

ORIGINAL ARTICLE

Atopic Dermatitis, Urticaria and Skin Disease

Environment-dependent alterations of immune mediators in urban and rural south African children with atopic dermatitis

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Abstract

Background: In order to improve targeted therapeutic approaches for children with atopic dermatitis (AD), novel insights into the molecular mechanisms and environmental exposures that differentially contribute to disease phenotypes are required. We wished to identify AD immunological endotypes in South African children from rural and urban environments.

Methods: We measured immunological, socio-economic and environmental factors in healthy children ($n = 74$) and children with AD ($n = 78$), in rural and urban settings from the same ethno-linguistic AmaXhosa background in South Africa.

Results: Circulating eosinophils, monocytes, TARC, MCP-4, IL-16 and allergen-specific IgE levels were elevated, while IL-17A and IL-23 levels were reduced, in children with

Abbreviations: AD, Atopic Dermatitis; bFGF, basic fibroblast growth factor; CCD, cross-reactive carbohydrate determinants; FLT1, FMS-like tyrosine kinase; HC, Healthy Control; IgE, Immunoglobulin E; IL, Interleukin; MCP, monocyte chemoattractant protein; MDC, macrophage-derived chemokine; MIP, macrophage inflammatory protein; PIGF, placental growth factor; SCORAD, SCORing Atopic Dermatitis index; sICAM, soluble inter-cellular adhesion molecule; SPT, skin prick test; sVCAM, soluble vascular cell adhesion molecule; TARC, thymus and activation regulated chemokine; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

O'Mahony and Akdis share senior authorship.

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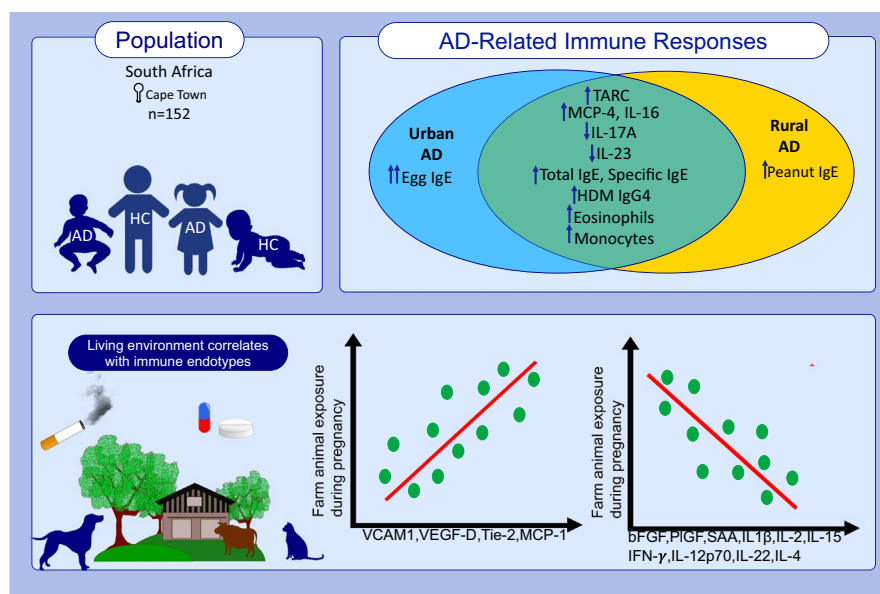
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AD regardless of their location. Independent of AD, children living in a rural environment had the highest levels of $\text{TNF}\alpha$, $\text{TNF}\beta$, IL-1 α , IL-6, IL-8, IL-21, MCP-1, MIP-1 α , MIP-1 β , MDC, sICAM1, sVCAM1, VEGFA, VEGFD and Tie2, suggesting a generalized microinflammation or a pattern of trained immunity without any specific T_H polarization. In contrast, IL-15, IL-22, Flt1, PIGF and β FGF were highest in urban children. Rural healthy children had the lowest levels of food allergen-specific IgG4. Early life nutritional factors, medications, animal exposures, indoor environment, sunlight exposure, household size, household income and parental education levels were associated with differences in circulating cytokine levels.

Conclusions: This study highlights the immunological impact of environmental exposures and socio-economic status in the manifestation of immune endotypes in children with AD living in urban and rural areas, which are important in selecting appropriately matched immunological therapies for treatment of AD.

KEYWORDS

atopic dermatitis, cytokines, endotypes, environment, personalized medicine



GRAPHICAL ABSTRACT

In this cohort of children with atopic dermatitis from South Africa, elevated T_H2 responses are accompanied by diminished T_H17 responses. Environmental and socio-economic factors dominate the functional reprogramming of immune cell reactivity in children from urban and rural settings. This data highlights the need to include patients from multiple ethnicities, environments and socio-economic backgrounds in research studies and clinical trials assessing novel immune targeting therapies.

1 | INTRODUCTION

Atopic dermatitis (AD) is the most common chronic pruritic inflammatory skin disease affecting 15–20% of children and 1–3% of adults globally and is often a foreshadow of other atopic disorders.¹ It is a complex disease with a wide spectrum of clinical phenotypes and is characterized by skin barrier defects, microbial dysbiosis (with *Staphylococcal aureus* colonization) and dysregulated immune responses.^{2–6} Several different molecular

mechanisms can lead to similar clinical outcomes, which has led to the concept of AD endotypes (an AD subtype defined by a distinct pathophysiological mechanism).⁷ In the era of personalized medicine, a systems biology approach merging the numerous immunological biomarkers and related environmental exposures will be important for stratified treatment of distinct immune and inflammatory subgroups.

Recent studies have recognized an increasing prevalence and severity of AD and asthma in individuals of African ancestry compared

with those of European descent, to the extent that children of African ancestry have a sixfold increased risk of severe AD compared to European children and African Americans are 5 times likely to die following acute asthma exacerbations compared to European Americans.^{8–12} Polymorphisms in genes associated with T_H2 immune responses have been linked to an increased susceptibility to allergic diseases among African populations.^{13,14} In addition to genetic factors, environmental exposures influence the development of AD.¹⁵ Within South Africa, a lower prevalence of atopy in rural areas compared with urban areas still exists but this rural-urban gradient is rapidly diminishing over time.¹⁶ However, while allergy rates might be different between rural and urban settings, children still do develop allergy in both these diverse environments. Thus, there is a need to characterize the immunological endotypes underpinning AD in rural and urban environments. To this end, we have studied the immune profiles of children from the same ethno-linguistical population with AD and healthy controls in a developing country with distinct environmental settings, that is rural and urban environments in South Africa.

In the present study, we analysed plasma samples of 152 children between 12 and 36 months of age from rural subsistent farming environment and urban township settings in South Africa for inflammatory responses in AD. We report a comprehensive analysis of total IgE, allergen-specific IgE and IgG4, peripheral blood immune cells, plasma cytokines, chemokines and growth factors that reflect immune imprinting in early childhood in AD and healthy children in two distinct environments. Both AD-related and environment-related factors were identified that significantly influence immunological polarization networks.

2 | METHODS

2.1 | Study design

This study was designed as a matched case-control study conducted at one time point in patients with moderate to severe AD. All participants were from the ethno-linguistic AmaXhosa population and aged 12–36 months. Urban children with moderate to severe atopic dermatitis ($n = 32$) were enrolled at the Paediatric Dermatology Clinic, Red Cross Children's Hospital, Cape Town. Healthy non-allergic and non-food sensitized (on SPT screening) participants ($n = 35$) were enrolled from randomly selected crèches in the Cape Town metropole. Similarly, children from a rural setting with AD ($n = 46$) were enrolled at the dermatology clinic at the Nelson Mandela Academic Hospital, Umthatha. Their non-allergic healthy control counterparts ($n = 39$) were randomly selected at study sites based at village clinics in the Mqanduli district of Umthatha. All participants were seen at one time point for questionnaire, SPT, clinical assessment and blood sample collection. This clinical study received Human Research Ethics Committee approval (HREC 451/2014) and was conducted in accordance with the declaration of Helsinki and written informed

consent was obtained from parents/guardians of all participants prior to inclusion in the study.

2.2 | Inclusion and exclusion criteria

Participants with moderate to severe atopic dermatitis were eligible for inclusion, if they had a dermatologist diagnosis of AD and fulfilled the UK Working Party Diagnostic criteria.¹⁷ Moderate to severe disease was graded according to the objective SCORAD (SCORing Atopic Dermatitis) index.^{18,19} All participants with AD were on soap substitutes, emollients and topical steroid therapy only. Healthy, non-allergic, age-matched and gender-matched participants were eligible as controls if they had no history of atopy and were non-food sensitized by objective SPT. Both AD and healthy participants were excluded, if they had any other skin or systemic disease. Any child with a positive stool test for helminths was excluded from the analysis.

2.3 | Skin prick test and allergen-specific IgE antibodies

We assessed all participants by skin prick test (SPT) with standardized reagents from ALK-Abello for sensitization to seven common allergenic foods, that is cow's milk, egg (white), peanut, hazelnut, wheat, soy, fish (cod) with positive and negative controls performed with 10 mg/mL histamine and saline, respectively. In addition, modified SPT of raw egg white, fresh cow's milk and fresh peanut was performed.²⁰ Total IgE, allergen-specific IgE, allergen-specific IgG4 and anti-CCD IgE were measured using singleplex ImmunoCap (Thermo Fisher Scientific/Phadia, Uppsala, Sweden).

