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Overlapping patterns and unique differences: a study into immunological variation within and between populations

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Chapter 4

Distinct immune profiles in children of high versus low socioeconomic status in Makassar, Indonesia

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ABSTRACT

Background. Responses to immune perturbations can vary greatly when comparing geographical regions such Europe versus South East Asia but also when comparing urban versus rural areas within a country. This can translate into differences in disease profiles or in responses to vaccines. However, even within an urban area, large differences in income settings can be seen. Therefore, it is also important to assess whether differences observed when comparing rural and urban residents can also be detected between high and low socioeconomic status (SES) within an urban center.

Aim. To examine the immune profile of children of high versus low SES

Methods. Using mass cytometry, we profiled immune cells in finger-prick blood samples of children attending high and low SES schools in Makassar, Indonesia.

Results. Significant differences were found in the immune profiles of children from low versus high SES. Increased frequencies of entire and CD11c+ B cells, CD161+ T helper 2 cells and CTLA-4+ Tregs and HLA-DRdimCD163+ monocytes were seen in low SES children, whereas a trend towards expansion of T helper 1 cells was observed in the high SES group.

Conclusion. Although differences in the immune system of populations living in rural versus urban areas have been documented, this study shows that within an urban center, the socioeconomic status can have a significant impact on the immune system of children. Such differences might contribute to variation seen in immune reactivity to allergens, autoantigens or vaccines, therefore SES should be factored in when studying responses to immune perturbations.

INTRODUCTION

The immune system evolves during life in response to environmental exposures [1, 2]. It should therefore not be surprising that a vast range of environmental factors have been identified that can impact immunity, including nutrition [3] and exposure to helminth infections [4, 5]. As a results of the differential environmental exposures, the immune system greatly varies when comparing geographical regions such as Europe versus South East Asia, but also when comparing urban versus rural areas within a country [5].

Several studies have observed differences in the phenotype and functionality of the immune system between geographical areas and high-dimensional single cell analysis allowed us to map these differences in detail. A study comparing the immune signatures of Europeans and Indonesians living in an urban or rural area, showed that the immune profile of urban Indonesians resembled that of Europeans rather than that of rural Indonesians. Moreover, rural Indonesians had an increased frequency of CD161⁺ Th₂ cells and regulatory T cells and an increased frequency of type 2 cytokine producing cells, which was associated with helminth infections, as their frequencies diminished after deworming [5]. Such differences already become apparent during early childhood as illustrated by a study mapping the immune profiles of children from Bangladesh and the United States in the first five years of life. By 2-3 years of age, Bangladeshi children already showed a significant altered activation and cytokine production profiles upon stimulation and their immune trajectory resembled those of American adults rather than of American children of the same age [6].

Variations in the immune system can translate into differences in disease profiles such as development of allergic and auto-immune diseases and responses to vaccines. The prevalence of allergies and auto-immunities have been increasing over the past decades, initially only in high income countries but more recently also in low- and middle income countries mainly due to an increase among urban populations [7, 8]. Upon urbanization and better infrastructure, the residents have a decreased exposure to certain microorganisms and parasites. For example, parasitic helminths, which has been shown to be protective against development of asthma and allergic diseases by the induction of regulatory responses [9, 10] are much less prevalent in urban settings. Conversely, the increased immune regulation and the continuous immune activation as a result of heightened pathogen exposure can compromise the response to vaccine [11]. A study comparing rural and semi-urban children in Gabon, showed reduced

influenza vaccine responses [12]. Moreover, comparison of the response to yellow fever vaccine in Swiss and Ugandan adults showed a significant heightened immune activation at baseline in Ugandans, which correlated with reduced humoral and cellular responses to yellow fever vaccine in Ugandan compared to in Switzerland [13].

Differences in the immune system of populations living in rural versus urban areas have been documented, however few studies have focused on those living within an urban area, where large differences in socioeconomic status (SES) can be observed. As SES is an important determinant of environmental exposures, it can be an important determinant for the shaping of the immune system of an individual and their immune response to allergens, autoantigens or vaccines. This is illustrated by a study among newborns in the urban city of Makassar in Indonesia, showing that infants from low SES communities had a significantly smaller BCG scar size, indicating a reduced vaccine response in low compared to high SES infants [14]. Therefore, it is important to further explore and profile the immune system of populations with high versus low SES within the same city.

Here, we analysed the peripheral blood immune profiles of high and low SES schoolchildren living in the urban city of Makassar and used mass cytometry to catch the diversity in detail. The results show that within an urban centre, socioeconomic status can have a significant impact on the immune system of children.

METHODS

Data collection

The data was collected as part of a larger study into the effect of SES on the immune system of school-aged children in Makassar, Indonesia. The study population comprised of children attending two primary schools in Makassar, the capital of South Sulawesi. The schools were both located in the city center of Makassar 2 km apart from each other. One of the primary schools was fully funded by the government and predominantly attended by low SES children, while the other school was a privately funded school and attended by children from families of high SES as published previously [15]. Data and samples were collected in September-October 2019 after written informed consent for participation was obtained from the primary caregivers. Children with recent use of antihistamines/ corticoids were excluded from the study. In total 360 children were included (n= 260 low SES and n=100 high SES) to study allergy related parameters which already have been published [15]. On one of the study days, the blood was also collected

to analyze cellular immune response profiles. Sufficient quantity of finger prick blood was available from 35 children (n=21 low SES and n=14 high SES). Ethical approval was obtained from the Health Research Ethical Committee, Faculty of Medicine, Hasanuddin University (No:703/H4.6.4.5.31/PP36/2019). Sociodemographic information was obtained via questionnaires.

Sample preparation

Approximately 100-200 μ L whole blood was collected from children's fingertips using Microvette® CB300 tubes (Fisher scientific, USA). These samples were centrifuged within 8 hours, resuspended and incubated for 15 minutes at room temperature in BD FACSLysis buffer (BD Biosciences, USA). After incubation, cells were centrifuged, resuspended in 1mL RPMI+10% dimethyl sulfoxide and stored at -80°C until further use. In contrast to isolation of peripheral blood mononuclear cells (PBMCs) for which sterile conditions and liquid nitrogen storage is required, fixation of whole blood requires few laboratory facilities and therefore can be performed in settings with limited laboratory facilities such as in low- and middle- income countries.

