

ORIGINAL PAPER

Retinol concentrations after birth are inversely associated with atopic manifestations in children and young adults

M. Pesonen*, M. J. T. Kallio†, M. A. Siimes†, A. Ranki*

*Department of Dermatology, the Skin and Allergy Hospital and †the Hospital for Children and Adolescents, Helsinki University Hospital, University of Helsinki, Helsinki, Finland

Clinical and Experimental Allergy

Summary

Background Vitamin A has anti-inflammatory and immunomodulatory effects, and its deficiency results in impaired specific and innate immunity. Vitamin A is essential for inducing the gut-homing specificity on T cells.

Objective As an impaired gut immune response in early infancy may contribute to the development of atopic sensitization, we looked for an association of plasma retinol concentrations and the subsequent development of allergic symptoms in healthy infants.

Methods A cohort of 200 unselected, full-term newborns were followed up from birth to age 20 years. The plasma retinol concentration was determined in cord blood ($n = 97$), at ages of 2, 4 and 12 months ($n = 95$), and at ages 5 years ($n = 155$) and 11 years ($n = 151$). The subjects were re-examined at the ages of 5, 11 and 20 years with assessment of the occurrence of allergic symptoms during the preceding year, skin prick testing and measurement of serum total IgE.

Results Subjects with allergic symptoms or a positive skin prick test (SPT) in childhood or adolescence had lower retinol concentrations in infancy and childhood than symptom-free subjects. The difference was most pronounced at age 2 months. Retinol concentration at 2 months correlated inversely with positive SPT at ages of 5 and 20 years, and with allergic symptoms at age 20 years.

Conclusion Retinol concentration in young infants is inversely associated with the subsequent development of allergic symptoms. We propose that an inborn regulation of retinol may play a role in atopic sensitization, possibly through regulating the intestinal T cell responses.

Keywords atopy, retinol, vitamin A

Submitted 16 May 2006; revised 29 September 2006; accepted 25 October 2006

Correspondence:

Maria Pesonen, Skin and Allergy Hospital, PO Box-160, FIN-00029 HUS, Helsinki, Finland.
E-mail: maria.pesonen@luukku.com

Introduction

Atopic diseases are showing a continuous increase in industrialized countries. One of the reasons for this increase may be a reduced exposure to environmental microbes, important for the maturation of the newborn's immune system towards T-helper type 1 (Th1) responses [1, 2]. However, the underlying cellular and molecular mechanisms have remained elusive. A genetic background provides susceptibility for atopy [3], but environmental factors are important as well.

In newborn mammals, the proper maturation of the gastrointestinal tract and the immune system is dependent on the presence of the commensal bacteria in the gut [4, 5]. The gut immune response, induced by microbial

antigens, is assumed to play an important role in maturing the peripheral immune response [6, 7]. The vast majority of the body's T cells reside in the gut, while the blood carries only 2–5% of the T cells.

To become activated, a naïve T cell must be introduced to a foreign antigen by an antigen-presenting cell, such as the dendritic cell (DC, [8]). Thereafter, the activated T cell migrates and homes to some specific compartment of the body, such as the skin or the intestine, guided by integrins and chemokine receptors expressed on the cell surface. Recently, vitamin A (retinol) was reported to have an important role in inducing the expression of the gut-homing chemokine receptor CCR9 and the integrin $\alpha 4\beta 7$ on T cells [9]. The DCs isolated from the gut-associated lymphoid tissue were shown to be able to convert vitamin

A into its active form, retinoic acid, which induced the expression of CCR9 and $\alpha 4\beta 7$ on the T cells. Furthermore, removing vitamin A from the diet caused a dramatic depletion of CD4-positive T cells from the intestinal mucosa but not from, e.g., the lung in mice.

Children with asthma may have lower concentrations of serum vitamin A and β -carotene than healthy controls [10, 11]. *In vitro*, retinoic acid seems to inhibit the production of eotaxin, an eosinophil chemoattractant highly expressed in patients with asthma [12]. Retinoic acid may also suppress the eosinophil/basophil and mast cell differentiation of progenitor cells in cord blood and in cultured human bone marrow cells [13, 14]. Atopic subjects have higher numbers of circulating eosinophil/basophil colony-forming units in the blood than non-atopics [15], and blood eosinophilia during infancy is associated with subsequent allergic disease in childhood [16]. *In vitro*, retinoic acid may also inhibit the IL-4-mediated proliferation and IgE production of B cells [17].

Against this background, we decided to explore the eventual association between the plasma retinol levels, determined since birth, and the development of atopic signs and symptoms in a cohort of unselected newborns followed up for 20 years.