2.4 | Questionnaires

Detailed standardized questionnaires were completed with the parent/guardian to obtain data on family history of atopy, household size, family income, infant exposures, medication use and environmental exposures, as previously described.²⁰

2.5 | Peripheral blood immune cells and cytokines

Blood was collected in lithium heparin tubes. Peripheral blood total leukocyte counts, eosinophil, neutrophil, monocyte and lymphocyte numbers were quantified using an automated cell counter at the routine hospital laboratory. Plasma samples were obtained following centrifugation and were stored at -80°C until batched analysis. Levels of 44 plasma analytes were quantified using the ultrasensitive Meso Scale Discovery multi-spot assay system (Meso Scale Discovery, Gaithersburg, MD, USA). Plasma cytokine levels were quantified using the MSD QuickPlex SQ 120 platform.

TABLE 1 Participant demographics

	Rural AD	Rural HC	Urban AD	Urban HC
Study population (n)	46	39	32	35
Age [mo]	21.0 (15.8–25.0)	20.1 (15.0–35.0)	22.5 (16.0–28.0)	24.4 (18.0–34.0)
Gender (F/M)	19/26	16/23	14/18	18/17
Height [cm]	80.0 (76.8–85.3)	84.0 (79.0–87.0)	81.5 (77.5–84.8)	85.0 (78.0–89.0)
Weight [kg]	11.7 (10.5–13.2)	12.0 (10.8–13.0)	12.0 (10.3–13.1)	12.5 (10.5–14.6)
SCORAD	43.0 (37.4–51.6)		43.5 (36.1–55.3)	

Note: Results are shown as median with the 25% - 75% percentile range in brackets.

Abbreviations: AD, Atopic Dermatitis; HC, Healthy Control.

2.6 | Statistical analysis

Determination of statistical significance between two groups was estimated using the Mann-Whitney test (Prism GraphPad Software version 7TM). Comparisons between multiple groups were performed using the Kruskal-Wallis test, while differences in proportions between groups for the demographic data were estimated using the Fisher's exact test. Associations between plasma cytokine levels and environmental exposures were examined using a linear regression model. Briefly, nearly zero variance, correlated and linear dependent environmental exposure measurements were discarded. Then, a recursive feature elimination algorithm was used to keep the most informative environmental exposure measurements. Finally, a linear regression model was built for each cytokine to explore both positive and negative associations. Data visualization using heatmaps was produced by R package *corrplot* (<https://cran.r-project.org/web/packages/corrplot/index.html>).

3 | RESULTS

3.1 | Study population

The urban AmaXhosa study recruits reside in urban townships, while the rural AmaXhosa study recruits reside in rural reserves and live in homesteads (cluster of houses with extended family) practising subsistence crop and pastoral farming. There were no significant differences between the groups regarding age, gender, SCORAD, height or weight (Table 1). SPT reactivity rates for urban and rural children with AD were similar for most of the allergens tested, except for reactions to fresh egg and egg white, which were more common in urban AD (Table S1). Interestingly, the wheal diameter in response to histamine was significantly increased ($p < .001$; ANOVA) in urban and rural children with AD (median 5.5 mm and 5.0 mm, respectively) compared to urban and rural healthy children (median 4.0 mm and 4.0 mm, respectively). None of the rural or urban healthy controls were SPT positive, as required for entry into this study. Parents of rural healthy children had a lower level of formal education (Table S2) and their children were less frequently consuming Amasi (a fermented cow's milk drink), compared to all other groups. For both rural AD and rural healthy children, average household income was

lower with a larger average family size, normal vaginal delivery was more frequent, they were exposed to more sunlight in summer and winter, received paracetamol at a younger age, were more frequently exposed to cats, dogs, farm animals antenatally and during early childhood compared to their urban counterparts. Rural AD and healthy control children were less likely to be exposed to cigarette smoke in the home, but the rural healthy controls were more likely to live in a home using a paraffin stove or an open fire for cooking, compared to their urban counterparts.

3.2 | AD-related systemic immune changes

Regardless of study site, whole blood eosinophil and monocyte counts were significantly higher in children with AD compared to healthy children (Figure 1A). In addition, circulating levels of TARC, MCP-4 and IL-16 were significantly elevated in AD patients compared to healthy controls (Figure 1B). Eosinophil counts, TARC, MCP-4 and IL-16 levels were positively correlated with SCORAD scores ($r^2 = 0.173$, $r^2 = 0.151$, $r^2 = 0.254$ and $r^2 = 0.228$, respectively; $p < .0001$). In contrast, IL-17A levels were significantly reduced with a trend ($p = .059$) towards reduced levels of the IL-17-associated cytokine IL-23, in children with AD (Figure 1C). No significant differences in total white cell counts, lymphocyte and neutrophil counts, or any of the other plasma cytokines measured were observed when comparing all children with AD to all healthy controls (Figure S1).

When the data were separated based on study site, a subgroup of urban children with AD displayed significantly elevated plasma level of IL-4, which were not observed for rural children with AD (Figure 2A). In addition, in this urban cohort, IL-4 levels were positively correlated with plasma levels of IL-13, IL-12p70, IFN- γ , IL-1 β , IL-2 and IL-10 (Figure 2B). None of the AD severity, demographic or exposure data significantly associated with this urban cytokine^{high} subgroup.

Total IgE levels were elevated in both rural and urban AD groups, compared to healthy controls (Figure 3A). Similarly, levels of peanut-, soybean-, hazelnut-, wheat-, cod- and *D. pteronyssinus*-specific IgE and anti-CCD IgE were elevated in both AD groups relative to healthy controls. Egg-specific IgE was also elevated in both AD groups, with higher levels in urban healthy controls compared to rural healthy controls (Figure 3B). In contrast, cow's milk-specific

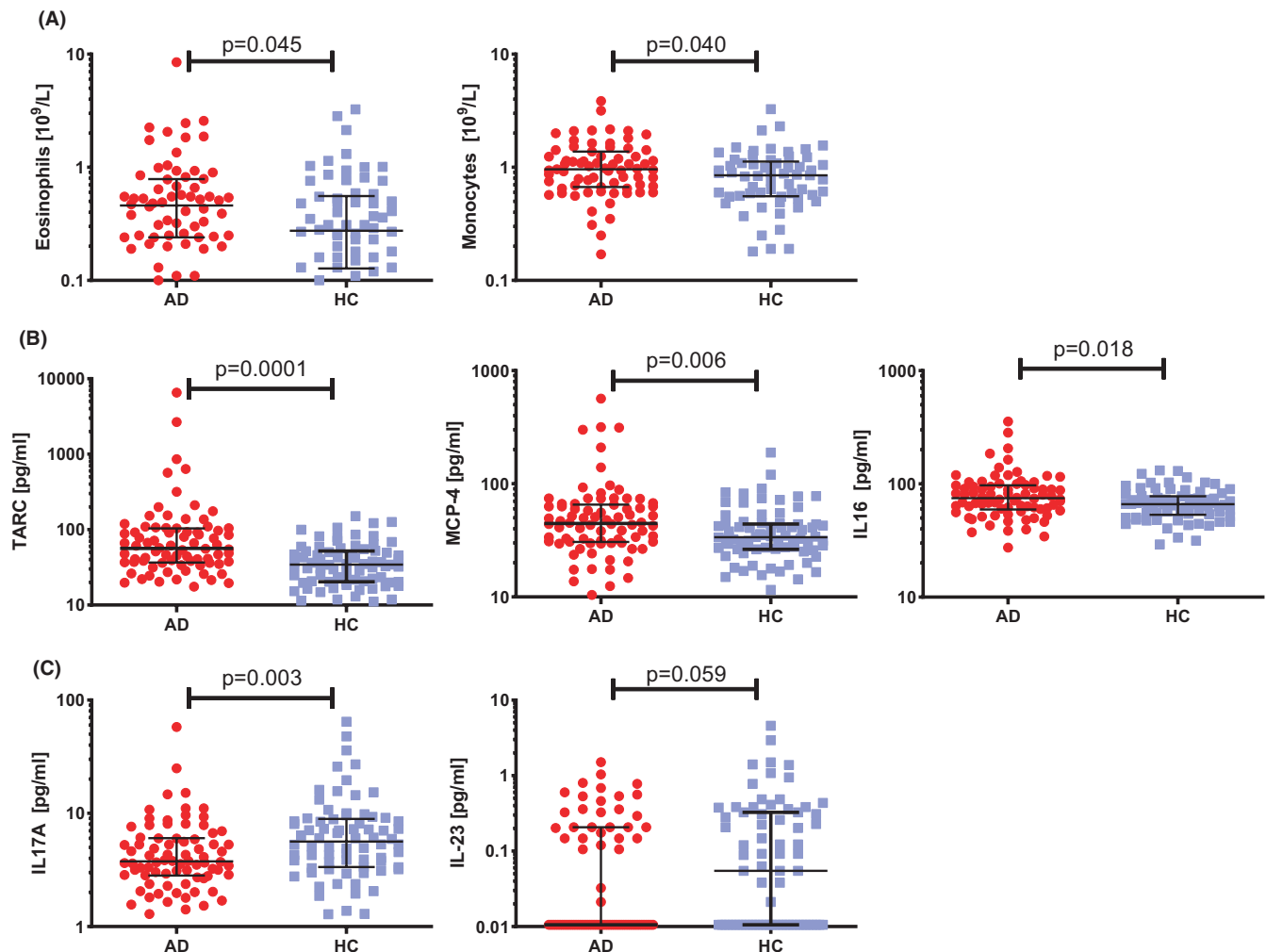


FIGURE 1 Circulating Immune Biomarkers Associated with AD. (A) Circulating eosinophils and monocytes are elevated in peripheral blood from children with atopic dermatitis (AD) compared to healthy controls (HC). (B) Plasma cytokine levels of TARC, MCP-4 and IL-16 are significantly elevated, while plasma levels of IL-17A and IL-23 are decreased in children with AD (C). Results are presented on a log scale and differences analysed using a Mann-Whitney test. The medians with interquartile ranges are indicated

