

- patients with IgE antibodies specific for galactose- α -1,3-galactose. *J Allergy Clin Immunol* 2009;123:426-33.
2. Commins SP, Platts-Mills TA. Delayed anaphylaxis to red meat in patients with IgE specific for galactose α -1,3-galactose (alpha-gal). *Curr Allergy Asthma Rep* 2013;13:72-7.
 3. Morisset M, Richard C, Astier C, Jacquenet S, Croizier A, Beaudouin E, et al. Anaphylaxis to pork kidney is related to IgE antibodies specific for galactose- α -1,3-galactose. *Allergy* 2012;67:699-704.
 4. Ring J, Messmer K. Incidence and severity of anaphylactoid reactions to colloid volume substitutes. *Lancet* 1977;1:466-9.
 5. Wölbing F, Fischer J, Köberle M, Kaesler S, Biedermann T. About the role and underlying mechanisms of co-factors in anaphylaxis. *Allergy* 2013;68:1085-92.
 6. Mullins RJ, James H, Platts-Mills TA, Commins S. Relationship between red meat allergy and sensitization to gelatin and galactose- α -1,3-galactose. *J Allergy Clin Immunol* 2012;129:1334-42.
 7. Caponetto P, Fischer J, Biedermann T. Gelatin-containing sweets can elicit anaphylaxis in a patient with sensitization to galactose- α -1,3-galactose. *J Allergy Clin Immunol Pract* 2013;1:302-3.
 8. Commins SP, James HR, Kelly LA, Pochan SL, Workman LJ, Perzanowski MS, et al. The relevance of tick bites to the production of IgE antibodies to the mammalian oligosaccharide galactose- α -1,3-galactose. *J Allergy Clin Immunol* 2011;127:1286-93.
 9. Hamsten C, Starkhammar M, Tran TA, Johansson M, Bengtsson U, Ahlén G, et al. Identification of galactose- α -1,3-galactose in the gastrointestinal tract of the tick *Ixodes ricinus*; possible relationship with red meat allergy. *Allergy* 2013;68:549-52.

<http://dx.doi.org/10.1016/j.jaci.2014.05.051>

Control lymphocyte subsets: Can one country's values serve for another's?

To the Editor:

Reliable interpretation of abnormal immunity depends on an understanding of what constitutes “normal.” We and others have reported that lymphocyte patterns are affected by ethnicity, sex, and environmental factors.¹⁻³ Differing exposure to infectious agents, such as malaria, tuberculosis, and cytomegalovirus, could underlie differences in lymphocyte subset patterns in contrasting settings.⁴⁻⁶ In this study we analyzed combined data from 3 studies of healthy children from Thailand,¹ Malawi,² and the United States³ who had used similar standard flow cytometric methods and markers, thus allowing assessment of immune heterogeneity across settings with differences in ethnicity and infectious disease exposure.

We used lymphocyte subset data from healthy children aged 0 to 15 years enrolled in our previously published studies¹⁻³; additional Thai subjects less than 2 years of age were recruited to allow comparisons with the youngest children. Exclusion criteria were febrile illness, clinical infection at screening, concomitant medical illnesses potentially resulting in abnormal immunity, or abnormal growth (defined as <3rd or >97th percentiles of the country's growth chart for Thai and US children or a weight-for-height *z* score <70% for Malawian children). Rapid HIV diagnostic kits were used with PCR to confirm discordant results. Statistical analyses were performed in a manner similar to previous publications (details are shown in the [Methods](#) section in this article's Online Repository at www.jacionline.org).¹⁻³

Of 1399 children enrolled (Thailand, *n* = 281; Malawi, *n* = 397; and the United States, *n* = 721), approximately 50% were male (see [Table E1](#) in this article's Online Repository at www.jacionline.org), and approximately 50% were between 0 and 2 years of age. The Thai and Malawian cohorts were ethnically homogenous; the US cohort had a mix of African American (53%), Hispanic (29%), and Euro-Caucasian (17%) subjects.

The following lymphocyte subpopulations were identified as percentages of the lymphocyte gate in all 3 cohorts: T cells (CD3⁺), CD4⁺ T cells (CD3+CD4⁺), CD8⁺ T cells (CD3⁺CD8⁺), B cells (CD19⁺), and B memory cells (CD19⁺CD27⁺). [Fig 1](#) compares cell counts of total T cells, CD4⁺ T cells, and CD8⁺ T and B cells by age and country. Graphs representing other cells and percentages are provided online (see [Figs E1 and E2](#) in this article's Online Repository at www.jacionline.org).

Thai children had higher total T-cell counts compared with the US and Malawian cohorts aged 6 months to 4 years and 0 to 24 months, respectively. Malawian children aged 0 to 5 months had lower total T-cell counts compared with US children. Compared with the US and Thai cohorts, Malawian children aged 0 to 11 and 0 to 17 months, respectively, had lower T-cell percentages.

Thai children aged 6 to 11 months had higher CD4⁺ T-cell counts but a lower percentage of CD4⁺ T cells compared with US children of the same age group. Compared with US children, Malawian children had lower CD4⁺ T-cell counts from 0 to 5 months of age and greater CD4⁺ T-cell counts from 8 to 15 years of age, whereas CD4⁺ T-cell percentages were lower from 0 to 17 months of age but greater from 8 to 11 years of age. Compared with Thai children, CD4⁺ T-cell counts were lower in Malawian children aged 0 to 11 and 18 to 24 months of age but greater in those aged 5 to 7 and 12 to 15 years of age. Percentages of CD4⁺ T cells were similarly lower in Malawian children aged 0 to 12 months of age but consistently higher than those seen in Thai children aged 2 to 15 years of age.

Higher CD8⁺ T-cell counts (birth to 4 years and 8-11 years) and percentages (birth to 18 months and 2-7 years) were observed in Thai compared with US children and also compared with Malawian children for absolute counts from 0 to 17 months and 8 to 11 years and percentages from 0 to 5 months and 8 to 11 years. These values were also higher among Malawian compared with US children for certain age groups (6-17 months for absolute CD8⁺ T-cell counts and percentages and 2-4 years for percentages only). B-cell and natural killer (NK) cell results are presented in the [Results](#) section in this article's Online Repository at www.jacionline.org.

In secondary analyses geometric mean ratios were predominantly consistent with the significance results from nonparametric tests, with few apparent sex differences, although the power to detect these differences was limited male (see [Table E2](#) in this article's Online Repository at www.jacionline.org).

