

ORIGINAL ARTICLE

Inflammatory response and IgE sensitization at early age

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To cite this article: Mustonen K, Orivuori L, Keski-Nisula L, Hyvärinen A, Pfefferle PI, Riedler J, Dalphin J-C, Genuneit J, Lauener R, Roduit C, Braun-Fahrlander C, Weber J, Schaub B, von Mutius E, Pekkanen J, Vaarala O. Inflammatory response and IgE sensitization at early age. *Pediatr Allergy Immunol* 2013; **00**.

Keywords

allergic sensitization; asthma; asthmatic bronchitis; atopic dermatitis; C-reactive protein; low-grade inflammation

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Abstract

Background: Microbial exposure may induce low-grade inflammation at an early age and decrease the risk of allergic diseases, as suggested by the hygiene hypothesis. We examined the associations between low-grade inflammation and the development of allergic sensitization, atopic dermatitis (AD), and asthma at the age of 4.5 yr.

Methods: We studied 636 children participating in the PASTURE study in Finland, Germany, Austria, France, and Switzerland. Data of environmental factors, doctor-diagnosed AD, and asthma were collected by questionnaire. The serum high-sensitivity C-reactive protein (CRP) values were measured at the age of 1 yr, and serum-specific IgE concentrations (sIgE) at the age of one and 4.5 yr. Analyses were made by logistic regression analysis.

Results: The risk of allergic sensitization at the age of 4.5 yr was decreased in children who had increased CRP levels at the age of 1 yr (level in the highest vs. lowest quartile: aOR 0.48, 95% CI 0.24–0.95; $p = 0.014$). The risk of AD and asthma was not significantly related to CRP.

Conclusion: The findings confirm that elevated levels of CRP at early age showed association with decreased allergic sensitization later in life. Our results suggest that poor inflammatory response could predispose for IgE sensitization.

Accepted for publication 20 February 2013

DOI:10.1111/pai.12072

It is over twenty years since Strachan first proposed that infections in early childhood might prevent allergic diseases later in life. He hypothesized that the increase in prevalence of allergic diseases during childhood might be due to declining family size, improvement in household amenities and higher standards of personal cleanliness, leading to fewer incidents of clinical and subclinical infections (1).

High-sensitivity C-reactive protein (CRP) is a marker of low-grade inflammation. Increased CRP levels have been associated with female sex (2), obesity (3), and respiratory impairment (4) in children. Marschan and associates have earlier shown that increased plasma CRP levels at the age of 6 months were associated with a decreased risk of eczema at the age of 2 yr (5). In their study, infants who were treated

with probiotic bacteria had higher plasma CRP levels, but the decreased risk of eczema was not only seen in children who received probiotics, but also in children with higher CRP levels on placebo (5). Also when infants with atopic eczema–dermatitis syndrome (AEDS) were treated with *Lactobacillus GG* (LGG), an increase in CRP levels was observed in the treatment group with alleviated symptoms of AEDS (6). Both of these studies suggest that a low-grade inflammation, induced by microbial exposure in infancy, would generate some protection against allergic diseases, at least against eczema.

A protective effect of a farming environment on the development of allergic diseases during childhood has been shown in several studies (7–12). Thus, our main hypothesis was to test whether systemic low-grade inflammation measured as the high-sensitivity CRP levels during early life, that is at 1 yr of age, would provide protection from the development of allergic sensitization and diseases later in childhood. We further evaluated whether different farming environmental factors associated with microbial exposure such as visiting stables or barns, farm milk consumption, and domestic animal contact, would be related to CRP levels at 1 yr of age.

Methods

Study population

The study population consisted of the PASTURE (Protection against Allergy Study in Rural Environments) birth cohort located in the rural areas of five European countries: Austria, Finland, France, Germany, and Switzerland (13). Farming and non-farming pregnant women living in these rural areas were recruited in the third trimester of pregnancy. Details of this recruitment have been described previously (14). Lifestyle factors and health issues as well as a broad range of agricultural exposures of the children and mothers were assessed via questionnaires. Data on high-sensitivity CRP levels were available for 636 (56.1%) of 1133 study children at the age of 1 yr. Among these children, 321 were living on a farm and 315 in rural areas at the time of the study.