Methods

Study population

This investigation is based on a 20-year follow-up study of 200 unselected newborns and their mothers, which was initiated in 1981 [18, 19]. Healthy, non-smoking mothers with an uncomplicated pregnancy and delivery and a full-term healthy infant with a birth weight appropriate for gestational age were invited to participate. The mothers were encouraged to breastfeed exclusively for as long as possible. During exclusive breastfeeding, the infants received exclusively human milk without any supplementary formula or solid foods. In accordance to a national recommendation, all the infants received vitamin A (1000 IU/day) and vitamin D (400 IU/day) supplementation (Vitol[®], Orion, Espoo, Finland) from birth through the second year of life. The number of infants on strictly exclusive breastfeeding was 167 at 2, 116 at 6, 36 at 9 and 7 at 12 months of age. When exclusive breastfeeding became insufficient for the infant, the infant was gradually weaned to a cow's-milk-based formula (Tutteli[®]; Valio, Helsinki, Finland) and after the age of 3 months to solid foods. Of the 200 infants, 84 (42%) had a family history of allergy, defined as the presence of at least one first-grade relative with allergic symptoms.

Of the 200 infants, two were lost to follow-up during the first year. Of the remaining 198 children, 163 (82%) were re-assessed at age 5 years, 150 (76%) at age 11 years and 164 (83%) at age 20 years with skin prick testing

(SPT), clinical examination and parental (at ages 5 and 11) and personal (at age 20) structured interviews by a physician. At each of the three re-examinations, all the subjects and their parents were personally interviewed by a single physician unaware of the early feeding history and laboratory parameters. The structured personal interview and the clinical examination were based on a form filled in by the interviewing physician, and the questions on allergic symptoms were similar at the three re-examinations. Based on the interview and the clinical examination, the present allergic symptoms and those during the preceding year were recorded at the re-examinations at ages 5, 11 and 20 years.

The study was approved by the Ethical Review Board of the Hospital for Children and Adolescents and the Ethical Review Board for Internal Medicine of Helsinki University Hospital. Written informed consent was obtained from the parents at the beginning of the study, and from the participants of the 20-year re-examination.

Definitions of allergic symptoms

Atopic dermatitis was defined as a chronic or relapsing itchy dermatitis with a characteristic morphology and distribution [20]. Atopic dermatitis was recorded if it was present at a follow-up examination, or if it had been diagnosed by a physician and there was a history of relapsing eczema with typical features and localization during the preceding year.

Allergic rhinoconjunctivitis was defined as recurrent non-infectious rhinitis at pollen seasons or on aeroallergen exposure, and/or itching and watery discharge of the eyes at pollen seasons or on aeroallergen exposure.

Recurrent wheezing was recorded if the subject had a diagnosis of bronchial asthma, or two or more separate episodes of respiratory distress and wheezing.

Symptoms of food hypersensitivity were defined as a repeated history of itching or swelling of the lips, the oral mucosa or the throat, urticarial eruption or severe vomiting after ingestion of a specific food. As performing double-blind placebo-controlled food challenges was not feasible, we chose to rely on the history of food hypersensitivity, which was recorded in a personal interview by a physician and based on reports of typical symptoms appearing after ingestion of a specific food. At age 20 years, symptoms of food hypersensitivity were no longer taken into account.

Verified atopy was recorded if the subject had one or several allergic symptoms and a positive SPT to at least one of the common allergens tested.

Skin prick testing

We performed SPTs at the ages of 5, 11 and 20 years with standardized allergen extracts (ALK, Copenhagen,

Denmark) of birch, alder, timothy, Kentucky blue, mugwort, cat, dog, *Dermatophagoides pteronyssinus*, cow's milk, egg and codfish; histamine hydrochloride (10 mg/mL) and a diluent control were included. A positive SPT was defined as at least one test reaction with a mean diameter equal to or greater than half the diameter of the histamine weal [21].

Serum total immunoglobulin E

Serum total IgE was determined by the Pharmacia UniCAP total IgE system (Pharmacia Diagnostics, Uppsala, Sweden) at the ages of 5, 11 and 20 years. Total IgE at the age of 5 years was determined in sera collected in 1986 and stored at -20°C until analysed in 2004. The cut-off for an elevated total IgE was set to 130 IU/L at ages of 11 and 20 years, and to 48 IU/L at the age of 5 years [22].

Blood sampling and determination of the plasma concentration of vitamin A

The retinol concentration of the cohort members was repeatedly measured in infancy in every other infant entering the study, and in childhood in all the participants of the re-examinations. Therefore, the plasma concentration of retinol was determined in cord blood at birth ($n=97$), in plasma samples at ages 2, 4 and 12 months ($n=95$) and at ages 5 ($n=155$) and 11 years ($n=151$). Blood plasma was separated and frozen within 30 min, stored at -18°C and transported in dry ice by air to the Department of Human Nutrition and Health, (F. Hoffmann-La Roche & Co. Ltd, Basel, Switzerland), where the vitamin A concentration in plasma was determined by high-performance liquid chromatography [23].