This analysis of lymphocyte subsets of children from countries within 3 different continents demonstrates a marked heterogeneity in subset patterns of HIV-uninfected children, the extent of which is most marked in children younger than 2 years. The differences in subset patterns might be driven by several factors, including ethnicity, with participants from the 3 countries being of different ethnicities. Previous healthy control studies in Asian and African subjects have reported smaller proportions of CD4⁺ T-cell subsets and larger proportions of CD8⁺ T-cell subsets in these populations compared with those observed in populations in the developed world.^{2,7-10} However, ethnic differences alone might not account for all subset differences because variations within specific ethnic groups have also been reported.^{2,8,11,12} Environmental factors, particularly prevalent infectious diseases, might affect cellular phenotypic patterns.¹³

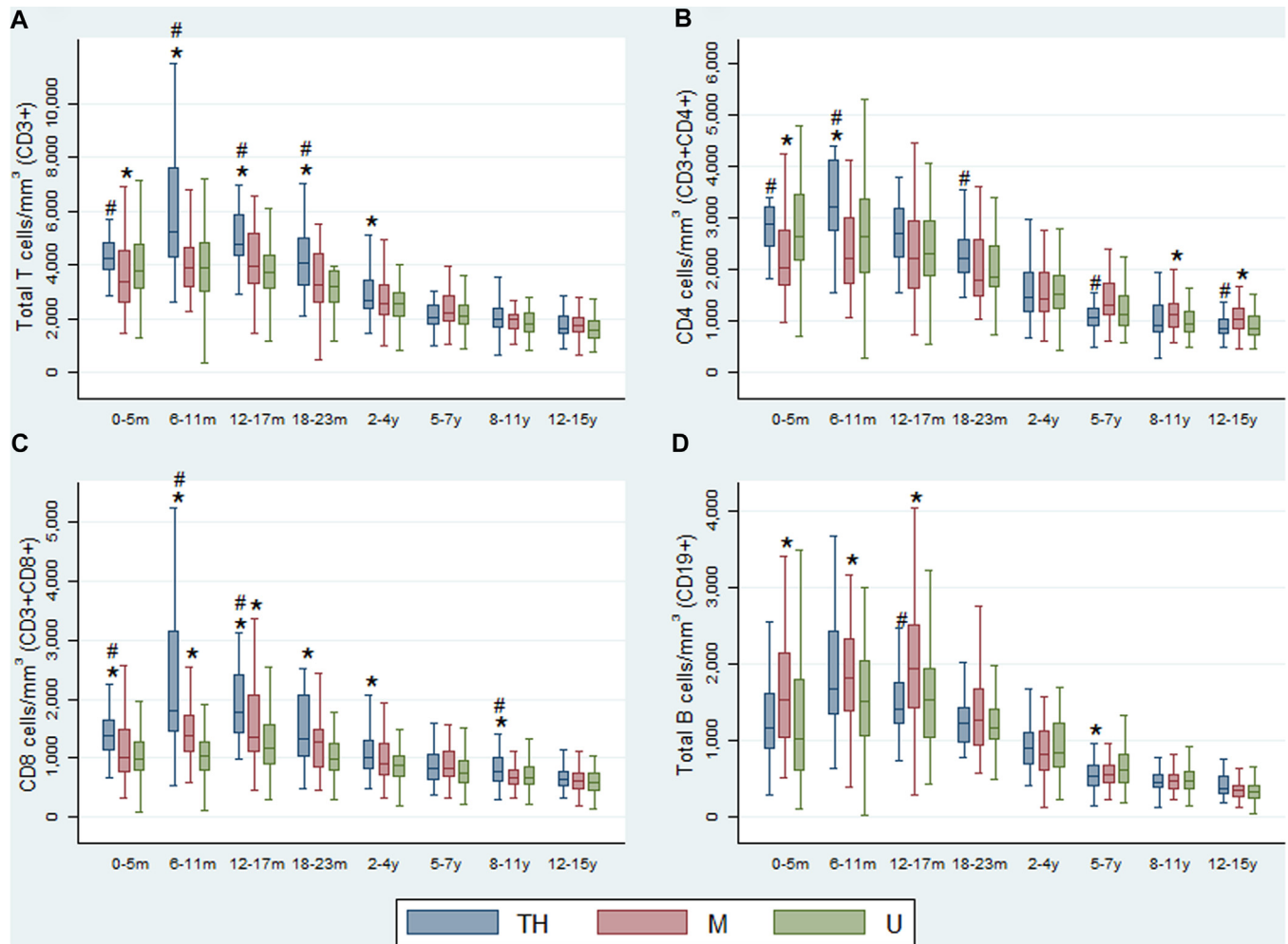


FIG 1. Box plots of total T-cell (A), CD4⁺ T-cell (B), CD8⁺ T-cell (C), and total B-cell counts (D) (box, 25th-75th percentiles; line, median; whiskers, lowest and highest points within 1.5 × the interquartile range) in children by country cohort and age group. Pairwise comparisons between lymphocyte subset distributions of children from each country were made in each age group by using a Mann-Whitney-Wilcoxon test, and *P* values of less than .05 were considered significant. *Significant difference in the Malawian or Thai (depending on the bar below the symbol) versus the US cohort. #Significant difference from the Malawian cohort versus the Thai cohort. M, Malawian cohort; TH, Thai cohort; U, US cohort.

The high median B-cell counts observed in Malawian children in younger age groups, which have been reported in other African cohorts,^{8,12} might be associated with malaria, which is prevalent in Malawi but less common in Thailand and absent in the United States. Children living in malaria-endemic areas are known to exhibit expansion of atypical memory B cells, which are thought to be driven by parasite antigens and could have beneficial immune-modulating effects for the host.¹⁴ In contrast, Thailand is a dengue-endemic area, with most Thai children being dengue seropositive by 5 years of age,¹⁵ which could explain the pattern of higher CD4⁺ and CD8⁺ T-cell and NK cell counts observed in Thai children. As a likely confounding factor, participants in all 3 cohorts were screened for HIV, and those who were HIV seropositive were not included in the analysis. It is also possible that the prominent CD8⁺ T cells and NK cells seen in Thai children could result partly from air pollution.¹³ The cause underlying the diversity between cohorts still remains speculative and deserves further investigation. Limitations to this study include some differences

in the details of the flow cytometric methodologies used by the 3 countries and possible variation in the nutritional status of the participants.

In conclusion, this study highlights that distinct patterns of lymphocyte subsets exist between populations from different countries with different ethnicities and exposed to different environmental and nutritional factors. Therefore population-specific healthy control subject reference ranges for different lymphocyte subsets should be developed for the diagnosis and monitoring of immunologic disorders.

We thank the children and families for their participation in the study, the staff from all participating sites for their contribution, and Ms Piraporn June Ohata for her assistance in preparing the manuscript. A complete list of the Study Team members can be found in this article's Online Repository at www.jacionline.org.

