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Analysis of specific antibody and cellular immune response to first-dose measles vaccine Edmonston-Zagreb in 9-month-old infants

Teni Nurlatifah HR[a,b], Wisnu Barlıanto[c*], I Wayan Arsana Wıyasa[d], H.M.S. Chandra Kusuma[c], Tita Luthfia Sari[c], Novilia Sjafri Bachtıar[e]

[a]*Applied Midwifery, Master's Study Program of STIKes Dharma Husada Bandung, West Java, Indonesia*
[b]*Doctoral Program of Medical Science, Faculty of Medicine Universitas Brawijaya Malang*
[c]*Pediatric Department, Faculty of Medicine, Brawijaya University, Veteran Street, Malang 65145, East Java, Indonesia*
[d]*Obstetric and Gynecology Department, Faculty of Medicine, Brawijaya University, Veteran Street, Malang 65145, East Java, Indonesia*
[e]*Bio Farma, Pasteur Street No. 28, Bandung 40161, West Java, Indonesia.*

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Abstract
Background: Measles vaccinations have been suggested to provide immune protection and decreased measles incidence. However, there was a limited study evaluating how the measles vaccine elicits specific immune responses.
Objective: This study aimed to evaluate both humoral and cellular immunity to first-dose measles vaccine Edmonston-Zagreb (EZ) in 9-month-old Indonesian infants.
Methods: A cohort study was conducted on 9-month-old infants who got the first-dose of measles vaccine EZ. Measles-specific immunoglobulin G (IgG) antibody serum levels were measured using plaque-reduction microneutralization assay. Peripheral blood mononuclear cells were stimulated with a measles-specific peptide to identify a cellular immune response. Quantification of $CD4^+$ and $CD8^+$ T-cells producing interferon-gamma (IFN-ɣ) and interleukin 17-A (IL-17A) were conducted by flow cytometry. Humoral and cellular immune response parameters were analyzed over time.
Results: The prevalence of seropositivity rates was 85.8% at 1-month after vaccination and 16.67% at 6-months postvaccination. Measles-specific IgG antibodies increased significantly at 1-month after measles vaccination. However, they decreased significantly 6-months after vaccination. IFN-ɣ and IL-17A secreting T-cells increased significantly at 1-month after measles vaccination. Interestingly, a significant decrease of IFN-ɣ and IL-17A secreting $CD4^+$ T cells was noticed 6-months postvaccination compared to IFN-ɣ and IL-17A secreting $CD8^+$ T cells.
Conclusion: Our study suggests that the first-dose measles vaccine on 9-months-old infants seems to induce both humoral and cellular immune responses that decline 6-months after vaccination.

***Corresponding author**: Wisnu Barlıanto, Pediatric Department, Faculty of Medicine, Brawijaya University, Veteran Street, Malang 65145, East Java, Indonesia, *Email address*: wisnu_barlian@ub.ac.id

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Introduction

Measles virus (MV) is the causative agent of measles, an acute systemic infection predominantly found in young children. It is a highly contagious disease associated with high rates of morbidity and mortality among children. The highest case-fatality rates occur in children <12 months old.[1] The average number of infections reported to the Centers for Disease Control and Prevention ranged from 3–4 million in the pre-vaccination era, and there were around 1.2 million deaths attributed to measles.[2] The global reductions in incidence from 2006 were due to the successful vaccination programs. Measles vaccinations are reportedly effective in providing immune protection and decreasing measles incidence. However, several countries still report measles outbreaks despite the vaccination program. Since 2006, the 2017–2019 period demonstrated the highest upwards fluctuation of measles incidence globally (401.024 cases in 2019).[3] Indonesia's coverage with the first dose of the measles vaccine increased from 75–90%.[4,5] However, there were over 12,000 reported measles cases annually in the past five years in Indonesia.[6] An outbreak of 800 in a single district earlier in 2017 also resulted in 72 child deaths.[7] Moreover, it has been reported that several measles infections still occurred in Indonesian children who had the measles vaccination history.[5] To date, no study evaluated the effectiveness of measles vaccination program in Indonesia. Hence, this study was the first pilot study that analyzed immune responses the following first dose of measles vaccination in Indonesia.

