

ORIGINAL ARTICLE

Chemokine levels in serum of children with atopic dermatitis with regard to severity and sensitization status

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Abstract

Background: Many infants with atopic dermatitis (AD) are sensitized against food or airborne allergens. The severity of AD, using the SCORAD, seems to correlate with elevated serum levels of TARC/CCL17. Other chemokines, such as CCL20 or CCL25, have been described in the context of allergic inflammation. The aim of this study was to analyze whether chemokine serum levels differ within a cohort of infants suffering from varying severities of AD with or without allergic sensitization.

Methods: Chemokine serum levels (CCL8, CCL17, CCL20, CCL25) as well as food and airborne allergen-specific IgE were analyzed in infants with AD.

Results: About 60.9% (78/128) infants with AD (median age 8.8 months, 49 (38%) girls and 79 (62%) boys) showed a positive screening test to common food allergens and 26.6% to common airborne allergens. There was a strong correlation between serum levels of CCL17 and SCORAD in food-sensitized infants ($r_s = 0.646$, $p = <1e-04$) and airborne-sensitized infants ($r_s = 0.587$, $p = 0.00065$) in contrast to non-sensitized ones. Moreover, food-sensitized infants showed significantly higher levels of CCL25 compared to non-food-sensitized ones ($p = 0.007$).

Conclusion: The strong correlation between TARC/CCL17 and SCORAD in infants with specific sensitizations may be accounted for by the impaired skin barrier. As TARC/CCL17 has been found mainly in the (inflamed) skin but not in the gut, the detection of significantly higher levels of CCL25, ligand of CCR9, localized primarily in the gastrointestinal tract, suggests its impact on food allergen-induced inflammation processes in food-sensitized infants.

Children with atopic dermatitis (AD) are more often sensitized against food or airborne allergens and suffer more often from subsequent allergies including food allergies (FA), asthma, or allergic rhinitis compared to children without AD. In this context, it has been hypothesized that allergens may be absorbed through an ineffective epithelial barrier and promote allergic sensitization (1). Nevertheless, not all children with AD develop sensitizations and allergies. The measurement of specific IgE (sIgE) detects allergic sensitization, but it does not give any information about epithelial or other upstream conditions regarding the sensitization process. We wanted to

look for further players in allergic inflammation which possibly serve as early biomarkers or help to distinguish between different phenotypes of AD.

The chemokine TARC/CCL17 has been reported as correlating with the disease severity in patients suffering from AD (2). Therefore, it has been suggested as a good clinical marker (3, 4). The thymus- and activation-regulated chemokine TARC/CCL17 is a ligand for the CC chemokine receptor 4 (CCR4), which is expressed predominantly by Th2 lymphocytes and signals in response to the ligands CCL22 and CCL17 (5). TARC/CCL17, also produced by keratinocytes, has been detected in the inflamed skin of patients and in an animal model of AD. It has been shown to recruit (CCR4+) T lymphocytes into inflammatory sites (6, 7).

Both CCL17 and CCL22 are reported to be up-regulated in the human lung following allergen challenge (8, 9). These

Abbreviations

AD, Atopic dermatitis; CM, Cow's milk; FA, Food allergy; GALT, Gut-associated lymphoid tissues; HE, Hen's egg; HDM, House dust mite; SCORAD, Scoring of atopic dermatitis; sIgE, specific IgE; TARC, Thymus and activation-regulated chemokine; TECK, Thymus-expressed chemokine.

findings are consistent with a role for CCR4 in T-cell recruitment to the allergic lung (8, 9). CCL17 is described as a pro-allergic factor which attracts Th2 cells and promotes Th2 cell differentiation (10).

In the course of an allergen-induced airway inflammation in asthma, the proteolytic activities of house dust mite extract (HDM) have been described as inducing an increase in CCL20 and a bronchial epithelial barrier dysfunction by the disruption of cell–cell contacts (11). The same group demonstrates in an *in vivo* mouse model the importance of the (airway) epithelial barrier in allergic sensitization, underlining the involvement of various chemokines and cytokines, including CCL20, a ‘critical chemokine in allergen-induced airway inflammation in asthma’ (11, 12).

