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The LINA cohort: indoor chemical exposure, circulating eosinophil/basophil (Eo/B) progenitors and early life skin manifestations

Condensed title:

Indoor chemical exposure and eosinophil/basophil progenitors in early infancy

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Abstract

BACKGROUND: Hematopoietic progenitor cells, especially those committed to the Eo/B lineage, are known to contribute to allergic inflammation.

OBJECTIVE: The aim of the present study was to investigate whether environmental factors are associated with changes in numbers of circulating Eo/B progenitors at one year of age.

METHODS: Peripheral blood from 60 one-year old children enrolled in the LINA (Lifestyle and environmental factors and their Influence on Newborns Allergy risk) birth cohort was assessed for Eo/B progenitor cells (Eo/B CFU) by standardized and validated methylcellulose assays. Frozen peripheral blood mononuclear cells (PBMC) were cultured in the presence of IL-3, IL-5 or GM-CSF, and Eo/B CFUs enumerated. Clinical outcomes and exposure to environmental tobacco smoke (ETS) were documented by standardized questionnaires, and indoor volatile organic compound (VOC) concentrations were assessed by passive sampling.

RESULTS: Children with skin manifestations (atopic dermatitis or cradle cap) within the first year of life had higher numbers of circulating IL-3-, IL-5- or GM-CSF-stimulated Eo/B CFUs ($p < 0.05$) at one year. In children with cradle cap, a positive correlation was found between Eo/B CFUs and exposure to ETS-related VOCs during pregnancy or at one year of age ($p < 0.05$).

CONCLUSIONS AND CLINICAL RELEVANCE: This is the first demonstration that environmental exposures are positively associated with levels of circulating Eo/B progenitors. The recruitment and differentiation of Eo/B progenitors in response to environmental triggers may play a role in the development of skin manifestations during the first year of life.

Accepted Article

Keywords:

atopic dermatitis, cradle cap, eosinophil/basophil progenitors, indoor chemical exposure, VOC, smoking, LINA, one year old children

Abbreviations:

AD	atopic dermatitis
CB	cord blood
CC	cradle cap
CFU	colony forming unit
Eo/B	eosinophil/basophil
ETS	environmental tobacco smoke
GM-CSF	granulocyte macrophage colony-stimulating factor
IL-3, IL-5	interleukin 3, interleukin 5
IL-5R α	interleukin 5 receptor alpha sub-unit
NAMNC	non adherent mononuclear cells
OR	odds ratio
pb	peripheral blood
PBMC	peripheral blood mononuclear cells
Th2	T helper cell type 2
VOC	volatile organic compounds

INTRODUCTION

Many risk factors for the development of atopic disease, including genetic background, individual immune response and environmental conditions, have been identified [1]. Eosinophils and basophils are also well known to contribute to tissue allergic inflammation [2]. For example, mature eosinophils are increased in tissue, blood and bone marrow in asthma and atopic dermatitis, their numbers positively correlating with disease severity [2] [3]. In addition to mature cells, bone marrow Eo/B progenitors themselves can be released into the blood and migrate to tissues, under the influence of hemopoietic cytokines and chemokines such as eotaxin (reviewed in [4] and [5]). Several studies have also demonstrated that the number of Eo/B progenitors is increased in patients with allergic rhinitis, nasal polyposis, asthma or atopic dermatitis [6], [7], [8], [9]. In addition, Eo/B progenitors can act as inflammatory effector cells producing a variety of pro-inflammatory cytokines and chemokines [10]. Altered phenotype and function of CD34+IL-5R α + Eo/B progenitors have recently been demonstrated in several studies of cord blood of newborns at risk of atopy [11] [12] with progenitor profiles predicting acute respiratory symptoms in one year old infants [13].

Environmental exposures can further contribute to the development of allergic inflammation, especially exposure to indoor air pollutants [14] [15] [16]. A number of studies indicate that children exposed to environmental tobacco smoke (ETS) in utero or in infancy are at a higher risk of developing respiratory tract infections [17], wheezing or asthma [18]. In addition, ETS exposure is associated with allergic skin manifestations such as atopic dermatitis [19] [20]. Indoor exposure to chemical pollutants such as volatile organic compounds (VOC), released from different sources including ETS [14] [21], can trigger allergic sensitization [15], allergic skin manifestations [22] and asthma [23] [24]. We hypothesized that a contributing mechanism underlying the association of indoor exposure with allergic manifestations was recruitment and differentiation of Eo/B progenitors. We therefore undertook an investigation of the association of pre- or postnatal

environmental ETS or VOC exposure with numbers and function of peripheral blood Eo/B progenitors and the development of skin manifestations in children enrolled in the LINA birth cohort.

METHODS

Study design and sample collection

The LINA cohort study (Lifestyle and environmental factors and their Influence on Newborns Allergy risk) recruited 622 mother-child-pairs between May 2006 and December 2008 in Leipzig, Germany, to investigate how environmental factors in the pre- and postnatal period influence the immune system and whether they are determinants of increased allergy risk in the newborn and early childhood. 606 mother-child-pairs (599 mothers and 606 children) participated in the one year follow-up examination. Mothers suffering from immune or infectious diseases during pregnancy were excluded from the study.