The measles vaccine is a live, attenuated virus that will induce both humoral and cellular immune responses.[8] The initial immune response of measles infection was characterized by an increase of interferon-ɣ (IFN-ɣ) producing T cells. A shift in cytokine production was noticed later, where the IFN-ɣ in the early phase was replaced by interleukin-4 (IL-4), IL-10, IL-13 and IL-17A.[9,10] This promoted B cell maturation and contributed to the production of antibodies.[11] A study by Martin et al.[12] reported that protective measles antibody levels were developed rapidly after vaccination and persisted even after the second dose of vaccine. But another study showed that measles vaccination of six months old infants revealed poor humoral immunogenicity,[13] This which was attributed to the interference of passively acquired antibodies from maternal and limitations of the developing immune system in infants.[14] It also has been speculated that measles vaccine failure can also be because of the induction of a polarized cytokine profile, which contributes to an immune response that may not be sufficiently protective.[11] However, there were conflicting results about cytokine production patterns and protective immunity in the measles vaccine.[15]

Studies have been reported that measles-specific T cell-mediated immunity was elicited by primary measles vaccination.[13,14,16] Early dose of measles vaccination of 6-months-old infants increases the T cell responses which may prime the humoral response. Previous studies also reported that the primary measles immunization was sustained for 5–10 years of age regardless of the time it was administered.[14] These results suggested a pivotal role of cellular immune responses to the measles vaccine. Only a limited study evaluating the cellular immune response induced by $CD4^+$ and $CD8^+$ T cells in the measles vaccination. This is partly because of the experimental difficulties of stimulation induction and limited technical methods to provide precise quantification of measles virus-specific T cells. Hence, this study aimed to evaluate the humoral and cellular immunity to first dose measles vaccine Edmonston-Zagreb (EZ) in 9-month-old Indonesian infants.

Material and methods

Research participants

An observational cohort study was conducted on 9-month-healthy infants who were not immunized with the measles vaccination or had not got measles infection before. This study was performed in the Primary Health Center, Plumbon, Cirebon, West Java, Indonesia. The EZ strain of the measles vaccine and the MR vaccine (Batch Number 0128W0780 and 0128W0800) was provided by the Serum Institute of India (Pune, India). This study used the obtained measles vaccination as the first dose of the routine vaccination program for 9-month-old infants in Indonesia. The study included 35 infants (19 males and 16 females) between 9–12 months. Participants who had concurrent medical problems (such as malnutrition, infection, malignancy, a congenital or genetic disease), had prior measles infections and had received measles vaccinations in the past were excluded from this study. Informed written consent was obtained from the parent or parents, and 3 ml of peripheral blood samples were obtained by venepuncture before vaccination and at one and six months after vaccination. The research design is shown in Figure 1.

Peripheral blood mononuclear cells isolation, culture and stimulation

The peripheral blood sample was separated by the Ficoll-Paque method to separate red blood cells. These red blood cells were separated for PBMC fraction and plasma. The cellular fractions were subjected to flowcytometry analysis (XL, Beckman Coulter, Miami, FL, USA) to obtain the absolute cell population numbers (10^5–10^6 cells per well). Later

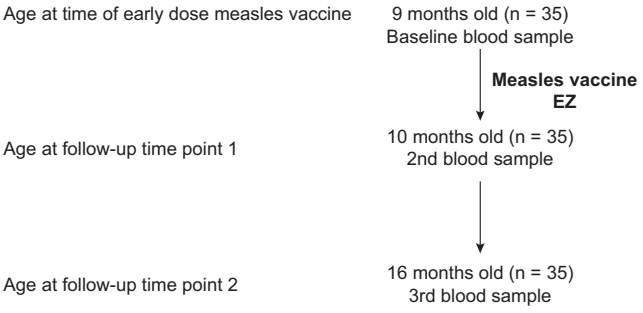

Figure 1 Research design representing the timing of the first measles vaccine and immune evaluations. Blood samples were collected at 1- and 6-months after the first-dose measles vaccine.

the cell suspension was incubated in a serum-free medium (Roswell Park Memorial Institute medium 1640, sodium bicarbonate, HEPES, and 1.25% penicillin-streptomycin), and complete medium at 5% carbon-di-oxide and 37°C for 24 hours. The cell suspension was then stimulated for 12 hours using H61 and H1 peptides (1 µg/ml) and peptide diluent dimethyl sulfoxide (DMSO). The H61 and H1 peptides were overlapping peptides, representing measles virus proteins, which were already synthesized and obtained from GENSCRIPT.[17]