High-sensitivity C-reactive protein

Venous blood samples were collected from study children at the age of 1 yr. CRP measurements were additional part of the total study and were made afterwards from venous blood samples. Blood samples were available for CRP analysis after other primary laboratory analyses only from 56.1% of the total study population. No significant differences were detected in the risk of developing asthma, atopic dermatitis, or sensitization between children who did and who did not have CRP levels analyzed. The CRP values were measured from serum samples using ELISA method (BenderMedSystems human CRP, Vienna, Austria). The sensitivity of the assay is 3.0×10^{-6} mg/l according to the manufacturer.

Specific IgE determination

Specific immunoglobulin E (sIgE) against 19 common allergens was tested by the Allergy Screen Test Panel for Atopy (Mediawiss Analytic, Moers, Germany) (15) in blood samples of one- and 4.5-yr-old study children. The 13 inhalation allergens tested were two house dust mites (*Dermaphagoides pteronyssinus* and *D. farinae*), seven types of pollen (alder, birch, European hazel, grass pollen mixture, rye, mugwort, and plantain), cat, horse and dog dander, and the mold *Alternaria alternata*. The six food allergens were hen's egg, cow's milk, peanut, hazelnut, carrot, and wheat. Any allergic sensitization was defined as a positive result [sIgE concentration of 0.35 kU/l or greater at the age of 1 yr (16) and 0.70 kU/l or greater at the age of 4.5 yr (17)] to any of the tested allergens. Inhalation or food atopy was defined as a positive result to any of the 13 inhalation or six food allergens tested, respectively. The following inhalation allergens were considered to be seasonal: alder, birch, hazel or rye pollen, grass pollen mix, mugwort, plantain, and *Alternaria* species, while all others were considered perennial (14). Both CRP concentrations and specific IgE values at the age of 1 yr were available from 634 study children (56.0% of total study population). Both the CRP concentrations at the age of 1 yr and specific IgE values at the age of 4.5 yr were available for 448 study children (39.5% of total study population). No significant differences were detected between living environment or IgE levels between those with and without CRP results available.

Questionnaires

The first questionnaire was administered to mothers during the third trimester of pregnancy, and follow-up data were collected 2, 12, 18, 24, 36, and 48 months after delivery. The extensive questionnaires focused on respiratory and allergic diseases and farm-related exposures, as has been described previously (14). We focused on exposures previously identified as major explanatory variables of the farming effect (7–12) and reported to associate with CRP levels (17): time spent in animal sheds and hay barns, consuming farm milk and maternal smoking. The ownership of cats and dogs was also included in the analysis.

Prenatal exposure and children's exposure to stables and hay barns were defined as a dichotomized variable (No/Yes): if children did not visit any stable defined as no, otherwise yes. Consumption of farm milk was also categorized as yes if children consumed more than 10 ml per day.

Data on doctor-diagnosed atopic dermatitis and asthma were also obtained from questionnaires where parents had reported if a doctor had diagnosed atopic dermatitis or asthma at least once or asthmatic bronchitis at least twice during the follow-up. In most children, atopic dermatitis occurs early in life, and to evaluate CRP levels measured prior to the disease, atopic dermatitis with onset after the 1st year of life was defined. Children with no reports of a doctor's diagnosis of atopic dermatitis in the 1-yr questionnaire but who developed it any time between ages 1 and 4 yr were defined as having

atopic dermatitis. Children who already had atopic dermatitis before the measurement of the CRP values ($n = 60$) were excluded from analyses. Children who did not have doctor-diagnosed asthma or asthmatic bronchitis more than once before the measurement of CRP value but developed such disease between 1 and 4 yr of age were defined as having asthma or asthmatic bronchitis; children who already had asthma or asthmatic bronchitis more than once before the measurement of CRP value ($n = 23$) were excluded from analyses.