An antibody panel to phenotype the immune cells *ex vivo* was designed and the antibodies are listed in **Table S1**. To facilitate staining and measurement of all samples in a single batch, barcoding was performed before samples were stained with the antibody panel to phenotype the cells. Antibodies for barcoding were produced by conjugating seven identical, purified antibody for Beta-2 microglobulin (Biolegend, USA) to seven different metals. Each sample was labelled with a combination of three of these antibody-metal conjugates, enabling us to pool 35 samples. Details of barcoding antibodies are listed in **Table S2**. The day before the staining the barcoding cocktail and antibody cocktail for phenotyping were prepared separately in Perm/Wash (BD Biosciences, USA) and stored overnight at 4 °C. On the day of the staining, the cryopreserved, fixed, whole blood samples were thawed in 2 mL thawing medium at 37 °C. The cells were spun down at 1600 rpm for 10 min and the supernatant was removed. Next, cells were resuspended in 1 mL 1x BD Perm/wash (BD Biosciences, USA) and moved to a new tube. Next, the cells were spun down at 800 g for 5 min at room temperature and the supernatant was discarded subsequently. The cells were resuspended in 50 μ L Perm/Wash and 50 μ L of the sample-specific barcode cocktail was added to each sample for a 30 min incubation at room temperature. After incubation, 4 mL MaxPar Cell Staining Buffer (Fluidigm, USA) was added to each sample and the cells spun down at 800 g for 5 min at room temperature, whereafter the supernatant was removed. Subsequently, the samples were pooled in 3 mL Perm/Wash and spun down at 800 g for 5

min at room temperature, whereafter the supernatant was discarded. The cells were resuspended in 45 μ L Perm/wash with 5 μ L FcR block and 0,5 μ L heparin for a 20 min incubation at room temperature. Next, the antibody mix was added and the samples were incubated with these antibodies for 45 min at room temperature. Following the incubation, the cells were washed twice by adding 2 mL of MaxPar Cell Staining Buffer, centrifuging at 800 g for 5 min at room temperature and discarding the supernatant. After washing twice, 1 mL of Intercalator prepared in Maxpar Fix and Perm Buffer (Fluidigm, USA) was added and the samples were stored overnight at 4° C before being measured.

Data acquisition

Before measurement by mass cytometry, the cells were washed twice by adding 2 mL MaxPar Cell Staining Buffer, centrifuging the cells at 800 g for 5 minutes and removing the supernatant. Samples were measured with a Helios TM mass cytometer (Fluidigm, USA), which was tuned according to the manufacturer's recommendations. Mass cytometry data were acquired by dual-count mode and the noise-reduction on. After data acquisition, the mass bead signal was used to normalize the short-term signal fluctuations with the reference EQ passport P13H2302 during the course of each measurement. When applicable, normalized FCS files were concatenated using Helios software (Fluidigm, USA), without removing beads.

Data analysis

To select single intact immune cells, manual gating was performed on the DNA stain and the CD45 marker. Subsequently, gating using the gaussian parameters of the normalization was performed manually and the calibration beads were gated out. Thereafter, the data were compensated based on a study-specific compensation matrix (**Figure S1**). The CATALYST [16] package was used to debarcode the data and we were able to assign 95% of the cells. Finally, the data were transformed by a hyperbolic arcsin with a cofactor of 5. All manual gating for pre-processing of the mass cytometry data was performed in RStudio (Rstudio, USA) and with the R software.

All normalized, pre-processed and assigned cells were selected and exported as FCS files. These FCS files were imported in Cytosplore [17] and within this software clustering was performed by Hierarchical Stochastic Neighbor Embedding (HSNE), a t-distributed Stochastic Neighbor Embedding (t-SNE) based method. As we obtained 5.6 million single intact immune cells, we created four hierarchical levels of embedding. The clusters produced in Cytosplore were further analyzed using R software (x64 version 3.5.1, R

Foundation for Statistical Computing) within the RStudio (Rstudio, USA) IDE. Core R packages, cytofast [18], CATALYST [16] were used.

Since some markers were dimly expressed due to the type of antibody-metal conjugate, populations that would express these markers could not clearly be distinguished and selected based on clustering. Therefore, manual gating was performed to select these populations by using FlowJo (FlowJo LCC, USA). In order to investigate the Th₂ cell population, all cells within the CD4⁺ T cell population with an CRTH2 expression of ≥ 2 were selected. To select TCRgd⁺ T cells, all T cells (expressing CD3 and CD7) were included in the manual gating process. From this T cell population only cells with CD8 and CD4 expression ≤ 1.8 and with TCRgd expression ≥ 1.8 were gated and identified as TCRgd⁺ T cells (**Figure S2**).

Statistical analysis

The characteristics of the participants were described employing descriptive statistics and compared between high- and low SES using Students' T-test and Pearson's Chi-square test. To examine the effect of SES on the immune profile, relative cell abundances were calculated using the CyTOF data. To do so, the frequencies of the population of interest was divided by the total frequency of CD45⁺ cells or of that of the parent or grandparent population, depending on the research question. For the comparison of cell abundance (relative to CD45⁺ cells, parent or grand-parent) between low and high SES, the t-test was performed comparing the mean frequency between the groups. Since this is an explorative study, p-values < 0.05 were considered significant (with no correction for multiple testing). All statistics were performed and all figures were created using R Software (x64 version 3.5.1, R Foundation for Statistical Computing) within RStudio (Rstudio, USA), and Excel (Microsoft Office, USA).

RESULTS

Characteristics of the study population

The study population comprised of 21 children attending the low SES school and 14 children attending high SES school (**Table 1**). The mean age tended to be lower in children attending the low SES school (8.35 years mean age in low SES, 9.00 year in high SES) and the percentage of females was higher in the low SES compared to the high SES (76.2% female in low SES and 35.7% in the high SES). As expected, the z-BMI, a measure for nutritional status, was significantly higher in the high SES compared to the low SES (-1.63 z-BMI score in the low SES and 0.43 in the high SES), while the education level of parents was also lower in the low SES group. Moreover, helminths

were detected in four low SES children (19%), whereas none of the high SES children were infected with helminths.