Flow cytometry analysis of T cells expressing IFN- γ and IL-17A

The cell suspensions were surface labeled with CD4 (FITC Anti-human CD4 Antibody (Biolegend, Cat.no. 317408) and CD8 antibodies (PE/Cy5 Anti-human CD8a Antibody) (Biolegend, Cat.no. 300910). Later, it was permeabilized for 10 min in 4% paraformaldehyde and 1% saponin buffer before intracellular cytokine staining. Intracellular cytokine staining identifies T cells expressing IFN-γ (PE Anti-human IFN-γ Antibody; Biolegend, Cat.no. 502509) and IL-17A (PE Anti-human IL-17a Antibody; Biolegend, Cat.no. 512306). Flow cytometry acquisition and analysis were performed on a FACSCalibur (BD Biosciences, Franklin Lakes, NJ, USA).

Measles-specific immunoglobulin-G antibody measurement

A neutralization test evaluated the Measles-specific immunoglobulin-G (IgG) antibody level. The sample was heat-inactivated in a 56°C water bath for 30 min. Two-fold serial dilutions (1:2 to 1:256) of specimens along with positive and negative control sera were prepared in Dulbecco's Modified Eagle Medium (DMEM) media (Sigma-Aldrich, St. Louis, MO, USA). Fifty microliters of the median tissue culture infectious dose were added to 50 µl of each serum dilution and controls in microtiter plates and were placed on a shaker before being transferred to an incubator at 36°C for an hour. Post incubation, 50 µl of the serum-virus mixture was transferred to appropriate wells of another microplate containing monolayers of VERO cells and incubated at 36°C for 1 h for the adsorption of the virus on the monolayers. Later, 100 µl of DMEM containing antibiotics and 2% fetal bovine serum were added to each well. A cellular (normal cells and medium without virus and serum) and viral controls (50 µl of the prepared virus in 50 µl DMEM without serum) were also maintained for each plate. The microtiter plates were then placed on a 36°C incubator and checked for seven days for the presence of cytopathic effect (CPE). The highest test serum dilution that could prevent CPE of the measles virus was recorded as a measles antibody titer.[18] A protective neutralizing antibody titer was >1:8, and the antibody level was converted in mIU/ml. According to the 2nd International Standard for measles antibody assigned by the World Health Organization (WHO) Expert Committee on Biological Standardization, an antibody level ≥120 mIU/mL was considered as seropositive and safe.[19] The minimum antibody concentration detectable in our study was 106.95 mIU/ml. Measles IgG-specific antibody measurement was conducted at the Biofarma Laboratory (Bandung, Indonesia).

Statistical analysis

Descriptive statistics were used to analyze the demographic characteristics of subjects. The dependent variables were measles-specific antibody level, CD4 and CD8 T cells expressing IFN-γ and IL-17A. Comparative analysis of variables between time of evaluation was performed by analysis of variance or Kruskal-Wallis test. The Pearson or Spearman test was used to measure the antibody level and cellular immune response association to the measles vaccine. Data analyses were performed using the software program IBM SPSS Statistics, Version 25.0 (IBM SPSS Statistics, Armonk, NY, USA). $P \leq 0.05$ were considered significant.

Results

Research participant characteristics

Demographic data including age, gender, weight, length and body mass index were collected at the first visit and are shown in Table 1.

The study included 35 infants (19 males and 16 females) with a median age of 10 (9–12) months who were in healthy condition and were due for their measles vaccination and had not contradicted measles natural infection. The mean weight and height at enrolment were 9.81 ± 4.27 kg and 71.67 ± 2.80 cm, respectively. From the anthropometric analysis, four (11.4%) infants were classified as underweight. Seven (20%) were categorized with short stature.

Profile of measles-specific IgG antibody

All the participants had a measles-specific IgG antibody level <120 mIU/mL at baseline which meant that they had

Table 1 Demographic data of research participants.