Wilson L. Mandala, PhD^{a,b,c,*}
Jintanat Ananworanich, MD, PhD^{d,e,f,*}

Tanakorn Apornpong, MSc^d
Stephen J. Kerr, PhD^{d,g}
Jenny M. MacLennan, MRCP, MRCPCH^{a,h}
Celine Hanson, MDⁱ
Tanyathip Jaimulwong, MSc^d
Esther N. Gondwe, PhD^{a,b}
Howard M. Rosenblatt, MD^j
Torsak Bunupuradah, MD^d
Malcolm E. Molyneux, FMedSci^{a,b}
Stephen A. Spector, MD^k
Chitsanu Pancharoen, MD^j
Rebecca S. Gelman, PhD^{l,m}
Calman A. MacLennan, DPhil, FRCP, FRCPath^{a,n,o}
William T. Shearer, MD, PhD^p

From ^athe Malawi-Liverpool-Wellcome Trust Clinical Research Programme and ^cthe Department of Basic Medical Sciences, College of Medicine, Blantyre, Malawi; ^bthe Liverpool School of Tropical Medicine, Liverpool, United Kingdom; ^dHIV Netherlands Australia Thailand Research Collaboration (HIV-NAT) and ^eSEARCH, Thai Red Cross AIDS Research Centre, Bangkok, Thailand; ^fthe Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; ^gthe Kirby Institute, UNSW Australia, Sydney, Australia; ^hthe Department of Zoology, University of Oxford, United Kingdom; ⁱBaylor College of Medicine, Texas Children's Hospital, Houston, Tex; ^jSpecialty for Children, Austin, Tex; ^kUniversity of California at San Diego and Rady Children's Hospital, San Diego, Calif; ^lHarvard Medical School, Boston, Mass; ^mthe Dana-Farber Cancer Institute, Boston, Mass; ⁿthe MRC Centre for Immune Regulation & Clinical Immunology Service, School of Immunity and Infection, College of Medicine, University of Birmingham, Birmingham, United Kingdom; ^oNovartis Vaccines Institute for Global Health, Siena, Italy; and ^pAllergy & Immunology, Texas Children's Hospital, Baylor College of Medicine, Houston, Tex. E-mail: wmandala2002@gmail.com.

*These authors contributed equally to this work.

The Thai study was supported by The Thailand Research Fund (grant BRG5280017) and the National Institute of Allergy and Infectious Diseases (grant R01AI075408-0, to J.A.). The Malawian study was supported by a Gates Malaria Partnership PhD studentship, funded by the Bill and Melinda Gates Foundation (to W.L.M.), a Wellcome Trust Research Fellowship (to C.A.M.), a Wellcome Trust Programme Grant (to M.E.M.), and a Clinical Research Fellowship from GlaxoSmithKline (to C.A.M.). The US study was supported by the American Academy of Allergy, Asthma, and Basic and Clinical Immunology Interest Section Research Award (to W.T.S.) and the National Institute of Allergy and Infectious Diseases (grants U01AI27551, R01 AI94029, P30 AI36211 [to W.T.S.]; U01 AI41110 [to R.S.G.], and by General Clinical Research Grants M01 RR-00188 [to W.T.S.]).

Disclosure of potential conflict of interest: J. Ananworanich has received compensation for board membership from ViiV Healthcare, and her institution has received funding from Gilead. C. A. MacLennan is employed by the Novartis Vaccines Institute for Global Health, which has received or has grants pending from GlaxoSmithKline. W. T. Shearer is employed by the Baylor College of Medicine/Texas Children's Hospital. The rest of the authors declare that they have no other relevant conflicts of interest.

REFERENCES

1. Ananworanich J, Apornpong T, Kosalaraksa P, Jaimulwong T, Hansudewechakul R, Pancharoen C, et al. Characteristics of lymphocyte subsets in HIV-infected, long-term nonprogressor, and healthy Asian children through 12 years of age. *J Allergy Clin Immunol* 2010;126:1294-301.e10.
2. Mandala WL, MacLennan JM, Gondwe EN, Ward SA, Molyneux ME, MacLennan CA. Lymphocyte subsets in healthy Malawians: implications for immunologic assessment of HIV infection in Africa. *J Allergy Clin Immunol* 2010;125:203-8.
3. Shearer WT, Rosenblatt HM, Gelman RS, Oyomopito R, Plaeger S, Stiehm ER, et al. Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study. *J Allergy Clin Immunol* 2003;112:973-80.
4. Ben-Smith A, Gorak-Stolinska P, Floyd S, Weir RE, Lalor MK, Mvula H, et al. Differences between naive and memory T cell phenotype in Malawian and UK adolescents: a role for Cytomegalovirus? *BMC Infect Dis* 2008;8:139.
5. Weiss GE, Crompton PD, Li S, Walsh LA, Moir S, Traore B, et al. Atypical memory B cells are greatly expanded in individuals living in a malaria-endemic area. *J Immunol* 2009;183:2176-82.
6. Sutherland JS, Hill PC, Adetifa IM, de Jong BC, Donkor S, Joosten SA, et al. Identification of probable early-onset biomarkers for tuberculosis disease progression. *PLoS One* 2011;6:e25230.
7. Jiang W, Kang L, Lu HZ, Pan X, Lin Q, Pan Q, et al. Normal values for CD4 and CD8 lymphocyte subsets in healthy Chinese adults from Shanghai. *Clin Diagn Lab Immunol* 2004;11:811-3.
8. Sagnia B, Ateba Ndongo F, Ndiang Moyo Tetang S, Ndongo Torimiro J, Cairo C, Domkam I, et al. Reference values of lymphocyte subsets in healthy, HIV-negative children in Cameroon. *Clin Vaccine Immunol* 2011;18:790-5.
9. Amatya R, Vajpayee M, Kaushik S, Kanswal S, Pandey RM, Seth P. Lymphocyte immunophenotype reference ranges in healthy Indian adults: implications for management of HIV/AIDS in India. *Clin Immunol* 2004;112:290-5.
10. Likansakul S, Wasi C, Thepthai C, Suthent R, Louisiriratchanakul S, Chearskul S, et al. The reference range of CD4+ and CD8+ T-lymphocytes in healthy non-infected infants born to HIV-1 seropositive mothers: a preliminary study at Siriraj Hospital. *Southeast Asian J Trop Med Public Health* 1998;29:453-63.
11. Lugada ES, Mermin J, Kaharuza F, Ulvestad E, Were W, Langeland N, et al. Population-based hematologic and immunologic reference values for a healthy Ugandan population. *Clin Diagn Lab Immunol* 2004;11:29-34.
12. Embree J, Bwayo J, Nagelkerke N, Njenga S, Nyange P, Ndinya-Achola J, et al. Lymphocyte subsets in human immunodeficiency virus type 1-infected and uninfected children in Nairobi. *Pediatr Infect Dis J* 2001;20:397-403.
13. Duramad P, Tager IB, Holland NT. Cytokines and other immunological biomarkers in children's environmental health studies. *Toxicol Lett* 2007;172:48-59.
14. Weiss GE, Crompton PD, Li S, Walsh LA, Moir S, Traore B, et al. Atypical memory B cells are greatly expanded in individuals living in a malaria-endemic area. *J Immunol* 2009;183:2176-82.
15. Dejnirattisai W, Duangchinda T, Lin CL, Vasanawathana S, Jones M, Jacobs M, et al. A complex interplay among virus, dendritic cells, T cells, and cytokines in dengue virus infections. *J Immunol* 2008;181:5865-74.