Table 1. Characteristics of the study population

	Low SES (n= 21)	High SES (n=14)	P-value
Age (in years, mean, SD)	8.35 ± 1.37	9.00 ± 0.88	0.095 ¹
Sex (female %, n/N)	76.2 (16/21)	35.7 (5/14)	0.017 ²
z-BMI (mean, SD)	-1.63 ± 1.66	0.43 ± 1.77	0.001 ¹
Education father (high ³ %, n/N)	9.5 (2/21)	85.7 (12/14)	<0.001 ²
Education mother (high ³ %, n/N)	9.5 (2/21)	85.7 (12/14)	<0.001 ²
Floor material (ceramics %, n /N)	28.6 (6/21)	100 (14/14)	<0.001 ²
House wall material (brick or concrete %, n/N)	61.9 (13/21)	100 (14/14)	0.016 ²
Toilet (private inside %, n/N)	90.5 (19/21)	100 (14/14)	0.234 ²
Helminth infection by microscope (%, n/N)	19.0 (4/21) ³	0.0 (0/14)	0.275 ²
Skin prick test for house dust mite (positive %, n/N)	4.76 (1/21)	28.6 (4/14)	0.056 ²

¹ Normally distributed, independent *t*-test. ² Pearson Chi-square test. Number of positives (n) of the total number (N). ³ High education refers to attending university or obtaining an academic degree. SD, standard deviation. SES, socioeconomic status. Z-BMI, standardized z-scores of body mass index

Differences in immunological profiles of high and low SES schoolchildren

Finger-prick blood was collected from children of high and low SES and preserved by fixation within 8 hours after collection. The samples were kept at -80 °C degrees until thawing for immune phenotyping. Using a panel of 39 antibodies the cells were stained and measured in one batch as indicated in materials and methods. The Hierarchical Stochastic Neighbor Embedding (HSNE), identified twelve major immune subsets; plasmacytoid dendritic cells (pDCs), basophils, myeloid dendritic cells, Mucosal Associated invariant T (MAIT) cells, unconventional T cells (double negative), TCRgd⁺ T cells, innate lymphoid cells (ILCs)/natural killer (NK) cells, B cells, monocytes, CD8⁺ and CD4⁺ T cells as well as granulocytes (**Figure 1A-1C**). Comparison of the frequency of these immune subsets as percentage of the total CD45⁺ cells between high and low SES, revealed that the frequency of total B cells was significantly increased (*p*=0.001) and the frequency of TCRgd⁺ T cells was reduced (*p*=0.049) in the low compared with the high SES (**Figure 1D-E**, **Figure S3**).

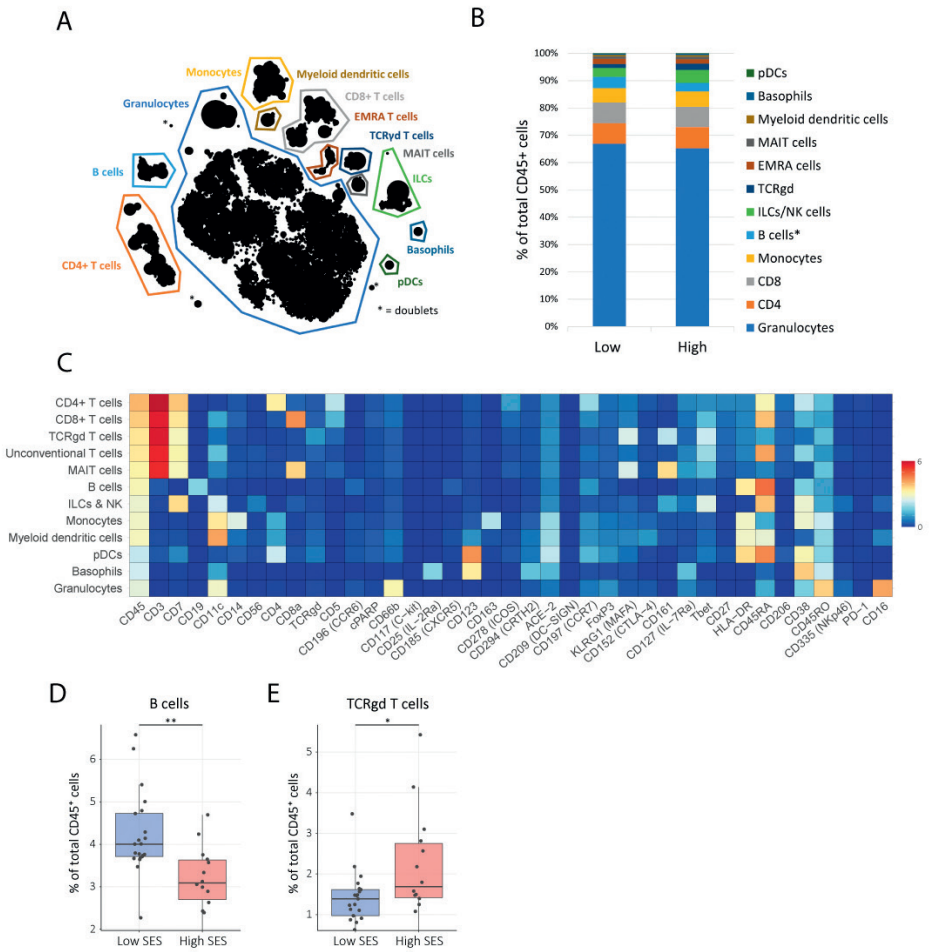


Figure 1. B cell population is expanded and TCRgd T cell population is diminished in low SES children.

(A) Hierarchical Stochastic Neighbor Embedding (HSNE) first level of embedding of 5.8 million peripheral immune cells of children from low SES (n=21) and high SES (n=14), with the major immune lineages annotated on the basis of lineage marker expression. (B) Bar graph showing the comparison of the lineage proportions as percentage of all CD45⁺ cells between low SES and high SES children. Colors correspond with the major immune lineages. Immune lineages that were significantly different between low and high SES are marked with *. (C) Heatmap of the median expression of all markers for all major immune lineages identified. In all figures, color represents arcsin5-transformed marker expression. (D) Boxplot depicting the comparison between the frequency of B cells relative to the total CD45⁺ cells between low and high SES children. The boxes represent the interquartile range, the line within represents the median and the whiskers represent the 1.5 IQ of the upper and lower quartile. ** *P* < 0.01. (E) Boxplot depicting the frequency of TCRgd T cells relative to the total CD45⁺ cells between low and high

SES children. The boxes represent the interquartile range, the line within represents the median and the whiskers represent the 1.5 IQ of the upper and lower quartile. * $p < 0.05$. All differences were tested statistically using a Student's t test.

To examine the immune cell profiles in more detail, immune subsets that have been described to be distinct between geographical regions were compared. To this end, the further examination of the B cell compartment revealed that the expression of CCR7 (95th percentile of MFI), a homing receptor presented by activated cells mediating the migration of mature B cells to the lymph node [19, 20], was higher on B cells of children from low SES compared with high SES ($p=0.008$) (**Figure 2A-B**). Furthermore, B cells expressing CD11c⁺ (**Figure 2A**), which are often increased upon intense antigen encounter and form a pool of memory B cells that can become antibody secreting cells [21], were expanded along with the entire B cell compartment in low SES.