Characteristics	
Sex, n (%)	
Male	19 (54.3)
Female	16 (45.7)
Age, months (Median (min-max))	10 (9-12)
Anthropometric data	
Weight, kg (mean ± SD)	9.81 ± 4.27
Height, cm (mean ± SD)	71.67 ± 3.80
BMI, kg/m^2 (mean ± SD)	18.34 ± 3.26
Z-score weight for age (n) (%)	
Normal	31 (88.6)
Underweight	4 (11.4)
Z-score height for age (n) (%)	
Normal	28 (80)
Short stature	7 (20)

no protective antibody against measles or were susceptible to infection and disease. There were changes in the mean of measles-specific IgG antibody level at 1- and 6-months postvaccination. The prevalence of seropositivity rates at 1-month was 85.8% and only 16.67% at 6-months postvaccination. The Kruskal-Wallis and Mann-Whitney test were performed on the measles-specific IgG antibody level mean to assess its dynamics. There was a significant increase in the measles-specific IgG antibody level between baseline and 1-month after vaccination (P < 0.001). At 6-months after vaccination, we found that the measles-specific IgG antibody level decreased significantly compared to 1-month after vaccination (P < 0.001). However, measles-specific IgG antibody level at 6-months was higher than baseline (P = 0.015; Figure 2; Table 2).

Characterization of CD4⁺ and CD8⁺ T cells producing IFN-g and IL-17A

This study evaluated the number of $CD4^+$ and $CD8^+$ T cells expressing IFN-g and IL-17A from PBMC after stimulation with measles-specific peptide. Both $CD4^+$ and $CD8^+$ T cells could be induced to produce IFN-g and IL-4, respectively. Table 2 shows the profile and range of the measles vaccine-induced cellular immune responses over time. We found a significant increase of $CD4^+$ T cells expressing IFN-g at 1-month after vaccination (P < 0.001). However, it was decreased significantly at 6-months compared to 1-month after vaccination (P = 0.003). No significant difference was observed in the number of $CD4^+$ T cells expressing IFN-g at 6-months and baseline, but it was still higher at 6-months after vaccination (P = 0.246). The number of $CD8^+$ T cells expressing IFN-g also increased significantly at 1-month (P < 0.001) and decreased at the 6-month but without any altered statistical difference (P = 0.329). Even though there was a decline in $CD8^+$ T cells expressing IFN-g, the number of $CD8^+$ T cells was still higher than that in the baseline (P < 0.001; Figure 3).

Both $CD4^+$ and $CD8^+$ T cells producing IL-17A increased significantly at 1-month after vaccination compared to baseline (P = 0.002, P < 0.001, respectively). The number of $CD4^+$ T cells producing IL-17A also decreased at 6-month versus 1-month after vaccination (P = 0.018), and no significant difference with baseline (p = 0.608) was observed. We also reported a decreasing number of $CD8^+$ T cells producing IL-17A at 6-months after vaccination, even though it was not significantly different (P = 0.464). The number of $CD8^+$ T cells producing IL-17A at 6-months was higher than that in the baseline (P = 0.015; Figure 4).

Correlation between measles-specific IgG antibody and cellular immune response.

This study demonstrated an increased measles-specific IgG antibody levels and T cells mediated immune response after the first dose of measles vaccination. From the