Whole blood samples were obtained from mothers at the 34th week of pregnancy, and cord blood was collected at delivery. Whole blood samples were also collected from each mother and child at one year of age. Standardized questionnaires were administered during pregnancy (at week 34) and annually thereafter, collecting information on family history of atopy as well as housing and environmental conditions (active or passive smoking, mould, traffic, noise, pets, renovation activities) and personal lifestyle. At the age of one year information about clinical outcomes were collected.

Atopic dermatitis was recorded as a parental report of a doctor-diagnosed dermatitis or of dermatitis symptoms. In detail, for a doctor-diagnosed dermatitis parents were asked whether a physician had diagnosed atopic dermatitis in the child in the last 12 months. Children were classified as having dermatitis symptoms when parents reported an intermittent itchy skin rash, which affected places other than the nappy area and lasted at least 2 weeks, or appeared repeatedly in the last 12 months.

Cradle cap was recorded as a parental report of a doctor-diagnosed cradle cap. All questionnaires were self administered by the parents. In addition to the survey data from the questionnaires,

individual chemical exposures to VOCs in the homes were assessed during pregnancy (passive sampling from 34th to 38th week) and at children's first birthday (passive sampling from 12th to 13th month of life). Participation in the study was voluntary and informed consent was obtained from all participants. The study was approved by the Ethics Committee of the University of Leipzig (046-2006).

Preparation of peripheral blood samples

In a sub-cohort of 60 randomly selected children progenitor analyses were performed. It was previously shown that a sample size in that range allows significant conclusions about Eo/B progenitor pathways [25]. Since it was described in earlier experiments that family history of atopy (FHA) may influence numbers of Eo/B progenitors in peripheral blood, samples were chosen with similar numbers in groups of no, single or double FHA to avoid unbalanced changes in progenitor cell numbers. The investigator, who performed the progenitor assays and analyses, was blinded to identification of subjects and other data collected by protocol.

Frozen peripheral blood mononuclear cells (PBMCs) were isolated from 2-3ml fresh heparinised peripheral blood via Ficoll Paque density centrifugation [26]. PBMCs were frozen in 1ml aliquots of 90% fetal bovine serum and 10% dimethyl sulfoxide with $10\text{--}30 \times 10^6$ cells. The cell thawing protocol was according to Reece et al [25]. Resulting non adherent mononuclear cells (NAMNC) after 2h incubation were used for methylcellulose assays. Viability of PBMCs after thawing and NAMNCs after 2h incubation averaged 90%.

Methylcellulose Assay

Methylcellulose assays were performed as described earlier [13], [25]. Each sample was analysed in duplicates and incubated in the presence of recombinant human cytokine IL-3, IL-5 or GM-CSF (R&D systems®) over 14 days to assess stage dependent progenitor cell differentiation by morphological distinguishable colony formation (see Fig SI1 in the supplementary information).

Measurement of VOC concentrations

Different VOC concentrations were measured for 4 weeks with passive samplers (3 M monitors, type OVM 3500; 3M GmbH, Neuss, Germany) as described earlier [14] [27]. For measurements during pregnancy passive samplers were placed in the room where mothers spent most of their time, for measurements at year one the sampler was placed in the child's bedroom. VOC concentrations were analysed by gas chromatography and afterwards corrected for seasonal variations. Additional details regarding VOC sample collection analytical methods and seasonal variations are described elsewhere [28], [29].

Measurement of Th2 cytokines

For cytokine measurements heparinised blood was prepared as described earlier. In brief, concentrations of the phytohemagglutinin (PHA) stimulated cytokines IL-4, IL-5, and IL-13 were measured by flow cytometry using a cytometric bead array (BD CBA Human Soluble Flex Set system, Becton Dickinson, Heidelberg, Germany) according to manufacturer's instructions; for more details see Herberth et al. [30].

Statistical analyses

Since the majority of parameters were not normally distributed, analyses were performed using non-parametric tests in general. To address the relationship between clinical outcomes and Eo/B CFUs medians are compared using Mann-Whitney U-test. In addition, multiple logistic regression models were used to consider possible confounding factors. Data are presented as odds ratios with 95% confidence interval and were adjusted for maternal atopic dermatitis, maternal school education, cat ownership and smoking during pregnancy. Spearman's rank correlation test was applied to analyse the association between VOCs or cytokine concentrations and numbers of Eo/B CFUs as well as between VOCs and the number of smoked cigarettes. Statistical power analyses for these correlation tests (highest estimated Rho 0.5, alpha 0.05 and beta 0.2) indicated a necessary sample size of 29 cases, suggesting that the sample size of 60 subjects, even when divided in sub-groups, ensure a

sufficient statistical power. To verify obtained Spearman results, analyses were repeated after excluding outliers (below and above the 1.5-fold 25 to 75 percentile range). Since no differences were seen after these exclusions data are presented as original data. All p-values <0.05 were considered to be significant. Adjustments due to multiple testing were not performed since our analyses were based on an *a priori* hypothesis [31]. The χ^2 -test for cross relationship was used to compare the analysed sub-cohort with the total cohort. Statistical analyses were performed with STATISTICA for Windows, Version 7 (Statsoft Inc.).