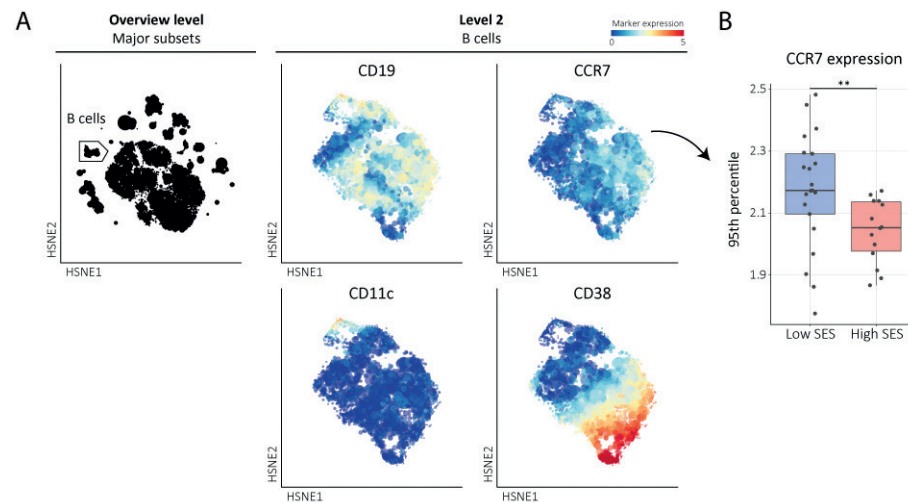


Figure 2. Characterization of B cell population expanded in the low SES compared to the high SES

(A) Second HSNE level embedding of B cells (CD19⁺) selected from the overview of total PBMCs, as indicated by the black outline. Markers that were heterogeneously expressed within the B cell population are depicted. (B) Boxplot depicting the comparison of the 95th percentile of mean fluorescent intensity (MFI) of CCR7 within the B cell population between low and high SES children. The boxes represent the interquartile range, the line within represents the median and the whiskers represent the 1.5 IQ of the upper and lower quartile. ** $p < 0.01$. All differences were tested statistically using a Student's t test

Within the CD4⁺ T cell compartment, we identified distinct clusters of Th₂ cells that were gated based on expression of CRTH2. Four clusters were distinguished based on the expression of CD7 and CD161 (**Figure 3A, 3C**). Comparison of the density plots of the Th₂ population in high and low SES children, revealed an increased density, resulting from a higher frequencies of cells in cluster 4, which is characterized by CD161⁺ and CD7⁻ (**Figure 3**). This was confirmed by quantification of the frequency of the cells within cluster 2, showing a highly significant increase in the number of CD161⁺ in the low SES compared to the high SES ($p=0.005$) (**Figure 3D**). These CD161⁺ Th₂ cells, which are considered more mature have been reported to be elevated in rural areas where helminth infections are highly prevalent [5].

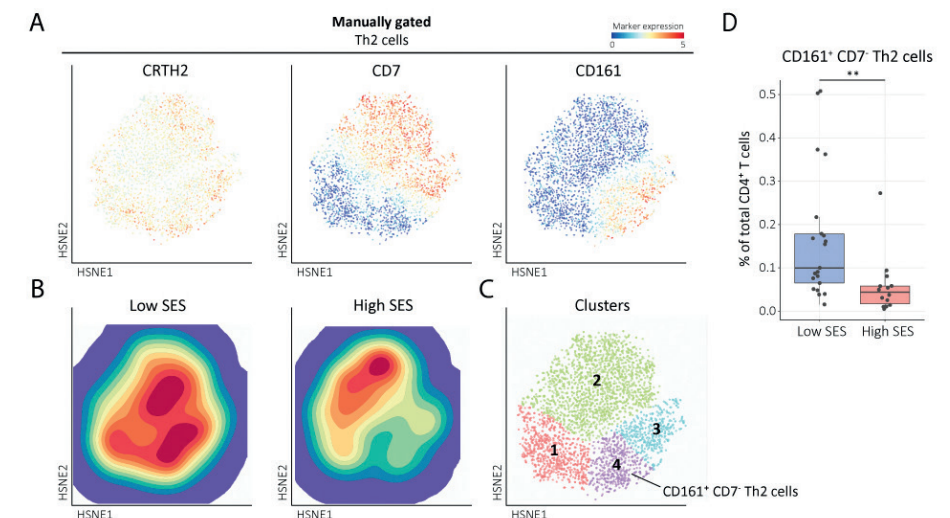


Figure 3. CD161⁺ CD7⁻ Th₂ cells are more abundant in low SES children

(A) T helper 2 (Th₂) cells were manually gated by selecting CD4⁺ T cells expressing CRTH2 and clustered using HSNE. The markers CD7 and CD161 were differently expressed within the Th₂ population as depicted. (B) Density features of the Th₂ population, stratified by SES showed reduced cell density of cells corresponding to cluster 4 (as identified in (B)). (C) Cluster partitions of Th₂ cells using GMS clustering. Four distinct clusters were identified within the Th₂ population. The cluster containing CD161⁺ CD7⁻ Th₂ cells is highlighted. (D) Boxplot depicting the comparison of the frequency of CD161⁺ CD7⁻ Th₂ cells (Cluster 5 of the Th₂ population in (B)) relative to number of total Th₂ in high and low SES. The boxes represent the interquartile range, the line within represents the median and the whiskers represent the 1.5 IQ of the upper and lower quartile. ** $P < 0.01$. Differences were tested using a Student's t test.

Next to Th2 cells, T helper 1 (Th1) cells could be identified on expression of Tbet and regulatory T cells formed a distinct cluster characterized by FoxP3 and CD25 expression (**Figure 4A**). In the high SES the frequency of Th₁ cells, as percentage of total CD4⁺ T cells, tended to be increased compared to the low SES ($p=0.078$) (**Figure 4B**). Within T_{regs} (FoxP3⁺), which did not significantly differ between high and low SES (**Figure 4C**), the cluster expressing CCR7, CTLA-4, ICOS and CD38 (**Figure 4D-E**) was increased significantly in low SES children. It is interesting that these regulatory T cells have been reported to have an increased regulatory activity [22] ($p=0.033$) (**Figure 4F**). Therefore, our analysis revealed that within CD4⁺ cells, a more skewed responses towards Th₂ and suppressive regulatory phenotype is seen the low SES children, while in the high SES a trend towards a more Th₁ phenotype.