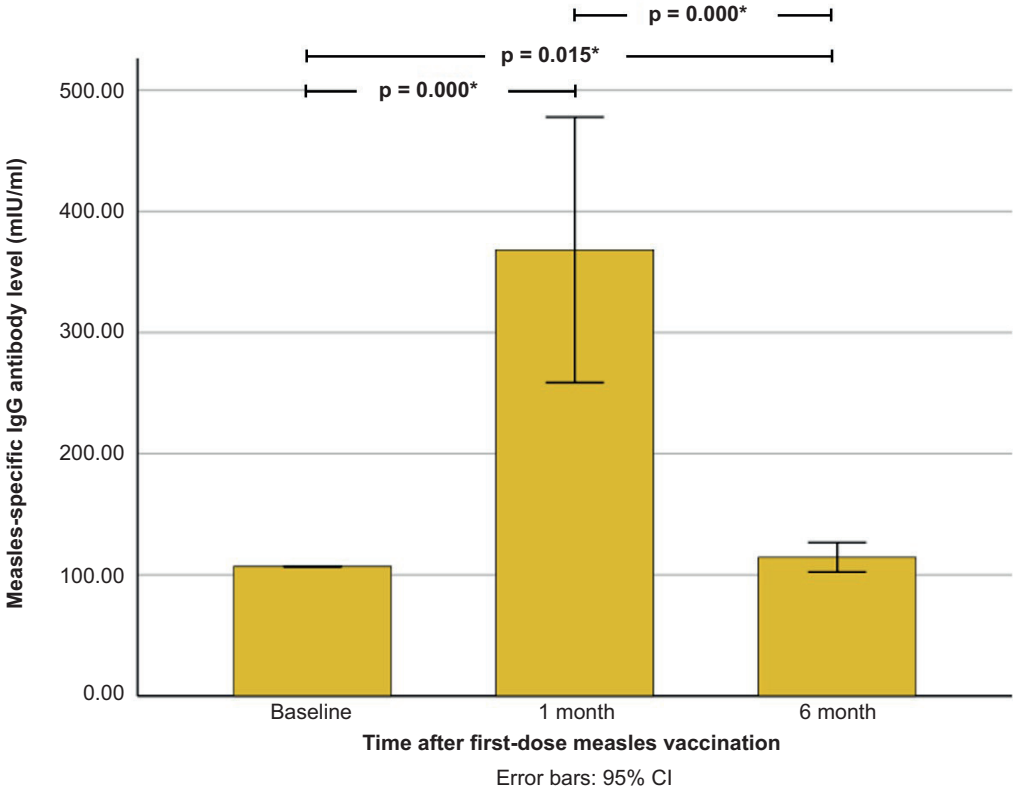

Figure 2 Comparison of measles-specific IgG antibody level over time of evaluation. *p <0.05 was statistically significant (Mann-Whitney test).

Table 2 Profile of measles-specific humoral and cellular immune responses.[a]

Immune response parameters	Baseline (n = 35)	1-month after first dose measles vaccine (n = 35)	6-months after first dose measles vaccine (n = 12)	p Value
IgG antibody (mIU/ml)	106.95	368.25 ± 319.06	114.48 ± 19.12	0.000*
Seronegativity rate, n (%)	35 (100)	5 (14.20)	10 (83.33)	
Seropositivity rate, n (%)	0	30 (85.80)	2 (16.67)	
$CD4^+IFN\text{-}\gamma^+$ (%)	3.45 ± 2.04	7.37 ± 3.72	4.22 ± 1.25	0.000*
$CD8^+IFN\text{-}\gamma^+$ (%)	1.78 ± 2.03	5.19 ± 3.43	3.84 ± 1.67	0.000*
$CD4^+IL\text{-}17A^+$ (%)	4.73 ± 1.47	6.76 ± 2.71	4.64 ± 2.09	0.003*
$CD8^+IL\text{-}17A^+$ (%)	2.58 ± 1.15	4.30 ± 1.96	3.74 ± 1.48	0.000*

[a] Data were shown as mean ± standard deviation.
*p <0.05 was statistically significant (Kruskal-Wallis test).

Spearman correlation analysis, a significant positive correlation between the number of $CD4^+$ and $CD8^+$ T cells producing IFN-γ and IL-17A and measles-specific IgG antibody level ($P < 0.05$) was established. However, from the correlation coefficients, we observed no significant correlation between variables (Table 3).

Discussion

Several studies have been conducted to assess the effectiveness and antibody response to measles vaccines. Only limited information observing both humoral and cellular immune response against the measles vaccine is available.[20] This study was a pilot study with cohort design conducted on 9-months-old Indonesian infants who received their first-dose measles vaccine EZ. Here relatively high seropositivity rates among vaccinated 9-month-old infants (85.8%) after 1-month of first-dose measles vaccine. Several studies reported that approximately 85% of children develop protective antibody levels when given one dose of measles vaccine at nine months of age, and 90–95% respond when vaccinated at 12 months of age.[8] A cohort study conducted in California reported similar results, the seroconversion rate in 9-month-old infants, 12 weeks after measles vaccination, was 97%.[13]

It was important to evaluate when the antibodies start to provide decline to provide protection. The study by LeBaron et al.[21] showed that involved as many as 364 participants in the United States showed that measles antibodies increased rapidly one month after vaccination, then begin to decrease gradually, but showed persistent protection levels for up to 10 years after immunization with two doses of the measles vaccine. Another study conducted in Pakistan reported that the early EZ measles vaccine in 9-month-old infants raised protective antibody levels in most children. The antibody concentrations increased dramatically within two weeks after immunization and faded slowly with time.[22] Our study also reported similar outcomes. There was a significant increase of measles-specific IgG antibody level at 1-month and a decline at 6-months post initial dose of measles vaccine. An indication of re-vaccination was required to increase the antibody titers, already reported in many previous studies. The cohort study by Gans et al.[9] showed that neutralizing antibody concentrations were lower following the first measles vaccine. However, responses could be boosted by subsequent doses. Several other studies showed that initiating two doses of the measles vaccine were significantly better at increasing antibody levels than a single dose.[23-25] A systematic review also suggested that administering a first-dose measles vaccine followed by additional measles vaccine doses result in high seropositivity and vaccine effectiveness.[20]