Statistical analyses

Statistical analyses were performed with StatView 5.0 (SAS Institute; Cary, NC, USA). The plasma retinol concentrations were log transformed before analyses. Significances were tested with analysis of variance for repeated measurements and two-tail unpaired Student's *t*-test. Logistic regression analyses were performed in order to control for the effect of potential covariates. The factors taken into account as potential covariates were gender, duration of exclusive breastfeeding (< 2 or ≥ 2 months), a family history of allergy, smoking in the household during the infant's first year, personal smoking at the age of 20 years, maternal educational level, maternal age, sibship size and daycare attendance during the infant's first year.

Results

Lower neonatal retinol concentrations in boys than in girls

The plasma retinol concentrations in cord blood and during the first year of life are presented in Fig. 1. The retinol concentrations were significantly lower in boys than in girls at birth and at age 2 months, whereas they were comparable from the age of 4 months onward (Table 1). Retinol concentrations in infancy and childhood tended to be lower in children with a family history of allergy than in those without, but the difference was not significant. Moreover, maternal atopy did not have an effect on the retinol concentrations in infancy.

We have previously published the prevalences of allergic symptoms at the ages of 5, 11 and 20 years in this cohort [19]. Throughout the 20-year follow-up, the boys had a higher frequency of allergic symptoms than the girls, although the differences were not statistically significant. The frequency of allergic symptoms at age 20 years was 54% in the subjects whose retinol concentrations had been measured in infancy, and 49% in those whose retinol concentrations had not been measured.

An inverse association between the retinol concentrations in infancy and subsequent atopic manifestations

We observed that the retinol concentrations in infancy were lower in subjects who subsequently developed atopic manifestations in childhood and adolescence than in those who remained symptom free. The difference in retinol concentrations was most striking at 2 months of age.

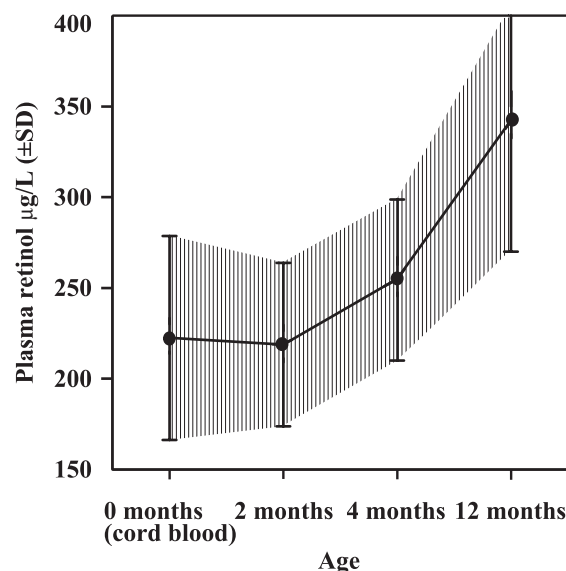


Fig. 1. Mean plasma retinol during the first year of life. Shaded area, SD.

Table 1. Plasma retinol concentrations ($\mu\text{g/L}$, mean $\pm$ SEM) of the total population, and in girls and boys

Age	Total P-retinol	Girls		Boys		<i>P</i> *
		<i>N</i>	P-retinol	<i>N</i>	P-retinol	
0 months	222 ($\pm$ 5.7)	55	240 ($\pm$ 8.0)	42	197 ($\pm$ 6.3)	0.0001
2 months	218 ($\pm$ 4.6)	52	226 ($\pm$ 5.8)	43	208 ($\pm$ 7.1)	0.03
4 months	254 ($\pm$ 4.6)	54	253 ($\pm$ 6.1)	41	256 ($\pm$ 7.1)	0.74
12 months	341 ($\pm$ 7.4)	54	347 ($\pm$ 9.3)	41	334 ($\pm$ 12.1)	0.32
5 years	320 ($\pm$ 4.6)	86	320 ($\pm$ 5.8)	69	319 ($\pm$ 7.4)	0.82
11 years	384 ($\pm$ 5.3)	94	385 ($\pm$ 6.8)	57	383 ($\pm$ 8.5)	0.86

**P* value calculated for the difference in retinol concentrations between girls and boys.

SEM, standard error of the mean.