Statistical analyses

The CRP values were not normally distributed, and the samples under the detection limit (1.4%) were grouped as zero values and included in the analysis. Log-transformed CRP levels were not either normally distributed. As we wanted to evaluate whether low-grade inflammation is prospectively related to the development of allergic diseases, we evaluated the associations between CRP levels and risks

of atopic dermatitis, asthma, and allergic sensitization separately in non-sensitized and sensitized children at the age of 1 yr. The risk of atopic dermatitis and asthma between ages 1 and 4 yr and the risk of developing allergic sensitization and persistence of allergic sensitization at the age of 4.5 yr were analyzed by logistic multivariate regression. CRP values were categorized into four groups using 25th, 50th, and 75th percentile cut points. Odds ratios were adjusted for farming status at study entry, study center, mother's history of allergic disease (No/Yes), and potential confounders, which were at least marginally significantly ($p < 0.10$) associated with CRP in the univariate analysis, that is gender, siblings (0, 1–2, 3 or more), and day care with other children and siblings (No/Yes).

In the analyses of associations between CRP values and environmental and farming factors, results are given as median values with interquartile ranges. p -values for the comparisons between different categories in the univariate analysis were calculated by the Mann–Whitney U -test and the Kruskal–Wallis test. For logistic multivariate regression analysis, the

Table 1 Development of allergic diseases during childhood and CRP levels at the age of 1 yr in children who have no signs of IgE sensitization and in children who have developed allergen-specific IgE response at the age of 1 yr.

CRP 1 yr	Non-sensitized at 1 yr		Sensitized at 1 yr	
	Atopic dermatitis n (%)	aOR (95% CI)	Atopic dermatitis n (%)	aOR (95% CI)
≤ 25th percentile	7 (7.8)	1	8 (25.8)	1
25–50th	13 (14.9)	1.53 (0.54–4.32)	5 (21.7)	0.27 (0.04–1.76)
50–75th	8 (9.3)	0.93 (0.31–2.81)	3 (9.7)	0.11 (0.02–0.77)
≥ 75th	11 (11.7)	1.41 (0.49–4.03)	5 (12.8)	0.20 (0.03–1.26)
p -value	0.449	0.780	0.291	0.060
CRP 1 yr	Asthma/asthmatic bronchitis n (%)		Asthma/asthmatic bronchitis n (%)	
	Asthma/asthmatic bronchitis n (%)	aOR (95% CI)	Asthma/asthmatic bronchitis n (%)	aOR (95% CI)
≤ 25th percentile	14 (14.4)	1	6 (15.8)	1
25–50th	15 (15.2)	0.91 (0.38–2.18)	4 (15.4)	1.02 (0.22–4.64)
50–75th	6 (6.8)	0.39 (0.13–1.19)	5 (13.9)	1.31 (0.31–5.43)
≥ 75th	9 (9.3)	0.65 (0.25–1.69)	9 (24.3)	2.20 (0.59–8.19)
p -value	0.215	0.183	0.643	0.213
CRP 1 yr	Sensitization to at least one allergen at the age of 4.5 yr n (%)		Sensitization to at least one allergen at the age of 4.5 yr n (%)	
	Sensitization to at least one allergen at the age of 4.5 yr n (%)	aOR (95% CI)	Sensitization to at least one allergen at the age of 4.5 yr n (%)	aOR (95% CI)
≤ 25th percentile	34 (45.9)	1	16 (57.1)	1
25–50th	48 (53.3)	1.04 (0.54–2.02)	16 (57.1)	0.62 (0.18–2.12)
50–75th	32 (40.0)	0.68 (0.35–1.34)	20 (71.4)	1.40 (0.40–4.92)
≥ 75th	26 (32.5)	0.48 (0.24–0.95)	22 (56.4)	0.91 (0.30–2.79)
p -value	0.045	0.014	0.588	0.781

Results are presented as odds ratios (OR) and their 95% confidence intervals (CI). Odds ratios are adjusted for farmer, country, gender, siblings, day care with other children than siblings and mother's history of allergic disease.

