Clin Exp All 2002; **32**:373–378

Increase of allergen-specific immunoglobulin E antibodies from 1973 to 1994 in a Finnish population and a possible relationship to *Helicobacter pylori* infections[1]

T. U. Kosunen*, J. Höök-Nikanne†, A. Salomaa*‡, S. Sarna§, A. Aromaa¶ and T. Haahtela†

*Department of Bacteriology and Immunology, Haartman Institute and Helsinki University Central Hospital, University of Helsinki, †Division of Allergy, Helsinki University Central Hospital, Helsinki, ‡Vammala Health Center, Vammala, §Department of Public Health, University of Helsinki, and ¶Social Insurance Institution and National Public Health Institute, Helsinki, Finland

Summary

Background The prevalence of atopic diseases – hayfever, asthma and eczema – has increased over the past decades. The increase may be associated with decreased rates of infections such as measles, hepatitis A, tuberculosis, toxoplasmosis, and, as recently suggested, *Helicobacter pylori* gastritis.

Objective Since the increase of atopy has been mainly based on clinical studies, we wanted to study the prevalence of allergen-specific Immunoglobulin (Ig)E antibodies in two cross-sectional, adult population-based serum samples two decades apart. Since the sera had been tested for *H. pylori* antibodies, we also had a chance to look for a possible relationship between these two findings.

Methods We determined the prevalence rate of allergen-specific serum IgE antibodies against birch and timothy pollen, and cat and dog epithelium allergens by the radioallergosorbent test in a 15–54-years-old Finnish population using 326 sera collected in 1973 and 319 sera collected in 1994 from randomly selected subjects.

Results From 1973 to 1994 allergen-specific IgE prevalence rates and IgE antibody levels rose. In 1994, the prevalence rate of positive findings in 15–24-year-old population had increased from 11 to 38% (3.5-fold increase, $P=0.0001$, OR 5.12, CI 95% 2.32–11.3). In older 10-year age groups similar trends did not reach significance, but the overall change was significant with all three cut-off levels of allergen-specific IgE analysed.

The percentage of IgE-positive persons rose mainly in the subgroup with no *H. pylori* antibodies. In 1994 21% of the *H. pylori*-negative subjects had IgE antibodies compared with 5% of the *H. pylori*-positive subjects (in 1973 11% in both subgroups).

Conclusions IgE-based evidence for an increase in IgE-mediated allergy was uncovered. The increase occurred mainly in the subgroup with no antibodies to *H. pylori*, which support the hypothesis that *H. pylori* could be one of the microbes counteracting atopy.

Keywords allergy, allergen-specific IgE, atopy, *Helicobacter pylori* antibodies, prevalence rates
Submitted 19 December 2000; revised 19 March 2001; accepted 20 September 2001

Introduction

Epidemiological and clinical evidence suggest that the prevalence of atopic diseases – hayfever, asthma and eczema – has increased over the past decades [1,2]. Evidence based on measurements of allergen-specific Immunoglobulin (Ig)E levels has remained scarce [3–5].

The search for causes of this increase has revealed associations with several factors, among them fetal growth [6], relationship of T helper (Th)1 and Th2 lymphocyte responses [7], family size and sibship order [8], the decrease in the rate of infections such as measles [9], hepatitis A [10], tuberculosis [11], and contact with Mycobacteria or BCG vaccination [12]. In the recent expansion of the original hepatitis A study from Italy [10] toxoplasmosis could be added to this list of infections. Even *H. pylori* infection seemed to have similar influence, but it was seen only when cumulative lifetime exposure indexes to these three foodborne and orofecal microbes were calculated [13]. In the United Kingdom, however, exposure to hepatitis A, toxoplasmosis and *H. pylori*, as measured by parental reports obtained at age 10–14 years and by serological tests carried out in adulthood, did not influence the rate of wheezing symptoms or atopy in adulthood [14].