In addition to measles-specific antibody titers, a study by Griffin[10] also assessed the role of cellular immunity in the immune response to measles vaccination. In measles virus infection, immune activation and lymphocyte proliferation, $CD4^+$ and $CD8^+$ T cells occur acutely and persist for several months after resolution. During this period, there was a change in the cytokine production pattern from Th1 cell cytokines (IFN-γ) to Th2 cell cytokines (IL-4, IL-10, IL-13) and the appearance of IL-17 production by Th17 cells. This change induces the maturation of B lymphocyte cells into a plasma that secretes specific IgG antibodies.[26] The *in vivo* study of Nelson et al.[17] evaluated the cellular immune responses specific to T lymphocyte cells on monkeys infected with wild-type strains of the measles virus. They reported an increase in $CD4^+$ T cells and $CD8^+$ T cells producing IFN-γ and IL-17 to hemagglutinin protein (H) and nucleocapsid (N) on days 2–3, 8, and 18–24 weeks after infection. T lymphocytes producing IFN-γ increase in the initial phase of infection while IL-17A increases in the final phase.

However, in this study we found that $CD4^+$ and $CD8^+$ T cells were equally prominent and produce both IFN-γ and IL-17A at 1-month postvaccination that agreed with the study evaluating cytokine secretion patterns following the measles vaccine in infants. It showed that cytokines (IL-2, IL-6 and IFN-γ) increased on day 30 postvaccination to levels greater than baseline levels.[15] These results showed that both Th1 and Th2 cytokine production were also detected after the first dose of measles vaccination. No study to date has evaluated the pattern of Th17 T cells in response to the measles vaccine. It has been suggested that Th1 cells have a direct role in the clearance of measles vaccine protein antigen, whereas Th2 and Th17 play indirect roles by induction of immune cells recruitment and antibody production.[27,28] Another study also reported an increase of measles-specific T cell proliferation in 3-months after the