<http://dx.doi.org/10.1016/j.jaci.2014.06.030>

METHODS

Flow cytometric analyses are widely used for the diagnosis of primary immune deficiencies^{E1} and, more recently, have been used more to facilitate assessment of immunodeficiency in HIV infection and progression. CD4 counts and percentages are essential in the management of HIV infection and initiation of antiretroviral therapy. CD4/CD8 ratios can be used to diagnosis HIV infection in HIV-exposed children less than 2 years of age instead of molecular testing.^{E2-E4} This study compared lymphocyte subset data from healthy children aged between 0 and 15 years recruited in previously published studies.^{E5-E7}

Lymphocyte subset data from a total of 1399 children (281 Thai children, 397 Malawian children, and 721 American children) aged between 0 and 2 years were collected and compared. Statistical analysis was conducted with STATA 12 software (StataCorp, College Station, Tex). Consistent with our previous work, categorical age groupings were made in 6-month intervals up to 24 months of age and then in 3- or 4-year age bands from 2 to 7 years and 8 to 15 years of age, respectively. Pairwise comparisons between lymphocyte subset count and percentage distributions of children from each country were made in each age group by using a Mann-Whitney-Wilcoxon test. In a secondary analysis log-transformed CD4⁺ T-cell, CD8⁺ T-cell, and B-cell counts and percentages were used to calculate pairwise geometric mean ratios within age bands and between countries, adjusting for sex. The proportion of children who met 2006 World Health Organization HIV treatment guideline CD4 count criteria for immune deficiency^{E8,E9} were determined by using a χ^2 test, using percentages for children 59 months of age or less, and counts or percentages for those aged 5 to 15 years. No adjustments were made for multiple comparisons; *P* values of less than .05 were considered significant.

RESULTS

Malawian children exhibited higher B-cell counts from 0 to 17 months of age (Fig 1, D) and from 0 to 11 months of age for percentages (Fig E1, D) than US children but had lower percentages from 5 to 7 years of age. Thai children had lower B-cell counts compared with American children from 5 to 7 years of age; in addition, they had lower B-cell percentages from 12 months to 11 years of age. From 0 to 2 years of age, Malawian children had higher B-cell percentages than Thai children and higher B-cell counts from 12 to 18 months of age.

Thai children had higher NK cell counts (in 5 different age groups) and percentages (in 5 different age groups) than American children (Fig E2, A and B), but Malawian children had higher NK cell counts and percentages compared with American children only in the 12- to 15-year age group. Relative to Malawian children, NK cell counts were higher in Thai children aged 1 to 4 and 8 to 15 years, and NK percentages were higher in Thai subjects aged greater than 12 months.

Compared with US children, Malawians from 0 to 18 months and 2 to 4 years of age tended to have significantly lower CD4/CD8 ratios (although the 8- to 11-year group had a significantly higher ratio), whereas Thai children had lower CD4/CD8 ratios than the US children for all age groups except aged 18 to 24 months (Fig E3). Although all study participants were HIV uninfected, a higher proportion of Malawian children less than 36 months of age and Thai children aged 12 to 35 months met CD4 count immune deficiency criterion for HIV-infected children than US children (Fig E4). This implies that in the Malawian and Thai context it would not be appropriate or accurate to assume

that a low CD4 percentage alone, based on the World Health Organization criteria, is indicative of HIV infection.

STUDY TEAM MEMBERS FOR THE THAILAND STUDY

HIV-NAT center:

Thanyawee Puthanakit
Wasana Prasitsuebsai
Oratai Butterworth
Thongsuai Chuanjaroen
Tawan Mengthaisong
Sasiwimol Ubolyam
Apicha Mahanontharit
Phonethipsavahn Nounthong
Channuwat Bouko
Bunruan Sopa

Pediatric Infectious Diseases Unit, Chulalongkorn University Hospital:

Arene Klinklom
Chareeya Thaneer
Nichada Naknoi
Napasri Kuljarusiri
Areeratana Piromwong

REFERENCES

- E1. Oliveira JB, Notarangelo LD, Fleisher TA. Applications of flow cytometry for the study of primary immune deficiencies. *Curr Opin Allergy Clin Immunol* 2008;8:499-509.
- E2. Pahwa S, Read JS, Yin W, Matthews Y, Shearer W, Diaz C, et al. CD4⁺/CD8⁺ T cell ratio for diagnosis of HIV-1 infection in infants: Women and Infants Transmission Study. *Pediatrics* 2008;122:331-9.
- E3. O'Gorman MR, Zijenah LS. CD4 T cell measurements in the management of antiretroviral therapy—a review with an emphasis on pediatric HIV-infected patients. *Cytometry B Clin Cytom* 2008;74(suppl 1):S19-26.
- E4. Zijenah L, Katzenstein DA, Nathoo KJ, Rusakaniko S, Tobaiwa O, Gwanzura C, et al. T lymphocytes among HIV-infected and -uninfected infants: CD4/CD8 ratio as a potential tool in diagnosis of infection in infants under the age of 2 years. *J Transl Med* 2005;3:6.
- E5. Ananworanich J, Apornpong T, Kosalaraksa P, Jaimulwong T, Hansudewechakul R, Pancharoen C, et al. Characteristics of lymphocyte subsets in HIV-infected, long-term nonprogressor, and healthy Asian children through 12 years of age. *J Allergy Clin Immunol* 2010;126:1294-301.e10.
- E6. Mandala WL, MacLennan JM, Gondwe EN, Ward SA, Molyneux ME, MacLennan CA. Lymphocyte subsets in healthy Malawians: implications for immunologic assessment of HIV infection in Africa. *J Allergy Clin Immunol* 2010;125:203-8.
- E7. Shearer WT, Rosenblatt HM, Gelman RS, Oyomopito R, Plaeger S, Stiehm ER, et al. Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study. *J Allergy Clin Immunol* 2003;112:973-80.
- E8. World Health Organization. HIV/AIDS Programme. Strengthening health services to fight HIV/AIDS. WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. Available at: www.who.int/hiv/pub/guidelines/HIVstaging150307.pdf. Accessed August 6, 2012.
- E9. Antiretroviral therapy of HIV infection in infants and children in resource-limited settings: towards universal access. Geneva: World Health Organization; 2006. Available at: <http://www.who.int/hiv/pub/guidelines/paediatric020907.pdf>. Accessed July 22, 2009.

