

Atopic sensitization and atopic dermatitis in Estonian and Swedish infants

T. Voor*, K. Julge*, M. F. Böttcher†, M. C. Jenmalm†, K. Duchén† and B. Björkstén‡

*Children's Clinic of Tartu University Clinics, Tartu, Estonia, †Department of Molecular and Clinical Medicine, Division of Paediatrics, Clinical Research Centre, Faculty of Health Sciences, Linköping University, Linköping, Sweden and ‡Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

Summary

Background Early life events seem to have a major impact on the development of tolerance or sensitization.

Objective The aim of the study was to compare the prevalence of sensitization and atopic dermatitis (AD) during the first 2 years of life in Estonia and in Sweden.

Methods Two groups comprising 110 Estonian and 123 Swedish infants were followed from birth up to 2 years of age. Data about symptoms of allergy, infections and use of antibiotics were obtained by questionnaires. Clinical examinations, skin prick tests (SPTs) with food and inhalant allergens, and blood sampling for IgE analyses were carried out at 3, 6, 12 and 24 months.

Results The cumulative incidence of AD and positive SPTs were lower in the Estonian than the Swedish infants (14% vs. 24%; $P=0.06$ and 13% vs. 24%; $P=0.03$), while circulating IgE antibodies were more common (39% vs. 27%; $P=0.06$) and often present without any clinical significance in Estonian children. Estonian infants had respiratory illnesses more often and they had received antibiotics more frequently. Use of antibiotics increased the risk for positive SPT in the Estonian (odds ratio = 1.7, 95% confidence interval = 1.1–2.5), but not in the Swedish infants. This may be explained by the use of broad-spectrum antibiotics in Estonia, while in Sweden mostly penicillin was prescribed.

Conclusions The prevalence of AD and positive SPTs was lower in the Estonian than the Swedish infants, while circulating IgE antibodies were more common and often present without any clinical significance. These differences cannot simply be explained by infections, or use of broad-spectrum antibiotics in the two countries, although more the natural lifestyle in Estonia may be contributing factor.

Keywords antibiotics, atopic dermatitis, IgE, infant, infection, skin prick test

Submitted 5 May 2004; revised 9 September 2004; accepted 11 November 2004

Introduction

There is considerable evidence that the prevalence of allergic diseases is increasing in industrialized high-income countries [1, 2]. Furthermore, there are large geographical differences in the prevalence of allergy, and in particular a low prevalence of atopy has been reported among the children in formerly socialist countries of Europe [3–5]. A significant increase in the prevalence of atopy, as determined by a positive skin prick test (SPT) over a 5-year period among 9–11-year-old children, was recently reported in the Eastern part of Germany [6]. An increase was not confirmed in another study, comprising 5–14-year-old children, in whom atopy was determined by the presence of circulating IgE antibodies [7], nor were any changes in the prevalence of allergic symptoms and SPTs observed over a 4-year period in a recent Estonian study [8].

The increasing prevalence of atopy is usually explained by a changed life style in the Western countries, including an overall decrease of exposure to microbes [9]. Studies have shown an inverse relation between faeco-orally transferred infections and allergic diseases [10, 11]. On the other hand, some respiratory infections, notably with respiratory syncytial virus, could lead to enhanced sensitization and respiratory allergy [12]. A low prevalence of allergic diseases has been reported in children who grew up in a farming environment, and it was suggested that this could be explained by more exposure to endotoxin as a marker of increased bacterial load in the environment [13]. We recently confirmed an inverse relationship between the presence of a positive SPT and exposure to endotoxin in Swedish children [14]. This was, however, not the case among the Estonian children, who were generally exposed to higher levels of endotoxin than the Swedish children. Furthermore, we recently reported that although the cumulative incidence of allergic diseases during the first 5 years of life was similar in Estonia and Scandinavia, the point prevalence of sensitization dropped after the age of

Correspondence: Tiia Voor, Children's Clinic of Tartu University Clinics, Lunini Street 6, 51014 Tartu, Estonia. E-mail: Tiia.Voor@kliinikum.ee

2 years [15]. This observation is in contrast with the studies from Western Europe, showing an increasing prevalence of allergy with age.