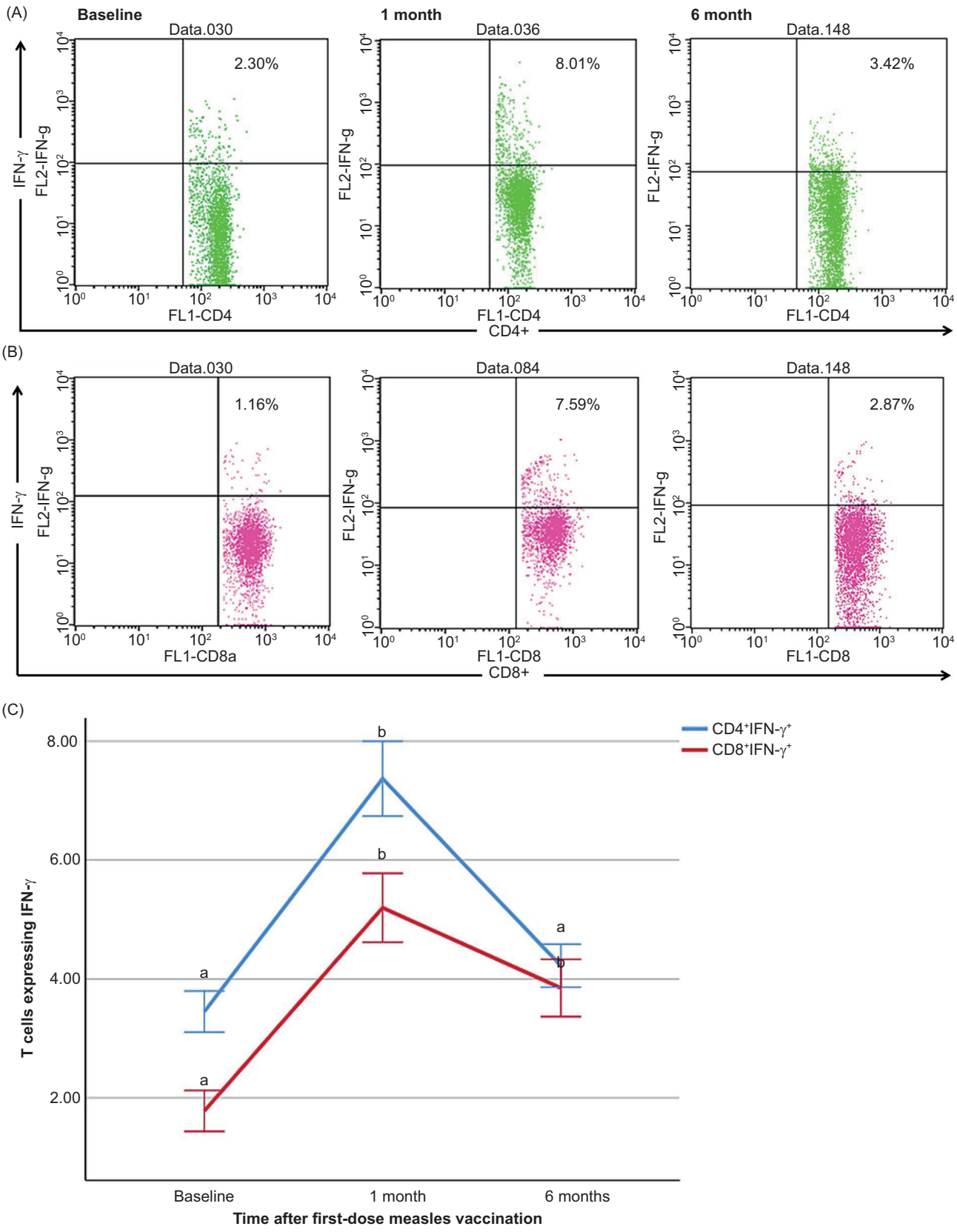

Figure 3 Flow cytometric analysis of T cell producing IFN-g. Gated CD4+ IFN-ɣ$^{+}$ cells at baseline, 1- and 6-months after measles EZ vaccination (A). Gated CD8+ IFN-ɣ$^{+}$ cells at baseline, 1- and 6-months after measles EZ vaccination (B). Comparison of CD4^{+} and CD8^{+} T cells expressing IFN-ɣ overtime of evaluation (C). *p < 0.05 was significant statistically (Mann-Whitney test).