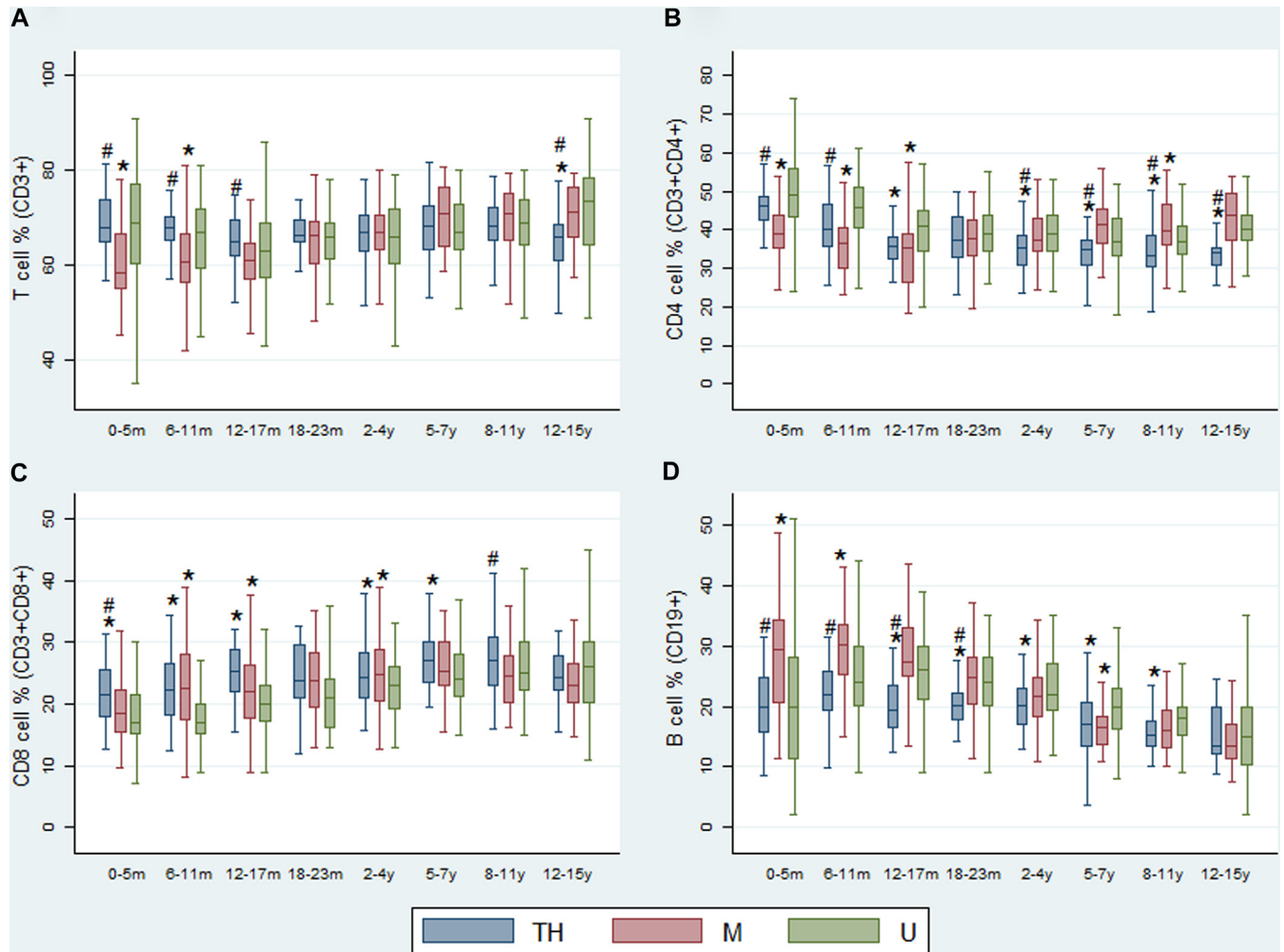


FIG E1. Box plots of total T-cell (A), CD4⁺ T-cell (B), CD8⁺ T-cell (C), and total B-cell (D) percentages in children by country cohort and age group. Pairwise comparisons between lymphocyte subset distributions of children from each country were made in each age group by using a Mann-Whitney-Wilcoxon test, and *P* values of less than .05 were considered significant. *Significant difference in the Malawian or Thai versus (depending on the bar below the symbol) the US cohort. #Significant difference from the Malawian cohort versus the Thai cohort. M, Malawian cohort; TH, Thai cohort; U, US cohort.

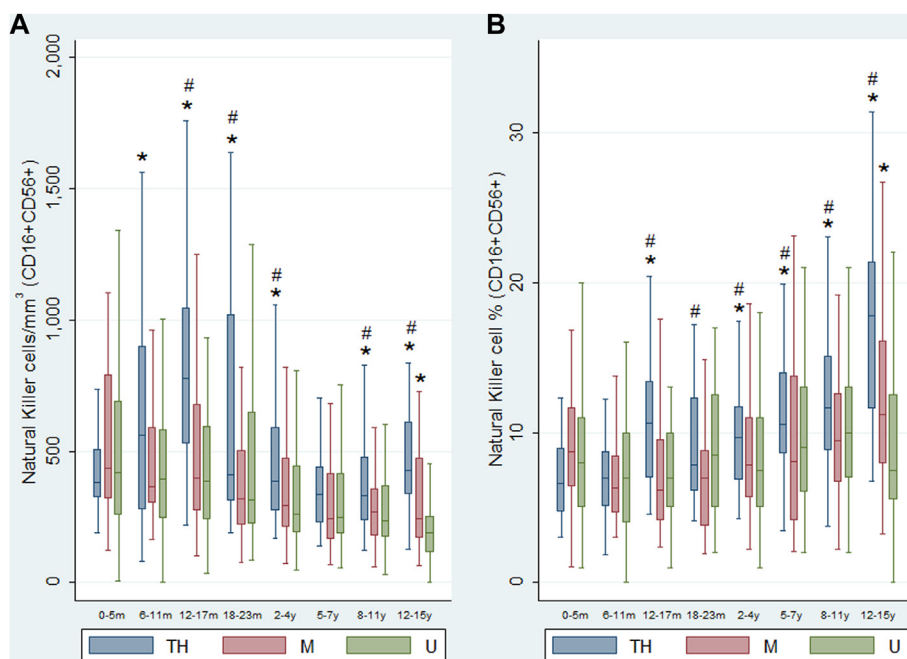


FIG E2. Box plots of NK cell counts and percentages in children by country cohort and age group. Statistical analysis was done as for Fig E1. M, Malawian cohort; TH, Thai cohort; U, US cohort.

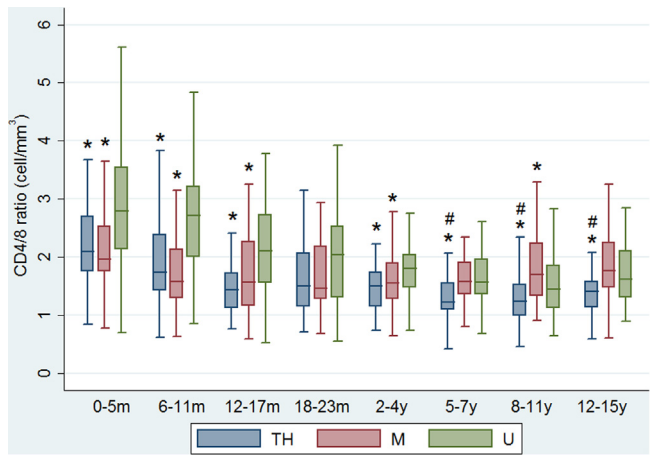


FIG E3. CD4/CD8 cell ratios in children by country cohort and age group. Statistical analysis was done as for [Fig E1](#). *M*, Malawian cohort; *TH*, Thai cohort; *U*, US cohort.