RESULTS

Characteristics of the analysed LINA sub-cohort

The frequencies of skin manifestations during the first year of life assessed via questionnaire are shown in Table 1. Within the analysed sub-cohort, 56.7% of the children were positive for cradle cap (CC) at one year. Atopic dermatitis (AD) was less prevalent, either when reported as a physician diagnosis (15.0%) or described by the parents as symptomatic atopic dermatitis (13.3%). For all skin manifestations there were no mean differences between the analysed sub-cohort (n=60) and the total cohort (n=606, p>0.05).

Eo/B CFUs and outcomes

One year old children positive for a skin manifestation had higher numbers of peripheral blood (pb) Eo/B CFUs (Fig 1). In detail, children positive for cradle cap had significantly more IL-5-stimulated Eo/B CFUs (p=0.012), children positive for diagnosed atopic dermatitis had more IL-5- (p=0.002) and GM-CSF- (p=0.044) stimulated Eo/B CFUs, and children positive for symptomatic atopic dermatitis had more IL-3- (p=0.045), IL-5- (p=0.007) and GM-CSF- (p=0.048) stimulated Eo/B CFUs, compared to children who were negative for these skin manifestations. This relationship was further calculated in a regression model adjusted for known confounding variables like maternal atopic dermatitis, maternal school education, cat ownership and smoking during pregnancy (Table 2). Here, children positive for cradle cap within the first year of life had higher amounts of IL-5-stimulated Eo/B CFUs

(OR: 2.19, 95% CI: 1.10 – 4.35) and GM-CSF-stimulated Eo/B CFUs (OR: 1.25, CI: 1.01 – 1.55) at one year of age. Children positive for symptomatic atopic dermatitis within the first year of life had more IL-5-stimulated Eo/B CFUs (OR: 2.72, CI: 1.12 – 6.64).

Indoor chemical exposure: ETS and VOC

In the analysed sub-cohort, 35% of the pregnant woman indicated their indoor exposure to ETS during pregnancy as “(almost) daily”, “once a week or more” or “occasionally”. During the first year of life 18.3% of the children were exposed to ETS (Table 3). Questionnaire data revealed that 10 families gave up (indoor) smoking within the first year of life compared to their smoking behaviour during pregnancy.

A significant correlation was found between the number of smoked cigarettes per day and different VOC concentrations in the home either during pregnancy or during the first year of life (Table 4).

Correlations were significant for single aromatic compounds like benzene, toluene and m,p-xylene, as well as for the total concentration of aromatic compounds and the total concentration of VOCs.

Coincident with the higher prevalence of questionnaire-documented smoking practice during pregnancy compared to the first year of life, absolute concentrations of these VOCs were also higher during pregnancy compared to the first year of life (see Table SI1 in the supplementary information).

Association between VOC / ETS and Eo/B CFUs

To investigate the association between smoking related VOCs and the numbers and differentiation of Eo/B progenitor cells, Spearman’s rank correlation test was performed for the total sub-cohort (n=60) and, in addition, separately for children positive or negative for skin manifestations. In detail, for the total sub-cohort there were no correlations between VOC concentrations and the number of Eo/B CFUs ($p>0.05$). However, in children diagnosed with cradle cap within the first year of life, a positive correlation was found between IL-3- or GM-CSF-stimulated Eo/B CFUs at one year of age and exposure to VOC (single aromatic compounds benzene, toluene and m,p-xylene, total concentration of aromatics and total VOC concentration) during pregnancy or at one year of age (Table 5). In

children with atopic dermatitis, either reported as a physician diagnosis or as AD symptoms, no correlation was seen between VOCs and Eo/B CFUs ($p>0.05$).

Exposure to prenatal ETS was associated with numbers of pb Eo/B CFUs, and also specific for children positive for cradle cap within the first year of life (CC+). In detail, only in CC+ children there was a borderline correlation of the number of smoked cigarettes per day during pregnancy and GM-CSF-stimulated Eo/B CFUs ($p=0.083$, $R=0.333$, $n=28$). In addition, when these CC+ children were subdivided into 'daily' exposure to ETS during pregnancy ($n=4$) vs. 'never' ($n=23$), 'daily' exposure was associated with 2.2 fold more GM-CSF-stimulated Eo/B CFUs compared to participants who documented their exposure to ETS as 'never' ($p=0.049$). For exposure to postnatal ETS, no effect was seen on Eo/B CFU formation. In children with atopic dermatitis no correlation was found between ETS and Eo/B CFUs ($p>0.05$).