CRP values were categorized into two groups using the 75th percentile cutoff point (0.19 mg/l). Odds ratios were adjusted for farming status at study entry, study center, and potential confounders, which were at least marginally significantly ($p < 0.10$) associated with CRP in the univariate analysis, that is gender, siblings, and day care with other children and siblings.

All statistical analyses were performed with PASW Statistics 18.0 (SPSS Inc., Chicago, IL, USA).

Results

All study children had CRP values lower than 5 mg/l at the age of 1 yr (median 0.06 mg/l, interquartile range 0.04–0.19). At the age of 1 yr, 27.6% ($N = 175$) of the study children were sensitized to at least one allergen. Between 1 and 4.5 yr 60 (12.4%) of the study children developed atopic dermatitis, 68 (13.1%) developed asthma or asthmatic bronchitis and at the age of 4.5 yr 215 (48.0%) were sensitized to at least one allergen.

Among non-sensitized 1-yr-old children (allergen-specific IgE below 0.35 kU/l at the age of 1 yr), those with a concentration of CRP in the highest quartile had a significantly lower risk of sensitization to any allergen at the age of 4.5 yr compared with those in the lowest quartile (Table 1). There was a significant inverse dose-dependent trend: The higher the CRP value at the age of 1 yr, the lower was the risk for allergic sensitization later (Table 1). No significant associations were observed between CRP levels at 1 yr of age and the risk of atopic dermatitis or asthma between ages 1 and 4 yr (Table 1). In sensitivity analyses, there was a significant inverse dose-dependent trend between CRP values and sensitization to inhalant allergens (Supplementary Table 1). Among children who had developed an allergen-specific IgE response by the age of 1 yr, the risk of atopic dermatitis later tended to be associated with low CRP values: The risk being significantly lower if the CRP value was from the 50–75th percentile compared with values lower than the 25th percentile (Table 1).

There were no significant associations between CRP values and the development of atopic dermatitis or asthma/asthmatic bronchitis between ages 1 and 4 yr in the non-stratified study population (Table 2). There tended to be an inverse dose-dependent trend: the higher the CRP value, the lower the risk of IgE sensitization at the age of 4.5 yr (Table 2).

Among children who had developed an allergen-specific IgE response by the age of 1 yr, drinking boiled or both unboiled and boiled farm milk, or having dogs or both dogs and cats at home, or having mother who had been visiting stables weekly during pregnancy or during the last 10 months implied significantly lower CRP values compared with children who did not have these exposures (Table 3). Similar associations were not detected between environmental factors and CRP levels among non-sensitized children at the age of 1 yr (Table 3).

If the child was sensitized at the age of 1 yr, the risk of allergic sensitization at the age of 4.5 yr was significantly higher (aOR 2.01 95% CI 1.39–2.90, p -value < 0.001) and the risk of atopic dermatitis and asthma/asthmatic bronchitis tended to be higher (aOR 1.57 95% CI 0.96–2.56, p -value = 0.072 and aOR 1.55

Table 2 Development of allergic diseases between ages 1 and 4 yr and CRP levels at the age of 1 yr

CRP 1 yr	Atopic dermatitis n (%)	aOR (95% CI)
≤ 25th percentile	15 (12.3)	1
25–50th	18 (13.7)	0.98 (0.43–2.20)
50–75th	11 (9.4)	0.56 (0.23–1.33)
≥ 75th	16 (11.9)	0.81 (0.36–1.83)
p -value	0.461	0.379

CRP 1 yr	Asthma/asthmatic bronchitis n (%)	aOR (95% CI)
≤ 25th percentile	20 (14.7)	1
25–50th	19 (15.2)	0.94 (0.45–1.96)
50–75th	11 (8.9)	0.63 (0.27–1.45)
≥ 75th	18 (13.4)	0.93 (0.45–1.95)
p -value	0.431	0.645

CRP 1 yr	Sensitization to at least one allergen at the age of 4.5 yr n (%)	aOR (95% CI)
≤ 25th percentile	50 (49.0)	1
25–50th	64 (54.2)	0.99 (0.56–1.74)
50–75th	52 (48.1)	0.87 (0.49–1.54)
≥ 75th	48 (40.3)	0.62 (0.36–1.09)
p -value	0.192	0.077

Results are presented as odds ratios (OR) and their 95% confidence intervals (CI). Odds ratios are adjusted for farmer, country, gender, siblings, day care with other children than siblings, and mother's history of allergic disease.