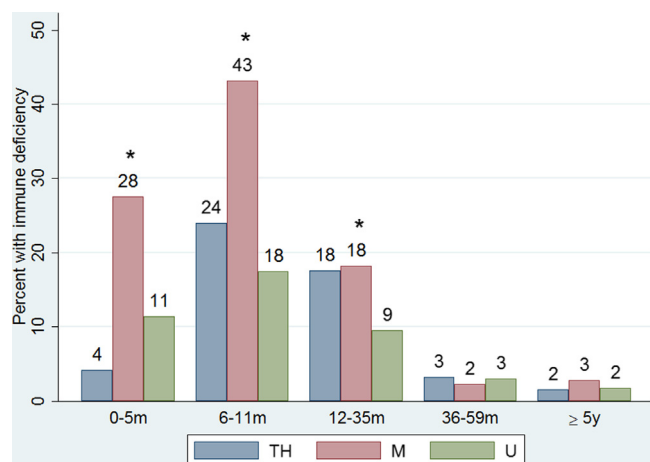


FIG E4. Proportion of healthy children from each country meeting World Health Organization 2006 treatment guideline case definitions for immune deficiency: age 11 months or less, CD4 <35%; age 12 to 35 months, CD4 <30%; age 36 to 59 months, CD4 <25%; and age 5 years or greater, CD4 <500 cells/mm³. **P* < .05 for the age group by using a χ^2 test.

TABLE E1. Demographics of children in the Thai, Malawian, and American cohorts

Characteristic	Thai cohort (n = 281)	Malawian cohort (n = 397)	US cohort (n = 721)
Male sex, no. (%)	125 (44.48)	190 (47.86)	373 (51.73)
Age (y)*			
Mean (SD)	5.1 (4.2)	3.9 (4.2)	3.5 (4.4)
Median (IQR)	4.3 (1.4, 8.3)	2.0 (0.9, 5.5)	1.3 (0.4, 5.2)
Range (minimum-maximum)	0.0-15.2	0.1-15.9	0.0-15.9
Age group, no. (%)			
0-5 mo	24 (8.54)	58 (14.61)	202 (28.02)
6-11 mo	25 (8.90)	51 (12.85)	113 (15.67)
12-17 mo	25 (8.90)	46 (11.59)	71 (9.85)
18-23 mo	26 (9.25)	45 (11.34)	36 (4.99)
2-4 y	54 (19.22)	89 (22.42)	76 (10.54)
5-7 y	52 (18.51)	36 (9.07)	77 (10.68)
8-11 y	52 (18.51)	42 (10.58)	89 (12.34)
12-15 y	23 (8.19)	30 (7.56)	57 (7.91)
Ethnicity	100% Thai	100% Black African	53% African American, 29% Hispanic, 17% Euro-Caucasian, 1% other

*Overall, Thai subjects were significantly older than those in the Malawian or US cohorts, and Malawian children were older than US children ($P < .05$ for all, pairwise Mann-Whitney-Wilcoxon U tests).

TABLE E2. Geometric mean ratios (95% CIs) for CD4, CD8, and B-cell counts and percentages