H. pylori causes active chronic gastritis, which is strongly associated with the development of peptic ulcers and is a risk factor for gastric cancer [15]. The infection is one of the most common bacterial diseases world-wide. We found a substantial decrease in the prevalence rate of *H. pylori* antibodies from 1973 to 1994 in Finland [16]. Using material from that study, we now searched for immunological proof for the increase of atopy

Correspondence: Dr Timo U. Kosunen, Department of Bacteriology and Immunology, PO Box 21, FIN 00014 University of Helsinki, Finland. E-mail: timo.kosunen@helsinki.fi

[1]Presented in part at the XIIth International Workshop on Gastroduodenal Pathology and Helicobacter pylori, 2–4 September 1999, Helsinki.

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from 1973 to 1994, which enabled us to look also into the suggested relationship with *H. pylori* infections.

Methods

We studied sera collected in 1973 and in 1994 from randomly selected persons, 15–54 years old, living in Vammala, Finland (Table 1). The participation rate in the original helicobacter study (15–75-year-old subjects) was 84% at both time-points [16]. In 1973 the sample size was defined to be one per 20 adults resident within Vammala, in 1994 50 females and 50 males were invited per each 10-year age group. Both random samples were selected by computer from the National Population Register.

Vammala is a small, semiurban community in south-west Finland. The town has had a relatively stable population size of approximately 16000 from 1973 to 1994; data on movements were not available. According to the national statistics, Finland has experienced a considerable demographic change [17,18]. In the early 1970s, 20% of the population earned their living from agriculture and 38% from services, in 1994 these had changed to 12% and 54%, respectively. In accordance with the development in the entire country, the level of education of the Vammala population has risen markedly. Young age groups in 1994 achieved a much higher educational level than in 1973.

The informed consent of all the participants was obtained. The study was approved by the Ethical Committee of the University Hospital, Tampere, Finland.

Sera collected in 1973 and 1994 were stored at -20°C until and between the tests. In 1994 the sera were assayed for helicobacter antibodies and in 1998 for IgE antibodies against birch and timothy pollen, and cat and dog epithelium allergens using a radioallergosorbent test (UniCAP, Pharmacia-Upjohn, Uppsala, Sweden) at the Division of Allergy, Helsinki University Central Hospital. The reference (normal) range was 0.00–0.35 IU/mL. Concentrations above 0.35 IU/mL were regarded as 'positive' results.

H. pylori antibody data (combined results of IgG and IgA tests) are from our earlier study [16].

For statistical analyses the chi square test or Fisher's exact test was used. Also odds ratios and their 95% confidence limits were calculated. Odds ratios were adjusted for age using the Mantel-Haenszel method. The homogeneity of odds ratios was tested with the Breslow-Day's test. The interaction between categorical variables was tested with log-linear model. All *P*-values given are two-sided and based on Robins-Breslow-Greenland-variance estimates. The calculations were done using the software package StatXact 4 [19].

Results

IgE antibodies

In the youngest 10-year age group of the 15–54 years-old population sample the prevalence rate of positive IgE antibody findings (at least one allergen-specific IgE result above the reference range 0.35 IU/mL per person) rose from 11% in 1973 to 38% in 1994. This means a 3.5-fold increase within two decades ($P=0.0001$). In the youngest age groups this increase was even more obvious when higher cut-off levels for specific-IgE (3.5 and 6 IU/mL) were applied in calculations. The changes in the three older age groups showed similar trend but did not reach significance (Table 1, Fig. 1). However, the overall change in the population studied was significant and the significance increased with higher cut-off levels (age-adjusted *P*-values were 0.0144, <0.0001 and <0.0001 for cut-off levels 0.35, 3.5 and 6 IU/mL, respectively).