Association between VOC / ETS and outcomes

To test whether exposure to VOCs during pregnancy or during the first year of life was directly associated with skin manifestations, a Mann Whitney U-test was performed. No positive association was found between VOC exposure during pregnancy or during the first year of life and atopic dermatitis or cradle cap.

Exposure to ETS during pregnancy or during the first year of life did not show any association with skin manifestations.

Association between prenatal VOC / ETS and cord blood cytokines

In CC+ children, a borderline significance was seen between VOC exposure during pregnancy (total concentration of aromatics ($p=0.096$, $R=0.340$, $n=25$) or total concentration of VOCs ($p=0.057$, $R=0.386$, $n=25$) and cord blood PHA-stimulated IL-13. For single VOCs as well as the Th2 cytokines IL-4 and IL-5 no correlation was found.

For exposure to ETS during pregnancy no effects were seen on CB Th2 cytokines IL-4, IL-5 or IL-13.

Association between cord blood cytokines and Eo/B CFUs

To test whether the immune status of the newborn was correlated with the number of pb Eo/B colonies a Spearman's rank correlation test was performed. No significant effects were seen for the total sub-cohort. However, for CC+ children an association was seen between PHA-stimulated cord blood Th2 cytokines, IL-4 and IL-13, and the number of Eo/B CFUs at year one, with a trend for an association with IL-5 (Table 6). This association was not seen in children negative for cradle cap within the first year of life.

In children positive for atopic dermatitis (either diagnosed or symptoms) a significant positive correlation was found between CB PHA-stimulated IL-4 levels, and IL-3- or GM-CSF-stimulated CFUs. No significant effect was seen in children negative for AD ($p>0.05$).

All shown associations between environmental exposures, Eo/B CFUs and clinical outcomes are summarised in Figure 2.

DISCUSSION

In this study we were able to analyse for the first time the numbers and function of pb Eo/B progenitors of one year old children in relation to skin manifestations and VOC exposure in the home.

Cradle cap (CC) is one of the most common dermatoses in early infancy [32], [33]. In the analysed LINA sub-cohort CC was the most prevalent skin manifestation within the first year of life (57%), followed by AD (13-15%) which is comparable to previous cohort studies of dermal outcomes in early infancy (CC: 35.7-56.3% [32], AD: 8-10% [34], [35]). Although, there is no clear association between cradle cap and atopic dermatitis, it has been reported that a certain number of neonates with cradle cap develop an atopic dermatitis [36]. Possible links from the present analyses which could point out the possibility of an atopic component in cradle cap are mentioned later in the discussion.

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It has been shown that numbers of pb Eo/B progenitors are elevated in atopic adult peripheral blood [37], [8], [7], [6]. Sehmi et al. demonstrated that numbers of CD34⁺ cells in bone marrow are higher in atopic compared to non-atopic subjects and that this was accompanied by elevated numbers of Eo/B CFUs in peripheral blood [8]. Eo/B progenitors are also altered in neonates at high risk of atopy [12], and changes in CB Eo/B CFUs can predict acute respiratory symptoms in the first year of life [13]. In our LINA sub-cohort, we could demonstrate that one year old children positive for CC or AD had significantly more IL-3-, IL-5- or GM-CSF-responsive pb Eo/B CFUs, compared to children who were negative for these skin manifestations. Although CC has to be distinguished from AD [38], the positive association of Eo/B progenitors with both skin manifestations, AD and CC, might suggest an atopic component for both outcomes. After adjusting for known confounders, the strongest elevation of CFUs in children positive for a skin manifestation was seen for IL-5-responsive cells. This IL-5 responsiveness indicates that children positive for AD or CC mainly had increased numbers of terminal differentiated circulating eosinophil progenitor cells. One possible mechanistic explanation for an elevated IL-5 responsiveness of pb Eo/B progenitors in relation to skin manifestations is an up regulation in the expression of the IL-5 receptor alpha subunit, as previously shown for bone marrow CD34⁺ cells in atopic asthmatics [39], [40], putatively in response to IL-5 itself [41] or to the two other cytokines (IL-3, GM-CSF) which bind to the common β subunit of the receptor [42]; however, we were not able to perform phenotyping (by flow cytometry or qPCR) to analyse differences of cell surface receptors.

In addition to stimulating terminal differentiation of Eo/B progenitors, IL-3, GM-CSF and IL-5 are essential in encouraging both early and terminal eosinophil development and differentiation, respectively [4]. Therefore, Eo/B progenitors which are responsive to either one or more of these cytokines might give valuable information about overall eosinophil lineage commitment.