Table 2. Retinol concentration ($\mu\text{g/L}$) at age 2 months (mean $\pm$ SEM) and allergic symptoms, skin prick test (SPT) positivity and verified atopy at ages of 5 and 11 years

	Plasma retinol at age 2 months		<i>P</i> (<i>t</i> -test)	<i>P</i> (adjusted)*
	Atopics	Controls [†]		
Outcomes at age 5 years				
SPT positivity (<i>n</i> = 14)	193 ($\pm$ 11.1)	223 ($\pm$ 5.3)	0.02	0.04
Any allergic symptom (<i>n</i> = 19)	205 ($\pm$ 10.8)	222 ($\pm$ 5.7)	0.14	0.64
Verified atopy [‡] (<i>n</i> = 7)	198 ($\pm$ 18.1)	220 ($\pm$ 5.2)	0.20	0.44
Outcomes at age 11 years				
SPT positivity (<i>n</i> = 32)	212 ($\pm$ 48.5)	222 ($\pm$ 5.6)	0.27	0.21
Any allergic symptom (<i>n</i> = 39)	212 ($\pm$ 6.6)	222 ($\pm$ 6.7)	0.31	0.66
Verified atopy [‡] (<i>n</i> = 25)	209 ($\pm$ 7.9)	221 ($\pm$ 5.8)	0.29	0.36

*Adjusted for gender, duration of exclusive breastfeeding (< 2 or $\geq$ 2 months), smoking in the household during the first year, and a family history of allergy.

[†]Controls: symptom-free/SPT negative subjects.

[‡]Verified atopy: subjects with an allergic symptom and positive SPT.

SEM, standard error of the mean.

At birth, the difference in plasma retinol concentrations between the subjects with and without subsequent atopic manifestations was not yet significant. However, the subjects with allergic symptoms, positive SPT or verified atopy (i.e. allergic symptoms and positive SPT) at age 5 years as well as those with an elevated total IgE at age 20 years tended to have a lower cord blood retinol concentration than the non-atopic subjects.

The retinol concentration at age 2 months correlated inversely with the subsequent atopic manifestations in childhood and adolescence (Tables 2 and 3). The 14 children with positive SPT at age 5 years had a significantly lower retinol concentration at age 2 months than the SPT-negative children ($P = 0.02$). The retinol concentration at age 2 months tended to be lower in subjects with allergic symptoms and verified atopy at ages of 5 and 11 years. Symptoms of food hypersensitivity at the ages of 5 years ($n = 10$) and 11 years ($n = 9$) were associated with a comparatively low retinol concentration at age 2 months ($P = 0.05$ and 0.04 , respectively). Serum total IgE elevated to 130 kU/L at age 11 years was associated with a low retinol concentration at age 2 months ($P = 0.05$), whereas

there was no significant association of total IgE levels at ages of 5 and 20 and retinol concentrations in infancy.

Allergic symptoms ($n = 46$), positive SPT ($n = 46$) and verified atopy ($n = 35$) at age 20 years were associated with a low retinol concentration at age 2 months ($P = 0.01$, 0.002 and 0.001 , respectively; Table 3, Figs 2 and 3). The difference in retinol concentrations was more pronounced in girls than in boys even though the boys had a higher frequency of allergic symptoms at age 20 years (56%) than the girls (50%). Subjects with allergic rhinoconjunctivitis ($n = 37$), recurrent wheezing ($n = 13$) and atopic dermatitis ($n = 19$) had a lower retinol concentration at age 2 months as compared with symptom-free subjects ($P = 0.002$, 0.01 and 0.01 , respectively). All these associations remained significant after adjusting for the potential covariates, i.e. gender, a family history of allergy, duration of exclusive breastfeeding, smoking in the household during the infant's first year and smoking at age 20 years (Tables 2 and 3). Furthermore, the results remained unaltered after adjusting for maternal educational level, maternal age, sibship size and daycare attendance during the infant's first year.

Table 3. Retinol concentration ($\mu\text{g/L}$) at age 2 months (mean $\pm$ SEM) and allergic symptoms, skin prick test (SPT) positivity and verified atopy at age 20 years

	Plasma retinol at age 2 months		<i>P</i> (<i>t</i> -test)	<i>P</i> (adjusted)*
	Atopics	Controls [†]		
Any allergic symptom (<i>n</i> = 46)	209 ($\pm$ 6.2)	236 ($\pm$ 7.0)	0.01	0.01
SPT positivity (<i>n</i> = 46)	208 ($\pm$ 6.4)	237 ($\pm$ 6.6)	0.002	0.004
Verified atopy [‡] (<i>n</i> = 35)	203 ($\pm$ 6.8)	235 ($\pm$ 6.1)	0.001	0.01
All. rhinoconjunctivitis (<i>n</i> = 37)	205 ($\pm$ 6.9)	236 ($\pm$ 7.0)	0.002	0.01
Atopic dermatitis (<i>n</i> = 19)	207 ($\pm$ 7.8)	236 ($\pm$ 7.0)	0.01	0.02
Recurrent wheezing (<i>n</i> = 13)	198 ($\pm$ 11.0)	236 ($\pm$ 7.0)	0.01	0.05

*Adjusted for gender, duration of exclusive breastfeeding (< 2 or $\geq$ 2 months), a family history of allergy, smoking in the household during the first year, and smoking at age 20 years.

[†]Controls: symptom-free/skin prick test negative subjects.

[‡]Verified atopy: subjects with an allergic symptom and positive SPT.
SEM, standard error of the mean.