In addition to the skin and the respiratory system, a role of chemokines in allergic reactions has also been described in the gastrointestinal tract. Food antigen-specific T cells are known to express certain chemokine receptors on their surface. CCR9 is described as being expressed by small intestinal lamina propria lymphocytes and intra-epithelial lymphocytes, and as binding the ligand TECK/CCL25 (13).

Blazquez et al. (14) examined the role of the mucosal chemokine CCL20/MIP-3a, and its receptor CCR6 in the development of experimental FA using CCR6^{−/−} mice. They found that CCR6 was necessary for allergen-induced diarrhea symptoms. Their results point to a critical role for CCR6 in both T-cell-homing and activation in the gastrointestinal tract (14).

A chemokine (mouse) receptor–ligand pair CCR8–CCL8 seems to promote AD by recruiting IL-5 + T(H)2 cells (15, 16). Little is as yet known about a human equivalent.

Localization and the described impact on allergic mechanisms differ between chemokine receptor–ligand pairs like TARC/CCL17 (CCR4) in the skin (17, 18), TECK/CCL25 (CCR9) gastrointestinally (13, 19), or CCL20 (CCR6) in both airway and gastrointestinal inflammation (11, 12, 14). In order to obtain a more differentiated phenotypical characterization, we investigated these chemokine serum levels in a cohort of infants with AD.

We hypothesize that children with AD and specific sensitization have higher levels of CCL17 than children who are not sensitized. Furthermore, we propose that food-sensitized children with AD have higher levels of CCL25. Additionally, we suggest an involvement of CCL20 in infants with sensitization against airborne and food allergens.

Methods

Study population

We retrospectively examined serum samples from 128 infants with AD presenting at our outpatient clinic. From these samples, 49 (38%) are from girls and 79 (62%) from boys. Blood samples had been routinely drawn for analysis due to the suspicion of FA or allergic airway disease. Due to limited amounts of serum, we were not able to analyze all laboratory

parameters from all children. A screening test for the 6 most common food allergens (fx5) was performed in sera of 128 children, and a screening test for the common airborne allergens (sx1) was performed in sera of 108 children. In some cases, the entire differentiation of a positive fx5 was not possible; however, sIgE to cow’s milk (CM) was tested in 119 samples, to hen’s egg (HE) in 120, to wheat in 115, to soy in 119, to fish in 111, and to peanut in 120, respectively. Furthermore, regarding the chemokines, CCL8 was measured in 110 samples, CCL17 in 128, CCL20 in 124, and CCL25 in 116, respectively. AD was evaluated by the investigator using the SCORAD index (Scoring of AD) according to established criteria (20). The study was approved by the local institutional review board.

Fluorescence enzyme immunoassays

Determination of sIgE antibodies to common food allergens (fx5) as well as against airborne allergens (sx1) was performed by fluorescence enzyme immunoassay using ImmunoCAP250 System® (ThermoFisher Scientific, Phadia GmbH, Freiburg, Germany). SIgE titers were quantified as kilounits of antibody per liter (kU/l) with a lower detection limit of 0.00 kU/l and an upper limit of 100 kU/l as proposed by the manufacturer. SIgE titers for single allergens were considered as positive when >0.1 kU/l, and screening test (sx1,fx5) was considered as positive when >0.35 kU/l.

TARC/CCL17 (CCL17 pg/ml range: 31.2–2000; R&D Systems Inc., Minnesota, MN, USA), TECK/CCL25 (CCL25 pg/ml range: 62.5–4000; R&D Systems Inc.), CCL20 (CCL20 pg/ml range: 7.8–500; R&D Systems Inc.), and MCP-2/CCL8 (CCL8 pg/ml range: 0.82–200; ELISA Kit RayBio®, Norcross, GA, USA) were measured by ELISA according to the manufacturer’s instructions.