95% CI 0.97–2.48, p -value = 0.069) compared with non-sensitized children.

Discussion

We observed a decreased risk of the development of allergic sensitization by the age of 4.5 yr when CRP levels were increased at the age of 1 yr among children who were non-sensitized at the age of 1 yr. This kind of association was not seen, however, between CRP values and risk of atopic dermatitis or asthma between ages 1 and 4 yr. Our results show the association to be with an allergic-related immune response, that is IgE sensitization, supporting the earlier findings of the protective effect of low-grade inflammation in the control of IgE-mediated allergic diseases (5). In the studies of CRP as a marker of low-grade inflammation, the use of probiotics induced an increase in the values of CRP (5, 6), which suggests that environmental microbial load might be an important factor in the induction of inflammation-related protection from an allergic immune response.

Because we have earlier found a protective effect of certain farming determinants on the development of allergic diseases in childhood (7–12), we were interested in environmental animal and farming factors which could induce low-grade inflamma-

Table 3 Associations between different environmental and farming factors and CRP at the age of 1 yr in children who have no signs of IgE sensitization and in children who have developed allergen-specific IgE response at the age of 1 yr

	Non-sensitized			Sensitized		
	n	CRP*	aOR [†]	n	CRP*	aOR [†]
<i>Mode of living at study entry</i>						
Non-farm	234	0.06 (0.04/0.13)	1	80	0.07 (0.04/0.20)	1
Farm	225	0.07 (0.04/0.20)	1.15 (0.72–1.84)	95	0.06 (0.04/0.22)	1.02 (0.49–2.12)
p-value		0.133	0.557		0.942	0.948
<i>Duration of breastfeeding (months)</i>						
No breastfeeding	23	0.08 (0.04/0.26)	1	19	0.08 (0.04/0.64)	1
0.5–2	40	0.05 (0.03/0.15)	0.82 (0.25–2.74)	11	0.06 (0.03/0.14)	0.56 (0.08–4.06)
2–6	114	0.06 (0.05/0.17)	0.90 (0.32–2.58)	45	0.06 (0.04/0.12)	0.66 (0.16–2.70)
6 or more	276	0.06 (0.04/0.15)	1.02 (0.38–2.77)	98	0.07 (0.05/0.28)	1.19 (0.31–4.51)
p-value		0.212	0.942		0.247	0.594
<i>Child's staying in stable weekly in last 10 months</i>						
No	246	0.06 (0.04/0.13)	1	91	0.07 (0.05/0.29)	1
Yes	213	0.07 (0.04/0.19)	0.87 (0.46–1.66)	84	0.06 (0.04/0.19)	0.36 (0.12–1.05)
p-value		0.586	0.673		0.355	0.060
<i>Child's regular contact with hay weekly</i>						
No	395	0.06 (0.04/0.15)	1	149	0.07 (0.04/0.24)	1
At least once	55	0.07 (0.04/0.22)	0.76 (0.37–1.56)	24	0.05 (0.03/0.11)	0.56 (0.17–1.81)
p-value		0.715	0.453		0.126	0.335
<i>Child's farm milk consumption</i>						
No	311	0.06 (0.04/0.14)	1	114	0.07 (0.04/0.30)	1
Only boiled	67	0.06 (0.04/0.26)	1.14 (0.58–2.22)	29	0.06 (0.04/0.08)	0.16 (0.04–0.60)
Both unboiled and boiled	34	0.06 (0.03/0.37)	1.16 (0.48–2.81)	18	0.06 (0.04/0.14)	0.18 (0.04–0.85)
Only unboiled	41	0.07 (0.05/0.20)	1.29 (0.57–2.91)	12	0.05 (0.03/0.18)	0.41 (0.09–1.97)
p-value		0.928	0.581		0.283	0.026
<i>Maternal smoking (during last 10 months)</i>						
No	395	0.06 (0.04/0.15)	1	151	0.06 (0.04/0.20)	1
Yes	58	0.06 (0.04/0.20)	1.31 (0.68–2.55)	22	0.08 (0.05/0.37)	2.21 (0.76–6.47)
p-value		0.715	0.420		0.387	0.147
<i>Mother's staying in stable weekly during pregnancy</i>						
No	180	0.06 (0.04/0.13)	1	60	0.07 (0.05/0.40)	1
Yes	279	0.07 (0.04/0.20)	1.27 (0.69–2.33)	115	0.06 (0.04/0.29)	0.24 (0.07–0.79)
p-value		0.183	0.444		0.129	0.019
<i>Mother's staying in stable weekly in last 10 months</i>						
No	257	0.06 (0.04/0.14)	1	86	0.07 (0.04/0.28)	1
Yes	202	0.07 (0.04/0.19)	0.88 (0.32–2.46)	89	0.06 (0.04/0.20)	0.13 (0.02–0.96)
p-value		0.322	0.808		0.675	0.045
<i>Pets at home at the age of 1 yr</i>						
Neither cats nor dogs	197	0.06 (0.04/0.13)	1	64	0.07 (0.04/0.40)	1
Only cat/cats	123	0.08 (0.04/0.23)	1.40 (0.76–2.58)	44	0.07 (0.04/0.30)	0.68 (0.24–1.93)
Only dog/dogs	43	0.06 (0.05/0.10)	1.11 (0.44–2.76)	21	0.06 (0.05/0.10)	0.15 (0.04–0.62)
Both cat(s) and dog(s)	89	0.07 (0.04/0.19)	1.33 (0.65–2.74)	44	0.06 (0.03/0.12)	0.22 (0.07–0.72)
p-value		0.220	0.548		0.336	0.003