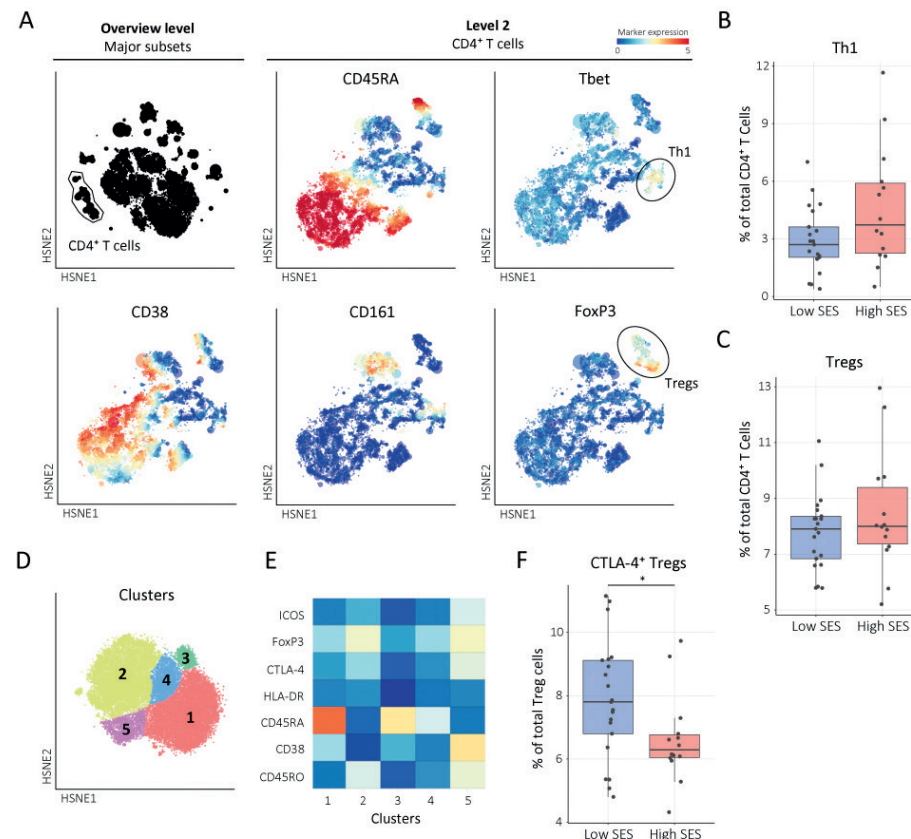


Figure 4. In depth analysis of CD4⁺ T cell population frequencies in low versus high SES children.

(A) Second HSNE level embedding of CD4⁺ cells (CD4⁺) selected from the overview of total PBMCs, as indicated by the black outline.

Markers that were heterogeneously expressed within the CD4⁺ T cell population are depicted. T helper 1 (Th₁) cells were identified as Tbet⁺ and regulatory T cells (T_{regs}) as FOXP3⁺ within the CD4⁺ T cell compartment and are encircled in black. (B) Boxplot depicting the comparison of the frequency of Th₁ cells relative to total number of CD4⁺ T cells in high and low SES children. The boxes represent the interquartile range, the line within represents the median and the whiskers represent the 1.5 IQ of the upper and lower quartile. (C) Boxplot depicting the comparison of the frequency of T_{regs} relative to number of CD4⁺ T cells in high and low SES. The boxes represent the interquartile range, the line within represents the median and the whiskers represent the 1.5 IQ of the upper and lower quartile. (D) Cluster partitions of T_{regs} using Gaussian mean-shift (GMS) clustering. Five distinct clusters were identified. (E) Heatmap summary of median expression values (same color coding as for the embedding) of cell markers expressed by FOXP3⁺ T_{reg} clusters identified in (D). (F) Boxplot depicting the comparison of the frequency of CTLA-4⁺ T_{regs} (Cluster 5 of the T_{reg} population in (D)) relative to number of total T_{regs} in high and low SES. The boxes represent the interquartile range, the line within represents the median and the whiskers represent the 1.5 IQ of the upper and lower quartile. * $p < 0.05$. Differences were tested using a Student's *t* test.

Monocytes are another group of cells that have shown variation across geographical areas and populations [6, 23, 24]. When examining the monocytes (**Figure 5A-B**), CD163⁺ HLA-DR^{dim} monocytes (cluster 3), were significantly higher in the low SES compared to the high SES (**Figure 5C**). Low expression of HLA-DR has been observed in monocytes with a suppressive phenotype [25] as has the expression of CD163 linked to anti-inflammatory responses [26, 27], which together indicate that at the innate immune cell level, there is skewing of monocytes with a more regulatory profile in the low SES group. In other compartments including those containing CD8⁺ T cells, unconventional T cells, ILCs/NK cells, eosinophils, basophils and dendritic cells, no differences were found between high and low SES children.

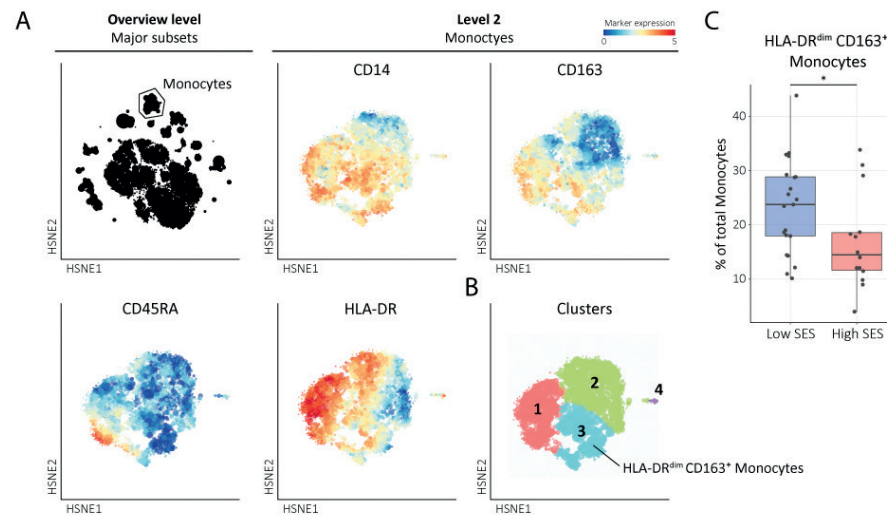


Figure 5. HLA-DR^{dim} CD163⁺ monocytes are elevated in the low SES

(A) Second HSNE level embedding of monocytes (CD11c⁺ CD14⁺) selected from the overview of total PBMCs, as indicated by the black outline. Markers that were heterogeneously expressed within the monocyte population are depicted. (B) Cluster partitions of monocytes using GMS clustering. Four distinct clusters were identified within the monocyte population. The cluster containing HLA-DR^{dim} CD163⁺ monocytes is highlighted. (C) Boxplot depicting the comparison of the frequency of HLA-DR^{dim} CD163⁺ monocytes (Cluster 3 of the monocyte population in (B)) relative to number of total monocytes in high and low SES. The boxes represent the interquartile range, the line within represents the median and the whiskers represent the 1.5 IQ of the upper and lower quartile. * $P < 0.05$. Differences were tested using a Student's t test.