Estonia is culturally similar to other Northern Europe countries, but because of 50 years of occupation, the lifestyle still resembles that in the Scandinavian countries some 30 years ago, including locally produced and home-made food, close contact with animals, smaller homes with more people. The prevalence of allergies is low in Estonia, as compared with industrialized countries in Western Europe, both in children [4, 8] and adults [16].

The aims of the present study were to compare the development of immune responses to allergens and atopic dermatitis (AD) over the first 2 years of life in Estonia, with a low, and in Sweden, with a high prevalence of allergic diseases, relating the findings to the incidence of infections and use of antibiotics. By comparing the development of immune responses to allergens and clinically manifested AD in the two countries, we addressed the hypothesis that allergic responses may be more rapidly down-regulated in Estonia as suggested by a previous study [15] and that the relationship between IgE as measured by SPTs and in serum differ in two countries, thus reconciling seemingly divergent observations in Eastern and Western Germany [6, 7].

Materials and methods

Study population

The study groups were recruited from the general population. The midwives of the maternity clinics in Tartu, Estonia, and Linköping, Sweden, informed pregnant women and their families about the study. The study groups comprised 110 (53% boys) Estonian and 123 Swedish (54% boys) children.

The Estonian children were born at Tartu University Women's Clinic during the period of February 1997–June 1998 and the Swedish children were born between March 1996 and October 1999 at Linköping University Hospital. Pregnancies and deliveries were uncomplicated and all the children were born at term. All the parents completed questionnaires about their symptoms of atopic diseases after the birth of child and consented to participate in the study.

Clinical examination

A clinical examination of the babies was made at 3, 6, 12 and 24 months. Questionnaires were completed on these occasions about symptoms of allergy, use of antibiotics and incidence of infections. The questionnaires were translated from Swedish into Estonian and then back into Swedish by two persons independently of each other. AD was defined as pruritic, chronic or chronically relapsing non-infectious dermatitis with typical features and distribution, as suggested by Hanifin and Rajka [17]. Respiratory infections were defined as cold symptoms with fever, with or without wheezing. In order to confirm consistency between the two study sites, the Estonian investigator visited the Clinical Research Centre in Linköping several times, and participated in the follow-up examinations of the Swedish children.

Skin prick tests

SPTs were done at all follow-ups in Estonia, whereas in the Swedish babies they were done either at 3 or 6 months, and then at 12 and 24 months. The tests were performed in duplicate on the volar aspects of the forearms, using lancets of ALK (Hørsholm, Denmark) with natural cow's milk (CM) and egg white (EW) at all ages. At 6, 12 and 24 months, the children were also tested with extracts of inhalant allergens (i.e. cat, dog, and in Estonia additionally *Dermatophagoides pteronyssinus*). At 12 months, birch was added to the panel and at 2 years timothy, and in Estonia also cockroach (*Blattella germanica*) allergen. Histamine hydrochloride, 10 mg/mL, was used as a positive and glycerol as a negative control. We used standardized allergen extracts (Solu-Prick SQ™, ALK), except the cockroach allergen extract, which was from Bayer (Spokane, WA, USA). The SPT was regarded as positive when the mean of the longest and right angle diameters of one of the weals was at least 3 mm. The same allergen extracts and lancets were used in the two study groups and the nurses, who made SPT, compared their technique carefully before the study as described in the manual of the International Study of Asthma and Allergy in Childhood (ISAAC) [18].

Immunoglobulin E measurements

Venous blood samples were drawn into heparinized vacutainers at 3, 6, 12 and 24 months. In the Swedish babies, blood samples were drawn either at 3 or 6 months, while in Estonia blood samples were obtained at both ages. Plasma and peripheral blood mononuclear cells were separated. The plasma samples were frozen immediately and kept at -20°C until analysed.