Bioinformatic analysis

Data were analyzed using the Mann–Whitney *U*-test for unpaired samples, and p-values were adjusted by the Holm method for multiple comparisons. Correlation coefficients were determined using the Spearman’s rank correlation test. By performing a simple logistic regression, the probability of having specific sensitizations for different chemokine serum levels was calculated, and no adjustment was made. A p-value <0.05 was considered as statistically significant. Data were analyzed using R (21). Figures were produced with ggplot2 (Wickham H, 2009).

Results

Description of the study population

The included children (n = 128) had a median age of 8.8 months. About 78 children (60.9%) were sensitized to at least one food allergen (CM, HE, wheat, soy, fish and/or peanut, screening test against common food allergens) (Table 1), whereas 50 children (39.1%) showed no food

Table 1 Baseline characteristics of participating infants

Food sensitized	Yes	No	Yes	No	Yes	No
Airborne sensitized	Yes	No	Yes	No	Yes	No
N (%)	78 (60.9)	50 (39.1)	34 (26.6)	74 (57.8)	32 (25)	39 (30.5)
Age in days (Median)	266.0	246.5	266.0	229.0	266.0	227.0
Age in days (25./75. Percentile)	197.0/37.02	177.7/404.0	197.0/437.0	184.5/362.2	198.0/425.0	190.0/438.5
SCORAD (Median)	32.0	25.0	35.0	29.0	33.5	27.0
SCORAD (25./75. Percentile)	22.0/4.06	18.0/35.0	26.3/46.0	20.0/40.0	25.8/48.3	18.0/36.0
CCL8 (Median)	59.1	63.7	72.1	57.1	68.1	60.5
CCL17 (Median)	1725.0	1400.8	1597.0	1777.7	1597.0	1544.3
CCL20 (Median)	33.0	38.5	31.6	39.1	31.6	39.3
CCL25 (Median)	112.3	84.6	100.9	96.2	100.9	86.2

Food sensitized = screening test for common food allergens, fx5.

Airborne sensitized = screening test for common airborne allergens, sx1.

sensitization. About 26.6% ($n = 34$ children) showed at least one specific sensitization against airborne allergens: timothy, birch, mugwort, HDM, cat, and dog (screening test against common airborne allergens). The sensitized children were slightly older (median 266 days of life) (Table 1).

Analysis of CCL17 in children with AD with or without specific sensitization

A distinct correlation was detected between serum levels of CCL17 and SCORAD (Spearman's correlation coefficient $r_s = 0.49$, $p = <1e-04$) (Fig. 1a). The correlation coefficient was even higher in food-sensitized ($r_s = 0.649$, $p = <1e-04$) than in non-food-sensitized children ($r_s = 0.25$, $p = 0.080$)

(Fig. 1a). Likewise in airborne-sensitized children, we observed a higher correlation coefficient ($r_s = 0.587$, $p = 0.00065$) than that in non-airborne-sensitized children ($r = 0.49$, $p = 0.00063$) (Fig. 1b). There was no significant correlation between SCORAD and levels of CCL8, CCL20, and CCL25 (data not shown).

Analysis of different chemokine serum levels in children with AD with or without specific sensitization

Children with at least one food-specific sensitization showed significant higher levels of CCL25 ($p = 0.007$, Holm corrected) compared to non-food-sensitized children (Fig. 2b). In further analysis, children sensitized against HE ($p = 0.046$), wheat ($p = 0.011$), and peanut ($p = 0.041$) had higher CCL25 levels