The overall increase of allergen-specific IgE was statistically significant in males (age-adjusted $P=0.0061$, 0.0002 and 0.0002 for cut-off levels 0.35, 3.5 and 6 IU/mL, respectively) and with higher cut-off levels also in females (age-adjusted $P=0.5268$, 0.0118 and 0.0287 for cut-off levels 0.35, 3.5 and 6 IU/mL, respectively).

The increased prevalence rates of positive allergen-specific IgE findings in the 1994 sample were accompanied with higher IgE concentrations. In 1973 the distribution of positive allergen-specific IgE concentrations peaked in the low (0.35–1.0 IU/mL) range, but in 1994 the peak was in the high (>6 IU/mL) range

Table 1. Prevalence of IgE antibodies on different concentration levels against at least one of the four inhalant allergens (birch, timothy, cat and dog) tested in different age groups in Vammala, Finland in 1973 and 1994. Values given are numbers (%) of participants unless stated otherwise

Age group (years)	IgE level (IU/mL)	IgE antibody prevalence (%)		Relative increase Odds ratio (CI 95%)	*P*-value
		1973	1994		
15–24	>0.35	11/102 (10.8)	26/68 (38.2)	5.12 (2.32–11.3)	0.0001
	>3.5	3/102 (2.9)	19/68 (27.9)	12.80 (3.61–45.3)	0.0001
	>6	3/102 (2.9)	17/68 (25.0)	11.00 (3.08–39.3)	0.0002
25–34	>0.35	19/97 (19.6)	15/78 (19.2)	0.98 (0.46–2.08)	0.9527
	>3.5	4/97 (4.1)	9/78 (11.5)	3.03 (0.90–10.3)	0.0743
	>6	3/97 (3.1)	8/78 (10.3)	3.58 (0.92–14.0)	0.0665
35–44	>0.35	3/67 (4.5)	11/84 (13.1)	3.21 (0.86–12.0)	0.0829
	>3.5	0/67 (0)	4/84 (4.8)	nc	nc
	>6	0/67 (0)	3/84 (3.6)	nc	nc
45–54	>0.35	3/60 (5.0)	5/89 (5.8)	1.13 (0.26–4.92)	0.8697
	>3.5	1/60 (1.7)	1/89 (1.1)	0.68 (0.04–11.1)	0.7880
	>6	0/60 (0)	1/89 (1.1)	nc	nc

nc = not calculable.

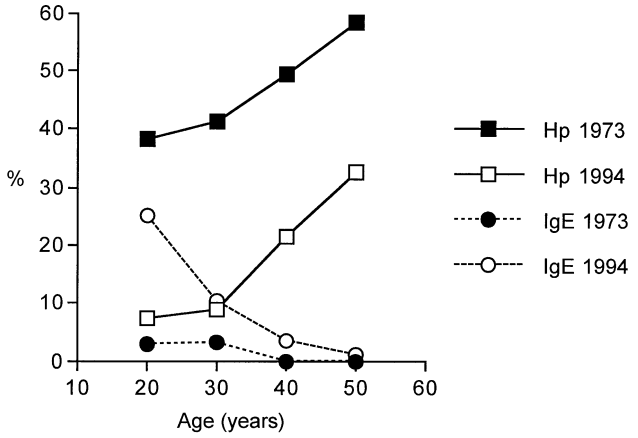

Fig. 1. Prevalence rates of *H. pylori* antibodies (IgG and IgA antibodies combined) and high (>6IU/mL) concentrations of IgE antibodies against at least one of the four inhalant allergens tested in 10-year age groups in 1973 and 1994.

