin and CD8-fluorescein isothiocyanate verified depletion of the CD8+ or CD4+ T cells (purity, >90%; data not shown).

**ELISPOT assay for the detection of gamma interferon (IFN-γ)- and interleukin-4 (IL-4)-producing cells.** The Edmonston-Enders vaccine strain of measles virus was grown in African green monkey kidney cells (Vero cells) in Dulbecco’s modified Eagle’s medium, supplemented with 5% fetal calf serum (virus stocks of 1.1 × 106 PFU/ml), and was used to infect autologous negatively depleted CD4+ or CD8+ antigen-presenting cells (APC). Irradiated (5,000 rads) T lymphocytes were incubated with the virus stock at a multiplicity of infection of 0.5 for 24 h at 37°C, in a humidified atmosphere with 5% CO2. At the same time, purified CD4+ and CD8+ cell subsets were also incubated separately in complete RPMI 1640 containing 10 IU of IL-2/ml and 20 ng of IL-7/ml for 24 h at 37°C. Lymphocytes (virus-infected stimulators plus titrated CD4+ or CD8+ T-cell responders) were pelleted and resuspended in culture medium containing 10% human AB serum and antibiotics. The ELISPOT assay used fractionated frozen PBMC, and assays were performed after 24 h in culture with live measles virus to rule out any artifacts due to long-term in vitro stimulation. IFN-γ and IL-4 ELISPOT assays (R&D Systems Inc., Minneapolis, Minn.) for the quantitative analysis of virus-specific CD8+ and CD4+ T cells were performed as previously described with slight modifications (12, 24, 25, 35). Briefly, 96-well nitrocellulose-based microtiter plates with a higher binding capacity coated with anti-IFN-γ or anti-IL-4 monoclonal antibody were blocked with complete culture medium for 30 min. After washing, 106 CD8+ or CD4+ T lymphocytes were added to the wells with and without 106 measles-infected APC and incubated for 24 h at 37°C. Stimulation with PHA (0.5 μg/ml) was used as a positive control. After the wells were washed, biotinylated anti-IFN-γ or anti-IL-4 polyclonal antibodies were added and incubated overnight at 4°C. After the wells were washed once more, streptavidin-conjugated alkaline phosphatase was added at room temperature for 2 h. Individual cytokine-producing cells were detected as dark spots after a 60-min reaction with 5-bromo-4-chloro-3-indolylphosphate/nitroblue tetrazolium chromogen. The background was <10 spots/well at 200,000 cells/well (data not shown). All assays were performed in duplicate. Spots were counted by using a specialized automated ELISPOT reader (Cellular Technology Ltd., Cleveland, Ohio). To calculate the number of measles virus-specific T cells, the mean number of spots induced by a control value (mean ± SD) was subtracted from the mean number of spots induced by measles virus-infected APC and normalized to numbers of cytokine spot-forming T-cell subsets per 2 × 106 cells. Measles virus vaccine-specific T-cell frequencies were calculated by percent for each subject and were analyzed separately.

**Statistical methods.** Descriptive statistics, including medians and ranges, were calculated for each of the four variables of interest (SI, antibody level, and IFN-γ or IL-4 precursor frequencies of demonstrating differences). Variables between seronegative and highly seropositive subjects were made by using Wilcoxon rank sum tests and Fisher exact tests. Spearman correlation coefficients were calculated for each of the four variables of interest with the antibody level and IFN-γ or IL-4 precursor frequencies of demonstrating differences.
were used to determine associations between the immune response variables, separately for seronegative and highly seropositive subjects.

RESULTS

Demographic variables. We recruited 15 individuals (5 males and 10 females) between the ages of 15 and 25 years, including 10 (66.7%) measles vaccine-seronegative subjects and 5 (33.3%) subjects who represented the highest 10th percentile of EIA index values. These subjects were grouped by the magnitude of antibody levels after the first dose of vaccine as seronegative or highly seropositive. Population characteristics did not differ between the subjects who were seronegative and those who were seropositive for measles virus antibodies. The vast majority of subjects were Caucasian (93.3%), and the median age at immunization was 15 months. The median age was 19.5 years (range, 15 to 25 years) for the seronegative group and 18 years (range, 16 to 19 years) for the highly seropositive group. The median time from immunization to antibody testing was 9.5 years for the seronegative group and 8 years for the highly seropositive group. Since the subjects were precategorized based on their EIA antibody values, we observed a lower median EIA value for seronegative subjects than for highly seropositive subjects (median EIA, 0.18 versus 3.70, \( P < 0.001 \)) as expected (Table 1). In addition, there were no significant differences between seronegative and highly seropositive subjects in age at initial immunization (\( P = 0.59 \)) or in time from initial immunization to measles virus antibody testing (\( P = 0.39 \)).

Detection of measles virus-responsive T cells after measles vaccination. Lymphoproliferative responses and the T-cell frequencies for each individual across antibody status were examined. Seven of 10 (70%) seronegative subjects had a low lymphocyte proliferative response (SI = \( \leq 3 \)) upon stimulation with measles vaccine virus. Three subjects in the seronegative group had SI values of \( > 3 \). Strong lymphoproliferative responses (SI = \( \geq 3 \)) were detected in five of five (100%) of the highly seropositive subjects. In addition, one of the two individuals with absent measles virus-specific antibody titers had a detectable lymphoproliferative response (SI = 3.4).