Total IgE levels and IgE antibodies to EW and β -lactoglobulin (β -LG) were determined at all ages, and the levels of IgE antibodies to cat and birch allergens were analysed at 12 and 24 months by a commercial chemiluminescence method, according to the recommendations of the manufacturer (Magic Lite™, ALK). The test results are given in classes corresponding to the concentration of allergen-specific IgE antibodies, i.e. class 1: 0.35–0.7 SU/mL and class ≥ 2 : above 0.7 SU/mL. Samples with total IgE levels above 100 IU/mL at 2 years were also analysed for IgE antibodies against *Ascaris*. All Estonian and Swedish serum samples for a particular test were analysed in the same laboratory, i.e. the measurements and circulating IgE antibodies to β -LG and EW and total IgE were done in Estonia and IgE antibodies to cat and birch were done in Sweden. Certain samples were analysed in both laboratories in order to confirm consistency.

Statistics

The statistical analyses were performed using the statistical package SAS 8.2. χ^2 test or *t*-test was used to test differences in prevalence and cumulative incidence of clinical allergy, SPT results and presence of circulating IgE antibodies and infections between the Estonian and the Swedish infants. As the number of prescribed antibiotics was not normally distributed among the infants, the Wilcoxon's test was employed for the comparisons. Where classical χ^2 test was

inappropriate because of small-expected cell counts, we used Fisher's exact test. The association between the development of atopic diseases and the use of antibiotics was analysed with logistic regression. A probability (P) level of $<5\%$ was considered to be statistically significant.

Ethical considerations

The study was approved by the Ethics Review Committee on Human Research of the University of Tartu and the Regional Ethics Committee for Human Research at Linköping University. The parents of all children gave their informed consent in writing.

Results

The cumulative incidence of AD at 2 years was lower in Estonia (15/110, 14%) than in Sweden (29/123, 24%; $P = 0.06$) and it was less common at all ages in the Estonian infants, although significantly so only at 3 months (Fig. 1).

Fourteen of 110 (13%) the Estonian and 30 of 123 (24%) the Swedish children had a positive SPT at least once during the first 2 years of life ($P = 0.03$). The prevalence of a positive SPT was lower in the Estonian than in the Swedish infants at all ages (Table 1). In addition, one Estonian infant had a positive SPT against cockroach and one against house dust mite (HDM) at 2 years. None of the children in either country had a positive SPT against dog and timothy. A positive SPT was recorded at least once during the first 2 years of life in

5/15 (33%) of the Estonian and 20/29 (69%; $P = 0.03$) of the Swedish children with AD, while positive SPTs were equally common among the infants without AD (8% in Estonia and 11% in Sweden).

The mean total serum-IgE levels were similar in two countries, except for a lower level in the Estonian infants at 12 months (Fig. 2). High levels of IgE, i.e. >100 IU/mL, were detected in 15 Estonian and 12 Swedish children at 24 months. These samples were tested for IgE antibodies against *Ascaris* and two Estonian samples were found to be weakly positive, i.e. class 1.

Despite the lower prevalence of AD and positive SPT at all ages, circulating IgE antibodies to any of the four allergens tended to be more common in the Estonian than in the Swedish children (cumulative incidence 39% vs. 27%; $P = 0.06$). As shown in Table 2, this difference was because of higher incidence of IgE antibodies to cat and birch in Estonia and apparent down-regulation of IgE antibodies to milk and egg in the Swedish children during the second year of life. The cumulative incidence of IgE antibodies was similar in the children with and without AD (7/15, 47% vs. 35/94, 37%; $P = 0.57$), in Estonia, while in Sweden IgE antibodies were detected in 17/29 (59%) of the children with, and in 16/93 (17%) in those without AD ($P < 0.001$, respectively). Thus, the prevalence of IgE antibodies among clinically non-allergic infants was higher in Estonia than in Sweden ($P = 0.003$). This was true for all the allergens when individually compared (data not shown).

