

Different effects of endotoxin versus mite and cat allergen exposure on T-cell differentiation in infants

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Background: Early exposure to bacterial endotoxin has been proposed to protect against allergy development in children. Whether endotoxin is able to direct T-cell differentiation into a predominance of type 1 immunity is still unresolved.

Objective: We sought to compare the effects of endotoxin and mite and cat allergens on T-cell differentiation in infants.

Methods: In a random population sample of 135 2-year-old children of an ongoing birth-cohort study, peripheral blood CD4⁺ and CD8⁺ T-cell subsets were defined by the expression of the chemokine receptors CCR5 and CCR3 as surrogate markers for type 1 and type 2 T cells, respectively. Endotoxin and mite and cat allergens were measured in house dust collected from the mother's mattress at the child's age of 3 months to assess early exposure.

Results: In the CD4⁺ T-cell subset, endotoxin levels were positively associated with high proportions of type 1 CCR5⁺ cells (odds ratio for fourth exposure quartile [OR_{Q4}], 7.68; 95% CI, 1.35-43.75), whereas cat allergen levels were associated with increased proportions of type 2 CCR3⁺ cells (OR_{Q4}, 4.07; 95% CI, 1.05-15.85). In contrast to endotoxin, allergen levels were associated with CD8⁺ T cells, showing an inverse relationship between mite allergen concentrations and high proportions of CCR5⁺ or CCR3⁺ cells (CCR5⁺ cells: OR_{Q4}, 0.14; 95% CI, 0.03-0.74; CCR3⁺ cells: OR_{Q4}, 0.16; 95% CI, 0.03-0.89) and a positive association of cat allergen levels with increased proportions of CCR5⁺ cells (OR_{Q4}, 9.24, 95% CI, 1.61-53.10), as well as CCR3⁺ cells (OR_{Q3}, 6.64; 95% CI, 1.21-36.51).

Conclusion: Our results indicate that endotoxin has the potential to promote the development of type 1 CD4⁺ T cells, where-

as mite and cat allergens primarily modify the proportion of CD8⁺ cells of both types. (J Allergy Clin Immunol 2002;110:634-40.)

Key words: Endotoxin, mite allergen, cat allergen, CCR5, CCR3, allergy

Strachan¹ introduced a theory to explain the high disease burden of asthma and allergies in affluent countries, later referred to as the "hygiene hypothesis." It implies that during infancy, the microbial environment has profound effects on the development of immune reactivities. T_H1-inducing infections, especially by bacteria, have been proposed to prevent the development of an atopic phenotype characterized by allergen-specific T_H2 responses.^{2,3} If stimulation by bacterial compounds is lacking as a result of improved hygiene and changes in the gastrointestinal microflora, a shift to predominant T_H2 response patterns might finally predispose to atopic disease.⁴ Several epidemiologic studies tested this hypothesis. Alm et al⁵ found that lifestyle factors related to anthroposophy, such as consumption of fermented vegetables containing live lactobacilli, were associated with a lowered prevalence of atopy in childhood. Moreover, a controlled trial demonstrated the protective effect of administration of the gram-positive probiotic *Lactobacillus* GG on the development of atopic eczema in infants.⁶

In addition to contact of probiotic bacteria with the gut-associated lymphoid tissue, exposure to airborne endotoxins, bacterial LPSs, was proposed to protect against allergies. These highly immunoreactive molecules are components of the outer membrane of gram-negative bacteria. Endotoxin concentrations in house dust are associated with the presence of a household cat or dog and are higher in homes of children living on farms or having regular contact with livestock.⁷⁻⁹ Accordingly, epidemiologic studies demonstrated a protective effect of living on a farm or keeping a pet on the development of atopic diseases in childhood.¹⁰⁻¹³

To date, only one small study investigated the effect of house dust endotoxin on immune reactivity in children at the cellular level.¹⁴ The authors analyzed the proportion of type 1 and type 2 cytokine-producing CD4⁺ and CD8⁺ T cells and reported a positive correlation of house dust endotoxin levels with IFN- γ -producing CD4⁺ T cells (T_H1 cells) and no association with T_H2 cells. This study

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Supported by grants of the Federal Ministry for Education, Science, Research, and Technology (No. 01 EG 9705/2) and the HSPIII, Ludwig-Maximilian-University, Munich, Germany.

