

Increased serum-soluble interleukin-5 receptor alpha level precedes the development of eczema in children

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Interleukin-5 receptor α -subunit expression may be implicated in the development of allergic diseases. In a population-based birth cohort, we investigated the relationship between IL-5R α and the development of allergic phenotypes in childhood, using soluble IL-5R α (s-IL-5R α) as a marker. Children ($n = 510$) were followed from birth and assessed at age 3, 5 and 8. Based on the onset and resolution of symptoms, we assigned children into the following wheeze and eczema phenotypes: never, transient, persistent, intermittent and late-onset. Specific IgE to common allergens, s-IL-5R α (ELISA) and urinary eosinophilic protein X (U-EPX) levels was measured at age 5. s-IL-5R α was significantly higher among atopic compared to non-atopic children (pg/ml, geometric means [95% CI], 152.4 [126.0–184.5] vs. 103.4 [94.0–113.9], $p < 0.0001$). While we found no association between s-IL-5R α and current eczema at age 5, there was a significant association between eczema phenotypes and s-IL-5R α (multiple ANOVA model adjusted for gender and atopy, $F = 2.56$, $p = 0.04$). After adjustment for multiple comparisons, we found that children with late-onset eczema had significantly higher s-IL-5R α compared to those who have never had eczema (mean difference [95% CI], 2.41 [1.03–5.62], $p = 0.04$) and those with intermittent eczema (2.63 [1.08–6.41], $p = 0.02$), with no difference between children who have never had eczema and other eczema phenotypes. We found no such association for wheeze phenotypes. There was a weak correlation between s-IL-5R α and U-EPX ($r = 0.16$, $p < 0.0001$). Increased serum s-IL-5R α level at age 5 was associated with contemporaneous atopic sensitization and with subsequent development of eczema by age 8.

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Eosinophils are involved in the pathogenesis of allergic disease, and increasing blood and tissue eosinophilia is related to the presence and severity of symptoms (1, 2). Eosinophil precursors in the bone marrow are differentiated and activated under the influence of several cytokines, mainly IL-3, IL-5 and GM-CSF. Eosinophil proliferation, homing and local survival in peripheral tissues depend on IL-5 stimulation. IL-5 binds to a high-affinity IL-5 receptor (IL-5R) found on the eosinophil membrane which is composed of two subunits: α -chain (IL-5R α), a primary IL-5 binding site, and cytoplasmic

β -chain (IL-5R β) which is responsible for cytoplasmic signal transduction. While the β -chain is common for IL-5, IL-3 and GM-CSF receptors, the IL-5R α chain is an exclusively IL-5 binding site (3). In humans, IL-5R α is predominantly expressed in eosinophils/basophils and their precursors.

Different IL-5R α transcripts encoding transmembrane and soluble isoforms have been identified in human and murine models (4, 5). The synthesis of these IL-5R α isoforms is determined by alternative splicing where genetic transcript either skips exon 11 and includes exon 12, 13,

and 14 (membrane-bound IL-5R α) or terminates at exon 11 (soluble-IL-5R α) (6). Expression of membrane-bound (m) or soluble (s) IL-5R α may have differing biological effects. When m-IL-5R α binds to its ligand, it forms a high-affinity complex with the IL-5R β chain, resulting in cytoplasmic signal transduction and a consequent activation of several kinase pathways that induce eosinophil proliferation, activation and survival, both in peripheral blood and bone marrow (7, 8).

The role of s-IL-5R α is less well elucidated, with different studies reporting conflicting findings. Several studies conducted *in vitro* (3) and *ex vivo* (9) suggested that s-IL-5R α binds IL-5 and prevents its effect on target cells (i.e. acts as a part of the regulatory mechanisms). Mature eosinophils express s-IL-5R α ubiquitously, suggesting that mature eosinophils may have control over their response to IL-5 stimuli (3). Generally, s-IL-5R α exceeds IL-5 levels by tenfold, but in a severe eosinophilic inflammation, s-IL-5R α levels are hundredfold higher than IL-5. We have recently demonstrated that *in vitro* IL-5 stimulation of mature eosinophils significantly upregulates s-IL-5R α protein but not its transcript level, indicating that the primary source of s-IL-5R α is cleavage of m-IL-5R α (10). In contrast, others have suggested that s-IL-5R α protects its ligand from proteolytic inactivation by binding to circulating IL-5, thus prolonging its serum half-life (11).