The number of measles virus-specific T cells secreting IFN-\( \gamma \) and IL-4 was determined in CD8\(^{+} \) and CD4\(^{+} \) T-cell subsets from subjects seronegative or seropositive for measles vaccine. Both CD8\(^{+} \) and CD4\(^{+} \) T cells could be induced to produce IFN-\( \gamma \)- and IL-4-producing T-cell frequencies were compared by using measles virus-infected autologous negatively depleted APC. As expected, the number of IL-4-producing CD4\(^{+} \)-enriched T cells was much lower in response to measles virus stimulation than was the number of IFN-\( \gamma \)-producing CD8\(^{+} \) T cells. All subjects produced IFN-\( \gamma \) and IL-4 in response to PHA (data not shown).

Comparison of immune responses in subjects seronegative and highly seropositive for measles vaccine virus. Table 1 presents the median values of the four immune response variables of interest for the seronegative and highly seropositive subjects. We compared the levels of measles virus-specific lymphoproliferative responses and determined their correlation with seronegative and highly seropositive subjects post-measles vaccination. The proliferation of T cells in the seronegative subjects was lower than that in the highly seropositive subjects (median SI of 2.32 versus 7.45, \( P = 0.005 \)). Measles virus-specific CD8\(^{+} \) and CD4\(^{+} \) lymphocytes were readily detectable by ELISPOT. Our results demonstrated that higher overall frequencies of measles virus-specific CD8\(^{+} \) and CD4\(^{+} \) T cells were detected in highly seropositive subjects than in seronegative subjects. In particular, IFN-\( \gamma \) secretion predominated over IL-4 secretion. The median frequency of measles virus-reactive CD8\(^{+} \) T cells secreting IFN-\( \gamma \)- and IL-4 production was observed in unstimulated cells (Table 2).

Association among variables of immune response post-measles immunization. Table 2 presents the association among the four variables of interest (measles virus-specific SI, antibody level, and IFN-\( \gamma \) and IL-4 precursor frequencies), separately for the seronegative and highly seropositive groups, in the form of a Spearman correlation matrix. Associations among seronegative subjects are shown in the upper right triangle of the matrix, while associations among highly seropositive subjects are in the lower left triangle portion. A significant negative correlation of \(-0.68 (P = 0.03)\) was observed between antibody level and IFN-\( \gamma \)-precursor frequencies in the seronegative group. No other significant associations were observed between the other variables of interest in either seronegative or seropositive subjects, possibly due in part to the limited sample size of our study groups.
The association between measles virus antibody titers and measures of CMI in age-matched subjects are needed.

**DISCUSSION**

Immune correlates of protection against measles virus infection and post-measles immunization are still not completely understood. The humoral arm of the immune system has been the focus as the prime contributor of immunoprotection, and most studies largely correlate serum antibodies with immunity (20, 21). However, a limited number of studies using both wild-type and vaccine strain measles viruses demonstrate that an insufficient or impaired cellular immune response may lead to progressive or even fatal infection (19, 22). These observations suggest that immunity to measles is a more complex orchestration between the cellular and humoral arms of immunity. These observations also raise concerns that antibody-based definitions of vaccine success and failure may be misleading, or at least simplistic and incomplete.

Our study demonstrated a significantly higher measles vaccine virus-specific proliferative response in highly seropositive individuals than in seronegative individuals. Both the proliferation of T cells and their effector functions, such as cytokine secretion, were greatly reduced in measles vaccine-seronegative subjects. Our study subjects were between 15 and 25 years of age; therefore, we were able to observe and measure long-term maintenance of proliferative response post-MMR vaccination.

These findings are in concordance with other reports that suggest an important role of persistent measles cellular immunity (13, 34). Bautista-López et al. (4) demonstrated that a measles virus-specific cellular response could be detected in roughly two-thirds of children during the first 6 months after MMR vaccination; this response was also observed in approximately 64% of the children studied between 5 and 13 years after vaccination. Ward et al. (34) demonstrated that cellular responses to measles virus might be better sustained than antibody responses after vaccination and revaccination in some subjects. We observed a positive (SI = >3) proliferative response in three seronegative individuals and did not find any correlation between the measles virus-specific antibody levels and SI in either group (Table 2). This finding is consistent with the data from two study populations in which no correlation was observed between the measles virus antibody titers and lymphoproliferative responses (4). Alternative explanations include the lack of proliferative assay sensitivity, a reflection of the true immune response generated by measles vaccination, or a low sample size, leading to low-power estimates of association.