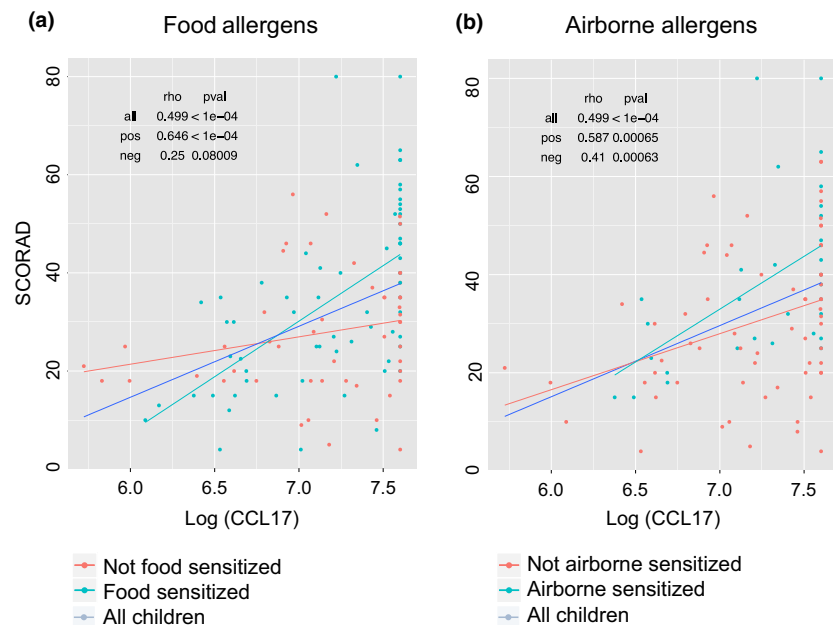


Figure 1 Correlation between SCORAD and CCL17. Correlation coefficients were determined using the Spearman's rank correlation test.

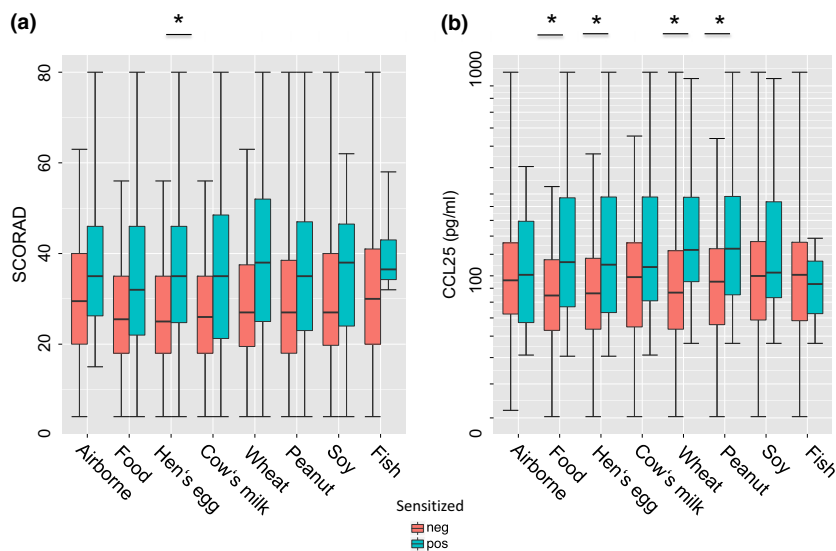


Figure 2 SCORAD (a) and CCL25 (b) levels in association with specific sensitization in screening tests against common airborne allergens and food allergens as well as against single food allergens. Data were analyzed using the Mann–Whitney *U*-test for unpaired samples, and *p*-values were adjusted by the Holm method for multiple comparisons. A *p*-value <0.05 was considered as statistically significant.

likewise (Fig. 2b), whereas no significant differences could be detected for CM, fish, or soy.

Children with at least one airborne-specific sensitization showed no significant differences in serum levels of CCL25 (Fig. 2b), CCL20, CCL17, or CCL8 (data not shown) compared to non-airborne-sensitized children.

SCORAD levels in children with AD with or without specific sensitization

SCORAD levels were higher in AD children with diverse allergen sensitization; nevertheless, significant differences after Holm's correction were just found in children sensitized against HE ($p = 0.041$, Fig. 2a).

Associated probability of having sensitizations against specific allergens for different chemokine serum levels and SCORAD

By performing logistic regression, we calculated the probability of having specific sensitizations for different chemokine serum levels. We detected a strong association between being food allergen sensitized and having elevated CCL25 levels (Fig. 3). However, no association is seen between levels of specific antibodies against common food or airborne allergens and levels of CCL8, CCL17, or CCL20 (Fig. 3).