Age group	Comparison	GMR (95% CI)	P value	Comparison	GMR (95% CI)	P value	Comparison	GMR (95% CI)	P value
CD4 cell count									
0-5 mo	US vs Thai	0.94 (0.79-1.11)	.44	US vs Malawi	1.21 (1.08-1.37)	.001	Malawi vs Thai	0.74 (0.62-0.89)	.001
	Female vs male	0.99 (0.89-1.1)	.83	Female vs male	1.03 (0.93-1.14)	.62	Female vs male	1.15 (0.98-1.36)	.09
6-11 mo	US vs Thai	0.73 (0.59-0.91)	.005	US vs Malawi	1.09 (0.94-1.28)	.25	Malawi vs Thai	0.66 (0.56-0.79)	<.001
	Female vs male	1.04 (0.87-1.23)	.69	Female vs male	1.01 (0.87-1.17)	.87	Female vs male	0.96 (0.8-1.13)	.60
12-17 mo	US vs Thai	0.86 (0.72-1.02)	.09	US vs Malawi	1.04 (0.88-1.22)	.65	Malawi vs Thai	0.81 (0.67-0.98)	.03
	Female vs male	1.11 (0.95-1.3)	.20	Female vs male	1.26 (1.08-1.48)	.004	Female vs male	1.26 (1.04-1.52)	.02
18-23 mo	US vs Thai	0.85 (0.71-1.02)	.08	US vs Malawi	1.01 (0.86-1.19)	.91	Malawi vs Thai	0.84 (0.73-0.97)	.02
	Female vs male	1.08 (0.9-1.29)	.39	Female vs male	1.1 (0.93-1.3)	.28	Female vs male	0.99 (0.85-1.14)	.85
2-4 y	US vs Thai	0.97 (0.83-1.13)	.67	US vs Malawi	0.99 (0.88-1.12)	.90	Malawi vs Thai	0.97 (0.86-1.11)	.67
	Female vs male	1.06 (0.91-1.23)	.46	Female vs male	1.08 (0.96-1.23)	.19	Female vs male	1.04 (0.92-1.18)	.54
5-7 y	US vs Thai	1.13 (1-1.28)	.06	US vs Malawi	0.87 (0.75-1.01)	.07	Malawi vs Thai	1.3 (1.13-1.5)	<.001
	Female vs male	1.11 (0.98-1.25)	.10	Female vs male	1.17 (1.01-1.35)	.03	Female vs male	1.13 (0.98-1.31)	.08
8-11 y	US vs Thai	0.98 (0.86-1.12)	.78	US vs Malawi	0.88 (0.77-1)	.04	Malawi vs Thai	1.11 (0.97-1.28)	.13
	Female vs male	1.04 (0.91-1.17)	.58	Female vs male	0.94 (0.84-1.07)	.35	Female vs male	1.14 (0.99-1.31)	.07
12-15 y	US vs Thai	1.03 (0.88-1.21)	.73	US vs Malawi	0.91 (0.78-1.05)	.20	Malawi vs Thai	1.15 (0.97-1.38)	.11
	Female vs male	1.11 (0.96-1.28)	.17	Female vs male	1.28 (1.1-1.48)	.001	Female vs male	1.22 (1.02-1.47)	.03
CD4 percentage									
0-5 mo	US vs Thai	1.07 (0.97-1.18)	.19	US vs Malawi	1.24 (1.16-1.33)	<.001	Malawi vs Thai	0.86 (0.78-0.94)	.002
	Female vs male	0.97 (0.91-1.03)	.29	Female vs male	0.98 (0.93-1.04)	.61	Female vs male	0.99 (0.91-1.08)	.83
6-11 mo	US vs Thai	1.09 (0.99-1.2)	.08	US vs Malawi	1.25 (1.16-1.34)	<.001	Malawi vs Thai	0.87 (0.79-0.96)	.006
	Female vs male	1.06 (0.98-1.15)	.13	Female vs male	1.05 (0.98-1.12)	.20	Female vs male	1 (0.91-1.1)	.97
12-17 mo	US vs Thai	1.1 (0.99-1.22)	.08	US vs Malawi	1.16 (1.05-1.27)	.002	Malawi vs Thai	0.95 (0.85-1.06)	.36
	Female vs male	1.13 (1.03-1.25)	.01	Female vs male	1.16 (1.05-1.27)	.003	Female vs male	1.11 (0.99-1.25)	.06
18-23 mo	US vs Thai	1.07 (0.97-1.19)	.18	US vs Malawi	1.05 (0.95-1.16)	.31	Malawi vs Thai	1.02 (0.92-1.14)	.68
	Female vs male	1.14 (1.02-1.26)	.02	Female vs male	1.09 (0.99-1.2)	.08	Female vs male	1.17 (1.05-1.3)	.006
2-4 y	US vs Thai	1.11 (1.04-1.18)	.002	US vs Malawi	1.01 (0.95-1.07)	.76	Malawi vs Thai	1.09 (1.03-1.15)	.004
	Female vs male	1.03 (0.96-1.09)	.45	Female vs male	1.04 (0.98-1.1)	.16	Female vs male	0.99 (0.94-1.05)	.74
5-7 y	US vs Thai	1.12 (1.04-1.2)	.003	US vs Malawi	0.95 (0.86-1.03)	.21	Malawi vs Thai	1.18 (1.07-1.3)	.001
	Female vs male	1.13 (1.05-1.22)	.001	Female vs male	1.11 (1.02-1.2)	.02	Female vs male	1.08 (0.98-1.19)	.10
8-11 y	US vs Thai	1.11 (1.04-1.18)	.003	US vs Malawi	0.92 (0.86-0.98)	.009	Malawi vs Thai	1.21 (1.12-1.3)	<.001
	Female vs male	1.03 (0.97-1.1)	.32	Female vs male	0.99 (0.93-1.06)	.82	Female vs male	1.09 (1.01-1.18)	.03
12-15 y	US vs Thai	1.24 (1.14-1.35)	<.001	US vs Malawi	1 (0.92-1.09)	.99	Malawi vs Thai	1.25 (1.14-1.37)	<.001
	Female vs male	1.05 (0.97-1.14)	.19	Female vs male	1.1 (1.01-1.19)	.03	Female vs male	1.08 (0.98-1.19)	.11
CD8 cell count									
0-5 mo	US vs Thai	0.73 (0.61-0.87)	.001	US vs Malawi	0.93 (0.81-1.06)	.26	Malawi vs Thai	0.75 (0.6-0.93)	.009
	Female vs male	0.99 (0.88-1.11)	.81	Female vs male	1 (0.89-1.12)	.97	Female vs male	1.17 (0.96-1.43)	.12
6-11 mo	US vs Thai	0.52 (0.41-0.65)	<.001	US vs Malawi	0.69 (0.58-0.8)	<.001	Malawi vs Thai	0.76 (0.61-0.94)	.01
	Female vs male	0.91 (0.75-1.1)	.31	Female vs male	0.91 (0.78-1.05)	.20	Female vs male	0.87 (0.7-1.07)	.18
12-17 mo	US vs Thai	0.63 (0.51-0.77)	<.001	US vs Malawi	0.81 (0.68-0.96)	.01	Malawi vs Thai	0.78 (0.62-0.96)	.02
	Female vs male	0.99 (0.83-1.19)	.93	Female vs male	1 (0.84-1.18)	.99	Female vs male	1 (0.81-1.24)	.98
18-23 mo	US vs Thai	0.71 (0.53-0.97)	.031	US vs Malawi	0.87 (0.69-1.09)	.23	Malawi vs Thai	0.81 (0.66-1.01)	.06
	Female vs male	0.83 (0.61-1.13)	.23	Female vs male	0.96 (0.76-1.21)	.71	Female vs male	0.76 (0.61-0.94)	.01
2-4 y	US vs Thai	0.8 (0.69-0.92)	.002	US vs Malawi	0.89 (0.79-1.01)	.07	Malawi vs Thai	0.89 (0.77-1.02)	.08
	Female vs male	1.06 (0.92-1.23)	.39	Female vs male	1.04 (0.92-1.18)	.51	Female vs male	1.05 (0.92-1.2)	.44
5-7 y	US vs Thai	0.9 (0.78-1.05)	.17	US vs Malawi	0.89 (0.76-1.05)	.17	Malawi vs Thai	1.01 (0.86-1.18)	.91
	Female vs male	0.98 (0.85-1.14)	.81	Female vs male	1 (0.86-1.17)	.97	Female vs male	0.95 (0.82-1.11)	.55
8-11 y	US vs Thai	0.85 (0.72-0.99)	.04	US vs Malawi	1.03 (0.88-1.2)	.71	Malawi vs Thai	0.82 (0.69-0.97)	.02
	Female vs male	1.02 (0.87-1.18)	.84	Female vs male	0.96 (0.83-1.11)	.55	Female vs male	1.01 (0.85-1.2)	.90
12-15 y	US vs Thai	0.84 (0.68-1.04)	.11	US vs Malawi	0.96 (0.78-1.17)	.66	Malawi vs Thai	0.9 (0.72-1.12)	.35
	Female vs male	1.18 (0.97-1.43)	.09	Female vs male	1.33 (1.09-1.61)	.005	Female vs male	1.19 (0.94-1.49)	.15
CD8 percentage									
0-5 mo	US vs Thai	0.83 (0.73-0.94)	.004	US vs Malawi	0.95 (0.87-1.03)	.21	Malawi vs Thai	0.86 (0.75-0.99)	.04
	Female vs male	0.96 (0.89-1.04)	.37	Female vs male	0.96 (0.89-1.03)	.26	Female vs male	1.01 (0.89-1.14)	.91
6-11 mo	US vs Thai	0.77 (0.69-0.87)	<.001	US vs Malawi	0.78 (0.71-0.87)	<.001	Malawi vs Thai	0.99 (0.84-1.16)	.88
	Female vs male	0.93 (0.84-1.03)	.14	Female vs male	0.94 (0.85-1.03)	.18	Female vs male	0.91 (0.78-1.06)	.22
12-17 mo	US vs Thai	0.8 (0.71-0.91)	.001	US vs Malawi	0.9 (0.8-1.01)	.06	Malawi vs Thai	0.91 (0.79-1.05)	.19
	Female vs male	1.01 (0.91-1.14)	.79	Female vs male	0.92 (0.82-1.02)	.12	Female vs male	0.89 (0.77-1.02)	.10
18-23 mo	US vs Thai	0.9 (0.77-1.04)	.16	US vs Malawi	0.9 (0.79-1.03)	.12	Malawi vs Thai	0.99 (0.87-1.13)	.89
	Female vs male	0.87 (0.75-1.01)	.07	Female vs male	0.95 (0.84-1.09)	.49	Female vs male	0.9 (0.79-1.03)	.11
2-4 y	US vs Thai	0.91 (0.84-0.99)	.03	US vs Malawi	0.91 (0.84-0.98)	.01	Malawi vs Thai	0.99 (0.92-1.08)	.86
	Female vs male	1.03 (0.95-1.12)	.45	Female vs male	1 (0.93-1.08)	.96	Female vs male	1 (0.93-1.08)	.94
5-7 y	US vs Thai	0.89 (0.83-0.97)	.007	US vs Malawi	0.97 (0.87-1.08)	.54	Malawi vs Thai	0.91 (0.82-1.02)	.09
	Female vs male	1 (0.92-1.09)	.97	Female vs male	0.95 (0.86-1.05)	.31	Female vs male	0.91 (0.82-1.01)	.08

(Continued)