Received for publication December 11, 2001; revised July 17, 2002; accepted for publication July 22, 2002.

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0091-6749/2002 \$35.00 + 0 1/83/128652

doi:10.1067/mai.2002.128652

Abbreviation used

EU: Endotoxin units

OR: Odds ratio

has several limitations, including the cross-sectional design, selectivity of the study population (13-20 asthma-prone infants), and characterization of T-cell subsets by means of intracellular cytokine staining, necessitating their in vitro stimulation before analysis.

T_H1 and T_H2 lymphocytes differentially express chemokine receptors that mediate cell migration. Both in vitro and in vivo studies have shown that CCR5 expression is associated with T_H1 responses, whereas CCR3 is expressed on a subset of T_H2 cells in human subjects.¹⁵⁻²⁰ In addition, differential expression of CCR5 and CCR3 on CD8⁺ T cells has been described.^{21,22} Moreover, expression of CCR5 and CCR3 seems to be a key feature of activated-memory type 1 and type 2 T cells, respectively, in contrast to naive T cells.²³

In an alternative approach we examined the effects of endotoxin exposure on T-cell differentiation in infants of a population-based study using surface chemokine receptor expression for T-cell characterization. This approach eliminated the necessity of culturing the T cells. The aim of our study was to compare the immunomodulatory effects of endotoxin, mite allergens, and cat allergen as different ubiquitous biologic agents in house dust.

METHODS

Study subjects

For analysis of chemokine receptor expression on T cells, a random subset of 144 children was selected from an ongoing birth-cohort study. This study on "Influences of life-style related factors on the immune system and the development of allergies in childhood" (LISA) recruited 1467 healthy neonates born in 6 obstetric clinics in Munich between January 1998 and January 1999 (response rate, 58%). Details of the inclusion criteria are given elsewhere.²⁴ Data on children's health, sociodemographic characteristics, health-related lifestyle of the families, housing conditions, and environmental exposures were obtained by means of self-administered questionnaires between birth and age 2 years. Three months after birth, house dust was collected on mothers' mattresses in 1337 families for measurement of endotoxin and mite and cat allergens. In each of these families, the child slept at least occasionally in the mother's bed. At the age of 2 years, children underwent a medical examination, and blood was drawn for serologic analyses in the whole cohort and for analysis of PBMCs in the subset of 144 children.

The final study population for this analysis consisted of 135 children with complete data on T cells, endotoxin concentrations in house dust, mite and cat allergen concentrations in house dust, and potential confounding variables. The study was approved by the ethics committee of the Landesärztekammer Bavaria and was carried out in accordance with the institutional guidelines for the protection of human subjects. Written informed consent was obtained from all parents of participating infants.

Analysis of endotoxin and allergens in house dust samples

Dust samples were obtained from mothers' mattresses and extracted as described previously.²⁴ Endotoxin concentration in dust samples was quantified by using a chromogenic kinetic Limulus

amoebocyte lysate test.⁷ Values were expressed as endotoxin units (EU) per square meter. The house dust mite allergens *Dermatophagoides pteronyssinus* (Der p 1) and *Dermatophagoides farinae* (Der f 1) and the cat allergen Fel d 1 were detected by using a 2-site monoclonal ELISA according to the manufacturer's instructions (Indoor Biotechnologies, Clwyd, United Kingdom). Values of less than the detection limit were assigned a value of one half of the detection limit. For further analyses, the sum of Der p 1 and Der f 1 was calculated, and all allergen concentrations were expressed as nanograms per square meter.

Chemokine receptor expression on T cells

Four-color flow cytometry was performed in freshly drawn whole-blood samples anticoagulated with EDTA, as described elsewhere.²⁵ The following primary antibodies were used: CD4-APC mouse IgG₁ (BD Pharmingen, Heidelberg, Germany); CD8-PC5 mouse IgG₁ (Immunotech, Marseille, France); CD19-PE mouse IgG₁ (Immunotech); CCR5-PE mouse IgG_{2a} (BD Pharmingen); and CCR3-FITC rat IgG_{2a} (R&D Systems, Wiesbaden, Germany).