CCL25: When we focused on single food allergens, we detected a strong association between the level of CCL25 and the probability of being sensitized against HE, and peanut, and a less marked association for the probability of being sensitized against wheat, CM, and soy. The probability of being sensitized against fish is not associated with the level of CCL25 (Fig. 4b).

SCORAD: When looking at the association between SCORAD and the probability of being sensitized against single food allergens, the strongest association was found for

HE, CM, wheat, and peanut, weak association for soy, and no association for fish (Fig. 4a).

Discussion

Chemokines and their corresponding receptors are largely involved in the pathogenesis of allergic diseases (22). Although this protein family is so fundamental to leukocyte trafficking within allergic tissues, we know of no data on chemokine levels in peripheral blood of infants in the context of specific sensitization or FA and allergic airway diseases.

We were able to elaborate that children with AD and specific sensitization against food or airborne allergens had an even higher correlation between CCL17 (ligand for CCR4) and their SCORAD value than children who were not sensitized (Fig. 1). Interestingly, in children with AD and specific sensitization against various food allergens, we observed significantly higher levels of CCL25, ligand of CCR9, localized primarily in the gastrointestinal tract, compared to children without food allergen sensitization (Fig. 2).

AD has been shown to correlate with the likelihood of development of FA (1). Therefore, it fits well that we observed a higher (and statistically highly significant) correlation between SCORAD and levels of CCL17 for food-sensitized than for non-food-sensitized children (Fig. 1a). Similar, AD represents a high risk factor for developing subsequent allergic airway diseases (23–25). We observed a higher correlation between SCORAD and levels of CCL17 for airborne-sensitized than for non-airborne-sensitized children (Fig. 1b). A possible impact of CCL17 on the skin barrier (or sensitization) has been suggested by Nakahigashi et al. (26). They describe that CCL17 seems to be able to induce aquaporin-3 in human keratinocytes (a water/glycerol-transporting protein expressed in keratinocytes of the epidermis), which in reverse enhances keratinocyte proliferation, which in turn is involved in hyperplasia and barrier disruption in AD (26).