IgE levels were higher in rural healthy controls compared to urban healthy controls (Figure 3B). Using 0.35 KU/L as a cut-off value to indicate sensitization, rural children with AD tended to be more frequently sensitized to peanut compared to urban children with AD (Table S3). Urban healthy controls were more likely to be sensitized to egg compared to their rural counterparts, while rural healthy controls were more likely to be sensitized to cow's milk, although none of these children had a positive SPT to egg or cow's milk, as described above.

All children had detectable specific IgG4s against milk, egg, peanut, hazelnut, soybean and wheat. Rural healthy control children had the lowest levels of peanut-specific IgG4, soybean-specific IgG4, hazelnut-specific IgG4, wheat-specific IgG4, egg-specific IgG4 and casein bos d8-specific IgG4 compared to all other groups (Figure 3C). Cod-specific IgG4 levels were lower in both rural groups compared to their urban counterparts (Figure 3D). In contrast to the food allergens, *D. pteronyssinus*-specific IgG4 levels were elevated in both AD groups (Figure 3E).

3.3 | Environment-related associations with circulating cytokines

Plasma levels of a large number of immunological markers were significantly affected by the environment that the children lived in, regardless of the presence of AD. Rural children had significantly elevated levels of the proinflammatory cytokines TNF- α and IL-6 compared to their urban counterparts (Figure 4A). Urban children with AD had higher levels of TNF- α and IL-6 compared to healthy urban children, but still at levels far lower than those observed in rural children. Rural children had the highest levels of IL-8, with particularly higher levels in AD. Circulating levels of other chemokines such as monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 α (MIP-1 α), MIP-1 β and macrophage-derived chemokine (MDC) were also significantly higher in the children living in the rural environment compared to children living in the urban environment (Figure 4B). Similarly, plasma IL-1 α , TNF- β , soluble inter-cellular adhesion molecule 1 (sICAM-1), soluble vascular cell adhesion molecule 1 (sVCAM1), vascular endothelial growth factor

A (VEGFA), VEGFD and Tie 2 levels were significantly higher in the rural children without showing any relationship to AD (Figure 4C and D). Circulating IL-21 was detected only in a subgroup of rural children, with rural healthy controls displaying the highest IL-21 levels.

In contrast, children living in an urban environment had the highest plasma levels of IL-22 and IL-15 (Figure 5A), accompanied by significantly increased levels of FMS-like tyrosine kinase (FLT1), placental growth factor (PIGF) and basic fibroblast growth factor (bFGF) (Figure 5B). A subpopulation of children with the highest

levels of PIGF and bFGF was noted in the urban group, but this subpopulation was not related to the presence of AD.

3.4 | Dominant environmental factors that influence circulating immune mediators

A wide range of environmental factors and exposures was associated with individual circulating cytokine levels (Figure 6).

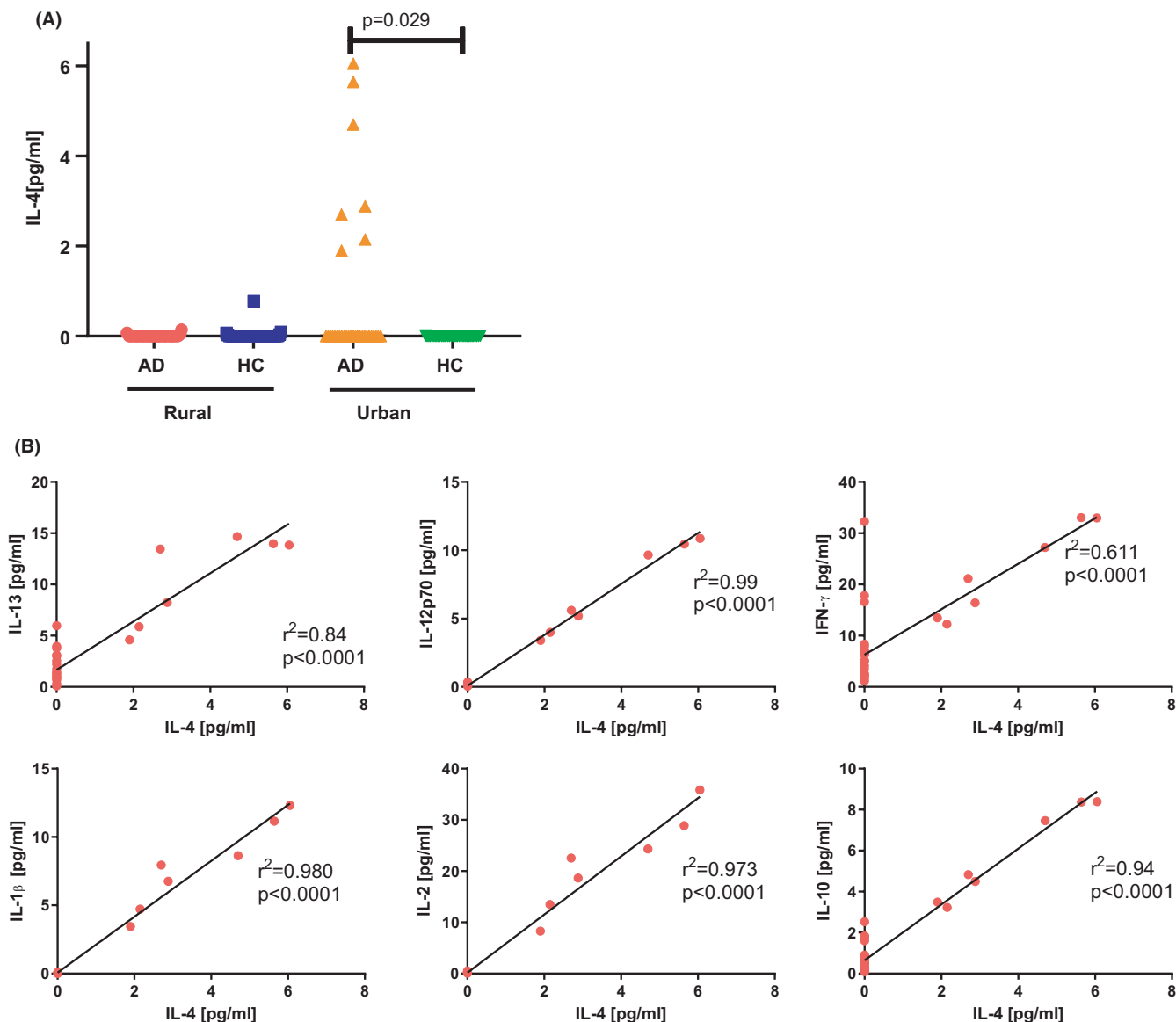
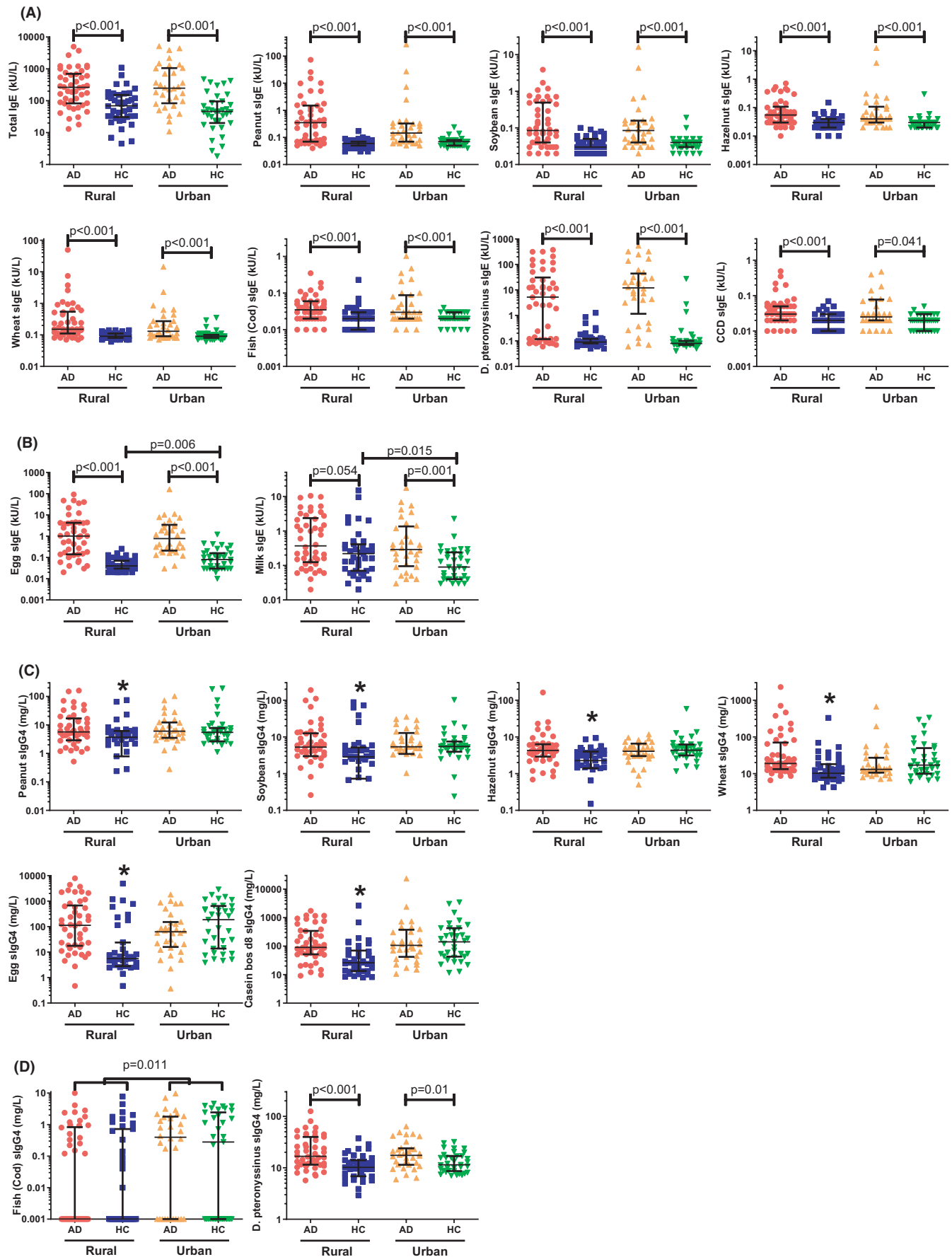