As isotype controls, mouse IgG₁-FITC (Immunotech), mouse IgG_{2a}-PE (Immunotech), and rat IgG_{2a}-FITC (kindly provided by Dr E. Kremmer, GSF-Institute for Molecular Immunology, Munich, Germany) were used. Cells were immediately analyzed by means of flow cytometry (Calibur; Beckton-Dickinson, Heidelberg, Germany), and calculations were performed with Cell Quest analysis software. Lymphocytes were first gated according to their forward-scatter/side-scatter characteristics to determine the percentage of chemokine receptor expression on T-cell subsets. On average, 13,000 events were counted for each sample (median, 12,313 events; range, 10,060-46,136 events). Whole CD4⁺ and CD8⁺ populations were used to determine the absolute numbers and the precise localization of these subsets. Because CCR3⁺ cells would be difficult to detect directly in the whole CD4 population, a second electronic gate was set on CCR3⁺ cells, and the absolute numbers of CCR3⁺ CD4⁺ cells were counted. Nonspecific staining was excluded by excluding all positive isotype control events for each sample. After that, the relative numbers of CCR3⁺ CD4⁺ cells within the CD4⁺ subset were calculated. The same procedure was applied for CCR3⁺ CD8⁺ and CCR5⁺ subsets. Usually, CCR5⁺ cells were not visible as a discrete population clearly separated from CCR5⁻ cells. Therefore only CCR5^{high} cells above a fluorescence intensity of 10² were included in the analysis.

Statistical analysis

For comparison of biocontaminant levels between 2 groups without assumption of normal data distribution, the Wilcoxon rank sum test was used. Correlations between endotoxin and allergen concentrations were assessed with the Spearman correlation coefficient.

In multivariate analysis of the association between biocontaminant exposure and percentage of T cells expressing a certain chemokine receptor (fourth quartile), we used logistic regression models in a 2-step approach. In all models adjustment was done for sex, season of blood collection, breast-feeding, parental atopy, and socioeconomic status and mutually for log-transformed endotoxin and mite and cat allergen levels as potential confounders.

As the first exploration, we applied generalized additive models²⁶ in a nonparametric approach to estimate the pattern of the relationship between the prevalence of elevated T-cell subsets and biocontaminant concentrations from the data with smooth functions. That is, the log odds of elevated proportions of chemokine receptor expressing T cells were modeled as the sum of smooth functions of the log concentrations of endotoxin and mite and cat allergens. The additive effects of the biocontaminants were estimated with loess smoothers by using a span of 0.8. This approach was chosen to

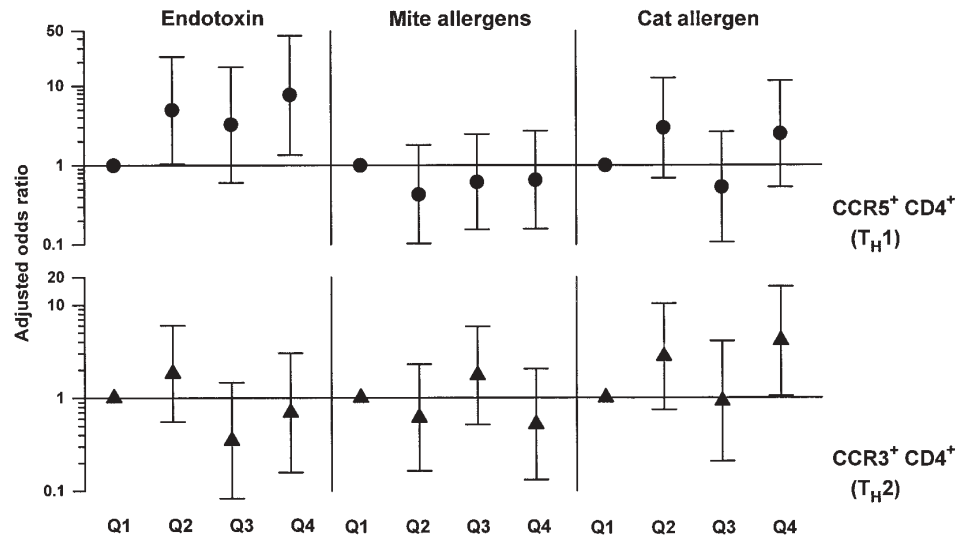


FIG 1. Association between T_H cell subsets (CD4⁺) and exposure to endotoxin, mite allergens (Der f 1 plus Der p 1), and cat allergen (Fel d 1). Adjusted ORs with 95% CIs are shown. Exposure to biocontaminants was categorized into quartiles (Q1-Q4).

explore whether it was justifiable from the form of the curves to analyze categories of exposure or whether mainly nonlinear associations existed.