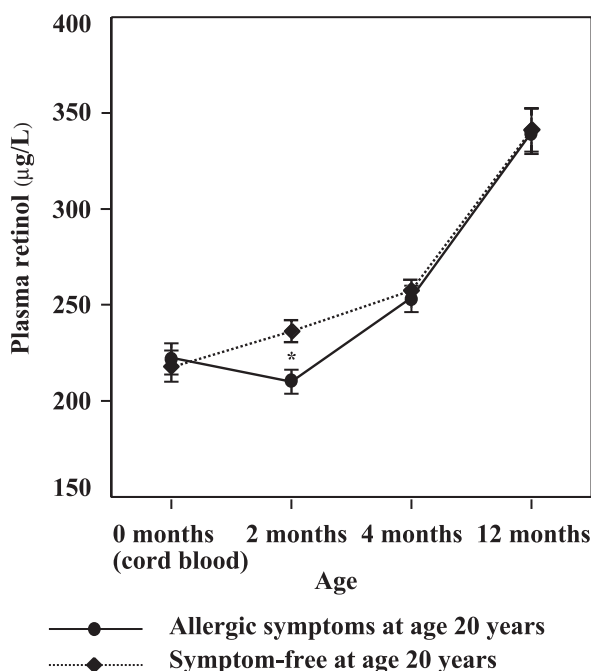


Fig. 2. Plasma retinol in subjects with and without allergic symptoms at age 20 years $\pm$ standard error of the mean. **P* = 0.01. For the numbers of subjects analysed, see 'Methods'.

After the age of 2 months, the difference in retinol concentration in subjects with and without subsequent atopic manifestations became less significant. The retinol concentration at age 4 months was inversely associated with a positive SPT and verified atopy at age 11 years (adjusted *P* = 0.03 and 0.02), and with atopic dermatitis at age 20 years (adjusted *P* = 0.04). Subjects with allergic symptoms, positive SPT or verified atopy at age 5 years tended to have lower retinol concentrations at age 4 months as compared with symptom-free subjects. A similar trend was still observable at age 12 months, i.e. the retinol concentrations remained lower in subjects with

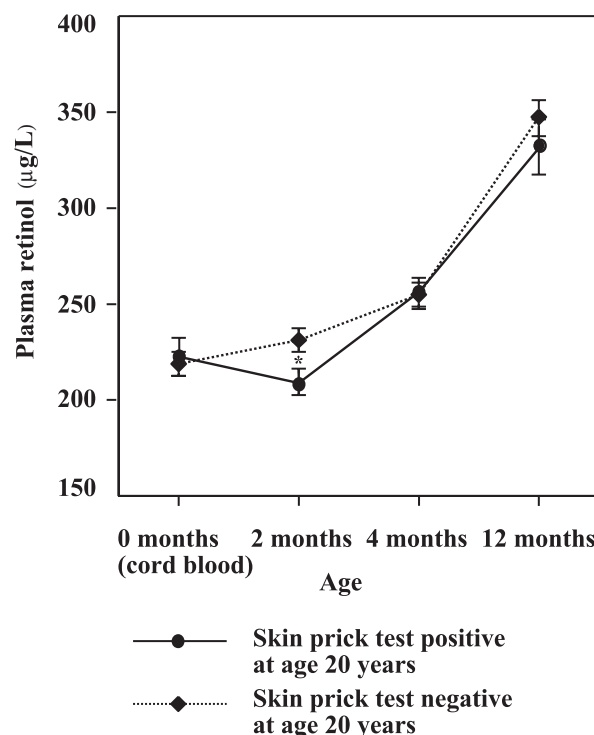


Fig. 3. Plasma retinol in infancy in subjects with and without a positive skin prick test at age 20 years $\pm$ standard error of the mean. **P* = 0.002.

subsequent atopic manifestations at the ages of 5, 11 and 20 years than in those without atopy.

An inverse association between the retinol concentrations in childhood and atopic manifestations

At age 5 years, children with atopic dermatitis (*n* = 26) had a lower retinol concentration, 297 $\mu\text{g/L}$, than the symptom-free subjects, 322 $\mu\text{g/L}$ (*P* = 0.03). Furthermore, children with allergic rhinoconjunctivitis, recurrent wheezing or food hypersensitivity and those with positive SPT at age

5 years had a lower retinol concentration than children without atopic manifestations. At age 11 years, the plasma retinol concentrations were similar in children with and without atopic manifestations at that age. However, subjects with allergic rhinoconjunctivitis ($n = 59$) or atopic dermatitis ($n = 31$) at age 20 years had a significantly lower retinol concentration at age 11 years as compared with those who remained symptom free ($P = 0.03$ and 0.01 , respectively).