One factor known to be involved in eosinophil lineage commitment and differentiation in the context of allergic disease development is an altered Th2 response. Previous studies have shown that inhaled allergens induce a trafficking of IL-5 producing Th2 cells to the bone marrow where they promote

eosinophilopoiesis [43]. Other groups have shown in mice that IL-4, which is recognised in allergy development, can direct bone marrow progenitor cell differentiation into Th2-cytokine-producing eosinophils [44]. Further data exist that eosinophils and basophils carry IL-4 and IL-13 transcripts during lineage differentiation, which enables rapid production of these Th2 cytokines [45]. These Th2 responses also seem to be involved, according to our present analyses, in the recruitment of Eo/B CFUs and subsequently in the following contribution to the development of skin manifestations. In our study, cord blood of children positive for CC within the first year of life revealed a significant positive correlation between electable CB Th2 cytokines (IL-13 and IL-4) and the number of IL-3- and GM-CSF-stimulated CFUs at one year. We hypothesise that these children were exposed to a stronger Th2 milieu at birth, or shortly thereafter, which played a role in shaping early Eo/B lineage commitment and skin inflammation later on. The observed association between a Th2 driven immune status at birth, the number of Eo/B progenitors and CC may further point to the possibility of an atopic component in CC.

In addition to Th2 milieu, another contributing factor to the development of allergic inflammation is environmental exposure. Environmental exposure during pregnancy or very early in life can have immunomodulatory effects [46] and affect clinical outcomes into infancy [47], [22], [17]. There are very limited data on the relationships among environmental factors, Eo/B progenitors and the development of atopy. Denburg et al. demonstrated that cord blood Eo/B progenitors can prenatally be modified by maternal diet during pregnancy [11] and that children at one year of age were less likely to develop wheeze and/or atopic dermatitis after maternal fish oil supplementation during gestation. However, the precise mechanism underlying the effects of environmental/life style factors in this context remains unclear.

In our analysed LINA cohort we show from questionnaire data that indoor exposure to ETS is present during pregnancy and during the first year of life. However, data suggest that the prevalence of indoor smoking seems to be reduced after delivery. Different studies critically discuss that information about indoor exposure to ETS or other environmental pollutants received from questionnaires are dependent on individual honesty and compliance by the study participants; actual exposures are further dependent on differences in housing conditions or ventilation [22]. Accordingly, associations between questionnaire documented smoking and Eo/B CFUs or skin outcomes were less prominent than expected. When we analyzed actual VOC concentrations within the homes by passive samplers which more directly reflect true exposures, we showed that exposure to specific VOCs significantly correlated with the number of smoked cigarettes/d, independent of the time point when measurements were performed. Further, the indoor VOC concentrations of benzene, toluene and xylene measured in our study were comparable to concentration shown in other cohorts [48], [49], [14], [28], [29].

In the present study we demonstrate that smoking-related VOC exposure, either during pregnancy or during the first year of life, correlates with Eo/B CFUs of CC+ one year old children. These interactions occurred only related to IL-3- and GM-CSF- responsive progenitor cells, but not to IL-5-responsive cells; most likely this is related to the power of our study, since subject numbers for sub-analyses were quite low. A more mechanistic possibility is that the cells associated with VOC exposure in CC+ children involve a less terminally-differentiated Eo/B progenitor, as was observed in the Fernandes study [13]. This may hint to a developmental stage dependent effect of VOCs on the infant's immune response. The increased VOC responsiveness of immature Eo/B progenitor cells may result via *in situ* hematopoiesis [10] in higher numbers of terminal differentiated IL-5-responsive progenitor cells. Nonetheless, conversely, Denburg et al. demonstrated that fish oil supplementation during pregnancy resulted in significantly more IL-5-, but not IL-3- and GM-CSF-stimulated CFUs in cord blood [11]. This suggests that the responsiveness of progenitor cells to environmental factors varies with the stage of their differentiation.

That we only observed a correlation between VOC exposure and Eo/B CFUs in cradle cap positive children, suggests that there might be genetic or gene- environment interactions at play in these children. Differing individual susceptibilities to environmental stimuli [50], [51], for example determined by polymorphisms in VOC detoxifying enzymes, could explain the observed result. Sun et al. hypothesized that deficient or altered phase I and II metabolic enzymes such as the cytochrome P450 (CYP) enzymes CYP1A1, CYP1A2, CYP1B1 or glutathione-S-transferases like GSTP1, which are involved for example in benzene metabolism, would affect individual susceptibility to benzene toxicity in bone marrow [52], which could, in turn, carry consequences for hematopoietic cell development even at low exposure concentrations. Consistent with that hypothesis, Wang et al. recently published data that genetic polymorphisms in GSTM1 and GSTP1 may be responsible for differences in the susceptibility to atopic dermatitis with regard to prenatal smoke exposure [53].

In summary, in this study we were able to analyse for the first time the numbers and functional differentiation of pb Eo/B progenitors of one year old children in relation to skin manifestations and ETS/VOC exposures. Our results suggest that ETS-exposure during pregnancy as well as ETS-related VOC exposure during pregnancy and the first year of life are associated with enhanced numbers of Eo/B CFUs at the age of one. Furthermore, we found a relationship between a Th2 milieu at birth and the presence of Eo/B CFUs at the age of one year. Therefore, we hypothesise that prenatal ETS/VOC exposure drives foetal T cell development toward Th2, and that this milieu contributes to downstream eosinophilic-basophilic differentiation and skin inflammation. In an earlier study we were in fact able to show, that VOC exposure during pregnancy correlates with higher amounts of Th2 or lower amounts of Th1 cells in cord blood [27]. Environmental exposures such as ETS-associated VOC may contribute via a Th2 bias to the recruitment and differentiation of Eo/B progenitors which, in turn, could play a role in the development of skin manifestations during the first year of life.