In the parametric approach we included endotoxin or allergen exposure quartiles as dummy variables into the models with the lowest quartile as a reference to calculate adjusted odds ratios (ORs) with 95% CIs as effect estimates.

Statistical significance was defined by using a 2-sided α level of 5%. The statistical software packages SAS for Windows version 8.2 (SAS Institute, Cary, NC) and S-PLUS 2000 (MathSoft Inc, Seattle, Wash) were used for all calculations.

RESULTS

Characteristics of the study population

The study population consisted of 59 girls and 76 boys. Parents of 81 (60%) children had a history of atopy. Fifty-two percent ($n = 70$) were exclusively breast-fed for at least 6 months. A dog or cat was kept as a pet in 8 (6%) and 17 (13%) families, respectively.

The endotoxin concentrations in dust samples of the mothers' mattresses ranged between 28 and 240,175 EU/m² (median, 1948 EU/m²; 25th percentile, 579 EU/m²; 75th percentile, 5815 EU/m²). Exposure to house dust mite allergens varied between levels below the detection limit ($n = 20$) and 99,560 ng/m² (median, 675 ng/m²; 25th percentile, 164 ng/m²; 75th percentile, 3204 ng/m²). Dust concentrations of cat allergen ranged between levels below the detection limit ($n = 6$) and 4,588,800 ng/m² (median, 139 ng/m²; 25th percentile, 62 ng/m²; 75th percentile, 722 ng/m²). Parental atopy did not influence the biocontaminant levels in house dust. Keeping a furred pet, especially a cat, significantly increased the dust levels of endotoxin and cat allergen, respectively (Table I). Mite allergen levels did not differ between households with or without furred pets.

Endotoxin concentrations were correlated with mite ($r = 0.43$, $P < .001$) and cat ($r = 0.39$, $P < .001$) allergen levels. Mite and cat allergen concentrations were also correlated ($r = 0.25$, $P = .004$).

Estimation of the pattern of the relationship between biocontaminants and T-cell subsets

Ranges of proportions of CCR5⁺ T cells were 0.1% to 16.2% in the CD4⁺ subset (median, 3.4%; 75th percentile, 4.8%) and 0% to 69.5% in the CD8⁺ subset (median, 5.4%; 75th percentile, 9.1%). The proportions of CCR3-expressing T cells were distinctly lower (CD4⁺: 0%-0.3%; median, 0.03%, 75th percentile, 0.06%; CD8⁺: 0%-1.1%; median, 0.06%; 75th percentile, 0.1%).

For a first evaluation of the potential association between biocontaminants and T-cell subsets, we used generalized additive models. The relation between log odds of elevated T-cell subsets and log-transformed biocontaminant levels was nonlinear only in the case of mite allergens and CCR3⁺ CD8⁺ cells and cat allergen and CCR5⁺ CD4⁺ cells ($P < .05$). Exposure to mite allergens was associated with CCR3⁺ CD8⁺ cells ($P < .05$). Furthermore, the curves indicated weak relationships between endotoxin and CCR5⁺ CD4⁺ cells ($P = .11$), mite allergens and CCR5⁺ CD8⁺ cells ($P = .09$), and cat allergen and CCR3⁺ CD8⁺ cells ($P = .14$).

Parametric analysis of the association between biocontaminants and T-cell subsets

For the parametric analysis, biocontaminant exposures were categorized into quartiles. Moderate-to-high endotoxin exposure was associated with increased proportions of CCR5⁺ CD4⁺ T cells (Fig 1). The OR for the highest endotoxin exposure (4th quartile [Q4]) was 7.68 (95% CI, 1.35-43.75). On the other hand, endotoxin lev-