Increased s-IL-5R α has been associated with atopic disease in adults (12, 13). However, at present, there is no information about the role of s-IL-5R α in the development of allergic disease in childhood. Within the context of a population-based birth cohort study, we investigated the association of IL-5R α and the development of allergic disease in childhood, using s-IL-5R α as a marker. In addition, to assess whether s-IL-5R α is a reflection of eosinophilic activation, we measured urinary eosinophilic protein X (U-EPX), an established marker of eosinophil-associated disease (14, 15).

Methods

Study population

The Manchester Asthma and Allergy Study is an unselected population-based birth cohort previously described elsewhere (16, 17). Subjects were recruited prenatally and followed prospectively, attending review clinic at age 3, 5 and 8 (± 4 wk from birthday). The study is registered as IS-RCTN72673620 and approved by the local

research ethics committee. Parents gave written informed consent, and children gave their assent if appropriate.

Definitions of outcomes

A validated questionnaire (18) was interviewer administered at each review clinic to collect information on parentally reported symptoms, physician-diagnosed illnesses and treatments received.

Rhinitis. We defined rhinitis as a runny or blocked nose in the absence of a cold or chest infection. *Current rhinitis* at age 5 and 8 years was defined as a presence of symptoms during the past 12 months and *rhinitis ever* as a parentally reported rhinitis during lifetime.

Wheeze. According to parentally reported history of wheeze, we defined *current wheeze* at age 5 and 8 as a positive response to the question 'Has your child had wheezing or whistling in the chest in the last 12 months?' and *wheeze ever* as a parentally reported wheeze during lifetime. Based on prospectively collected data, children were assigned to the following wheeze phenotypes: *No wheezing* – no wheeze ever during the first 8 yr of life; *Transient early wheezing* – wheezing during the first 5 yr, no wheezing after the age of 5; *Late-onset wheezing* – wheeze onset after the child's fifth birthday; *Persistent wheezing* – wheezing at all time points (3, 5 and 8 yr); *Intermittent wheezing* – wheezing at one time point during the first 5 yr, wheezing at age 8.

Eczema. We defined *current eczema* as a positive answer to two questions: 'Has your child had an itchy rash coming and going for the period of at least 6 months?' and 'Has your child had this itchy rash in the last 12 months?' *Eczema ever* was defined as a parentally reported history of doctor-diagnosed eczema. Based on prospectively collected data on current eczema, children were assigned as: *No eczema* – no eczema during the first 8 yr of life; *Transient eczema* – Eczema during the first 5 yr of life, no eczema after age 5; *Persistent eczema* – eczema at all time points (3, 5 and 8 yr); *Intermittent eczema* – eczema at one time point during the first 5 yr, eczema at age 8; *Late-onset eczema* – no eczema during the first 5 yr, eczema at age 8.

Atopic sensitization. Ascertained by the measurement of specific IgE to mite, cat, dog grasses, milk, egg and peanut (ImmunoCAP; Phadia AB, Uppsala, Sweden) at age 5. Sensitization was

defined as allergen-specific IgE > 0.35 kU_A/l to at least one allergen.

Soluble IL-5 Receptor α and U-EPX measurement

Soluble IL-5R α was measured using a sandwich ELISA, with a carefully selected combination of monoclonal antibodies (mAbs) against s-IL-5R α (Innogenetics, Ghent, Belgium). Detailed methodology on mAbs selection and ELISA is described elsewhere (11). Serum s-IL-5R α levels were expressed in picograms/millilitre (pg/ml).

Urinary EPX was measured in urine sample collected at age 5 years using immunofluorometric assay method (Phadia), with a lower detection limit < 0.5 μ g/l. Urinary creatinine level was also measured (Jaffe reaction HiCo Creatinine; Boehringer Mannheim GmbH, Mannheim, Germany), and results were expressed as the U-EPX/Creatinine ratio in micrograms/millimole (μ g/mmol).

Statistical methods

Statistical analysis was performed by using SPSS for Windows 15.0 (SPSS Inc, Chicago, IL, USA). Soluble IL-5R α and U-EPX/Creatinine ratio data were normalized by log_e transformation, and results are presented as geometric means (GM) with 95% confidence intervals (CI). The comparison between the groups was conducted using independent *t* test. Significant differences were further tested using multiple ANOVA model adjusted for gender and atopy, with Bonferroni correction for multiple comparisons. Correlation between s-IL-5R α and U-EPX/Creatinine ratio was tested using Pearson's correlation test.