Furthermore, there was poor agreement between the presence of circulating IgE antibodies and the outcome of SPT and the disagreement increased with age. Thus, SPT-negative Estonian infants 32/95 (34%) had circulating IgE

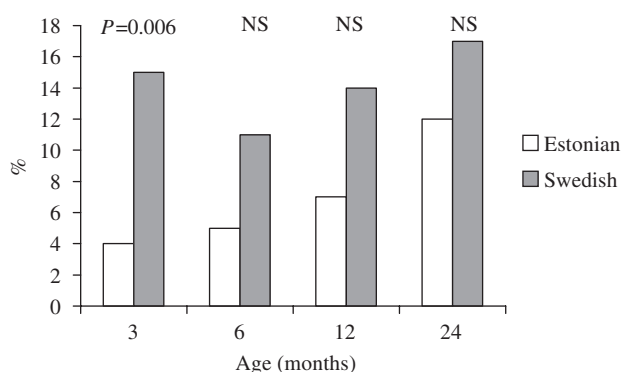


Fig. 1. Prevalence of atopic dermatitis in the Estonian and Swedish infants during the first 2 years of life.

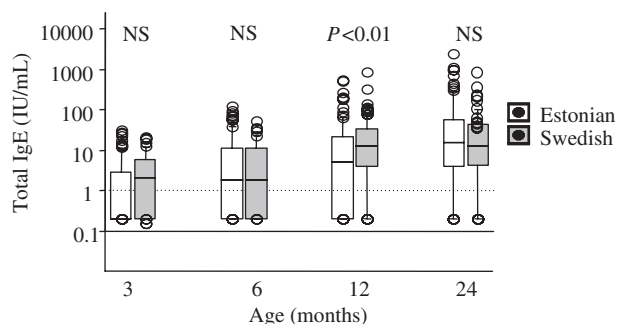


Fig. 2. The levels of total IgE (IU/mL) in Estonian and Swedish infants during the first 2 years of life.

Table 1. Prevalence (%) of positive skin prick test results in Estonian (Est) and the Swedish (Sw) infants during the first 2 years of life

Allergen	Age (months)							
	3		6		12		24	
	Est (N = 109)	Sw (N = 65)	Est (N = 108)	Sw (N = 69)	Est (N = 109)	Sw (N = 122)	Est (N = 103)	Sw (N = 120)
Cow's milk	0	3	0	9	1	4	0	3
Egg white	4	11	6	16	6*	18	4*	12
Cat			1	1	0	1	4	3
Birch					0	0	0*	6
Any	4*	12	6*	17	7*	20	6*	18

* $P < 0.05$ Estonian vs. Swedish infants at the same age.

Table 2. Prevalence (%) of circulating IgE antibodies in Estonian (Est) and Swedish (Sw) children during the first 2 years of life

Allergen	Age in months							
	3		6		12		24	
	Est (N = 104)	Sw (N = 53)	Est (N = 101)	Sw (N = 51)	Est (N = 100)	Sw (N = 114)	Est (N = 96)	Sw (N = 117)
β-LG								
All classes	9	6	8	14	6	7	10	5
Class ≥ 2	2	4	1*	8	0	4	1	2
Egg white								
All classes	11	13	15	14	10	18	18	13
Class ≥ 2	0	2	6	8	6	11	2	8
Cat								
All classes					6*	0	6	2
Class ≥ 2					3	0	2	1
Birch								
All classes					4	1	3	3
Class ≥ 2					1	0	1	2
Any								
All classes	16	15	18	21	26	21	30*	18
Class ≥ 2	2	6	6	12	8	13	5	9

* $P < 0.05$ Estonian vs. Swedish infants at the same age. β -LG, β -lactoglobulin.

antibodies at least once through the first 2 years of life, as compared with only 13/93 (14%) of the Swedish children ($P = 0.002$). In contrast, 15 Swedish infants had 25 positive SPTs without corresponding circulating IgE antibodies. In Estonia, only four infants had a positive SPT without the concurrent IgE antibodies. The poor agreement between circulating IgE antibodies and positive SPT were particularly obvious for inhalant allergens. Twelve Estonian children had circulating IgE antibodies to inhalant allergens and eight of them were SPT negative and only one child had a positive SPT to cat without corresponding circulating IgE antibodies. In contrast, six Swedish infants had IgE antibodies to inhalant allergens and four of them also had positive SPTs, whereas seven children had a positive SPT without the corresponding circulating IgE antibodies.