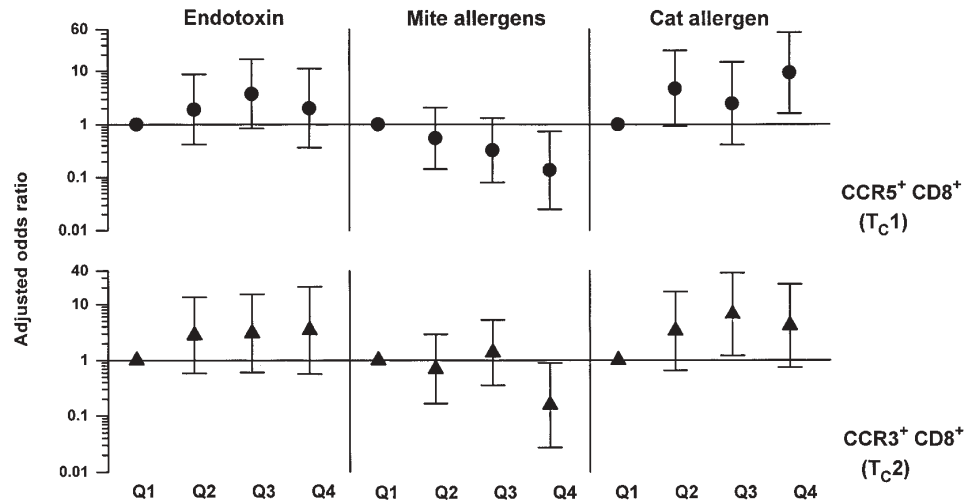


FIG 2. Association between T-effector-cell subsets (CD8⁺) and exposure to endotoxin, mite allergens (Der f 1 plus Der p 1), and cat allergen (Fel d 1). Adjusted ORs with 95% CIs are shown. Exposure to biocontaminants was categorized into quartiles (Q1-Q4).

TABLE I. Influence of pet keeping on biocontaminant levels in mothers' mattress dust

	n	Endotoxin (EU/m ²)		Mite allergens, Der p 1 + Der f 1 (ng/m ²)		Cat allergen, Fel d 1 (ng/m ²)	
		Median	P value*	Median	P value*	Median	P value*
Any furred pet†	28	5086	.047	622	.756	9894	<.001
No furred pet†	107	1740		696		107	
Cat as pet	17	4364	.195	773	.971	30,015	<.001
No cat as pet	118	1857		611		105	
Dog as pet	8	5062	.256	319	.351	112	.693
No dog as pet	127	1899		721		145	

*Wilcoxon test.

†Dog, cat, rabbit, hamster, or guinea pig.

TABLE II. Interaction between exposure to biocontaminants and being at risk because of parental atopy

T-cell subset	Exposure	Total		Parental atopy			
		OR	95% CI	No		Yes	
				OR	95% CI	OR	95% CI
CCR5 ⁺ CD4 ⁺	Endotoxin, ≥25th percentile	4.34	1.04-18.12	5.42	1.14-25.73	3.72	0.85-16.36
CCR3 ⁺ CD4 ⁺	Endotoxin, ≥50th percentile	0.34	0.13-0.89	0.60	0.19-1.93	0.21	0.06-0.73
CCR3 ⁺ CD4 ⁺	Cat allergen, ≥75th percentile	2.80	1.06-7.40	6.14	1.57-23.97	1.63	0.49-5.45
CCR5 ⁺ CD8 ⁺	Cat allergen, ≥75th percentile	3.14	1.10-8.99	5.21	1.30-20.87	2.00	0.51-7.92

ORs with 95% CIs were calculated for the proportion of chemokine receptor expressing T cells in the upper quartile. They were adjusted for sex, season of blood collection, breast-feeding, parental history of atopy, socioeconomic status, and mutually for the biocontaminants. Exposure to biocontaminants was dichotomized as indicated, with the lower exposure category as the reference group.

els of greater than the median gave reduced effect estimates for CCR3⁺ CD4⁺ T cells. In contrast to endotoxin, neither exposure to mite allergens nor to cat allergen were related to proportions of CD4⁺ T cells expressing CCR5. However, high cat allergen levels were associated with high proportions of CCR3⁺ CD4⁺ T cells (OR_{Q4}, 4.07; 95% CI, 1.05-15.85).