*CRP values are presented as median values with interquartile range. p-value estimated by Mann–Whitney or Kruskal–Wallis test.

[†]CRP values are presented in two groups according to 75th cutoff points (≥ 0.19 mg/l and < 0.19 mg/l) and comparison between two groups is shown values as odds ratios (OR) with their 95% confidence intervals (CI). p-values are obtained from the trend test (Wald) in logistic regression models. Odds ratios are adjusted for country, gender, farmer, siblings, and day care with other children than siblings.

tion. When analyzing the significance of environmental factors related to the microbial load, we did not find associations between CRP values and farming environment, exposure to farm milk or pets at home in the children who were not sensitized at the age of 1 yr. Thus, farming environment did not show significant effects in inducing systemic low-grade inflammation in this study.

It is possible that the level of CRP reflects not only the microbial load but also the individual ability to response to diverse inflammatory stimuli. Thus, the CRP level as an indicator of low-grade inflammation could be at least partly explained by diverse responders (good vs. poor), and not necessarily only by the presence or lack of environmental microbial exposure. It has been shown that impaired epithelial

cell response to environmental antigens is associated with asthma (18). According to these studies, the poor response of the innate immune system, which is also reflected as impaired induction of CRP, could be a determinant for the later development of an allergen-specific immune response. Our findings are in line with these observations and suggest that induction of inflammatory response in the host is of importance in the control of IgE response. This does not exclude the possibility that the individuals with poor inflammatory response get benefit from treatment that stimulates inflammation, such as shown for some probiotic preparations (5), especially in an environment with low inflammatory pressure. The individuals with impaired capability for inflammatory response may be protected from allergies in an environment with high microbial load, but may be prone to allergic immune responses in high hygiene environment.