FIGURE 2 A Subgroup of Urban Children with AD Display Elevated Cytokine Levels. (A) Plasma IL-4 levels are highly elevated in a subgroup of urban children with AD. Results are presented on a linear scale and differences analysed using ANOVA. (B) Linear regression plots illustrate that the high levels of IL-4 in the urban AD children correlates significantly with increased plasma levels of IL-13, IL-12p70, IFN- γ , IL-1 β , IL-2 and IL-10

FIGURE 3 Allergen-Specific IgE and IgG4 Levels. (A) and (B) Total IgE and allergen-specific IgE levels are increased in the AD groups (Mann-Whitney test). (C) Allergen-specific IgG4 levels are lowest in the rural healthy children group (ANOVA). (D) Cod-specific IgG4 are highest in the urban group (Mann-Whitney). (E) D. pteronyssinus-specific IgG4 levels are elevated in children with AD (Mann-Whitney). All results are presented on a log scale with median and interquartile ranges indicated per group. * $p < .05$ versus all other groups



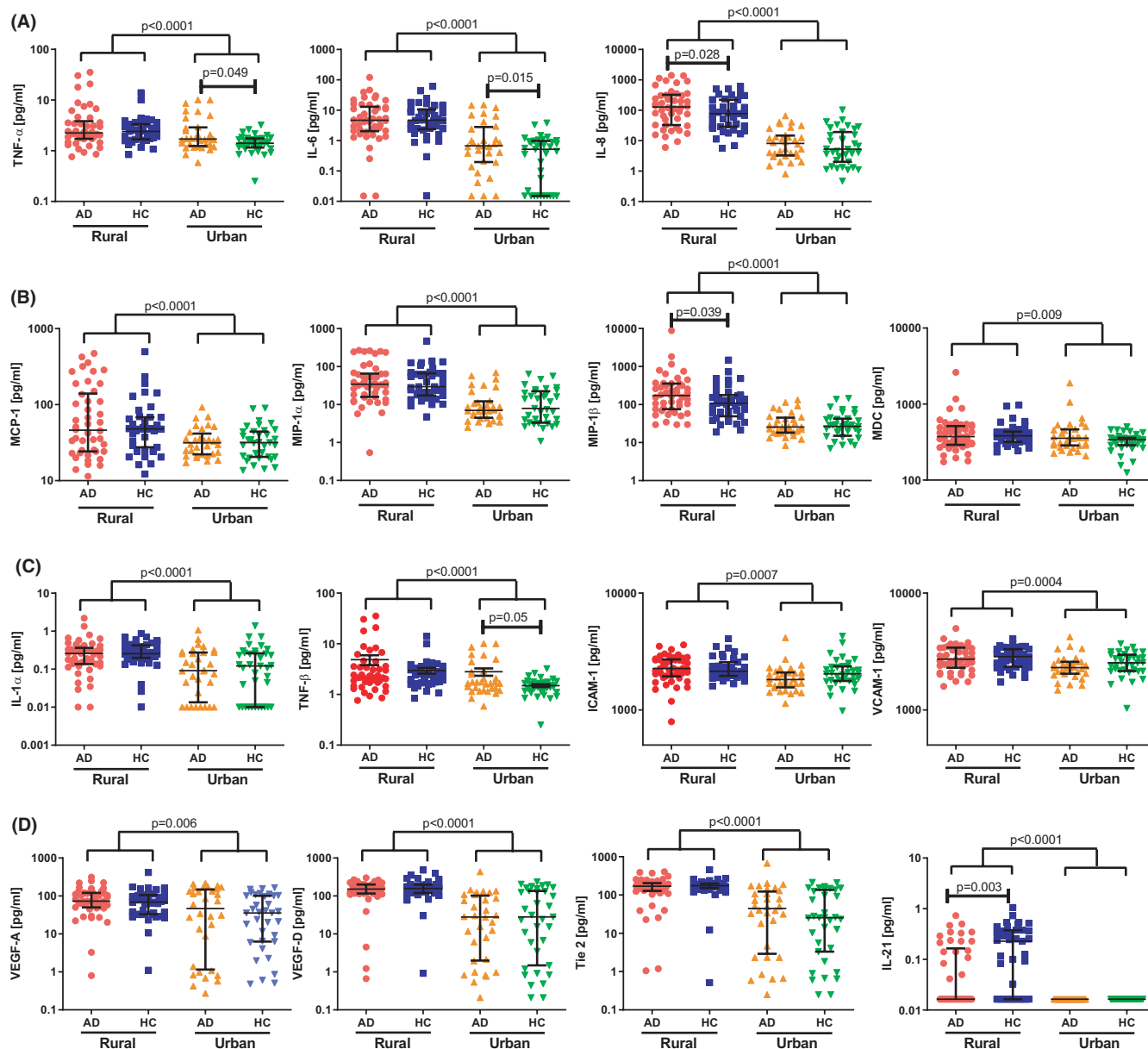


FIGURE 4 Increased Circulating Cytokines in Rural Children. Proinflammatory cytokines, chemokines, soluble adhesion molecules and growth factors (A–D) are significantly higher in plasma from rural children compared to urban children. Results are presented on a log scale and differences analysed using a Mann-Whitney test. Median and interquartile ranges are indicated per group

These included early life nutritional factors (such as age of solid food introduction and consumption of the fermented milk drink Amasi), use of medications (such as anti-histamines, steroid cream and paracetamol), animal exposures (in particular exposure to farm animals during pregnancy, which correlated with sVCAM1, VEGFD, bFGF, PIGF, Tie 2, IL-15, IFN- γ , IL-12p70, IL-22, IL-4 and MCP-1 levels), the indoor environment (including the type of fuel used for indoor heating and cooking), the outdoor environment (including sunlight exposure) and socio-economic factors (including the number of siblings, household size, household income and parental education level). When each group was analysed separately (Figures S2–S5), many of the associations were related to the

location of the child (ie rural versus urban) and the presence of AD also significantly altered the associations. For example, in urban children with AD, plasma IL-4 and IL-13 levels were positively correlated with the age of solid food introduction, use of antibiotics in the first year of life and household family number, while these cytokines were negatively correlated with cat exposure, farm animal exposure during pregnancy and the age of paracetamol first use. None of these factors were associated with IL-4 and IL-13 levels in rural children with AD. Similarly, IL-12/IL-23p40 plasma levels in urban healthy controls were positively correlated with Amasi consumption, household income, use of paracetamol in the first year and smoking in the home, while significant negative correlations