DISCUSSION

In this study, we compared the immune profile in finger-prick blood of schoolchildren from low and high SES using mass cytometry. The results show that even within an urban center differences in the immune profiles is seen between children attending high and low SES schools. The immune profile of low SES children shows an expanded B cell compartment with more antigen experienced B cells, higher frequency of CD4⁺ cells that have a regulatory or Th₂ phenotype, and an increase in monocytes with low HLA-DR and increased CD163 expression, while in high SES, a trend towards an increase in Th₁ cells can be seen which indicates a stronger Th2 skewing. Overall, this illustrates that differential environmental and lifestyle factors such as exposure to microorganisms and parasites or dietary nutrients that are associated with SES, have a significant impact on the immune profiles of

school-aged children and that this can be studied using a minimum amount of whole blood sampling that allows analysis by mass cytometry (CyTOF).

We confirmed that the observed expansion of Th₂ and T_{reg} clusters in rural areas of Indonesia where helminth infections are endemic, [5, 28, 29] is also seen in children of low SES. The study analyzing the immune system of rural helminth-infected Indonesians before and after deworming, along with urban Indonesians and Europeans, showed that CD161⁺ Th₂ cells and a Treg subset expressing CTLA-4 were expanded in rural Indonesians and decreased after deworming [5]. In our current study, the school serving the low SES areas, had more children infected with intestinal helminths. However, the prevalence of helminths found in the current study is lower than what has been reported before in the same school [42-43], which can be accounted for by the recent regular deworming programs at these schools. It is possible that light infections not detectable by Kato Katz method were accounting for the skewing towards Th₂ and T_{reg}. In addition, alterations of the immune system by helminths can persist, even after deworming [44,45]. It is clear that, the children of low SES show immune profiles that are similar to what has been seen in rural areas where helminth infections are highly prevalent. Other studies of helminth infected subjects, have shown that the frequency of CTLA-4⁺ T cells decreases upon treatment with albendazole in helminth-infected individuals from a rural area of Indonesia [28] and in another part of the world, CTLA-4⁺ Tregs have been shown to be involved in hookworm-induced immunosuppression [29]. Thus, not only the environmental exposure in rural areas can drive the expansion of Th₂ and Treg but also within one urban center, where large differences in SES is seen. Regarding differences in B cells, the percentage of CD11c⁺ B cells within the total B cell compartment was similar between high and low SES groups. However, the total increase in total B cells and CD11c⁺ B cells, which have been reported to be associated with recent antigen-driven stimulation [21, 30-36], can reflect the higher exposure of low SES children to an environment richer in microorganisms and parasites than their high SES counterparts.

Regarding the myeloid compartment, an increased number of CD163⁺ monocytes dimly expressing HLA-DR, were seen in the low SES. Low expression of HLA-DR is one of the hallmarks of myeloid derived suppressor cells (M-MDSCs), [25, 37]. Expansion of MDSCs has been observed in individuals with chronic bacterial, viral and parasitic infections [38, 39] and therefore might be reflective of the higher burden of microbes in low SES children. Interestingly, M-MDSCs at baseline have been shown to be associated with lower IFN- γ responses to vaccines in African infants [40].

However, identification of MDSC is challenging and requires functional assays to confirm their suppressive activity [41], therefore future studies in which the functionality of these subset is assessed, are needed to further characterize them and validate our findings.

Altogether, it is interesting to capture some of the reported differences in immunological profiles of rural versus urban populations, in the current study comparing high and low SES children within one city. By using mass cytometry and 39 antibodies in the immune cell profiling panel, it was possible to get a broad picture of the immune cell profiles within a single finger prick sample. It is important to note that using whole blood samples obtained by finger-prick and preserved by fixation, allowed blood sampling without requirement for complex equipment, which is in contrast to peripheral blood mononuclear cell sampling. This simplifies the ability to collect blood for profiling from diverse geographical areas and populations in a standardized manner. However, the limitation is that the small volume limits the functional analysis of immune cells or profiling of very rare events, although in the current study we were able to identify rare cell populations, such as innate lymphoid cells (ILCs).

To conclude, the immune profile of low SES children has a more regulatory and Th₂ phenotype and an expanded B cell compartment characterized by expression of CD11c, whereas in the high SES there is a trend towards a shift to Th₁. These findings indicate that environmental exposure associated with SES, has a substantial impact on the phenotype of the immune system of children within one city. Therefore, SES should be factored in during evaluations of immunogenicity and efficacy studies of vaccines targeting infectious diseases that afflict LMICs.