Conflict of interest:

The LINA study was supported by Helmholtz institutional funding (Helmholtz Centre for Environmental Research – UFZ). McMaster University supported the analyses of Eo/B progenitor cells in one year old children. The German Academic Exchange Service (DAAD) and AllerGen NCE Inc. provided support for the exchange of the postdoctoral fellow (K.W.).

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Figure legends

Fig. 1: IL-3, IL-5 or GM-CSF-stimulated Eo/B CFUs in peripheral blood of 1 year old children with or without **A** cradle cap (diagnosed) **B** atopic dermatitis (diagnosed) or **C** atopic dermatitis (symptoms) within the first year of life. Data are presented as box plots with median and 25th to 75th percentile. P-values<0.05 are considered to be significant (Mann-Whitney U-test).

Fig. 2: Associations among exposure to environmental factors, Eo/B CFUs and clinical outcomes of 1 year old children

Table 1: Prevalence of dermal outcomes within the first year of life

<i>Outcomes within the first year of life</i>				
		analysed sub-cohort (n=60)	total cohort (n=606)*	χ^2 -test
		n (%)	n (%)	p-value
Cradle cap (diagnosed)				
	positive	34 (56.7)	354 (58.4)	0.808
	negative	26 (43.3)	252 (41.6)	
Atopic dermatitis (diagnosed)				
	positive	9 (15.0)	60 (9.9)	0.275
	negative	51 (85.0)	546 (90.1)	
Atopic dermatitis (symptoms)				
	positive	8 (13.3)	59 (9.7)	0.432
	negative	52 (86.7)	544 (89.8)	

* total numbers may vary due to missing data (n=603 to 606)

Table 2: Relationship between cradle cap or atopic dermatitis and IL-3, IL-5 or GM-CSF-stimulated Eo/B CFUs in peripheral blood of one year old children. Data are presented as odds ratios (OR*) with 95% CI (analysed sub-cohort n=60). Significant results are presented in bold.

* OR are adjusted for maternal atopic dermatitis, maternal school education, cat ownership and smoking during pregnancy

	Cradle cap (diagnosed)	Atopic dermatitis (diagnosed)	Atopic dermatitis (symptoms)
IL-3-stimulated Eo/B CFUs	1.06 (0.96 – 1.18)	1.09 (0.97 – 1.24)	1.11 (0.97 – 1.26)
IL-5-stimulated Eo/B CFUs	2.19 (1.10 – 4.35)	1.14 (0.80 – 1.62)	2.72 (1.12 – 6.64)
GM-CSF-stimulated Eo/B CFUs	1.25 (1.01 – 1.55)	1.18 (0.98 – 1.42)	1.14 (0.95 – 1.37)

Table 3: Questionnaire documented indoor exposures to ETS during pregnancy and during the first year of life (analysed sub-cohort n=60)

ETS = environmental tobacco smoke (including smoking of the mother, father or others),

* = 3 missings

<i>during pregnancy</i>		n (%)
Exposure to indoor ETS		
(almost) daily		15 (25.0)
once a week or more		1 (1.7)
occasionally		5 (8.3)
never		39 (65.0)
Number of smoked cigarettes/d*		
≥ 20		5 (8.8)
10-19		4 (7.0)
1-9		8 (14.0)
0		40 (70.2)
<i>during first year of life</i>		
Exposure to indoor ETS		
(almost) daily		8 (13.3)
once a week or more		0 (0.0)
occasionally		3 (5.0)
never		49 (81.7)
Number of smoked cigarettes/d		
≥ 20		2 (3.3)
10-19		4 (6.7)
1-9		4 (6.7)
0		50 (83.3)

Table 4: Correlation of the number of smoked cigarettes per day with different VOC exposures, both either during pregnancy or during the first year of life. Data are presented as Spearman's rank correlations, p-values<0.05 are considered to be significant and printed in bold (analysed sub-cohort n=60).