Endotoxin exposure was not significantly related to proportions of CCR5- or CCR3-expressing CD8⁺ T cells,

but effect estimates were increased (Fig 2). In contrast, mite allergen concentrations were inversely associated with high proportions of CD8⁺ T cells expressing CCR5, with the lowest OR for the highest exposure category (OR_{Q4}, 0.14; 95% CI, 0.03-0.74). Moreover, high mite allergen exposure was inversely associated with CCR3⁺ CD8⁺ cells (OR_{Q4}, 0.16; 95% CI, 0.03-0.89). On the other hand, cat allergen levels were positively associated with increased CCR5⁺ CD8⁺ cells (OR_{Q4}, 9.24; 95% CI,

1.61-53.10) and CCR3⁺ CD8⁺ cells (OR_{Q3}, 6.64; 95% CI, 1.21-36.51).

Taken together, our data suggested an association of moderate-to-high endotoxin exposure with increased T_H1 cells (CCR5⁺ CD4⁺). High mite allergen exposure was associated with a profound decrease of type 1 and type 2 CD8⁺ T cells in contrast to cat allergen exposure, which was associated with increased proportions of T_H2 cells and both types of CD8⁺ cells.

Stratification by parental atopy

To test whether being at risk because of parental atopy and biocontaminant exposure have interdependent effects, we performed stratified logistic regression analyses. Because of the small numbers of infants in the strata (81 with and 54 without parental atopy), exposure to biocontaminants had to be dichotomized at selected cut-off points instead of categorization into quartiles. We chose the cut-off points according to the associations between biocontaminants and T cells in the whole study population, as described above. As shown in Table II, moderate-to-high endotoxin exposure (≥ 25 th percentile) seemed to be less strongly associated with increased proportions of CCR5⁺ CD4⁺ cells in infants with atopic parents compared with in those infants without risk. On the other hand, in children with parental atopy, the lowering effect of endotoxin exposure of the 50th percentile or greater on CCR3⁺ CD4⁺ cells was stronger. The enhancing effect of high cat allergen levels (≥ 75 th percentile) on proportions of CCR5⁺ CD8⁺ and CCR3⁺ CD4⁺ cells predominated in children without parental atopy. The associations between mite allergens and proportions of CCR5⁺ CD8⁺ or CCR3⁺ CD8⁺ T cells and cat allergen and CCR3⁺ CD8⁺ cells seemed to be independent of parental atopy (data not shown).

DISCUSSION

We analyzed the relation between exposure to biocontaminants in house dust during the first months of life and the proportion of effector T cells in the peripheral blood at age 2 years. For characterization of functionally varying peripheral blood T-cell populations, we used cell-surface detection of chemokine receptors without prior *in vitro* stimulation.

Endotoxin effects

Several studies have suggested that endotoxin might influence the proportion of T_H1 cells and that the timing of exposure to endotoxin might be responsible for the different effects observed.²⁷⁻³¹ Experimental studies in a rat model showed that exposure to aerosolized LPS, before or soon after sensitization, inhibited an increase in allergen-specific IgE, whereas later exposure promoted IgE production and allergic response.²⁷ The authors hypothesized that the early LPS effect was due to the upregulation of T_H1 cytokines, especially IFN- γ . A recent study gave evidence for an LPS-stimulated increase of CCR5 expression on human circulating CD4⁺ T cells through 2 mechanisms, a direct stimulation of CCR5 and an indirect stim-

ulation of this receptor through the cytokines IFN- γ and TNF.²⁸ The use of CCR5 and CCR3 as surrogate markers for type 1 and type 2 T cells allowed us to demonstrate that early exposure to endotoxin stimulated a T_H1, but not a T_H2, increase in healthy infants with and without parental atopy.

Thus our study supports the results of Gereda et al,¹⁴ who reported a correlation between endotoxin levels and proportion of T_H1 cells in asthma-prone infants detected by intracellular cytokine staining after *in vitro* stimulation. In contrast to the data of Gereda et al, however, our data suggest that endotoxin exposure might effect CD8⁺ T cells of both types. Our results support the contention that shifts in functionally distinct lymphocyte subsets can be detected in unstimulated peripheral blood samples through the measurement of chemokine receptor expression.