Episodes of respiratory illnesses were more common during the first 2 years of life in the Estonian than Swedish infants (median 6.2 [range 1–12] and 3.6 [range 0–15] episodes, respectively; $P < 0.001$). All the Estonian infants had at least one episode, while 13 Swedish children (11%) did not have any recorded infections during the first 2 years of life. There was no correlation between the numbers of episodes of infections and allergy in either country.

Antibiotics were more frequently prescribed to the Estonian than the Swedish infants during the first 2 years, i.e. mean 2.1 (SD 0–3.9) vs. 1.2 (SD 0–2.6) times; $P < 0.001$. Twenty-three (21%) Estonian and 40 (33%) Swedish children did not receive any antibiotics during this time period ($P = 0.03$). Already by the age of 1 year, 25% of the Estonian children had got antibacterial therapy at least twice, as compared with 9% in Sweden ($P = 0.002$). In Sweden, the most used antibiotics were β -lactams, mostly penicillin and only six children received erythromycin, cephalosporins or trimethoprim-sulphamethoxazol (TMP-SM). In Estonia, 17 children were prescribed only β -lactams, whereas 70 children received also erythromycin, cephalosporins and/or TMP-SM. In

Estonia, SPT positive children had been treated more often with antibiotics during the first year of life, as compared with the SPT negative children (mean 1.8 (SD 0.4–3.2) vs. 0.8 (SD 0–1.9) courses; $P = 0.007$). The use of antibiotics during the first year of life increased the risk for positive SPT (odds ratio (OR) = 1.7, 95% confidence interval (CI), 1.1–2.5) in Estonia. This was not the case in the Swedish infants (OR = 0.8, 95% CI, 0.5–1.4). There was no correlation between the use of antibiotics during the second year of life and SPT and AD in either country.

A history of allergic diseases was less common among Estonian than Swedish parents, i.e. 22/107 (21%) vs. 50/123 (41%) of the mothers ($P = 0.001$) and 18/107 (17%) vs. 47/123 (38%) of the fathers ($P = 0.001$). Data were missing for the parents of three Estonian children. There was no correlation between parental allergy and prevalence of AD, nor sensitization in the children.

Discussion

Most of the symptomatic Swedish children had positive SPT, but this was not the case in Estonia. Similar observations have been reported in cross-sectional studies in the German pre-school children showing that eczema in Eastern Germany was not related to allergy, while this was the case in Western Germany [19].

In contrast to the low prevalence of positive SPT, circulating IgE antibodies to foods and inhalants seemed to be more common in the Estonian than the Swedish children. This was particularly true among the infants without any allergic symptoms during their second year of life. The findings cannot be explained by differences in exposure to cat and birch, as this is comparable in the two countries [14, 20].

The cumulative incidence of dermatitis was lower in the Estonian than the Swedish infants during the first 2 years of life. A lower number of infants with a positive family history of allergy in Estonia would at first sight be suspected as a confounding factor. However, differences in family history in populations living in a low and a high prevalence country are not informative since the genotype is not phenotypically expressed in the former. Numerous studies of migrant populations have shown that geographic differences in prevalence are not because of genetic differences on a population level [21, 22].

Studies made in Western countries have reported a good correlation between positive SPT results and the presence of circulating IgE antibodies. Skin tests are considered to be more sensitive [23, 24] and circulating IgE antibodies to be more specific [25]. Low level of IgE antibody responses to foods have been previously reported in infancy, whereas the antibodies were transient in non-allergic children [26] and therefore the specificity of IgE antibodies might be low. Our results in the Swedish children agree with these reports. In the Estonian infants, however, low levels of circulating IgE antibodies were commonly detected and they were poorly related to allergy and positive SPTs. A poor correlation between circulating IgE antibodies to pollen and positive SPT results has been observed previously among Estonian school-children [5], and recently a positive association between AD and SPTs responses and high IgE values was observed among adults in Finland, but not in Russia [27]. We have suggested that the lack of correlation in Estonia between the two methods to detect sensitization might be because of transient sensitization during infancy, which is then followed by down-regulation of skin reactivity [15].