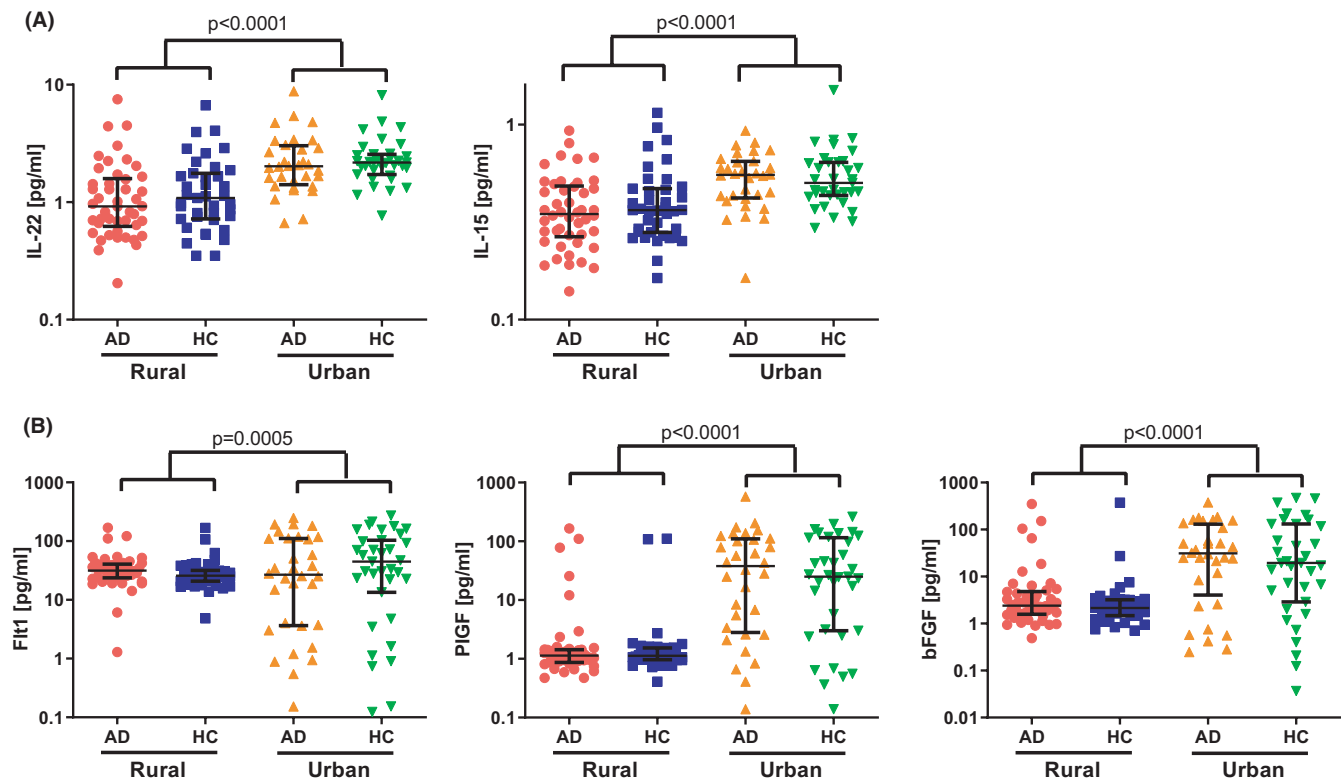


FIGURE 5 Increased Plasma Cytokines in Urban Children. Cytokines and growth factors (A-B) are significantly higher in plasma from urban children compared to rural children. Results are presented on a log scale and differences analysed using a Mann-Whitney test. Median and interquartile ranges are indicated per group

were observed for the type of heating fuel, age of paracetamol first exposure and the use of probiotics. However, there were no significant associations for IL-12/IL-23p40 levels with any of the measured exposures in rural children.

4 | DISCUSSION

In this report, we describe a range of circulating immunological mediators in South African children that (i) are altered due to the presence of an inflammatory atopic skin disease, that is AD or (ii) are dependent on the living environment of the child. Collectively, these data highlight the intersection of various environmental exposures including socio-economic status in the manifestation of inflammatory responses, which become particularly important in the context of disease (AD) and selecting appropriate disease targeting immunological therapies.

Several findings in this South African paediatric AD cohort are in keeping with results observed in other settings. Increased eosinophil and monocyte counts in the circulation of AD patients is in line with the well-described role of eosinophils and monocytes in skin inflammatory responses.^{21,22} TARC, IL-16 and MCP-4 are associated with inflammatory cell homing to the skin.^{19,23,24} The use of TARC and IL-16 as disease severity biomarkers may be particularly relevant for African AD patients as widely used disease severity scores such as Scoring Atopic Dermatitis Index (SCORAD), Six Area Six Sign

Atopic Dermatitis (SASSAD) and Eczema Area Severity Index (EASI), which are reliant on detection of erythema, grossly underestimate disease severity in darkly pigmented skin.¹² In contrast to the T_H2 cytokines, the reduced levels of IL-17A and IL-23 in this African paediatric cohort observed here are antithetical to previous observations in patients of European or Asian descent including paediatric AD.^{25,26} One possibility is that reduced IL-17A and IL-23 levels are related to the suppressive effects of T_H2 -related cytokines on T_H17 responses in AD²⁷ or this may be further evidence of a genetically predetermined robust predisposition to T_H2 skewing in populations of African ancestry^{13,14} and possibly concomitant Th17 suppression. Of note, the T_H17 /IL-17A axis is central to psoriasis pathogenesis and psoriasis is relatively rare in individuals of African descent.^{28,29} Taken together our observation may reflect population-specific immune responses. It will be interesting to determine in future studies if these cytokine differences are also observed in South African AD patients with mild disease, as our current data are limited to patients with moderate to severe AD.

The cytokine^{high} group described in this cohort represents an urban AD immune endotype with multipolar activation involving T_H2 (IL-4 and IL-13), T_H1 (IL-2, IL-12p70 and IFN- γ), proinflammatory (IL-1 β) and regulatory (IL-10) responses, indicating a hyper-inflamed subgroup. The levels of IL-10 induced by this inflammatory milieu do not seem sufficient to dampen this hyper-inflamed state. While the importance of T_H2 responses in AD pathogenesis is well established, providing pruritogenic cytokines and disrupting the skin

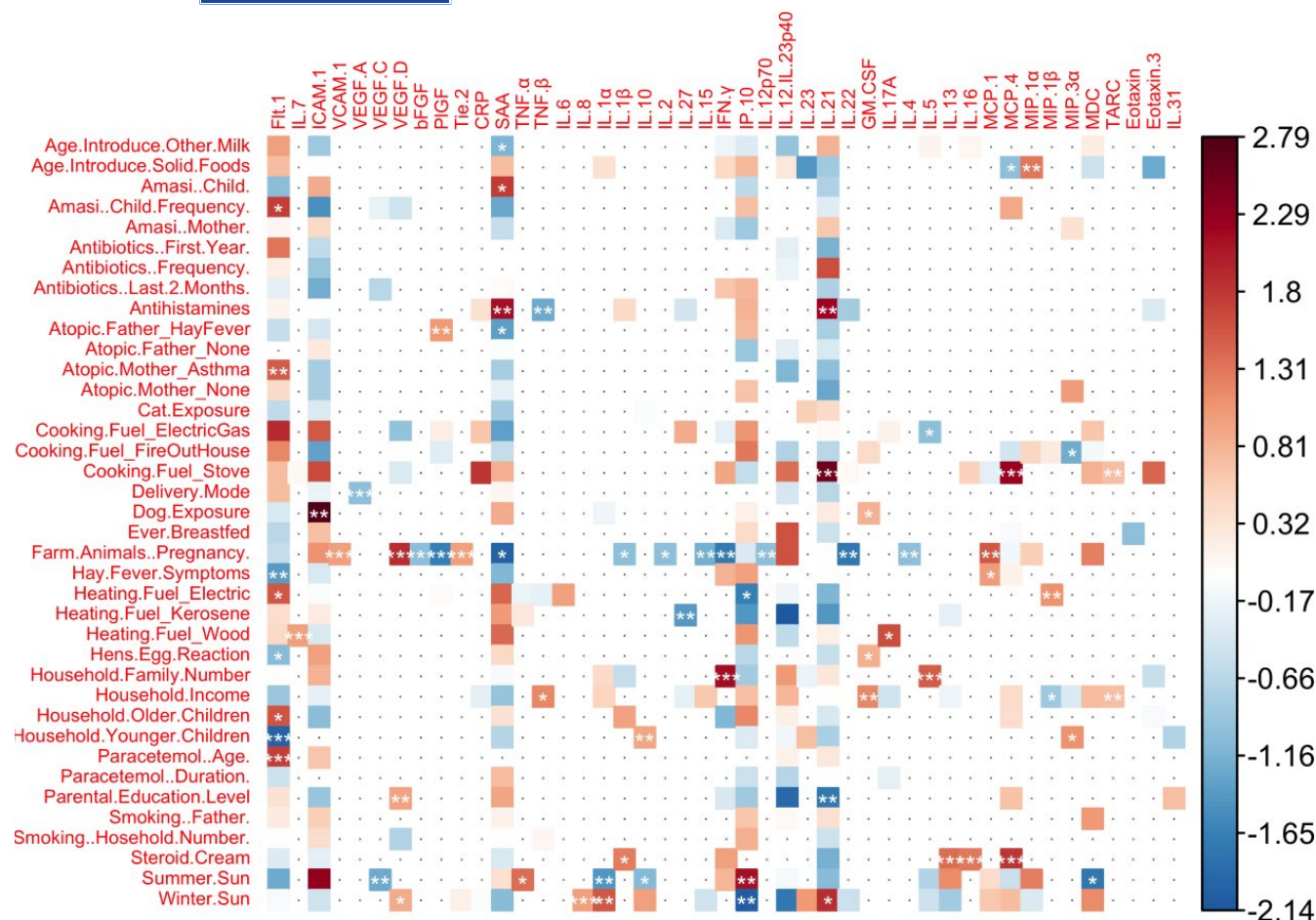


FIGURE 6 Environmental Factors that Associate with Plasma Cytokine Levels. Heatmaps illustrate the association between individual exposure and cytokine levels. **p* < .10, ***p* < .05, ****p* < .01