	Benzene	Toluene	Xylene	Aromatics	total VOCs
Number of smoked cigarettes/d					
during pregnancy	p=0.000	p=0.019	p=0.065	p=0.013	p=0.007
vs. VOC exposure during pregnancy	R=0.556	R=0.312	R=0.248	R=0.329	R=0.356
Number of smoked cigarettes/d					
during the first year of life	p=0.002	p=0.396	p=0.014	p=0.043	p=0.050
vs. VOC exposure during the first year of life	R=0.393	R=0.114	R=0.321	R=0.267	R=0.258

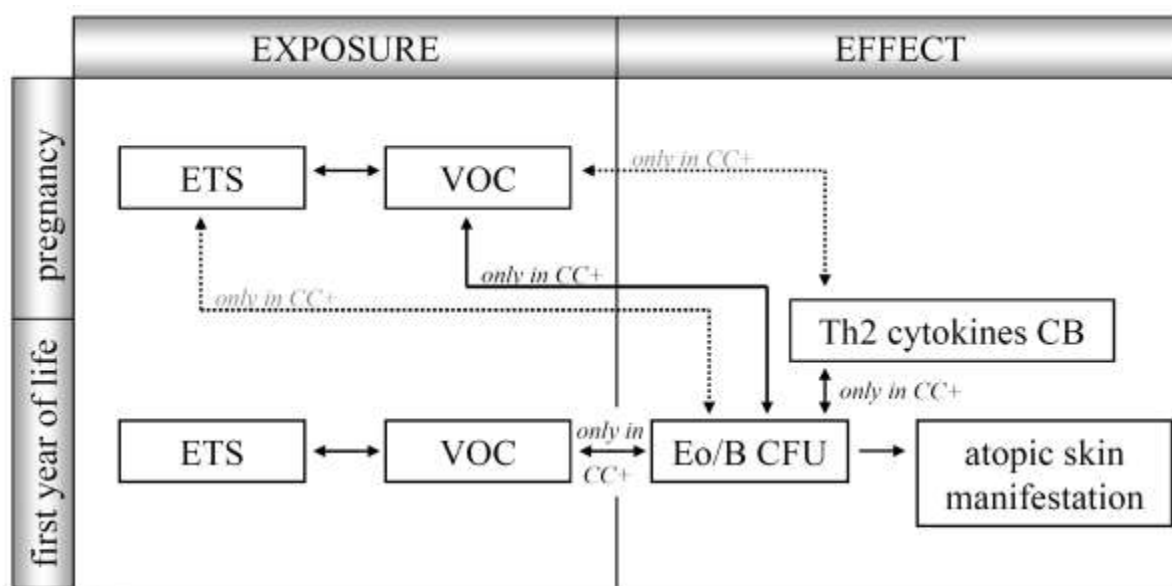
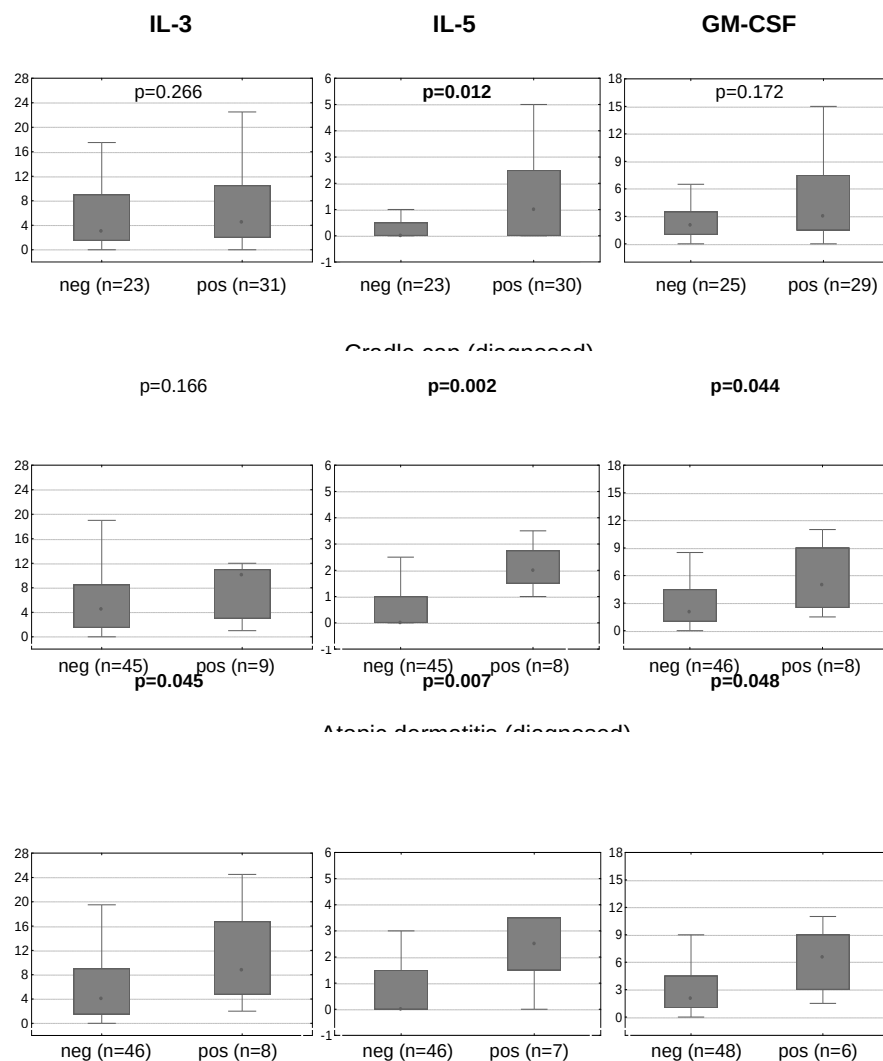
Table 5: Correlation of different VOC exposures during pregnancy or during the first year of life with the number of IL-3-, IL-5- or GM-CSF-stimulated Eo/B CFUs in peripheral blood of one year old children. Correlations are shown for cradle cap positive children within the first year of life (n=34). Data are presented as Spearman's rank correlations, p-values<0.05 are considered to be significant and printed in bold.