The disagreement between the IgE and SPT results in the Estonian children is not reasonably because of technical factors, as the same allergen extracts and lancets were used in the two study groups and the nurses who made SPT compared their technique carefully before the study. One explanation for the low incidence of SPT in the infants with circulating IgE antibodies could possibly be because of a lower IgE affinity, as suggested by a recent report that most of the low affinity IgE antibodies are seen in young children, which could suggest a critical period of time during early life when the affinity responses mature [28]. Hypothetically, this time period could be different in the Estonian and Swedish environments early in life.

In a recent study from Gabon, urinary schistosomiasis was associated with a low prevalence of positive SPTs to HDM [29]. The authors suggested that the anti-inflammatory cytokine IL-10, which is induced in chronic schistosomiasis, could suppress atopy. The same phenomena have been described among the children of low socio-economic class in Venezuela, where the majority had low level of specific IgE antibodies against a variety of inhalant allergens, but relatively few had positive SPTs [30]. As only two children in our study had IgE antibodies to *Ascaris* and the levels of total IgE were similar in the Estonian and the Swedish children, we believe that parasitic infections cannot explain negative SPTs in the Estonian children with circulating IgE antibodies.

Infections during early childhood are suggested to protect against allergy [31, 32]. The Estonian infants had more

respiratory infections and all of them had at least one infection during the study period. In contrast, 11% of the Swedish children did not have any respiratory infection recorded during the first 2 years of life. This figure is similar to that reported from Western Germany, where 14% of infants did not have any viral infection during the first year of life [33]. The Estonian children received more often antibiotics prescribed by the family doctors. There was no correlation between infections and the development of allergy; however, although the use of antibiotics was associated with positive SPT results in the Estonian infants. The lack of association between antibiotic treatment and sensitization in Sweden could be explained by the fact that mostly penicillin was used. This compound does not affect the intestinal microflora to the same extent as broader spectrum antibiotics.

We have previously demonstrated differences in the composition of the intestinal microflora between the Estonian and the Swedish children [34, 35] and between allergic and non-allergic children [36, 37]. Treatment with antibiotics with a broad microbial spectrum could affect a putatively 'allergy protective' gnotobiota in the Estonian children. Animal studies have shown that antibiotics during infancy may enhance T-helper type 2 (Th2) polarized immunity and prevent postnatal Th1 cell maturation, thus resulting in Th2 polarized immune deviation [38]. An alternative explanation could be that atopic children are more likely to have severe infections in early childhood and therefore be more likely to receive antibacterial treatment. However, this explanation is not supported by our study. Furthermore, atopic children had not been admitted more frequently to the hospital than non-allergic children.

It was recently suggested that the inverse relationship between allergy and parasitic infections is caused by stimulation of an immune regulatory network [39]. Our findings could suggest that not only parasitic infections, but also microbial pressure could do that. The Estonian children appear to have more contact with different micro-organisms than the Swedish children in early life, as demonstrated in comparative studies of the gut microbiota [34, 35], higher endotoxin levels in house dust [14], and more respiratory illnesses as observed in this study.

In conclusion, this prospective study has shown that in Estonia, in contrast to Sweden, circulating IgE antibodies are poorly related to clinical allergy and positive SPTs and appear to be general markers of immune stimulation. The findings cannot simply be explained by any certain factor, although more respiratory infections, higher exposure to endotoxin and a higher diversity of the gut flora in Estonia may all be contributing factors.

Acknowledgements

We thank Tiina Rebane, Ülle Kuld and Svetlana Svarõš from Children's Clinic of Tartu University Clinics, Estonia, and Anne-Marie Fornander and Lena Lindell, Department of Health and Environment, Division of Paediatrics, Linköping Hospital, Sweden for excellent technical assistance and Märt Möls, Institute of Mathematical Statistics, University of Tartu, for help with statistical analyses.

Glaxo-Wellcome, Swedish Heart and Lung Association, The Swedish Foundation for Health Care Science and Allergy Research, The National Swedish Association against Allergic Diseases and The Swedish Research Council (#7510) are acknowledged for financial support.