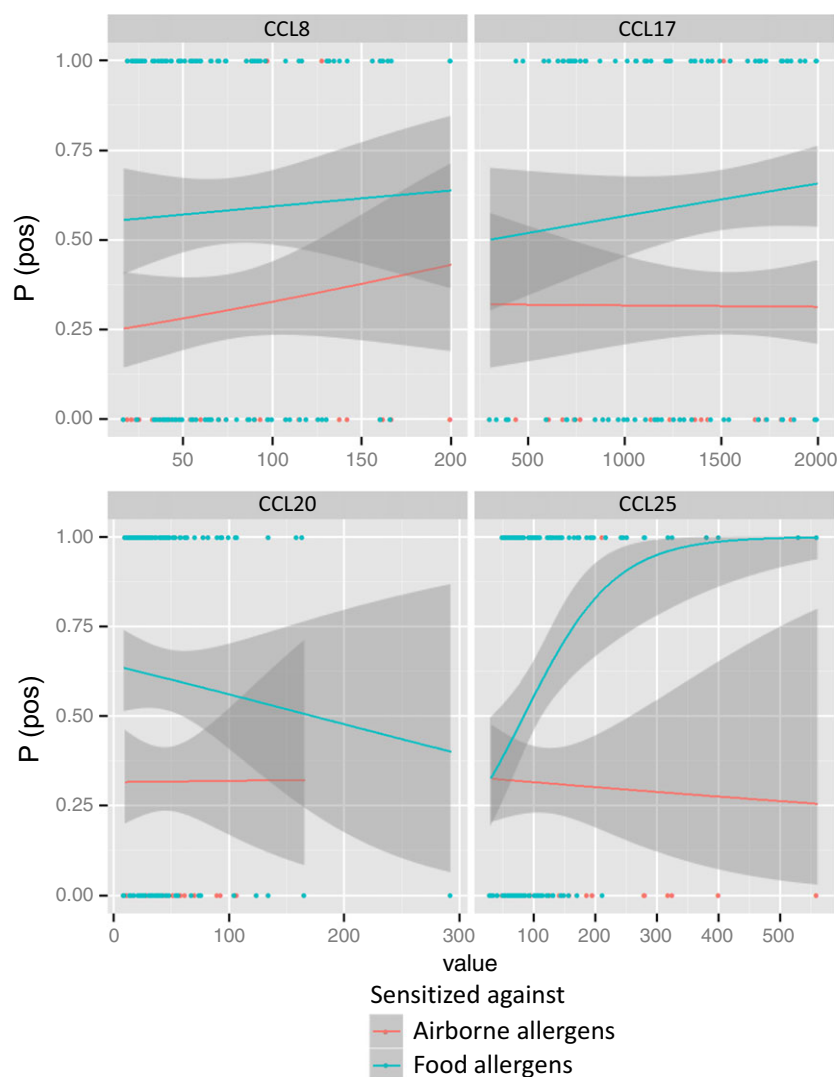


Figure 3 Associated probability of having sensitizations against food or airborne allergens for different chemokine serum levels of CCL8, CCL17, CCL20, and CCL25 by performing logistic regression.

In addition to skin descriptive qualities, the predictive qualities of CCL17 have been discussed. One study notes increased levels of CCL17 and CCL22 in cord blood of children who develop allergic symptoms and sensitization during the first 6 years of life, and another describes elevated umbilical cord serum levels of CCL17 as predicting the development of AD in infancy (27, 28).

In contrast to TARC/CCL17, for which ‘immunohistochemistry studies have revealed a strong signal in the skin, while it was mostly absent in the gut’ (17, 18), TECK/CCL25 with its receptor CCR9 is discussed as being involved in T-cell chemotaxis to the gut. CCR9 is selectively expressed on intestinal homing T lymphocytes (intestinal homing marker), mucosal lymphocytes, and thymocytes. The chemokine TECK (thymus-expressed chemokine) is particularly expressed in the thymus and the small intestine (13, 19). Clinically, a role for the CCR9-CCL25 axis is postulated in Crohn’s disease (12, 29). We wanted to look for the

involvement of CCL25 in the context of food sensitization. Even systemically, in peripheral blood, we were able to confirm our hypothesis and observed significantly higher CCL25 serum levels in food-sensitized compared to non-food-sensitized children (Fig. 2b). A subgroup analysis revealed significantly higher CCL25 levels in infants sensitized against HE, wheat, and peanut, whereas elevated but not statistically significant levels were found in infants with sensitization to CM and soy (Fig. 2b), which might be due in part to the small numbers of cases.

Cassini et al. have shown that the expression of CCR9 and integrin $\alpha 4\beta 7$ on T cells and their subsequent localization to the gut are required for the induction of oral immune tolerance in mice (30). Consequently, it would be important to look for differences in levels of CCL25 between allergic and tolerant children. Unfortunately, we do not have the corresponding information from our study population, so we cannot determine whether CCL25 is connected to FA and/or only to food