Discussion

We report that the plasma retinol concentration at age 2 months correlates inversely with the subsequent development of allergic symptoms and positive SPT in childhood and adolescence. Furthermore, the retinol concentration at age 5 years was inversely associated with the prevalence of atopic dermatitis at that age.

In addition, we observed that the cord blood retinol concentration was significantly lower in boys than in girls. The boys had a higher frequency of atopic manifestations than the girls, which is in accordance with the previous literature [24]. To our knowledge, this is the first report describing the sex difference in retinol concentration at birth. The difference was still detectable at age 2 months, whereas at age 4 months and onwards, the retinol concentrations in boys and girls were comparable (Table 1). In our study population, the mean retinol concentration in cord blood was $221.6 \mu\text{g/L}$, which is of the same magnitude as the values previously reported from European populations [25, 26].

Our study cohort consists of randomly selected healthy, full-term newborns prospectively followed up from birth to age 20 years. This setting enabled us to examine the association of retinol levels in infancy and the development of atopic manifestations later in childhood and adolescence, and to take into account the effects of several atopy-related potential confounding factors.

At age 2 months, nearly all the infants (94%) were on a similar diet, i.e. on breastfeeding, and the majority of the infants (84%) were on exclusive breastfeeding. All the infants received vitamin A supplementation; therefore, a dietary deficiency of vitamin A was unlikely. The mothers were encouraged to maintain exclusive breastfeeding as long as it was nutritionally sufficient for the infant; as a result, 58% of the infants were exclusively breastfed for at least the first 6 months of life. As we have reported previously [19], atopic dermatitis at age 5 years and food hypersensitivity at the ages of 5 and 11 years were over-represented among the children who had received exclusive breastfeeding for 9 months or more.

Retinol and its derivatives are required in various biological functions, including epidermal growth and differentiation, immunity, vision, bone development, reproduction and maintenance of the gut integrity [27–29].

In vitamin A deficiency, both the innate and the adaptive immune systems are compromised, the Th1/Th2 balance is disturbed and the T cell-dependent antibody responses are impaired [30–32]. Vitamin A deficiency is associated with elevated morbidity and mortality from infectious diarrhoea, measles and childhood respiratory illnesses [33].

Vitamin A is acquired from the diet as carotenoids in fruit and vegetables, and as retinyl esters in foods of animal origin. The absorption of dietary vitamin A involves the hydrolysis of dietary retinyl esters in the intestine, re-esterification in enterocytes and incorporation into chylomicrons containing dietary lipids [34]. Besides the chylomicron route, some of the free, unesterified retinol is transported directly to the portal circulation [35]. Vitamin A is stored in the liver, and the plasma vitamin A levels are kept constant through homeostatic regulation from the liver stores. In our study, the differences in the retinol concentrations were observable at age 2 months when nearly all the infants were on a similar diet, i.e. on breastfeeding. Thus, we suggest that the differences in the retinol concentrations between the atopic and the non-atopic subjects are not dependent on the diet; instead, the retinol concentration is subject to genetic control, in which there may be atopy-related differences.

Vitamin A derivatives modulate the host inflammatory responses by increasing the prostaglandin biosynthesis *in vivo* [36] and by regulating the expression and activity of several enzymes involved in the host defence against pathogens [37, 38]. *In vitro*, retinoic acid inhibits the IL-4-mediated proliferation and IgE production of CD40-positive B cells [17]. Retinoic acid inhibited IgE production more effectively in B cells from atopic dermatitis patients with low total IgE levels than in B cells from patients with high total IgE, whereas in an *in vivo* model using mice sensitized with ovalbumin, the retinoids did not inhibit IgE production [39].

It was recently shown in mice that low levels of vitamin A inhibit CD4-positive T cell homing to the gut [9]. Under such circumstances, the T cells rather home to the lung, the liver and the skin. Retinoic acid increases the expression of $\alpha 4\beta 7$ integrin and chemokine receptor CCR9 on T lymphocytes, which provides them with tropism for the gut, and a T-helper 1/T-regulatory 1 cytokine profile [9, 40, 41]. Of the DCs, only those isolated in the gut-associated lymphatic tissue were shown to be able to convert retinol to retinoic acid [9]. The DCs have been shown to regulate the T cell homing, and to be able to reprogram the homing receptor expression on T cells previously polarized *in vitro* for homing to the skin or to the small intestine [42].

In the prenatal human intestine, a population of precursor T cells differentiate into mature T cells *in situ* [43]. Thus, the most important contact between the environmental antigens and the immune system is likely to occur

through the mucosal membranes of the gastrointestinal tract. It has been assumed that the intestinal microflora and especially the bacterial polysaccharides [44] may play a particular role in this event, as it is the major external driving force in the postnatal maturation of the immune system, and animal experiments have shown it to be a prerequisite for the normal development of oral tolerance [45, 46].