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Supplementary materials

Table S1. Antibody panel CyTOF

Label	Specificity	Clone	Antibody vendor	Catalogue number	Dilution
⁸⁹ Y	CD45	HI30	Fluidigm ^a	3089003B	1/200
¹¹⁵ In	CD5	UCHT2	Biolegend ^b	300602	1/100
¹⁴¹ Pr	CD196 (CCR6)	G034E3	Fluidigm	3141003A	1/200
¹⁴² Nd	CD19	HIB19	Fluidigm	3142001B	1/200
¹⁴³ Nd	cPARP	F21-852	Fluidigm	3143011A	1/50
¹⁴⁴ Nd	CD66b	REA306	Miltenyi ^c	130-108-019	1/50
¹⁴⁵ Nd	CD4	RPA-T4	Biolegend	300541	1/100
¹⁴⁶ Nd	CD8a	RPA-T8	Biolegend	301053	1/200
¹⁴⁷ Sm	CD117 (C-kit)	104D2	BioLegend	313202	1/100
¹⁴⁸ Nd	CD14	M5E2	Biolegend	301843	1/100
¹⁴⁹ Sm	CD25 (IL-2Ra)	2A3	Fluidigm	3149010B	1/100
¹⁵⁰ Nd	CD185 (CXCR5)	J252D4	BioLegend	356902	1/100
¹⁵¹ Eu	CD123	6H6	Fluidigm	3151001B	1/100
¹⁵² Sm	TCRγδ	11F2	Fluidigm	3152008B	1/50
¹⁵³ Eu	CD7	CD7-6B7	Biolegend	343111	1/100
¹⁵⁴ Sm	CD163	GHI/61	Biolegend	333602	1/100
¹⁵⁵ Gd	CD278 (ICOS)	C398.4A	BioLegend	313502	1/50
¹⁵⁶ Gd	CD294 (CRTH2)	BM16	Biolegend	350102	1/50
¹⁵⁷ Gd	ACE-2	AC18F	Novus Biologicals ^d	NBP2-80035-100UG	1/100
¹⁵⁸ Gd	CD209 (DC-SIGN)	9E9A8	Biolegend	330102	1/100
¹⁵⁹ Tb	CD197 (CCR7)	G043H7	Biolegend	353237	1/200
¹⁶⁰ Gd	FoxP3	PCH101	eBioscience ^e	14-4776-82	1/50
¹⁶¹ Dy	KLRG1 (MAFA)	REA261	Miltenyi	Special order	1/100
¹⁶² Dy	CD11c	Bu15	Biolegend	337221	1/200

Table S1. Antibody panel CyTOF - continued

Label	Specificity	Clone	Antibody vendor	Catalogue number	Dilution
¹⁶³ Dy	CD152 (CTLA-4)	BNI3	BioLegend	369602	1/100
¹⁶⁴ Dy	CD161	HP-3G10	Biolegend	339919	1/100
¹⁶⁵ Ho	CD127 (IL-7Ra)	A019D5	Fluidigm	3165008B	1/200
¹⁶⁶ Er	Tbet	4B10	BioLegend	644825	1/100
¹⁶⁷ Er	CD27	O323	Biolegend	302839	1/200
¹⁶⁸ Er	HLA-DR	L243	Biolegend	307651	1/200
¹⁶⁹ Tm	CD45RA	HI100	Fluidigm	3169008B	1/100
¹⁷⁰ Er	CD3	UCHT1	Biolegend	300443	1/100
¹⁷¹ Yb	CD206	15-2	Biolegend	321127	1/200
¹⁷² Yb	CD38	HIT2	Biolegend	303535	1/200
¹⁷³ Yb	CD45RO	UCHL1	Biolegend	304239	1/100
¹⁷⁴ Yb	CD335 (NKp46)	92E	Biolegend	331902	1/100
¹⁷⁵ Yb	PD-1	EH12.2H7	Biolegend	329941	1/100
¹⁷⁶ Yb	CD56	B159	BD Biosciences ^f	555514	1/100
²⁰⁹ Bi	CD16	3G8	Fluidigm	3209002B	1/200

^a Fluidigm, South San Francisco, CA, USA. ^b Biolegend, San Diego, CA, USA. ^c Miltenyi Biotech, Bergisch Gladbach, Germany. ^d Novus Biologicals Centennial, CO, USA ^e eBioscience, San Diego, CA, USA. ^f BD Biosciences Franklin Lakes, NJ, USA. ACE2, Angiotensin-converting enzyme 2. CCR, C-C chemokine receptor. CD, cluster of differentiation. CRTH2, prostaglandin D2 receptor 2. CXCR, CXC chemokine receptor. FOXP3, forkhead box P3. HLA-DR, human leukocyte antigen-D-related. IL-2R, interleukin-2 receptor. IL-7Ra, interleukin-7 receptor α. KLRG1, killer cell lectin-like receptor subfamily G member 1. MAFA, mast cell function-associated antigen. PD-1, programmed cell death protein. Tbet, T-box transcription factor TBX21. TCR, T-cell receptor. All markers were stained on the cell surface.

Table S2. Barcoding panel

Label	Specificity	Clone	Antibody vendor	Catalogue number	End dilution
¹⁰⁶ Cd	B2M	2M2	Biolegend ^a	316302	1/50
¹¹⁰ Cd	B2M	2M2	Biolegend	316302	1/50
¹¹¹ Cd	B2M	2M2	Biolegend	316302	1/50
¹¹² Cd	B2M	2M2	Biolegend	316302	1/50
¹¹⁴ Cd	B2M	2M2	Biolegend	316302	1/50
¹¹⁶ Cd	B2M	2M2	Biolegend	316302	1/50
¹⁹⁸ Pt	B2M	2M2	Biolegend	316302	1/50

^a Biolegend, San Diego, CA, USA. B2M, Beta-2 microglobulin.

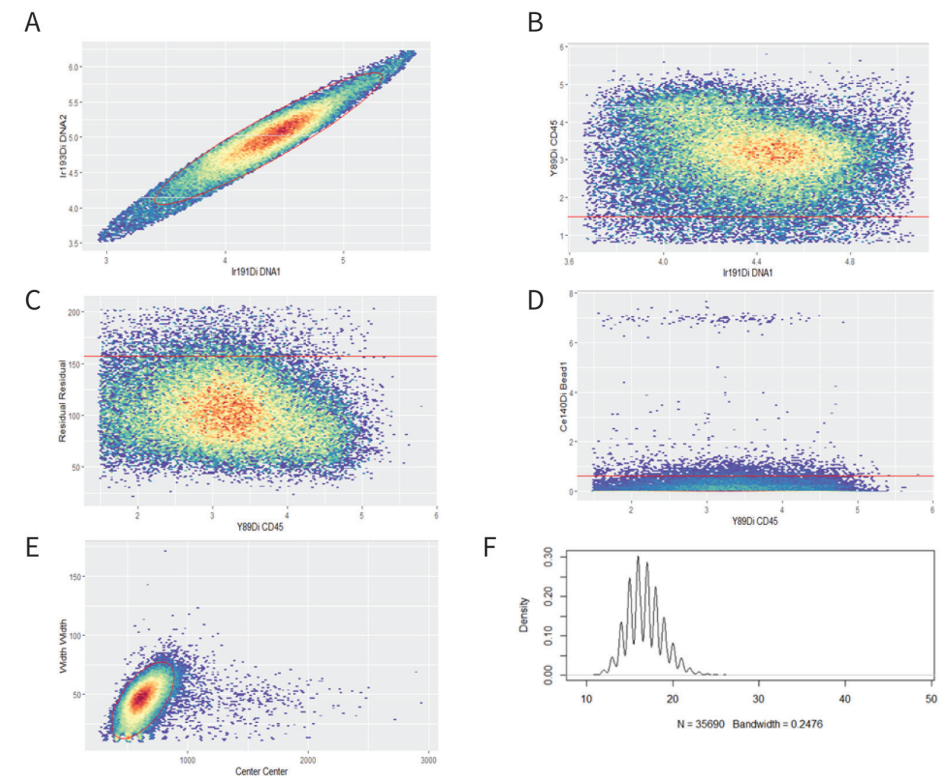


Figure S1. Pre-processing mass cytometry data. (A) Selection events with positive DNA staining. For further analysis 95% of all events with a positive DNA staining were selected, encircled in red. (B) Selection CD45⁺ events. Red line represents the cut-off point used during the pre-processing, all events above this threshold were selected. (C) Removal of events with high residual value, a Gaussian parameter created during normalization. All events below the red

