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Control Lymphocyte Subsets: Can one country's values serve for another's?

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Abstract

Lymphocyte subsets can be affected by host and environmental factors, yet direct comparisons of their patterns across continents are lacking. This work compares proportions and counts of lymphocyte subsets between healthy children from Thailand, Malawi and the USA. We analyzed subsets of 1,399 healthy children aged between 0 and 15 years: 281 Thai, 397 Malawian and 721 American children. Existing data for five subsets were available for all three cohorts (Total T, CD4+ T, CD8+ T, natural killer (NK) and B cells), with data for another six subsets from the Thai

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Additional references are included in the online E-text.

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and American cohorts (naïve, memory and activated CD4+ and CD8+ T cells). Cellular patterns between cohorts differed mainly in children under two years. Compared to American children, Thai children had higher median numbers of total T cells, CD8+ T cells and NK cells while Malawian children under 18 months, on average, had more CD8+ T cells and B cells. Both Thai and Malawian children had lower median CD4+ T cell percentages and CD4/CD8 ratios than American children. Thai children had more memory and activated CD8+ T cells than American children. Approximately one-fifth of Thai and Malawian HIV-uninfected healthy children aged 0-3 years met WHO-defined CD4+ count criteria for immune-deficiency in HIV-infected children. Healthy children from Thailand, Malawi and the USA have differences in lymphocyte subsets that are likely to be due to differences in ethnicity, exposure to infectious diseases and environmental factors. These results indicate the need for country-specific reference ranges for diagnosis and management of immunologic disorders.

Keywords

lymphocyte; immunophenotyping; children; Thailand; Malawi; USA; healthy controls; immunologic disorders; HIV

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, gender and environmental factors¹⁻³. Differing exposure to infectious agents such as malaria, tuberculosis and cytomegalovirus could underlie differences in lymphocyte subsets patterns in contrasting settings⁴⁻⁶. In this study, we analyzed combined data from three studies of healthy children from Thailand¹, Malawi² and the USA³ that 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 < 2 years 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 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 publications¹⁻³ (details in online E-text).

Of 1,399 children enrolled, (Thailand, n=281, Malawi, n=397 and the USA n = 721), approximately 50% were male (Online Table E-1) and approximately 50% were aged between 0-2 years. The Thai and Malawian cohorts were ethnically homogenous; the US cohort had a mix of African-Americans (53%), Hispanics (29%), and Euro-Caucasians (17%).

The following lymphocyte subpopulations were identified as percentages of the lymphocyte gate in all three cohorts: T cells (CD3+), CD4+ T cells (CD3+CD4+), CD8+ T cells (CD3+CD8+), B cells (CD19+), B memory cells (CD19+CD27+), Figure 1A-D compares cell counts of total T cells, CD4+ T, CD8+ T and B cells by age and country. Graphs representing other cells and percents are provided online (Online Figures E-1A-1D, E-2A-2B).

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

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

Higher CD8+ T cell counts (birth to 4 years, 8-11 years) and percentages (birth to 18 months, 2-7 years) were observed in Thais compared to US children and also compared to Malawian children for absolute counts from 0-17 months, and 8-11 years, and percents from 0-5 months and 8-11 years. These values were also higher among Malawian compared to US children for certain age groups (6-17 months for absolute CD8+ T cell counts and percentages; 2-4 years for percentages only). B and NK cell results are presented in the online E-text.

In secondary analyses geometric mean ratios were predominantly consistent with the significance results from non-parametric tests, with few apparent gender differences although power to detect these differences was limited (Online Table E-2).

This analysis of lymphocyte subsets of children from countries within three 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 may be driven by several factors including ethnicity with participants from the three countries being of different ethnicities. Previous healthy control studies in Asians and Africans have reported smaller proportions of CD4+ T cell 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 may not account for all subset differences since variations within specific ethnic groups have also been reported^{2,8,11,12}. Environmental factors, particularly prevalent infectious diseases, may affect cellular phenotypic patterns¹⁴.

The high median B cell counts observed in Malawian children in younger age groups, which have been reported in other African cohorts^{8,12} may be associated with malaria, which is

prevalent in Malawi, but less common in Thailand and absent in the USA. Children living in malaria-endemic areas are known to exhibit expansion of atypical memory B cells, thought to be driven by parasite antigens and could have beneficial immune modulating effects for the host²¹. In contrast, Thailand is a dengue-endemic area with most Thai children being dengue-seropositive by 5 years¹³ which could explain the pattern of higher CD4+ and CD8+ T cells and NK cells observed in Thai children. Being a likely confounding factor, participants in all three cohorts were screened for HIV and those that 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 pollution¹⁴. The etiology 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 three countries, and possible variation in the nutritional status of the participants. In conclusion, the 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. Population-specific healthy controls reference ranges for different lymphocyte subsets should therefore be developed for the diagnosis and monitoring of immunologic disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

HIV	Human Immunodeficiency Virus
NK	Natural Killer
WHO	World Health Organization

Figure 1.

A-D: Box plots of total T cell, CD4+ T cell, CD8+ T cell and total B cell counts (box goes from 25th-75th percentile, median is line in the box, and whiskers extend from 10th-90th percentiles) 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, using a Mann-Whitney-Wilcoxon test, and P values < 0.05 were considered significant. * indicates a significant difference in the Malawian or Thai (depending on the bar below the symbol) versus the US cohort; # indicates a significant difference from the Malawian cohort versus the Thai cohort.