barrier,^{25,30–35} parallel T_H1 activation can also be present and may associate with chronic AD.^{36,37} This endotype displaying multipolar activation will need to be carefully explored from a therapeutic perspective as blocking the T_H2 axis alone may have limited efficacy. This finding is in keeping with the observation in adults and adolescents that Dupilumab is not efficacious in all AD cases.^{38,39} In addition, high IL-4 and IL-13 levels in urban children with AD were associated with less exposure to animals but greater antibiotic and paracetamol use within the first year of life, which suggests that a lack of appropriate immune educating stimuli in early life may predispose to this endotype. Alternatively, infants born with more pronounced T_H2 polarized responses may require more frequent antibiotics due to potentially limited anti-microbial defence responses.

AD is often associated with increased risk for sensitization to food and aeroallergens and increased risk of developing other allergic diseases later in life⁴⁰ most likely mediated through the defective epidermal barrier. We observed high levels of total IgE and multi-allergen IgE sensitization in South African children with AD, which was not dependent on their urban or rural location (with the notable exception of peanut). Anti-house dust mite (HDM) IgE and IgG4 levels were significantly elevated in both rural and urban children with AD, suggesting a greater exposure to HDM allergens via the disrupted AD skin barrier,

which might be even more susceptible to the deleterious effects of HDM proteases. Previous observations of high IgE levels to major allergens in developing countries have been dismissed as epiphenomena due to high parasite infection rates and cross-reactivities to ubiquitous environmental exposures. However, the relatively low anti-CCD IgE reactivity in this cohort (perhaps due to their young age and regular prophylactic antihelminth treatment) suggests genuine IgE sensitization to food and aeroallergens in these South African children with AD.

The dominant effect of rural and urban exposures on modifying levels of circulating cytokines, independent of AD, is striking in this South African cohort. The long-term functional reprogramming of innate immune cells evoked by exogenous or endogenous exposures can be mediated via epigenetic tuning of cellular reactivity, as recently incorporated in the concept of trained immunity.⁴¹ Enhanced innate immune responses were observed in rural participants, consistent with previous studies that have also linked rural or traditional farm living environments with immune activation. Farm exposures were associated with higher expression of *toll-like* receptor (TLR) and CD14 genes and enhanced expression of innate immunity-related genes IRAK-4 and RIPK1.^{42,43} High levels of endotoxin exposure in Amish children were shown to influence the innate immune response.⁴⁴ While these studies showed innate immune system activation associated with

asthma protection, our study shows a high level of innate activation in both healthy and AD children within the rural setting. This suggests that the protective effects observed for respiratory allergy may not be equally protective for AD; or may not be able to mitigate against putative risk factors for AD that exert their effects via non-immune mechanisms; or our observations may be different due to qualitative and quantitative differences in rural/farming environmental exposures in our setting from those previously reported.

Exposure to animals, cooking or heating fuel type and lower socio-economic status were associated with the rural immune profile. Both microbial and non-microbial factors can contribute to the effects of animal exposures on inflammatory immune responses.^{45–47} The use of biomass and kerosene fuel combustion can be relevant as indoor and outdoor air pollutants. Traffic-related air pollution has been associated with AD development, AD exacerbations and disease persistence.⁴⁸ Biomass and kerosene fuel emissions are associated with adverse health outcomes, and it has been shown that particles from wood smoke have comparable proinflammatory effects to traffic emissions albeit mediated by different particle characteristics.⁴⁹ Studies looking at direct immune effects of kerosene combustion are sparse however kerosene particles are demonstrably similar to that of diesel.⁵⁰ Socio-economic status or socio-economic disadvantage (and its correlates) has been consistently associated with higher levels of circulating inflammatory mediators throughout the life span, via multiple interacting mechanisms.^{51–54} In addition to environmental exposures, dietary habits can be significantly different between rural and urban families, which heavily influence microbiota composition, metabolism and subsequent immune function.^{55,56} Rural South African children were shown to have a *Prevotella*-dominated microbiota associated with high fibre consumption, which is lost in the urban setting.^{57–60} *In vitro*, *Prevotella* strains are strong stimulators of innate immune activation, while a recent study in adults showed that individuals with a *Prevotella*-dominated microbiota had the highest levels of circulating cytokines.^{61,62} Future studies are required to determine the contribution of specific dietary components, microbiota composition and metabolism on the high levels of circulating cytokines in rural children.

This study describes disease- and environment-specific immune pathways associated with African paediatric AD. These observations represent a significant progression in our understanding of the immune endotypes present in this underrepresented population in clinical trials of endotype-targeting biological therapies.⁶³ Additionally, the use of molecular biomarkers identified in this study for objective assessment and monitoring of disease severity and response to treatment in clinical trials is of special relevance to this population (where disease severity may be difficult to discern clinically). Collectively, this study highlights the intersection of host genetics, environmental exposures, and socio-economic status in modifying immune processes in children with AD living in rural and urban settings.

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CONFLICT OF INTEREST

Dr. Sokolowska reports grants from Swiss National Science Foundation, grants from GSK, outside the submitted work. Dr. Nadeau reports grants from National Institute of Allergy and Infectious Diseases (NIAID), National Heart, Lung and Blood Institute (NHLBI), National Institute of Environmental Health Sciences (NIEHS) and Food Allergy Research & Education (FARE); Director of World Allergy Organization (WAO), Advisor at Cour Pharma, co-founder of Before Brands, Alladapt, Latitude and IgGenix; and National Scientific Committee member at Immune Tolerance Network (ITN), and National Institutes of Health (NIH) clinical research centres, outside the submitted work. In addition, Dr. Nadeau has the following patents: 'Special Oral Formula for Decreasing Food Allergy Risk and Treatment for Food Allergy', (with royalties paid to Before Brands and Alladapt), 'Granulocyte-based methods for detecting and monitoring immune system disorders', (issued), 'Methods and Assays for Detecting and Quantifying Pure Subpopulations of White Blood Cells in Immune System Disorders', (issued), 'Microfluidic Device and Diagnostic Methods for Allergy Testing Based on Detection of Basophil Activation', (pending). Dr. O'Mahony reports grants from Science Foundation Ireland, during the conduct of the study; grants from GSK, personal fees from PrecisionBiotics, personal fees from Nestle, personal fees from Nutricia, outside the submitted work. Dr. Akdis reports grants from Allergopharma, Idorsia, Swiss National Science Foundation, Christine Kühne-Center for Allergy Research and Education, European Commission's Horizon's 2020 Framework Programme, Cure, Novartis Research Institutes, Basel, Astra Zeneca, Switzerland, Scibase, Stockholm, advisory role for Sanofi/Regeneron, Glaxo Smith-Kline, Novartis. The remaining authors declare no competing interests.