References

- Butland BK, Strachan DP, Lewis S, Bynner J, Butler N, Britton J. Investigation into the increase in hay fever and eczema at age 16 observed between the 1958 and 1970 British birth cohorts. *BMJ* 1997; 315:717–21.
- Åberg N, Hesselmar B, Åberg B, Eriksson B. Increase of asthma, allergic rhinitis and eczema in Swedish schoolchildren between 1979 and 1991. *Clin Exp Allergy* 1995; 25:815–9.
- ISAAC Steering Committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema. The International Study of Asthma and Allergies in Childhood (ISAAC). *Lancet* 1998; 351:1225–32.
- Björkstén B, Dumitrescu D, Foucard T et al. Prevalence of childhood asthma, rhinitis and eczema in Scandinavia and Eastern Europe. *Eur Respir J* 1998; 12:432–7.
- Riikjäär MA, Julge K, Vasar M, Bråbäck L, Knutsson A, Björkstén B. The prevalence of atopic sensitisation and respiratory symptoms among Estonian schoolchildren. *Clin Exp Allergy* 1995; 25:1198–204.
- Weiland SK, von Mutius E, Hirsch T et al. Prevalence of respiratory and atopic disorders among children in the East and West of Germany five years after unification. *Eur Respir J* 1999; 14:862–70.
- Heinrich J, Hoelscher B, Jacob B, Wjst M, Wichmann HE. Trends in allergies among children in a region of former East Germany between 1992–1993 and 1995–1996. *Eur J Med Res* 1999; 26: 107–13.
- Riikjäär MA, Annus T, Bråbäck L, Rahu K, Björkstén B. Similar prevalence of respiratory symptoms and atopy in Estonian schoolchildren with changing lifestyle over 4 yrs. *Eur Respir J* 2000; 16:86–90.
- Holt PG, Sly PD, Björkstén B. Atopic versus infectious diseases in childhood: a question of balance? *Pediatr Allergy Immunol* 1997; 8:53–8.
- Matricardi PM, Rosmini F, Riondino S et al. Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. *BMJ* 2000; 12:412–7.
- Linneberg A, Ostergaard C, Tvede M et al. IgG antibodies against micro-organisms and atopic disease in Danish adults: the Copenhagen Allergy Study. *J Allergy Clin Immunol* 2003; 111: 847–53.
- Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. *Am J Respir Crit Care Med* 2000; 161:1501–7.
- von Mutius E, Braun-Fährlander C, Schierl R et al. Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clin Exp Allergy* 2000; 30:1230–4.
- Böttcher MF, Björkstén B, Gustafson S, Voor T, Jenmalm MC. Endotoxin level in Estonian and Swedish house dust and atopy in infancy. *Clin Exp Allergy* 2003; 33:295–300.
- Julge K, Vasar M, Björkstén B. Development of allergy and IgE antibodies during the first five years of life in Estonian children. *Clin Exp Allergy* 2001; 31:1854–61.
- Jõgi R, Janson C, Björnsson E, Boman G, Björkstén B. Atopy and allergic disorders among adults in Tartu, Estonia compared with Uppsala, Sweden. *Clin Exp Allergy* 1998; 28:1072–80.
- Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Dermatol Venerol* 1980; 92 (Suppl.):44–7.
- ISAAC Steering Committee. Phase II Modules of the International Study of Asthma and Allergies in Childhood (ISAAC). Münster: Institute of Epidemiology and Social Medicine, University of Münster, 1998.
- Schafer T, Kramer U, Vieluf D, Abeck D, Behrendt H, Ring J. The excess of atopic eczema in East Germany is related to the intrinsic type. *Br J Dermatol* 2000; 143:992–8.
- Ekeboom A, Nilsson S, Saar M, van Hage-Hamsten M. A comparative study of airborne pollen concentrations of three allergenic pollen types in Tartu (Estonia), Roma/Gotland and Stockholm (Sweden) 1990–1996. *Grana* 1998; 36: 366–72.