Results

Participant flow

Of 1211 couples recruited during pregnancy, 1085 had a successful full-term pregnancy; 128 children were prenatally randomized to take part in the environmental intervention study and were excluded from this analysis (Fig. 1) (19, 20). Of 957 children who were followed in the observational cohort, 840 (87.8%) attended the 5-yr follow-up (a further 84 returned postal questionnaires). Of 521 (62%) children who provided blood sample, 510 children had sufficient sample for s-IL-5R α measurement. There was no difference in personal atopy and parental history of allergic disease between children who gave blood and those who did not (data available on request).

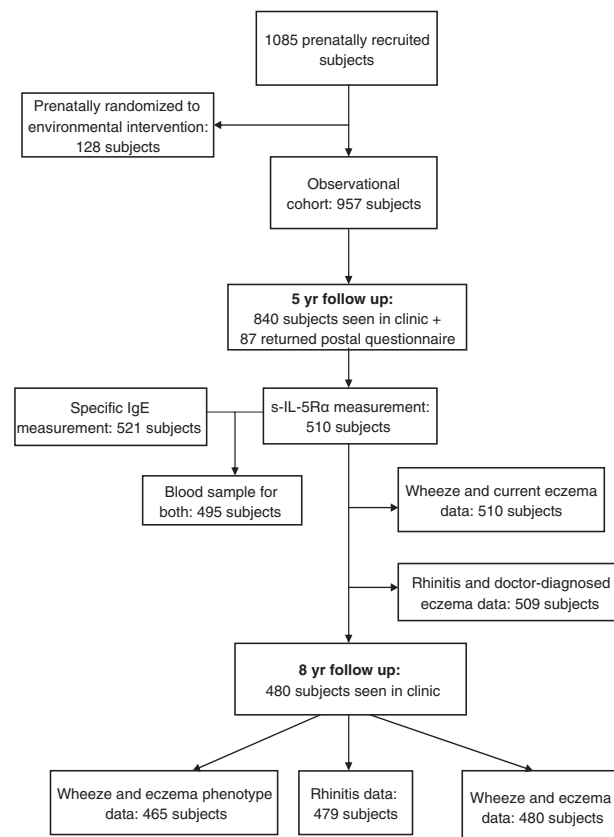


Fig. 1. Study consort.

Soluble IL-5R α , sensitization and allergic disease

Atopic sensitization. Among 495 children who had data on both specific IgE levels and s-IL-5R α at age 5, s-IL-5R α was significantly higher among atopic compared to non-atopic children (pg/ml, GM [95% CI], 152.4 [126.0–184.5] vs. 103.4 [94.0–113.9], $p < 0.0001$).

Rhinitis. s-IL-5R α was significantly higher among children with rhinitis at age 5 and 8 years (both ever and current) compared to those without rhinitis (Tables 1 and 2). However, in a multiple ANOVA model adjusted for gender and atopy, the association between current rhinitis at age 5 and s-IL-5R α failed to reach statistical significance ($F = 3.42$, $p = 0.07$).

Wheeze. There was no relationship between current wheeze or wheeze ever either at age 5 or 8 years and s-IL-5R α . In a multiple ANOVA model adjusted for gender and atopy, there was no significant difference in s-IL-5R α levels between different wheeze phenotype groups ($F = 0.48$, $p = 0.8$, Table 3).

Eczema. We found no association between s-IL-5R α levels and current eczema at age 5 or eczema

Table 1. s-IL-5R α levels and clinical outcomes at age 5 years

Clinical outcome at age 5	Number	s-IL-5R α GM pg/ml	95% CI of Mean	p Value
Rhinitis ever, n = 509				
Yes	154	139.1	114.7–168.6	0.03
No	355	108.7	98.8–119.7	
Rhinitis current, n = 509				
Yes	144	141.8	115.8–173.6	0.02
No	365	108.6	98.8–119.4	
Doctor-diagnosed eczema ever, n = 509				
Yes	198	123.2	107.2–141.5	0.4
No	311	113.4	101.0–127.4	
Eczema current, n = 510				
Yes	156	121.7	102.9–143.8	0.6
No	354	115.0	103.5–127.7	
Wheeze ever, n = 510				
Yes	201	110.7	97.0–126.2	0.3
No	309	121.2	107.6–136.6	
Wheeze current, n = 510				
Yes	115	110.0	93.6–129.3	0.4
No	395	119.1	107.2–132.2	

p-values significant if < 0.05.