AUTHOR CONTRIBUTIONS

NL, GT, AD, MS, DG, MW, CA, PW, WB and LOM performed laboratory assays and contributed to data analysis. NL, LH, MB, CG, AM, CG, CH and ML recruited subjects and obtained samples. NL, KN, CH, ML, LOM and CAA obtained funding, supervised the work and wrote the manuscript. All authors have read and approved the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Environment-Dependent Alterations of Immune Mediators in Urban and Rural South African
Children with Atopic Dermatitis

Online Supplementary Tables and Figures

Table S1. Skin Prick Test Positivity

	Rural AD (n=46)	Urban AD (n=32)
Fresh Egg*	18 (39%)	22 (68%)
Egg White Reagent*	10 (22%)	16 (50%)
Peanut Reagent	13 (28%)	5 (16%)
Fresh Peanut	10 (21%)	4 (13%)
Fresh Cow Milk	3 (7%)	3 (10%)
Cow Milk Reagent	0	2 (6%)
Wheat	1 (2%)	2 (6%)
Hazelnut	2 (5%)	1 (3%)
Soy	1 (2%)	1 (3%)
Fish	0	0
Any Food SPT+ Response	26 (57%)	23 (72%)

*p<0.05 Urban Atopic Dermatitis (AD) versus Rural AD

Table S2. Rural and Urban Living Conditions and Environmental Exposures

	Rural AD (n=46)	Rural HC (n=39)	Urban AD (n=32)	Urban HC (n=35)
Parental Education Level ⁺⁺⁺	14.7 (1.8)	11.9 (3.4)	14.9 (1.8)	14.6 (1.6)
Household Income (USD per month) ^{***}	442 (641)	170 (158)	723(581)	643 (652)
Household size*	6.1 (2.7)	6.9 (3.0)	5.2 (2.0)	5.5 (2.6)
Family history of allergy*	14 (30%)	3 (8%)	15 (46%)	8 (23%)
Breastfeeding (Ever)	36 (78%)	35 (90%)	25 (78%)	30 (86%)
Duration of exclusive breastfeeding (Months)	2.6 (2.4)	3.2 (2.4)	2.9(2.4)	2.5 (2.3)
Duration of breastfeeding (Months)	6.3 (6.5)	6.3 (6.4)	7.1 (7.6)	8.2 (9.0)
Delivery Mode*	37 (80%) NVD	31 (79%) NVD	17 (53%) NVD	20 (57%) NVD
Sunlight Exposure (Hours per day): Winter ^{***}	3.1 (2.2)	4.5 (2.4)	0.8 (1.7)	0.9 (1.1)
Sunlight Exposure (Hours per day): Summer ^{***}	4.8 (2.8)	6.4 (3.2)	2.1 (1.5)	1.9 (1.6)
Antibiotics exposure (Ever)	38 (83%)	33 (85%)	25 (78%)	26 (74%)
Age of first antibiotic exposure (Months)	4.5 (2.1)	5.8 (3.2)	6.4 (2.5)	5.5 (3.5)
Paracetamol exposure (Ever)	44 (96%)	39 (100%)	32 (100%)	31 (89%)
Age of first paracetamol exposure (Months)*	4.1 (1.9)	4.5 (2.5)	4.8 (1.9)	5.8 (3.1)
Amasi exposure (Ever) ⁺⁺⁺	42 (91%)	23 (59%)	32 (100%)	31 (89%)
Age of Amasi exposure (Months)	10.8 (3.7)	10.5 (2.9)	10.8 (4.7)	12.7 (5.8)
Unpasteurised Milk	0	0	1 (3%)	2 (6%)
Cat exposure*	8 (17%)	14 (36%)	3 (10%)	5 (14%)
Dog exposure ^{***}	25 (54%)	31 (79%)	9 (28%)	11 (31%)
Farm animal exposure as a Child ^{***}	31 (67%)	39 (100%)	1 (3%)	0
Farm animal exposure during Pregnancy ^{***}	36 (78%)	39 (100%)	2 (6%)	0
Smoking in the Home ^{**}	8 (17%)	9 (23%)	11 (34%)	18 (51%)
Cooking – Electricity/Gas ⁺⁺⁺	45 (98%)	7 (18%)	32 (100%)	35 (100%)

Cooking – Paraffin Stove ⁺⁺⁺	6 (13%)	25 (64%)	0	0
Cooking – Open Fire Outside House ⁺⁺⁺	10 (22%)	31 (79%)	0	0
Heating – Kerosene/Paraffin ⁺⁺⁺	37 (80%)	13 (33%)	24 (75%)	25 (71%)
Heating – Wood/Coal ⁺⁺⁺	1 (2%)	20 (51%)	0	0
Heating - Electricity	6 (13%)	3 (8%)	8 (25%)	10 (29%)
Heating - Gas	0	0	1 (3%)	2 (6%)
Heating - None	2 (4%)	4 (10%)	0	2 (6%)

*p<0.05 All Rural Children (AD and healthy) versus All Urban Children (AD and healthy)

**p<0.01 All Rural Children (AD and healthy) versus All Urban Children (AD and healthy)

***p<0.001 All Rural Children (AD and healthy) versus All Urban Children (AD and healthy)

⁺⁺⁺ p<0.001 Rural Healthy Children versus Urban Healthy Children

NVD – normal vaginal delivery

Table S3. Proportion of children with an IgE result >0.35 KU/L

	Rural AD	Rural HC	Urban AD	Urban HC
Peanut ^{1**}	52%	0%	19%	0%
Soybean ¹	28%	0%	13%	0%
Hazelnut ¹	15%	0%	6%	0%
Wheat ¹	30%	0%	22%	3%
Fish	2%	0%	9%	0%
D. pteronysinus ¹	65%	8%	81%	9%
Egg ^{1*}	63%	0%	59%	15%
Cow's Milk ^{1*}	50%	31%	44%	9%
Any IgE+ Response	86%	33%	97%	23%

¹p<0.05 Atopic Dermatitis (AD) versus Healthy Control (HC)

**p<0.01 Rural AD versus Urban AD

*p<0.05 Rural HC versus Urban HC

Figure S1. Circulating Immune Biomarkers Not Associated with AD

Plasma cytokine levels and whole blood immune cell counts for children with AD or healthy control children (HC). Results are presented on a log scale and differences analysed using a Mann Whitney test.

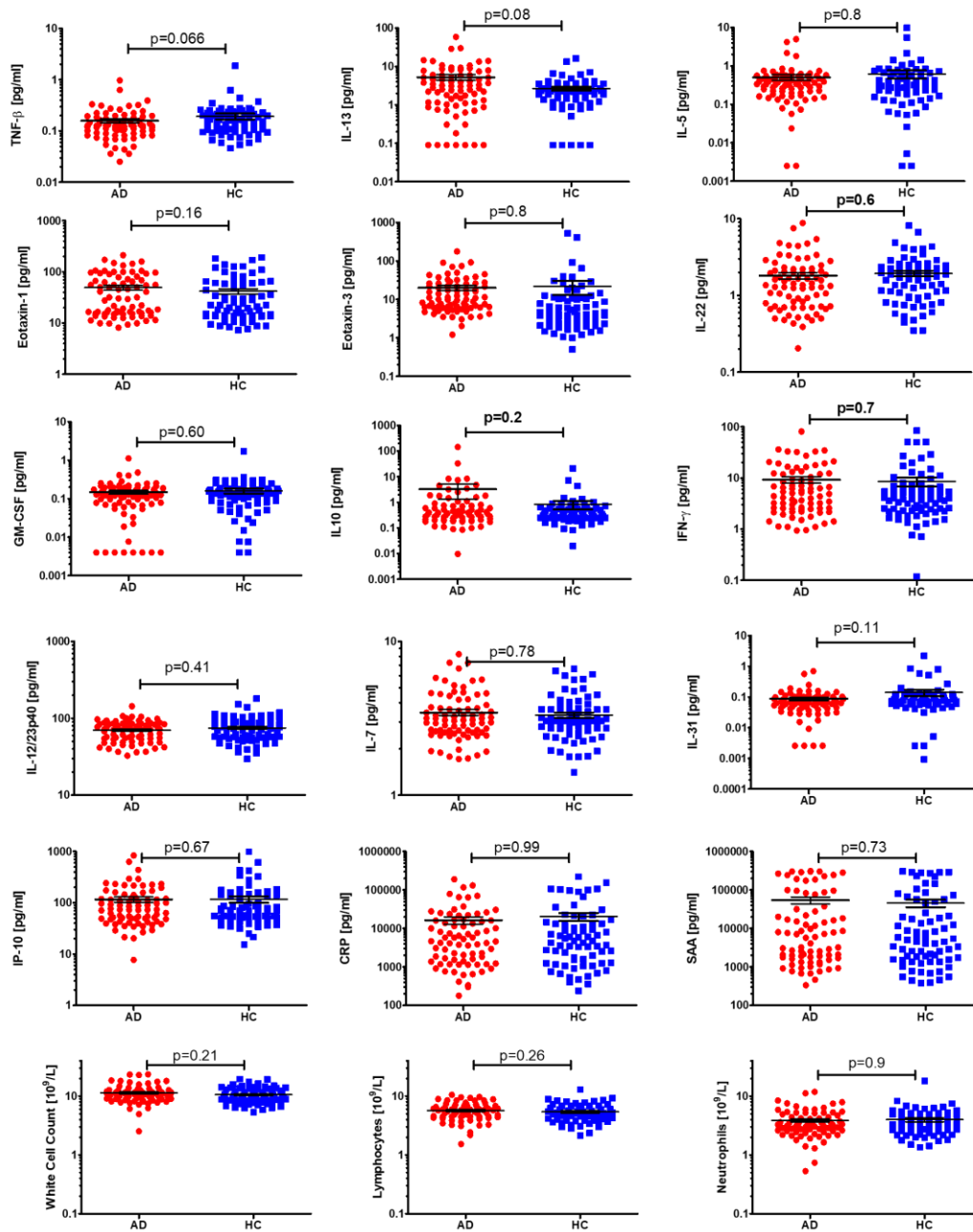


Figure S2. Environmental Factors that Associate with Plasma Cytokine Levels in Rural Healthy Children.

Heatmaps illustrate the association between individual exposure and cytokine levels.