Mite allergen effects

Exposure to house dust mite allergens is a prerequisite and risk factor for atopic sensitization for these allergens, particularly in early life. It has been reported that Der p 1 cleaves the IL-2 receptor (CD25), thus impairing proliferation and IFN- γ secretion of type 1 T cells, favoring type 2 development, and finally enhancing total IgE production.³² In accordance with these findings, we observed a profound decrease in CCR5⁺ CD8⁺ T cells in case of high mite allergen levels. We used the only commercially available antibody for surface staining of type 2 chemokine receptors directed against CCR3. Because CCR3 is expressed only on a subset of type 2 cells,²³ we cannot rule out that mite allergen exposure might influence the proportion of type 2 cells not expressing CCR3.

Cat allergen effects

Currently, the effect of early exposure to cats on atopic sensitization is controversial. Roost et al³³ argued that exposure to cats during childhood decreased the risk for sensitization to cat allergen in adulthood. The authors suggested that exposure to high allergen levels in the first years of life might induce deviation of immune responses toward the T_H1 cytokine phenotype. Platts-Mills et al³⁴ proposed that high cat allergen, in contrast to mite allergen exposure, results in a tolerant type 2 immune response by inducing IgG₄ instead of IgE antibodies against Fel d 1. They argued that the potential protective effect of close contact to a cat is due to a modified type 2 reactivity and does not imply a shift toward T_H1. Comparable with their findings, we did not observe an association of cat allergen levels with an increase in T_H1 cells in our study. In clear contrast to mite allergens, cat allergen exposure was strongly associated with the proportion of CCR5- or CCR3-expressing CD8⁺ T cells. Our data suggest that a potential protective effect of high cat allergen levels caused by increased proportions of CCR5⁺ CD8⁺ T cells would be more pronounced in children without parental atopy. Whether these cells function as regulatory T cells that have the capacity to influence the isotype produced by B lymphocytes and favor IgG₄ synthesis remains to be further investigated.

Strengths and limitations

The strength of our study lies in its prospective design. Exposure assessment was performed in children at the age of 3 months to analyze early effects on immune reactivities at the age of 2 years. Allergens and endotoxin in bed dust are considered to be significant sources of exposure for infants. In addition, endotoxin in bed dust samples was reported to be the most reliable marker to distinguish between homes and was not influenced by season.³⁵ We measured biocontaminants in dust of the mothers' mattresses because standardized dust collection on children's mattresses was not feasible.²⁴ Because all the children analyzed slept in their mother's bed during their first months of life, biocontaminant levels in mothers' mattresses dust might be interpreted as an indicator for prenatal, as well as early postnatal, exposure.

In the interpretation of data regarding different T-cell subsets, defined either by cytokine production or chemokine receptor expression, it has to be taken into account that the concept of immune response regulation by the balance of 2 discrete T-cell subsets, known as the T_H1/T_H2 model, is a simplification. This concept has been challenged by the detection of further cytokine-producing CD4⁺ T-cell phenotypes; T regulatory cells 1 or T_H3 cells, which counterregulate immune responses mediated by T_H1 and T_H2 ; and by the notion that a continuum of phenotypes might exist.^{36,37} We have interpreted our data in terms of CCR5 and CCR3 as surrogate markers to distinguish between predominant type 1 or type 2 activities in effector T cells. Clearly, using chemokine receptor expression for characterization of T cells, we were not able to further distinguish between different effector cell types that shared migration characteristics.

The major limitation of our study is the small number of infants. It has to be kept in mind that results of both logistic regression approaches are dependent on the choice of the number of exposure categories and of the smoothing parameter, respectively. Therefore our results should be valued in terms of an explorative analysis and need to be confirmed by further analyses. Moreover, the different effects of the biocontaminants on type 2 cells should be verified by using a suitable marker for the whole type 2 cell subset.

Conclusion

In conclusion, our data provide the first insight into the expression of CCR5 and CCR3 on CD4⁺ and CD8⁺ T-cell subsets in healthy infants and reveal for the first time that early exposure to endotoxin and mite and cat allergens in house dust might have different effects on effector type 1 and type 2 T-cell subsets, as defined by their chemokine receptor expression. Our results are in accordance with those of previous reports of potential protective effects of endotoxin, as well as cat allergen exposure early in life, on atopic immune reactivities in contrast to mite allergens.

We thank all the families for their participation. We thank Bernd Hoelscher for statistical assistance and Ulrike Gehring for critical reading of the manuscript.

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APPENDIX

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