Interestingly, in children who had already developed IgE sensitization at the age of 1 yr, we observed that decreased CRP levels at the age of 1 yr were associated with farm milk consumption, exposure to dogs or both cats and dogs at home and mother's visiting stables during pregnancy and recently. All these environmental factors are considered to be associated with increased microbial load. Thus, our findings further suggest that down-regulation of inflammation as a response to microbial load could be characteristic of children who develop early sensitization. The clinical importance of early IgE response to environmental allergens has been discussed, and it does not necessarily imply allergic disease, but may be considered as a physiological response to environmental antigens in infants (19). In our study, sensitization at 1 yr of age was, however, associated with sensitization at 4.5 yr and tended to be linked to atopic dermatitis and asthma or asthmatic bronchitis between the ages of one and four. Thus, it seems that sensitization at 1 yr of age was a marker for allergic

predisposition in our study population. The down-regulation of inflammatory response to environmental triggers of inflammation may be a fundamental characteristic of the hosts' immune response, which may predispose to the development of allergy. In the group of children with early allergic response at the age of 1 yr, it could be a factor related to genetic or epigenetic variation. The production of acute phase proteins in hepatocytes is controlled by a variety of different cytokines released during the inflammatory process with IL-1- and IL-6-type cytokines as the leading regulators and mediating their effects via STAT3 and NF- κ B signaling (20).

We conclude that the poor response to known inflammatory determinants of the host at early age appeared to be of importance in the control of later IgE sensitization. These results emphasize the role of inflammation and innate immunity in the regulation of an allergic immune response and thus predisposition to IgE-mediated allergy. The low number of children developing atopic dermatitis and asthma or asthmatic bronchitis between ages one and four is the main limitation to make conclusions of the relationship between CRP values and allergic diseases.

Acknowledgment

We would like to thank the families, all the fieldworkers, data management, and laboratory technicians for enthusiastic collaboration. This study was supported by the EU through the FP6-PASTURE contract (QLRT-2001-00250); follow-up of the children between ages 18 month and 5 yr has been ensured through the FP6-FORALLVENT contract (SSA. Contract no. 3170), by the National Institute for Health and Welfare Finland, the foundation of the Finnish Anti-Tuberculosis Association and the foundation of Väinö and Laina Kivi.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Development of IgE sensitization to inhaled and food allergens during childhood and CRP levels at the age of

1 yr in children who have no signs of IgE sensitization and in children who have developed allergen-specific IgE response at the age of 1 yr.

SUPPLEMENTARY TABLE 1. Development of IgE sensitization to inhaled and food allergens during childhood and CRP levels at the age of one year in children who have no signs of IgE sensitization and in children who have developed allergen specific IgE response at the age of one year.

CRP 1yr	Non-sensitized at 1y		Sensitized at 1y	
	Sensitization to at least one inhalant allergen at the age of 4.5 years n(%)	aOR (95% CI)	Sensitization to at least one inhalant allergen at the age of 4.5 years n(%)	aOR (95% CI)
≤25th percentile	26 (35.1)	1	13 (46.4)	1
25-50th	40 (44.4)	1.17 (0.59-2.31)	8 (28.6)	0.30 (0.08-1.08)
50-75	21 (26.3)	0.60 (0.29-1.24)	14 (50.0)	1.07 (0.32-3.58)
≥75th	18 (22.5)	0.50 (0.24-1.05)	19 (48.7)	1.15 (0.38-3.48)
p-value	0.011	0.017	0.316	0.284

CRP 1yr	Sensitization to at least one food allergen at the age of 4.5 years n(%)		Sensitization to at least one food allergen at the age of 4.5 years n(%)	
≤25th percentile	21 (28.4)	1	11 (39.3)	1
25-50th	25 (27.8)	0.82 (0.39-1.71)	11 (39.3)	0.82 (0.24-2.83)
50-75	18 (22.5)	0.63 (0.29-1.36)	16 (57.1)	1.49 (0.44-5.07)
≥75th	19 (23.8)	0.65 (0.31-1.38)	12 (30.8)	0.69 (0.22-2.20)
p-value	0.784	0.205	0.189	0.750

Results are presented as odds ratios (OR) and their 95% confidence intervals (CI). Odds ratios are adjusted for farmer, country, gender, siblings, day care with other children than siblings and mother's history of allergic disease.