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$

Association between exposure data and cytokine levels

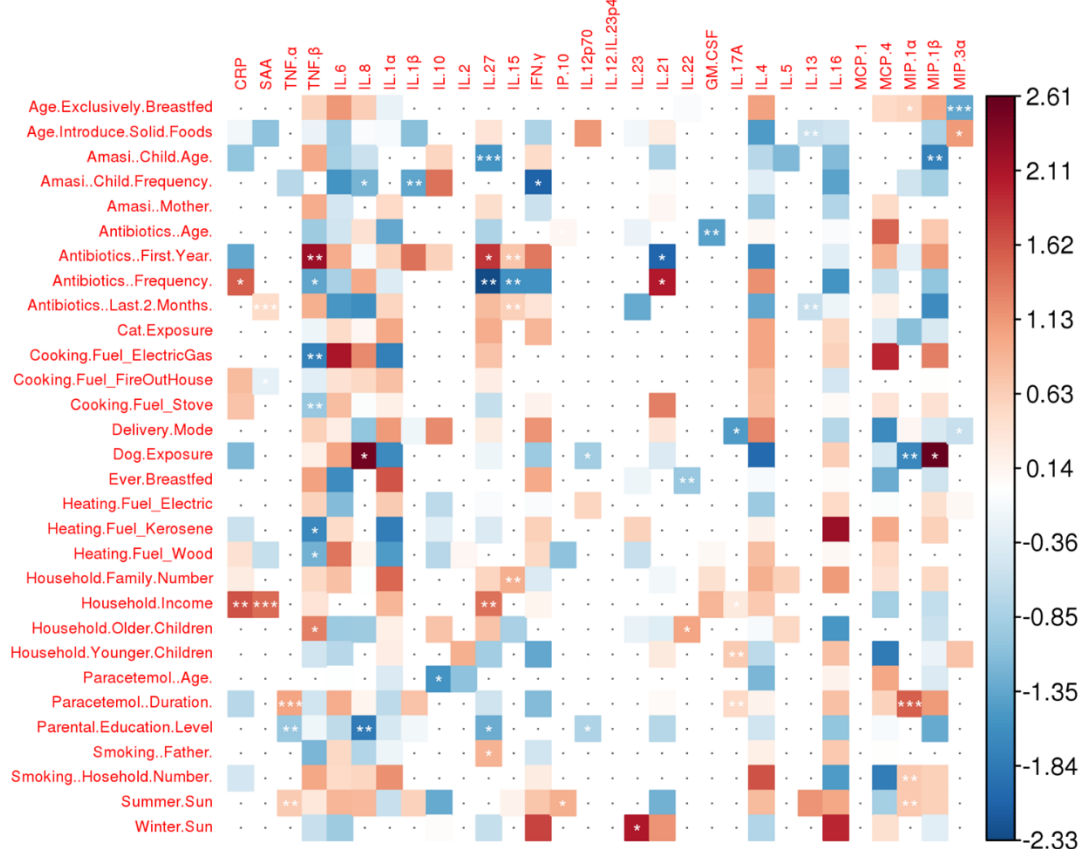


Figure S3. Environmental Factors that Associate with Plasma Cytokine Levels in Rural Children with AD.

Heatmaps illustrate the association between individual exposure and cytokine levels.

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$

Association between exposure data and cytokine levels

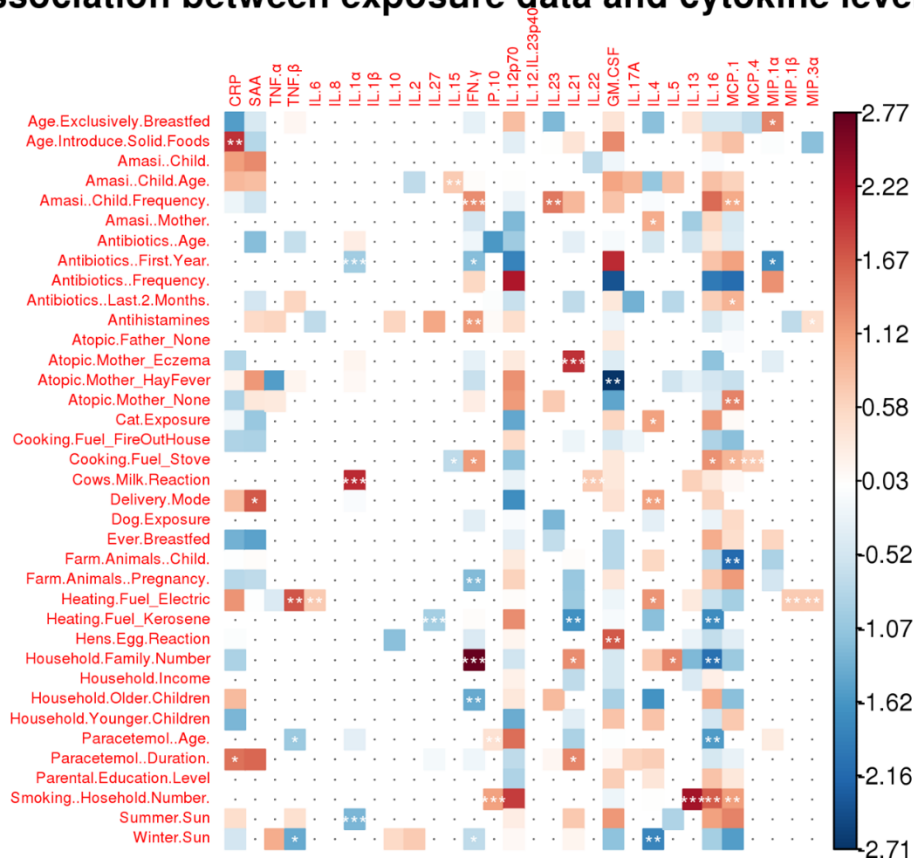


Figure S4. Environmental Factors that Associate with Plasma Cytokine Levels in Urban Healthy Children.

Heatmaps illustrate the association between individual exposure and cytokine levels.

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$

Association between exposure data and cytokine levels

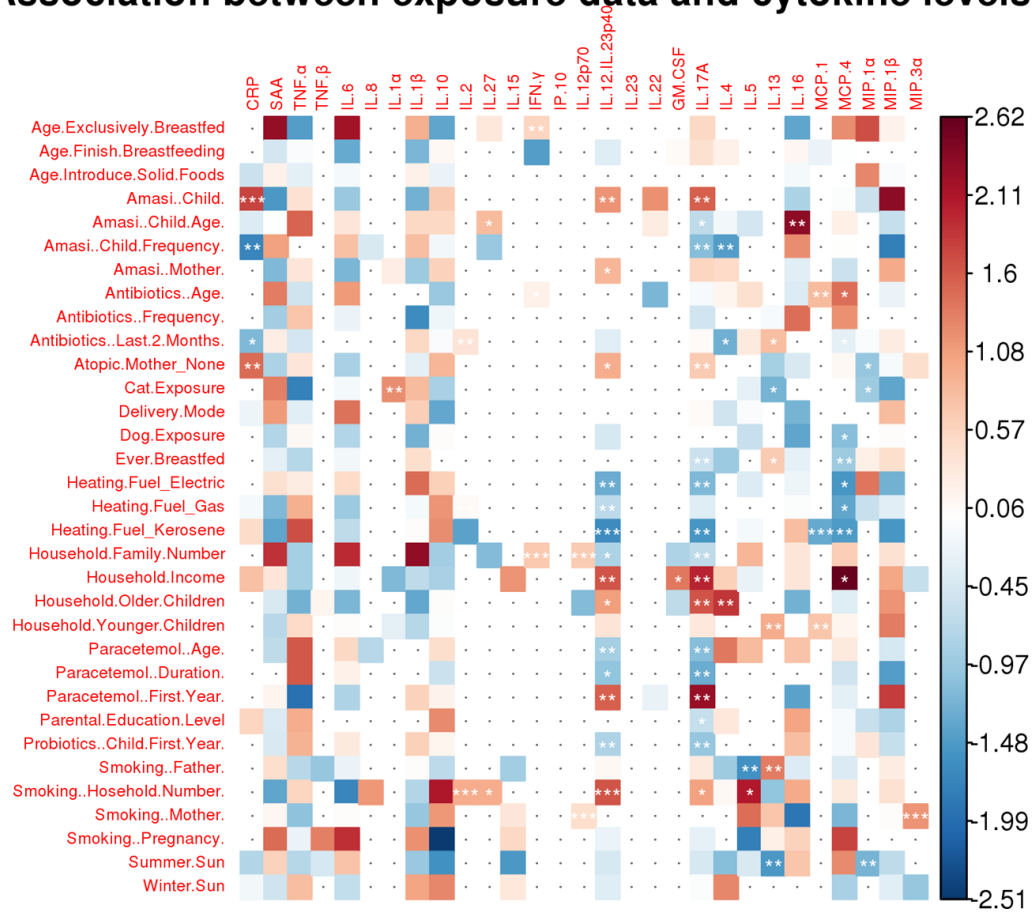


Figure S5. Environmental Factors that Associate with Plasma Cytokine Levels in Urban Children with AD.

Heatmaps illustrate the association between individual exposure and cytokine levels.

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$

Association between exposure data and cytokine levels