Thus, the above and our present findings taken together, we can assume that if the newborn infant has a low concentration of retinol, the CD4-positive T cell homing to the gut will be inhibited. Under such circumstances, the Th1-type responses initiated by the intestinal microflora and other antigens become weak and an environment favourable for the development of allergic sensitization may ensue. This may lead to the expression of the atopic phenotype and to the subsequent development of allergic symptoms.

Acknowledgements

Supported by the Foundation for Pediatric Research, the Finnish Medical Foundation, the National Graduate School of Clinical Investigation and the Helsinki University Hospital Research Funds.

References

- Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1999; 319:1259–60.
- von Mutius E, Martinez FD, Fritzsche C, Nicolai T, Reitmeir P, Thiermann HH. Skin test reactivity and number of siblings. *BMJ* 1994; 308:692–5.
- Cookson W. The immunogenetics of asthma and eczema: a new focus on the epithelium. *Nat Rev Immunol* 2004; 4:978–88.
- Hooper LV. Bacterial contributions to mammalian gut development. *Trends Microbiol* 2004; 12:129–34.
- Rhee KJ, Sethupathi P, Driks A, Lanning DK, Knight K. Role of commensal bacteria in development of gut-associated lymphoid tissues and preimmune antibody repertoire. *J Immunol* 2004; 172:1118–24.
- Macpherson AJ, Harris NL. Interactions between commensal intestinal bacteria and the immune system. *Nat Rev Immunol* 2004; 4:478–84.
- Noverr MC, Huffnagle GB. Does the microbiota regulate immune responses outside the gut? *Trends Microbiol* 2004; 12:562–8.
- Steinman RM, Bonifaz L, Fujii S *et al*. The innate functions of dendritic cells in peripheral lymphoid tissues. *Adv Exp Med Biol* 2005; 560:83–97.
- Iwata M, Hirakiyama A, Eshima Y, Kagechika H, Kato C, Song SY. Retinoic acid imprints gut-homing specificity on T cells. *Immunity* 2004; 21:527–38.
- Arora P, Kumar V, Batra S. Vitamin A status in children with asthma. *Pediatr Allergy Immunol* 2002; 13:223–6.
- Rubin RN, Navon L, Cassano PA. Relationship of serum antioxidants to asthma prevalence in youth. *Am J Respir Crit Care Med* 2004; 169:393–8.
- Takamura K, Nasuhara Y, Kobayashi M *et al*. Retinoic acid inhibits interleukin-4-induced eotaxin production in a human bronchial epithelial cell line. *Am J Physiol Lung Cell Mol Physiol* 2004; 286:L777–85.
- Denburg JA, Sehmi R, Upham J. Regulation of IL-5 receptor on eosinophil progenitors in allergic inflammation: role of retinoic acid. *Int Arch Allergy Immunol* 2001; 124:246–8.
- Kinoshita T, Koike K, Mwamtemi HH *et al*. Retinoic acid is a negative regulator for the differentiation of cord blood-derived human mast cell progenitors. *Blood* 2000; 95:2821–8.
- Denburg JA, Telizyn S, Belda A, Dolovich J, Bienenstock J. Increased numbers of circulating basophil progenitors in atopic patients. *J Allergy Clin Immunol* 1985; 76:466–72.
- Borres MP, Björkstén B. Peripheral blood eosinophils and IL-4 in infancy in relation to the appearance of allergic disease during the first 6 years of life. *Pediatr Allergy Immunol* 2004; 15:216–20.
- Worm M, Krah JM, Manz R, Henz BM. Retinoic acid inhibits CD40+IL-4-mediated IgE production in vitro. *Blood* 1998; 92:1713.
- Salmenperä L, Perheentupa J, Siimes MA. Exclusively breast-fed healthy infants grow slower than reference infants. *Pediatr Res* 1985; 19:307–12.
- Pesonen M, Kallio MJT, Ranki A, Siimes MA. Prolonged exclusive breastfeeding is associated with increased atopic dermatitis. A prospective follow-up study of unselected healthy newborns from birth to age 20 years. *Clin Exp Allergy* 2006; 36:1011–8.
- Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Dermatol Venereol* 1980; 92 (suppl):44–7.
- Meinert R, Frischer T, Karmaus W, Kuehr J. Influence of skin prick test criteria on estimation of prevalence and incidence of allergic skin prick positivity in children. *Allergy* 1994; 49:526–32.
- Johansson SGO, Yman L. In vitro assays for Immunoglobulin E. *Clin Rev Allergy* 1988; 6:93–139.
- Vuilleumier JP, Keller HE, Gysel D, Hunziker F. Clinical chemical methods for the routine assessment of the vitamin status in human populations. Part I: the fat-soluble vitamins A and E, and beta-carotene. *Int J Vitam Nutr Res* 1983; 53:265–72.
- PausJenssen ES, Cockcroft DW. Sex differences in asthma, atopy, and airway hyperresponsiveness in a university population. *Ann Allergy Asthma Immunol* 2003; 91:34–7.
- Sapin V, Alexandre MC, Chaib S *et al*. Effect of vitamin A status at the end of term pregnancy on the saturation of retinol binding protein with retinol. *Am J Clin Nutr* 2000; 71:537–43.
- Schulpis KH, Michalakakou K, Gavrili S *et al*. Maternal-neonatal retinol and alpha-tocopherol serum concentrations in Greeks and Albanians. *Acta Paediatr* 2004; 93:1075–80.
- Napoli JL. Interactions of retinoid binding proteins and enzymes in retinoid metabolism. *Biochim Biophys Acta* 1999; 1440:139–62.
- Kastner P, Mark M, Chambon P. Nonsteroid nuclear receptors: what are genetic studies telling us about their role in real life? *Cell* 1995; 83:859–69.
- Quadro L, Gamble MV, Vogel S *et al*. Retinol and retinol-binding protein: gut integrity and circulating immunoglobulins. *J Infect Dis* 2000; 182:S97–102.
- Stephensen CB, Jiang X, Freytag T. Vitamin A deficiency increases the in vivo development of IL-10-positive Th2 cells