Table 2. s-IL-5R α levels at age 5 and clinical outcomes at age 8 years

Clinical outcome at age 8	Number	s-IL-5R α GM pg/ml	95% CI of Mean	p Value
Rhinitis ever, n = 479				
Yes	131	145.6	118.1–179.6	0.02
No	348	109.7	99.0–121.4	
Rhinitis current, n = 479				
Yes	127	146.3	118.0–181.5	0.02
No	352	109.8	99.3–121.5	
Doctor-diagnosed eczema ever, n = 480				
Yes	215	136.7	116.4–160.6	0.008
No	265	105.2	94.6–117.1	
Eczema current, n = 480				
Yes	117	134.8	107.1–169.8	0.1
No	363	113.4	102.7–125.2	
Wheeze ever, n = 480				
Yes	197	120.0	105.2–137.0	0.8
No	283	117.1	102.8–133.4	
Wheeze current, n = 480				
Yes	89	131.9	108.5–160.5	0.3
No	391	115.4	103.8–128.3	

p-values significant if < 0.05.

ever in the first 5 yr of life (Table 1). However, children with doctor-diagnosed eczema ever at age 8 years had significantly higher s-IL-5R α levels at age 5 compared to those without eczema (pg/ml, GM [95% CI], 136.7 [116.4–160.6] vs. 105.2 [94.6–117.1], $p = 0.008$, Table 2).

We then explored the relationship between s-IL-5R α and different phenotypes of eczema during the first 8 yr of life. In the multiple ANOVA model adjusted for gender and atopy, we found a significant association between eczema phenotypes and s-IL-5R α ($F = 2.56$, $p = 0.04$, Table 3), with children with late-onset eczema having significantly higher s-IL-5R α levels

Table 3. s-IL-5R α at age 5 and phenotypes of wheeze and eczema derived at age 8 years. Multivariate model adjusted for gender and atopy

	n	s-IL-5R α (pg/ml) Estimated marginal mean 95% CI	F	p Value
Eczema	465		2.56	0.04
<i>never</i>	203	119.2 100.7–141.3		
<i>transient</i>	91	133.1 107.1–165.7		
<i>intermittent</i>	71	109.2 84.6–140.9		
<i>persistent</i>	81	133.0 106.3–166.2		
<i>late-onset</i>	19	287.1 162.9–505.7		
Wheeze	465		0.48	0.8
<i>never</i>	210	138.8 117.2–164.4		
<i>transient</i>	109	120.8 95.9–152.2		
<i>intermittent</i>	61	138.2 105.2–181.8		
<i>persistent</i>	57	121.9 81.7–181.8		
<i>late-onset</i>	28	115.1 86.4–181.8		

p-values significant if < 0.05.

compared to other groups (Table 3, Fig. 2). After adjustment for multiple comparisons (Bonferroni), we found that children with late-onset eczema had significantly higher s-IL-5R α levels compared to those who have never had eczema (mean difference [95% CI], 2.41 [1.03–5.62], $p = 0.04$) and those with intermittent eczema (2.63 [1.08–6.41], $p = 0.02$), with no difference between children who have never had eczema and other eczema phenotypes. Although there was no formal statistically significant interaction between atopy and eczema phenotypes, the data suggested that the association between s-IL-5R α at age 5 and the development of eczema after the age 5 years was strongest among children who

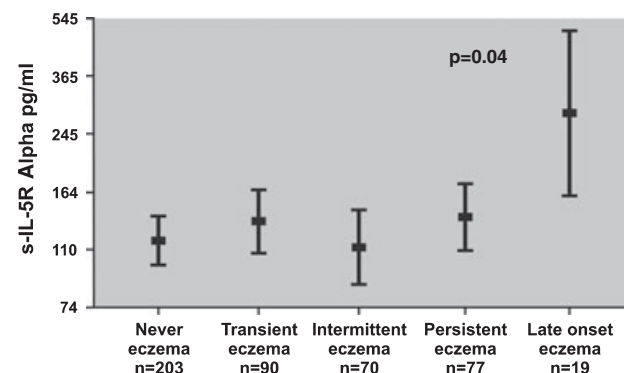


Fig. 2. Eczema phenotypes at age 8 and s-IL-5R α (pg/ml) levels at age 5 expressed as geometric means (GM, 95% CI).