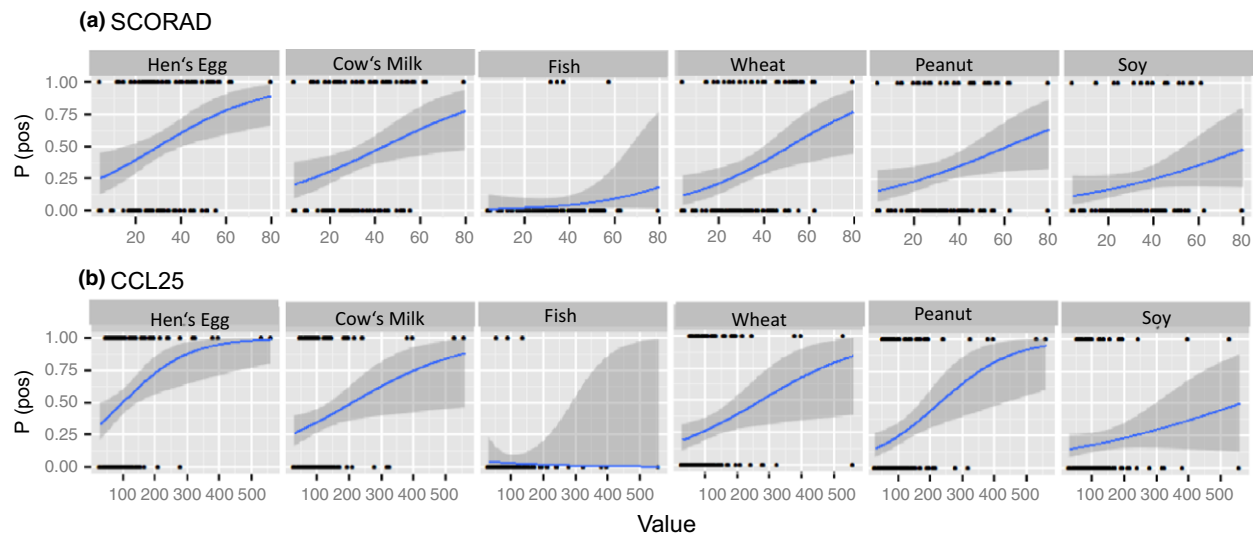


Figure 4 Associated probability of having specific food sensitizations for SCORAD (b) and different chemokine serum levels of CCL25 (a) by performing logistic regression.

sensitization. Another possibility is that CCL25 might be linked with an early (intestinal) barrier dysfunction in the context of (a first) allergen contact and/or specific sensitization, so that elevated CCL25 levels could function as an early predictor for developing FA.

Apart from TECK/CCL25, MIP-3a/CCL20 among others is expressed in the gut-associated lymphoid tissues (GALT) and/or the intestines and also seems to play a complex role in intestinal lymphocyte localization and intestinal immunity (31). CCL20, like CCL25, is known to be overexpressed in inflammatory bowel disease (14). CCR6, the cognate receptor for CCL20, is expressed on memory T cells, B lymphocytes, and dendritic cells. Nevertheless, we found no significant differences for either food or airborne allergens. Likewise, we did not find any statistically significant differences for CCL8, which is described in binding to CCR 1,2,3,5 (32).

The relevance of the CCL25-CCR9 axis in FA becomes even more apparent when using logistic regression to calculate the probability of having specific sensitizations in association with analyzed serum levels. Here, we find a strong association between being food allergen sensitized (especially against HE and peanut) and having elevated CCL25 levels (Figs 3 and 4b). Regarding the missing association between CCL25 and fish, we may have to consider the small number of cases. Moreover, this might be explained by the fact that our study population had a median age of 8.8 months, but the median age of onset of fish allergy has recently been estimated with 8.5 years (33).

Our results are derived from the analysis of peripheral blood. We found significant differences regarding serum levels and food sensitization for CCL25, but not for CCL20. One explanation might be the distance from the 'scene of event', in

particular the intestine (most other cited studies used histological tissue samples). An analysis of the sputum or mouth mucosa as being 'closer' to allergen contact might be promising. Another reason might be that the level of CCL20 (or of other chemokines) might be higher after a direct allergen contact. Therefore, an interesting setting would be the measurement of CCL25 and CCL20 both before and after an elective food challenge in sensitized children. In contrast to an animal model of FA, it will not be possible with children to analyze biopsy samples of the intestine or GALT for the expression of these markers.

In summary, our data validate the correlation between severity of AD (SCORAD) and CCL17 and moreover demonstrate that the correlation between CCL17 and SCORAD is even more pronounced in AD children with specific sensitization to food or airborne allergens. Therefore, our results highlight the link between skin inflammation, barrier dysfunction, and sensitization process. In food-sensitized infants, we have detected significantly higher serum levels of CCL25 than in non-food-sensitized infants. Although the differences in CCL25 levels do not offer sufficient predictive potential for sensitization against food allergens for individuals, these data strongly indicate an impact on CCL25, with its ligand CCR9, localized primarily in the gastrointestinal tract, in the context of food allergen-induced inflammation. The role of CCL25-CCR9 axis and its predictive potential for food allergies should be addressed in future studies.

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