- Grüber C, Illi S, Plieth A, Sommerfeld C, Wahn U. Cultural adaptation is associated with atopy and wheezing among children of Turkish origin living in Germany. *Clin Exp Allergy* 2002; 32:526–31.
- Leung RC, Carlin JB, Burdon JG, Czarny D. Asthma, allergy and atopy in Asian immigrants in Melbourne. *Med J Aust* 1994; 161:418–25.
- Kelso JM, Sodhi N, Gosselin VA, Yunginger JW. Diagnostic performance characteristics of the standard Phadebas RAST, modified RAST, and Pharmacia CAP system versus skin testing. *Ann Allergy* 1991; 67:511–4.
- Pastorello EA, Incorvaia C, Ortolani C et al. Studies on the relationship between the level of specific IgE antibodies and the clinical expression of allergy: I. Definition of levels distinguishing patients with symptomatic from patients with asymptomatic allergy to common aeroallergens. *J Allergy Clin Immunol* 1995; 9:580–7.
- Dekker FW, Mulder JD, Kramps JA, Vandenbroucke JP, Dijkman JH. The Phadiatop in vitro test for allergy in general practice: is it useful? *Fam Pract* 1990; 7:144–8.
- Hattevig G, Kjellman B, Björkstén B. Appearance of IgE antibodies to ingested and inhaled allergens during the first 12 years of life in atopic and non-atopic children. *Pediatr Allergy Immunol* 1993; 4:182–6.
- Vartiainen E, Petays T, Haahtela T, Jousilahti P, Pekkanen J. Allergic diseases, skin prick test responses, and IgE levels in North Karelia, Finland, and the Republic of Karelia, Russia. *J Allergy Clin Immunol* 2002; 109:643–8.
- Pierson-Mullany LK, Jackola DR, Blumenthal MN, Rosenberg A. Evidence of an affinity threshold for IgE-allergen binding in the percutaneous skin test reaction. *Clin Exp Allergy* 2002; 32:107–16.
- van den Biggelaar AH, van Ree R, Rodrigues LC et al. Decreased atopy in children infected with *Schistosoma haematobium*: a role for parasite-induced interleukin-10. *Lancet* 2000; 356:1723–7.
- Lynch NR, Lopez RI, Di Prisco-Fuenmayor MC et al. Allergic reactivity and socio-economic level in a tropical environment. *Clin Allergy* 1987; 17:199–207.
- Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989; 299:1259–60.
- Illi S, von Mutius E, Lau S et al. Early childhood infectious diseases and the development of asthma up to school age: a birth cohort study. *BMJ* 2001; 322:390–5.
- Lau S, Nickel R, Niggemann B et al. The development of childhood asthma: lesson from German Multicentre Allergy Study (MAS). *Pediatr Respir Rev* 2002; 3:265–72.
- Sepp E, Julge K, Vasar M, Naaber P, Björkstén B, Mikelsaar M. Intestinal microflora of Estonian and Swedish infants. *Acta Paediatr* 1997; 86:956–61.
- Sepp E, Naaber P, Voor T, Mikelsaar M, Björkstén B. Development of intestinal microflora during the first month of life in Estonian and Swedish infants. *Microb Ecol Health Dis* 2000; 12:22–26.

- 36 Björkstén B, Sepp E, Julge K, Voor T, Mikelsaar M. Allergy development and the intestinal microflora during the first year of life. *J Allergy Clin Immunol* 2001; 108:516–20.
- 37 Böttcher MF, Nordin EK, Sandin A, Midtvedt T, Björkstén B. Microflora-associated characteristics in faeces from allergic and nonallergic infants. *Clin Exp Allergy* 2000; 30:1590–6.
- 38 Oyama N, Sudo N, Sogawa H, Kubo C. Antibiotic use during infancy promotes a shift in the T(H)1/T(H)2 balance toward T(H)2-dominant immunity in mice. *J Allergy Clin Immunol* 2001; 107:153–9.
- 39 Yazdanbakhsh M, Kremsner PG, van Ree R. Allergy, parasites, and the hygiene hypothesis. *Science* 2002; 296:490–4.