- and decreases development of Th1 cells in mice. *J Nutr* 2004; 134:2660–6.
- 31 Pasatiempo AM, Kinoshita M, Taylor CE, Ross AC. Antibody production in vitamin A-depleted rats is impaired after the immunization with bacterial polysaccharide or protein antigens. *FASEB J* 1990; 4:2518–27.
 - 32 Wiedermann U, Hanson LA, Kahu H, Dahlgren UI. Aberrant T-cell function in vitro and impaired T-cell dependent antibody response in vivo in vitamin A-deficient rats. *Immunology* 1993; 80:581–6.
 - 33 Ross AC, Stephensen CB. Vitamin A and retinoids in antiviral responses. *FASEB J* 1996; 10:979–85.
 - 34 Harrison EH, Hussain MM. Mechanisms involved in the intestinal digestion and absorption of dietary vitamin A. *J Nutr* 2001; 131:1405–8.
 - 35 Nayak N, Harrison EH, Hussain MM. Retinyl ester secretion by intestinal cells: a specific and regulated process dependent on assembly and secretion of chylomicrons. *J Lipid Res* 2001; 42:272–80.
 - 36 Devaux Y, Seguin C, Grosjean S *et al.* Retinoic acid and lipopolysaccharide act synergistically to increase prostanoic concentrations in rats in vivo. *J Nutr* 2001; 131:2628–35.
 - 37 Grosjean S, Devaux Y, Seguin C *et al.* Retinoic acid attenuates inducible nitric oxide synthase (NO2) activation in cultured rat cardiac myocytes and microvascular endothelial cells. *J Mol Cell Cardiol* 2001; 33:933–45.
 - 38 Hill EM, Bader T, Nettesheim P, Eling TE. Retinoid-induced differentiation regulates prostaglandin H synthase and cPLA2 expression in tracheal epithelium. *Am J Physiol* 1996; 270:L854–62.
 - 39 Worm M, Herz U, Krah JM, Henz BM. Effects of retinoids on in vitro and in vivo IgE production. *Int Arch Allergy Immunol* 2001; 124:233–6.
 - 40 Svensson M, Johansson-Lindbom B, Wurbel MA, Malissen B, Marquez G, Agace W. Selective generation of gut-tropic T cells in gut-associated lymphoid tissues: requirement for GALT dendritic cells and adjuvant. *Ann NY Acad Sci* 2004; 1029:405–7.
 - 41 Papadakis KA, Zhu D, Prehn JL *et al.* Dominant role for TL1A/DR3 pathway in IL-12 plus IL-18-induced IFN-gamma production by peripheral blood and mucosal CCR9+T lymphocytes. *J Immunol* 2005; 174:4985–90.
 - 42 Dudda JC, Lembo A, Bachtanian E *et al.* Dendritic cells govern induction and reprogramming of polarized tissue-selective homing receptor patterns of T cells: important roles for soluble factors and tissue microenvironments. *Eur J Immunol* 2005; 35:1056–65.
 - 43 Howie D, Spencer J, DeLord D *et al.* Extrathymic T cell differentiation in the human intestine early in life. *J Immunol* 1998; 161:5862–72.
 - 44 Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* 2005; 122:107–18.
 - 45 Priault G, Fliss I, Pecquet S. Effect of probiotic bacteria on induction and maintenance of oral tolerance to beta-lactoglobulin in gnotobiotic mice. *Clin Diagn Lab Immunol* 2003; 10:787–92.
 - 46 Sudo N, Sawamura SA, Tanaka K, Aiba Y, Kubo C, Koga Y. The requirement of intestinal bacteria flora for the development of an IgE production system fully susceptible to oral tolerance induction. *J Immunol* 1997; 159:1739–45.