To further characterize the cellular immune response, we determined measles virus-specific T-cell subset frequencies by an ELISPOT technique that detects T cells secreting specific cytokines (IL-4 and IFN-γ) in response to antigenic stimulation (5). These two cytokines are important in the immune response to measles (17) and are signature cytokines for humoral immunity (which regulates B cells and antibody production) and CMI (recall lymphoproliferation) (14, 23, 31). The ELISPOT assay is remarkably well adapted for monitoring immune response to vaccines (3, 33). Since it is highly sensitive and versatile, it can be performed ex vivo and uses a relatively small number of cells. Despite the limited number of observations in this study, we readily demonstrated that higher frequencies of measles virus-specific CD8+ and CD4+ T cells were detected in highly seropositive subjects than in seronegative subjects. In particular, IFN-γ secretion predominated over IL-4 secretion in response to measles virus antigens. These data suggest that there is immunologic longevity of CMI post-measles immunization, and the detection of measles virus-induced IFN-γ and IL-4 production measured by ELISPOT assay at the single-cell level was successfully used to identify measles virus-specific low-frequency memory T cells. Further, these differences correlate with an observed antibody response phenotype. Thus, the detection of measles virus-induced IFN-γ and IL-4 secretion at the single-cell level can be used to identify measles virus-specific memory T cells in subjects previously vaccinated with measles vaccine. We were also able to detect memory T cells in subjects seronegative for measles vaccine, which suggests the possibility that the ELISPOT technique may be useful in the analysis of low-frequency responses. However, whether these low frequencies of cytokine-secreting cells are sufficient to allow protection against measles is currently unknown.

Even decades after measles immunization, both the CD4+ and CD8+ T-cell pools contain high levels of measles virus-specific memory T cells. This indicates that measles vaccination induces a long-lived T-cell memory response. These results are in agreement with and extend the previous report of Nanan et al. (24), where the ELISPOT technique was used to determine the frequency of measles virus-specific memory T lymphocytes in healthy adults with a childhood history of natural measles. Nanan et al. (24) report a high frequency of CD3+ CD4+ and

### TABLE 2. Association of measurements of humoral immunity and CMI in response to measles vaccine virus in seronegative and highly seropositive subjects as represented by a Spearman correlation matrix

| Measurement                        | SI (median = 2.32) | EIA antibody level (median = 0.18) | IFN-γ-producing CD8+ T cells (median = 0.09)a | IL-4-producing CD4+ T cells (median = 0.03)a |
|-----------------------------------|--------------------|-----------------------------------|-----------------------------------------------|-----------------------------------------------|
| SI                                | 1.00               | -0.31a                            | 0.01a                                         | -0.12a                                        |
| EIA antibody level                | 0.50b             | 1.00                              | -0.68c                                        | 0.51b                                         |
| IFN-γ-producing CD8+ T cells      | 0.70b             | 0.60b                             | 1.00                                          | -0.14b                                        |
| IL-4-producing CD4+ T cells       | 0.60b             | 0.30b                             | 0.10b                                         | 1.00                                          |

*a Associations among seronegative subjects.
*b Associations among highly seropositive subjects.
*c Statistically significant association (P = 0.03).
*d Values are Spearman correlation coefficients.
CD3+ CD8+ measles virus-specific T lymphocytes in PBMC that were clearly antigen specific and correlated with seropositivity after natural infection. Moreover, frequencies as low as 0.01% for measles virus-specific CD8+ or CD4+ T lymphocytes were determined by this assay (24). To our knowledge, this is the first report demonstrating that measles immunization leads to the development of a measles virus-specific memory CD8+ and CD4+ T-cell pool that is related to antibody seropositivity and sustained for a prolonged period after immunization. Moreover, our study demonstrated for the first time that the ELISPOT assay could be successfully used to monitor immunological memory responses in previously vaccinated subjects without in vitro expansion of specific T cells.

There are several limitations to this study. First, because this is a pilot study, the sample size is small. Despite the limited sample size, the results of our study suggest that measles virus-specific CD8+ and CD4+ T cells can be detected in both seronegative and highly seropositive subjects previously immunized with measles vaccine. Second, a specific and sensitive whole-measles-virus EIA for the detection of subjects’ measles virus-specific IgG antibody levels in serum was used in this study (1, 11). This assay is now widely used for the detection of measles virus-specific antibody and either complements or replaces the virus neutralization and hemagglutination-inhibition tests (7, 10, 36). For the above reasons, we evaluated the circulating measles virus antibody levels following measles immunization in the subjects by using a commercial measles virus EIA. However, the plaque reduction neutralization test is still considered the most sensitive assay and is often used as a “gold standard” serologic assay for measles (16, 30). Because this work may provide information for other vaccine-preventable diseases, further work is necessary to evaluate the interval between the last MMR-II dose and the present immune responses of immunized individuals. This information may increase our understanding of the nature of secondary vaccine failure.

In conclusion, both cellular and humoral immune responses play a critical role in protecting against measles, and both responses should be considered in the definition of vaccine success or failure. We also found evidence for immunologic longevity of the CMI post-measles vaccination in humans. Further characterization of CMI demonstrated the presence of both CD4+ and CD8+ T cells specific for measles virus in subjects immunized with measles vaccine, which suggests that a balance between measles virus-specific T-helper and cytotoxic memory T cells is critical to mount a protective response. Our study may have additional implications for studies of currently available and new candidate vaccines, particularly for monitoring T-cell induction following vaccination.

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