	CFUs	IL-3	IL-5	GM-CSF
<i>VOC exposure during pregnancy</i>				
Benzene	p=0.184	p=0.906	p=0.024	
	R=0.245	R=-0.023	R=0.419	
Toluene	p=0.043	p=0.591	p=0.253	
	R=0.366	R=-0.102	R=0.219	
Xylene	p=0.005	p=0.920	p=0.855	
	R=0.487	R=0.019	R=0.036	
total concentration of aromatics	p=0.003	p=0.768	p=0.681	
	R=0.511	R=0.056	R=0.078	
total concentration of VOCs	p=0.012	p=0.471	p=0.063	
	R=0.447	R=0.137	R=0.350	
<i>VOC exposure during the first year of life</i>				
Benzene	p=0.621	p=0.572	p=0.021	
	R=0.096	R=0.112	R=0.441	
Toluene	p=0.482	p=0.896	p=0.013	
	R=0.136	R=0.026	R=0.471	
Xylene	p=0.111	p=0.722	p=0.081	
	R=0.302	R=0.070	R=0.342	
total concentration of aromatics	p=0.150	p=0.521	p=0.022	
	R=0.274	R=0.126	R=0.440	
total concentration of VOCs	p=0.075	p=0.072	p=0.016	
	R=0.335	R=0.346	R=0.460	

Table 6: Correlation of different PHA-stimulated Th2 cytokines in cord blood with the number of IL-3, IL-5 or GM-CSF-stimulated Eo/B CFUs in peripheral blood of one year old children. Correlations are shown for cradle cap positive children within the first year of life (n=34). Data are presented as Spearman's rank correlations, p-values<0.05 are considered to be significant and printed in bold.

	CFUs	IL-3	IL-5	GM-CSF
<i>Th2 cytokines in cord blood</i>				
IL-4		p=0.045	p=0.782	p=0.024
		R=0.431	R=0.064	R=0.480
IL-5		p=0.845	p=0.078	p=0.728
		R=-0.039	R=-0.394	R=0.079
IL-13		p=0.122	p=0.993	p=0.036
		R=0.340	R=0.002	R=0.450

-----Eo/B CFU in peripheral blood (per 5×10^5 cells)-----



ETS = environmental tobacco smoke, VOC = volatile organic compounds, Eo/B CFU = eosinophil/basophil colony forming units, CC+ = children positive for cradle cap within the first year of life, CB = cord blood

Supporting information

The LINA cohort: indoor chemical exposure, circulating eosinophil/basophil (Eo/B) progenitors and early life atopic skin manifestations

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Table SI1: VOC concentrations measured in the homes of the study participants during pregnancy or during first year of life. Data are presented as median with 25th and 75th percentile (analysed sub-cohort n=60).

<i>VOC concentration during pregnancy</i>	$\mu\text{g}/\text{m}^3$
Benzene	1.82 (1.37 – 2.60)
Toluene	13.03 (7.67 – 25.47)
Xylene	1.32 (0.95 – 2.13)
total concentration of aromatics	28.68 (19.25 – 48.11)
total concentration of VOCs	295.90 (197.48 – 438.06)
<i>VOC concentration during the first year</i>	
Benzene	1.27 (0.86 – 2.38)
Toluene	7.81 (4.49 – 15.13)
Xylene	1.91 (1.29 – 3.52)
total concentration of aromatics	18.12 (12.93 – 32.65)
total concentration of VOCs	183.60 (117.19 – 278.25)

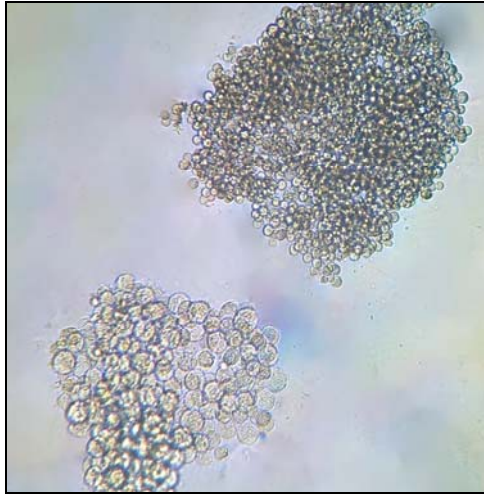


Figure SI1: Eosinophil/basophil colony forming unit (CFU; upper right corner) and granulocyte/macrophage colony forming unit (down left corner)