line were selected **(D)** Removal of calibrating beads from the data. Red line represents the cut-off point, all events below the threshold were selected. **(E)** Selection of events with appropriate width and center values, Gaussian parameters created during normalization. Events within the red circle represent the events that were selected. **(F)** Removal events with an increased event length. All events with an event length of 24 or beneath were selected.

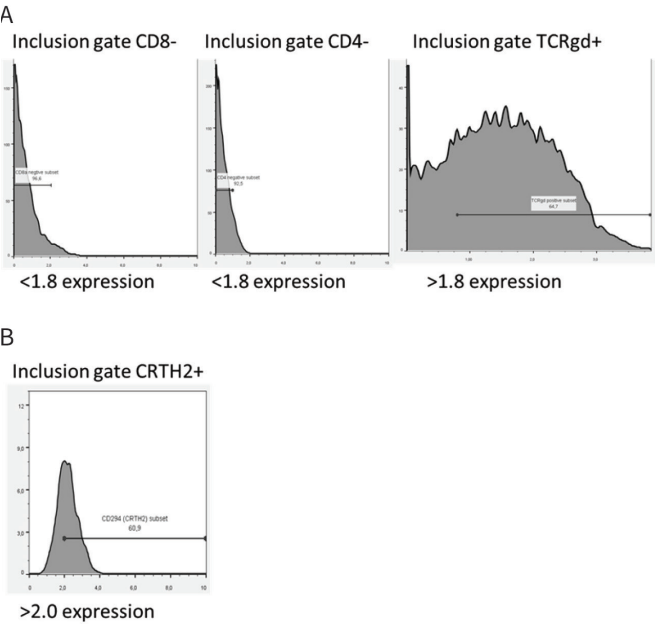


Figure S2. Manual gating strategy for (A)Th2 cells (B) for TCRgd T cells.

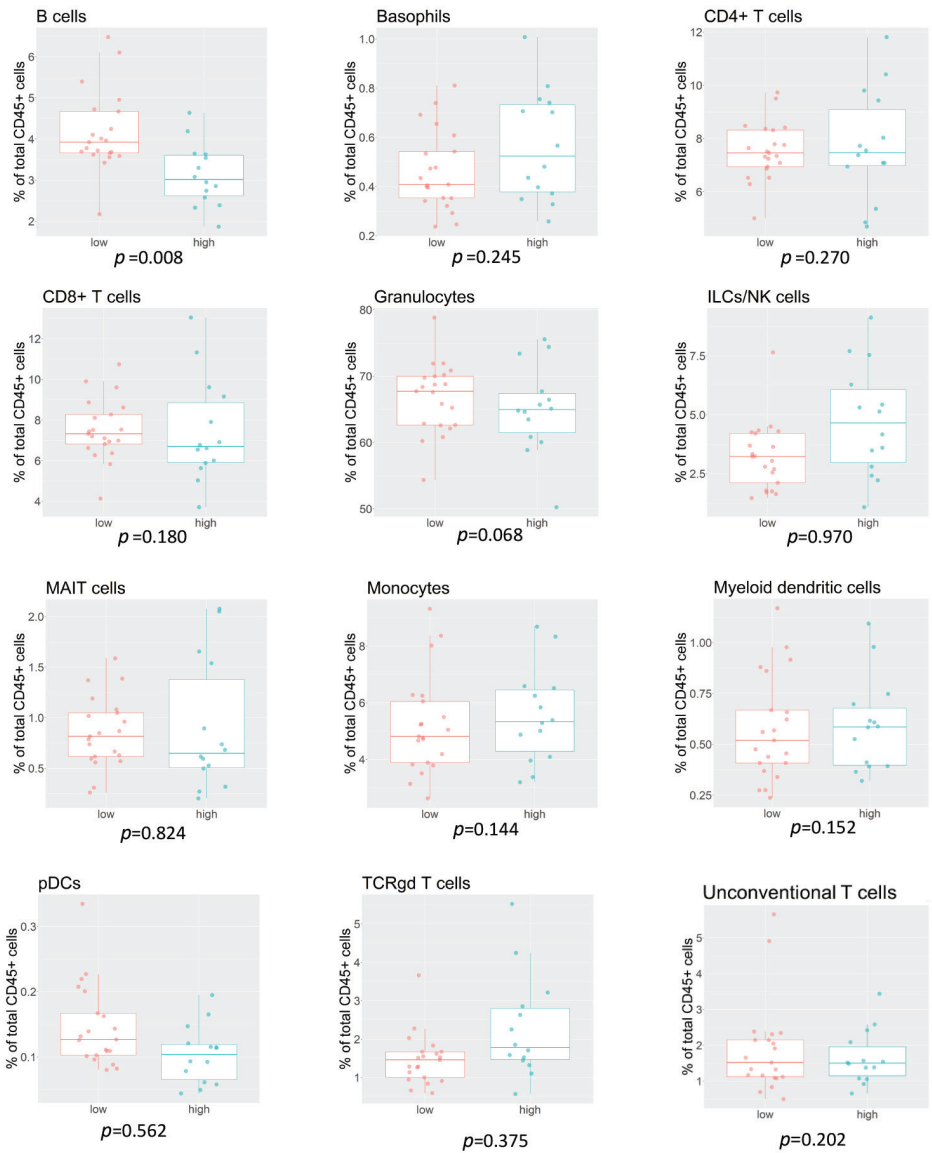


Figure S3. Major immune lineages annotated on the basis of lineage marker expression. Boxplots depicting the comparison of the frequency of these major immune lineage relative to number of total CD45+ cells in high and low SES. Differences were tested using a Student's *t* test.