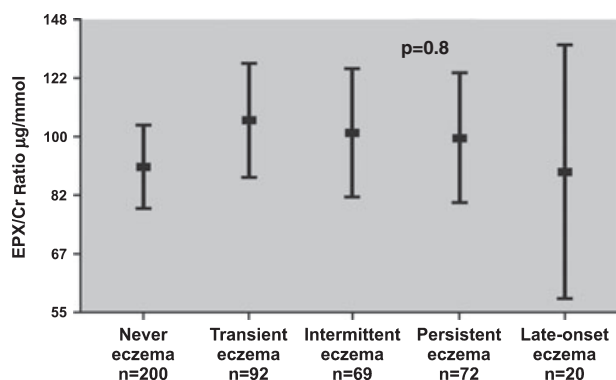


Fig. 3. Eczema phenotypes at age 8 and U- eosinophilic protein X (U-EPX/Creatinine ratio, micrograms/mmol) at age 5 expressed as geometric means (GM, 95% CI).

were atopic (atopic vs. non-atopic children who developed late-onset eczema: pg/ml, GM [95% CI], 665.8 [242.5–1828.0] vs. 123.8 [73.5–208.5]). It is of note that of 19 children who developed late-onset eczema, four were atopic and 15 were non-atopic at age 5. Of four atopic children, three were sensitized to inhalant allergens only (mite and grass sensitization in single child; mite, grass and milk sensitization- one child each).

To investigate whether high s-IL-5R α level was simply a marker of eosinophil activation, we investigated the association between s-IL-5R α level and U-EPX ($n = 480$). We observed only a weak correlation between s-IL-5R α and U-EPX levels ($r = 0.16$, $p < 0.0001$) which did not change after excluding outliers ($r = 0.13$, $p = 0.004$). We then analysed the relationship between U-EPX and eczema phenotypes in a model adjusted for gender and atopy and found no significant association between U-EPX levels at age 5 and different eczema phenotypes ($F = 0.21$, $p = 0.9$, Fig. 3). In this model, atopy was the only significant associate of U-EPX ($F = 11.7$, $p = 0.001$). In a further analysis, we found that U-EPX was significantly associated with current rhinitis at age 5 years, but not with current wheeze or eczema (data not shown, available on request).

Discussion

Principal findings

To our knowledge, this is the first study to report that the level of serum s-IL-5R α is markedly higher among atopic children at age 5 years compared to those who are not atopic. In addition, we found that s-IL-5R α levels measured at age 5 were significantly higher in children who went on to develop eczema after age 5 years (i.e. increased serum s-IL-5R α level

preceded the development of eczema). This relationship remained strong after adjusting for child's gender and atopic status. Although not statistically significant, the association between increased s-IL-5R α and the subsequent development of eczema appeared strongest among atopic children.

Strengths and limitations

The major strength of our study is its setting – the population-based birth cohort with high follow-up rate (> 90% by age 8). The prospective nature of the study enabled us to accurately identify those children who developed eczema after the age we measured s-IL-5R α levels.

One of the weaknesses is that we only analysed the data in approximately two thirds of the subjects, as the remainder refused venepuncture at age 5 years. However, we wish to emphasize that there was no difference in personal atopy and parental history of allergic disease between children who gave blood and those who did not, making it unlikely that any bias was introduced because of the refusal of venepuncture.

It is important to note that our findings are derived from a relatively small group of subjects ($n = 19$) who developed eczema for the first time after the age of 5, and the results need to be interpreted with caution. However, this is inevitable consequence of the fact that most cases of childhood eczema start during infancy, and the phenotype of the late-onset eczema is comparatively rare. It is also worth noting that the observed association remained strong after adjustment for possible confounders.

We do not have the data on serum eosinophil counts at age 5 years (when we measured serum s-IL-5R α levels); thus, we could not answer the question whether the increased s-IL-5R α levels were markers of eosinophilia. Instead, we used available data on U-EPX levels. We would argue that the weak association between s-IL-5R α and U-EPX suggested that it is unlikely that high s-IL-5R α level is a simple marker of activated mature eosinophils.

Finally, we do not have the data to explain the functional mechanism behind the association between the increased s-IL-5R α and subsequent development of eczema in childhood.

Interpretation

There is limited data on the relationship between IL-5R α and allergic diseases in adults. Subjects with nasal polyposis have significantly higher tissue s-IL-5R α levels compared to healthy

controls, and s-IL-5R α correlates with tissue concentration of IL-5, eosinophil cationic protein (ECP) and eosinophil counts (11). Patients with nasal polyposis who have concomitant airway disease have significantly higher serum s-IL-5R α compared to subjects with nasal polyposis only. This finding suggests that s-IL-5R α may be a marker of the extensiveness of the eosinophil-associated disease (localized vs. systemic) (11). We have recently reported differential expression of s-IL-5R α and m-IL-5R α at protein and mRNA level between subjects with nasal polyposis with and without asthma compared to controls (10); while both subjects with and without asthma had equally high m-IL-5R α and low CD69 levels, those who had concomitant asthma had significantly higher serum s-IL-5R α .