TABLE E2. (Continued)

Age group	Comparison	GMR (95% CI)	P value	Comparison	GMR (95% CI)	P value	Comparison	GMR (95% CI)	P value
8-11 y	US vs Thai	0.95 (0.88-1.04)	.27	US vs Malawi	1.07 (0.98-1.17)	.11	Malawi vs Thai	0.89 (0.81-0.97)	.01
	Female vs male	1.01 (0.93-1.1)	.75	Female vs male	1 (0.92-1.09)	.92	Female vs male	0.97 (0.88-1.06)	.50
12-15 y	US vs Thai	1.01 (0.88-1.16)	.86	US vs Malawi	1.07 (0.94-1.21)	.31	Malawi vs Thai	0.96 (0.84-1.09)	.49
	Female vs male	1.13 (1-1.28)	.06	Female vs male	1.14 (1-1.29)	.04	Female vs male	1.04 (0.91-1.19)	.60
B-cell count									
0-5 mo	US vs Thai	0.8 (0.58-1.11)	.18	US vs Malawi	0.65 (0.52-0.8)	<.001	Malawi vs Thai	1.24 (0.96-1.6)	.10
	Female vs male	1.09 (0.89-1.35)	.40	Female vs male	1.11 (0.92-1.33)	.28	Female vs male	1.09 (0.86-1.38)	.46
6-11 mo	US vs Thai	0.73 (0.53-0.99)	.04	US vs Malawi	0.72 (0.57-0.9)	.004	Malawi vs Thai	1.02 (0.81-1.28)	.86
	Female vs male	0.91 (0.71-1.16)	.45	Female vs male	0.93 (0.75-1.15)	.51	Female vs male	1.07 (0.86-1.33)	.55
12-17 mo	US vs Thai	1.03 (0.84-1.27)	.74	US vs Malawi	0.81 (0.67-0.98)	.03	Malawi vs Thai	1.22 (0.96-1.56)	.11
	Female vs male	0.82 (0.68-0.98)	.03	Female vs male	0.99 (0.81-1.19)	.88	Female vs male	1.11 (0.87-1.41)	.40
18-23 mo	US vs Thai	0.9 (0.73-1.11)	.34	US vs Malawi	0.86 (0.7-1.06)	.15	Malawi vs Thai	1.05 (0.86-1.27)	.64
	Female vs male	1.01 (0.81-1.24)	.96	Female vs male	0.98 (0.79-1.22)	.87	Female vs male	0.84 (0.69-1.03)	.09
2-4 y	US vs Thai	1 (0.85-1.19)	.97	US vs Malawi	1.07 (0.92-1.24)	.40	Malawi vs Thai	0.94 (0.8-1.11)	.47
	Female vs male	1.07 (0.91-1.26)	.43	Female vs male	1.04 (0.9-1.21)	.58	Female vs male	1.09 (0.93-1.27)	.30
5-7 y	US vs Thai	1.2 (1.01-1.41)	.03	US vs Malawi	1.1 (0.91-1.34)	.34	Malawi vs Thai	1.1 (0.92-1.32)	.28
	Female vs male	0.95 (0.81-1.12)	.55	Female vs male	0.99 (0.83-1.2)	.96	Female vs male	1.05 (0.88-1.25)	.59
8-11 y	US vs Thai	1.03 (0.89-1.19)	.73	US vs Malawi	1.05 (0.9-1.23)	.52	Malawi vs Thai	0.97 (0.83-1.14)	.72
	Female vs male	0.96 (0.83-1.11)	.57	Female vs male	0.91 (0.78-1.05)	.19	Female vs male	0.99 (0.85-1.16)	.93
12-15 y	US vs Thai	0.74 (0.54-1.01)	.06	US vs Malawi	0.85 (0.64-1.13)	.25	Malawi vs Thai	0.86 (0.7-1.07)	.17
	Female vs male	0.92 (0.69-1.22)	.55	Female vs male	0.98 (0.74-1.28)	.87	Female vs male	1.21 (0.97-1.51)	.10
B-cell percentage									
0-5 mo	US vs Thai	0.91 (0.7-1.18)	.49	US vs Malawi	0.67 (0.56-0.79)	<.001	US vs Malawi	1.41 (1.18-1.68)	<.001
	Female vs male	1.07 (0.91-1.26)	.43	Female vs male	1.07 (0.92-1.24)	.36	Female vs male	0.96 (0.82-1.12)	.58
6-11 mo	US vs Thai	1.08 (0.88-1.34)	.46	US vs Malawi	0.82 (0.7-0.96)	.01	US vs Malawi	1.33 (1.13-1.56)	.001
	Female vs male	0.93 (0.78-1.1)	.40	Female vs male	0.96 (0.83-1.12)	.62	Female vs male	1.12 (0.96-1.31)	.16
12-17 mo	US vs Thai	1.33 (1.16-1.52)	<.001	US vs Malawi	0.91 (0.81-1.02)	.10	US vs Malawi	1.43 (1.25-1.64)	<.001
	Female vs male	0.83 (0.74-0.94)	.005	Female vs male	0.9 (0.8-1.01)	.08	Female vs male	0.98 (0.86-1.12)	.75
18-23 mo	US vs Thai	1.14 (0.99-1.31)	.07	US vs Malawi	0.93 (0.81-1.07)	.28	US vs Malawi	1.23 (1.08-1.4)	.002
	Female vs male	1.06 (0.92-1.22)	.43	Female vs male	0.93 (0.81-1.07)	.31	Female vs male	0.95 (0.83-1.08)	.39
2-4 y	US vs Thai	1.15 (1.04-1.26)	.005	US vs Malawi	1.09 (0.99-1.19)	.07	US vs Malawi	1.06 (0.96-1.16)	.27
	Female vs male	1.04 (0.94-1.14)	.46	Female vs male	1 (0.92-1.09)	.98	Female vs male	1.04 (0.94-1.14)	.46
5-7 y	US vs Thai	1.19 (1.05-1.34)	.006	US vs Malawi	1.19 (1.04-1.37)	.01	US vs Malawi	1 (0.86-1.15)	.97
	Female vs male	0.97 (0.86-1.1)	.62	Female vs male	0.94 (0.83-1.07)	.36	Female vs male	1 (0.87-1.16)	.95
8-11 y	US vs Thai	1.16 (1.06-1.27)	.002	US vs Malawi	1.1 (0.99-1.22)	.07	US vs Malawi	1.05 (0.95-1.16)	.31
	Female vs male	0.96 (0.88-1.05)	.33	Female vs male	0.95 (0.87-1.05)	.34	Female vs male	0.95 (0.86-1.05)	.34
12-15 y	US vs Thai	0.89 (0.68-1.17)	.41	US vs Malawi	0.94 (0.74-1.2)	.61	US vs Malawi	0.92 (0.78-1.09)	.35
	Female vs male	0.88 (0.68-1.13)	.31	Female vs male	0.84 (0.66-1.06)	.14	Female vs male	1.06 (0.89-1.27)	.49

Pairwise comparisons between country groups in each age group are shown, along with coefficients comparing female with male subjects.

GMR, Geometric mean ratio.