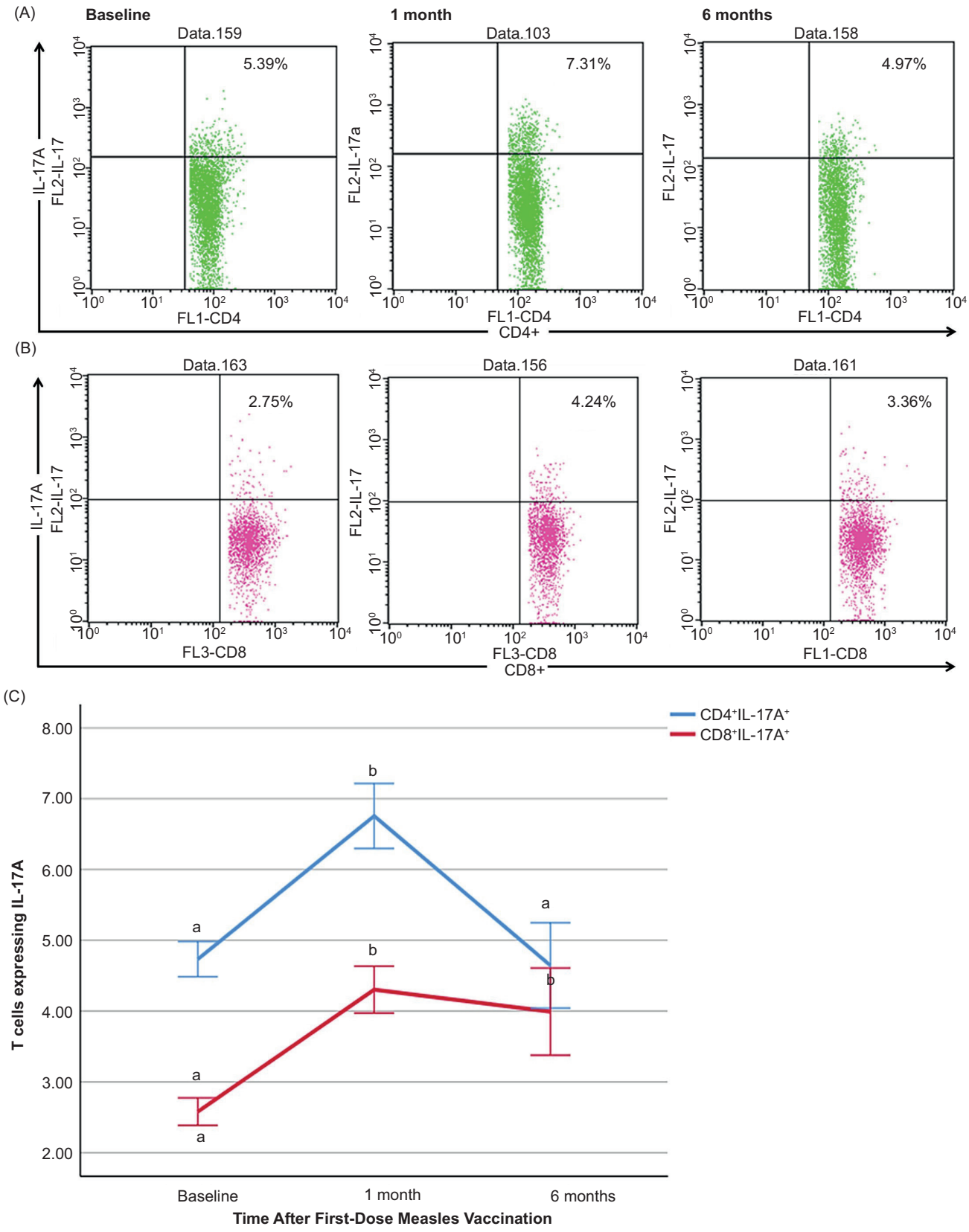

Figure 4 Flow cytometric analysis of T cell producing IL-17A. Gated CD4+ IL-17A+ cells at baseline, 1- and 6-months after measles EZ vaccination (A). Gated CD8+ IL-17A+ cells at baseline, 1- and 6-months after measles EZ vaccination (B). Comparison of $CD4^+$ and $CD8^+$ T cells expressing IL-17A over time of evaluation (C). *$p < 0.05$ was statistically significant (Mann-Whitney test).

Table 3 Correlation analysis of cellular immune response and measles antibody level.

Immune response parameters to measles antibody level	p Value	r
$CD4^+$ IFN-γ^+	0.003*	0.320
$CD8^+$ IFN-γ^+	0.004*	0.315
$CD4^+$ IL-17A$^+$	0.002*	0.331
$CD8^+$ IL-17A$^+$	0.000*	0.376

*p<0.05 was significant statistically (Spearman Correlation test).
r: correlation coefficient.

initial measles vaccine and 6-months after the second-dose measles vaccine in 6- or 9-month-old infants.[14] In this study, $CD4^+$ and $CD8^+$ T cells producing IFN-γ and IL-17A decrease at 6-months after vaccination. This outcome supports the necessity of the second dose of the measles vaccine to increase cellular immune response. There were limited studies that explained the evolution of $CD4^+$ or $CD8^+$ T cells in the measles vaccine response. Hence this is not fully understood.[29]

Our study demonstrated that the measles vaccine T-cell responses follow a similar pattern with humoral immunity. The number of $CD4^+$ and $CD8^+$ T cells producing IFN-γ and IL-17A were correlated with measles-specific IgG antibody levels. Another study also indicated high antibody titers and cell-mediated immune responses at 3–4 weeks after measles vaccination.[30] The study by Laksono et al.[11] reported no relationship between any specific cytokine level and measles antibody level even though there was an increase of humoral and cellular immune responses after the early-dose measles vaccine.

Strengths and limitations

This study has several limitations. First, this study was conducted with a short time of evaluation. Long-term evaluation of immune response against measles vaccine can be pivotal in evaluating the measles vaccine immune response for deciding on its booster dose administration. Second, this study was single centered, with limitations such as minimal sample size, which can hinder the outcome interpretation. Lastly, many confounding factors such as nutritional status associated with vaccine immune response were not studied.

Being the first pilot study evaluating specific immune responses to the EZ measles vaccine in Indonesian infants was the strength of this study.

Conclusion

In conclusion, the first dose of the EZ measles vaccine in 9-months-old infants increased humoral and cellular immune responses at 1-month after vaccination. However, it tended to decrease at 6-months. This pilot study conducted in Indonesia evaluated the specific immune response to measles vaccine. Further multicenter studies with large samples should evaluate this study's outcomes.

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