Additional data suggesting a potential relationship between sIL-5R α and eczema have come from genetic association studies. A recent case-control study of over 600 adults demonstrated a link between the single-nucleotide polymorphism (SNP) in IL-5R α (rs334809) and eczema, with a similar effect among atopic and non-atopic subjects. Another SNP in IL-5R α (rs6771148) has been linked to blood eosinophilia, high-ECP levels and eczema in adults, pointing to the direct implication of IL-5R α expression in the pathophysiology of allergic skin disease (21).

The factors which regulate differential splicing of IL-5R α gene to form two distinctive isoforms are not known (6). More importantly, the biological role of these two isoforms is not well elucidated. The m-IL-5R α , a membrane-bound isoform is responsible for IL-5-induced cytoplasmic signal transduction, whereas circulating s-IL-5R α isoform has been shown to have IL-5 neutralizing effects (3, 22). The study of human eosinophil development using human umbilical cord-derived CD34⁺ eosinophil progenitor cells has demonstrated a changing pattern of IL-5R α isoform expression upon continuous IL-5 stimulation; in the first week of IL-5 stimulus, cell cultures from predominantly m-IL-5R α mRNA expression switch to s-IL-5R α mRNA expression during the second week, suggesting that factors controlling IL-5R α gene splicing are dependent on the duration and stage of signalling (6). While m-IL-5R α is expressed on the cell membrane of progenitor cells and promotes the IL-5-stimulated eosinophilic differentiation and activation cascade, the s-IL-5R α is a major form present in mature eosinophils, and its secretion is considered as a possible self feedback mechanism to control excessive eosinophil activation (3, 23). Indeed, exposure of activated and mature serum eosinophils to recombinant IL-5 induces cleavage

of m-IL-5R α and release of s-IL-5R α probably by activation of membrane-associated metalloproteinases (10).

The expression of the IL-5R α isoforms differs according to the eosinophil activation state, maturation and localization in the body and may therefore be involved in the fine-tuning of the eosinophil homeostasis. Liu and co-workers demonstrated a different control mechanism for expression of the m-IL-5R α on the airway or circulating eosinophils. After allergen challenge, m-IL-5R α and β chain were markedly reduced on the airway eosinophils compared to the circulating cells. In addition, s-IL-5R α concentration was significantly elevated in bronchoalveolar lavage (BAL), but compared with blood eosinophils, the steady-state s-IL-5R α mRNA level was not increased in BAL; interestingly, airway eosinophils exhibited a lack of responsiveness to IL-5 (12). These findings suggest that when eosinophils are recruited to the airway, regulation of their function may become IL-5 independent.

In our study, we did not explore mechanism behind the link between high s-IL-5R α and subsequent development of eczema. Therefore, we are not able to comment on possible function behind the increase in s-IL-5R α secretion in children who are going to develop eczema. From nasal polyposis with asthma, we learned that s-IL-5R α exceeds IL-5 levels by a hundredfold; the increased s-IL-5R α level at the age of 5 may reflect imminent eosinophilia, with still undetectable other markers of eosinophilic inflammation. It is possible that profuse s-IL-5R α secretion may have a protective function against aberrant eosinophil differentiation and activation and hence may have anti-inflammatory properties even months before actual onset of the disease.

The association between U-EPX levels at age 5 years and concurrent atopy and rhinitis, but not wheeze, indirectly suggests that U-EPX is a marker of eosinophilia. Although a single case-control study of children with eczema found that the increase in U-EPX levels significantly correlated with atopy and disease severity (15), there is no data on the association with the natural history of eczema.

Conclusion

Increased serum s-IL-5R α level at age 5 years was associated with contemporaneous atopic sensitization and with a subsequent development of eczema by age 8; although there was no significant interaction between atopy at age

5 years and eczema development, s-IL-5R α levels tended to be the highest among atopic children who went on to develop eczema after that age. The lack of association between serum s-IL-5R α and U-EPX suggest that high s-IL-5R α level is not a simple marker of eosinophil activation. Further work needs to be carried out to explain the functional mechanism behind the observed association. If s-IL-5R α is to be used as an early marker of eczema development, these findings will need to be replicated in other populations.

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