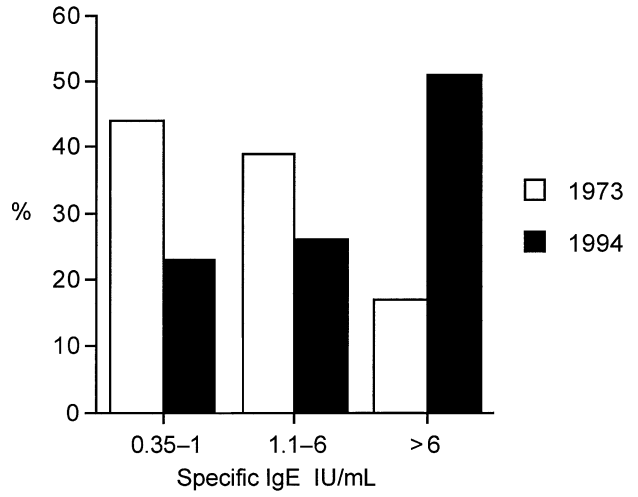

Fig. 2. Distribution of IgE-positive subjects by the highest observed allergen-specific IgE concentration in 1973 and 1994.

(Fig. 2). The proportion of IgE-positive subjects with high levels (>6IU/mL) of IgE antibodies against at least one of the allergens tested was higher in 1994 ($P=0.001$, OR=5.18, CI 95% 1.87–14.4).

In 1973 the prevalence rate of IgE-positive subjects was the same 11–12% in both genders, but in 1994 the rate was higher in males (23% vs. 13%, $P=0.0220$). The same was apparent when results for individual allergens were examined (Table 2).

IgE antibody findings showed a decreasing trend with age both in 1973 and 1994. This was seen particularly as low prevalence rates of both low- and high-specific IgE concentrations in subjects aged 35years or more (Table 1, Fig. 1).

H. pylori *antibodies*

The prevalence rate of *H. pylori* antibodies increased by age both in the 1973 and in the 1994 population sample (Fig. 1). In 1994 the seroprevalence rates of the 10-year subgroups tested for IgE were 24–32% units lower than in 1973 [16]. The interaction between the age and the decrease was not significant ($P=0.228$), which justified the use of age-adjusted odds ratios. The overall decrease (data not shown) was significant both in females (age-adjusted $P=0.0001$, OR 0.33, CI 95% 0.20–0.32) and males (age-adjusted $P>0.0001$, OR 0.14, CI 95% 0.07–0.25). These findings suggests a falling childhood infection rate.

IgE antibodies in H. pylori*-positive and -negative subjects*

In 1973 the occurrence of IgE antibodies (at least one specific IgE result above the reference range 0.35IU/mL per person) was equally common (11%) in *H. pylori* antibody-positive and -negative subjects, but in 1994 IgE antibodies were more common in subjects who did not have *H. pylori* antibodies (21% vs. 5%, $P=0.0095$) (Table 3). When subjects were grouped by the presence of *H. pylori* antibodies, the percentage of IgE-positive persons had increased in 1994 mainly in the subgroup with no *H. pylori* antibodies. The association was not strengthened by the use of higher cut-off values for specific IgE. The phenomenon was significant in males ($P=0.0047$) and approached significance in females ($P=0.0864$, data not shown).

Discussion

We determined the presence of allergen-specific IgE in sera collected in 1973 and in 1994 from subjects selected randomly from the National Population Register to represent the population of a Finnish semiurban community. The slightly different randomization procedures applied (in 1973 the number of subjects selected was one per 20 in adult population, in 1994 it was the same in each 10-year age group) did not influence the level of prevalence rates since they were calculated age specifically. Thus the study was epidemiologically sound and the influence of differences in the sample size per age group remained limited to the accuracy of the standard error of estimate only.

The IgE assays were made in 1998. Direct evidence that long-term storage at −20°C does not affect serum IgE has been reported in two studies [5,20]. Paganelli and coworkers summarized six clinical studies [20]. In one of them UniCAP and Pharmacia CAP System data were shown to agree in 266/274 cases (97%), which justified a comparison of the sensitivity and specificity data obtained in 1995 by the newer UniCAP-specific IgE with those obtained from the same material by the Pharmacia CAP System RAST in 1987. The results of more than 1200 tests on nine allergens indicated equivalent performance of the tests without change of efficacy or degradation of IgE antibodies after 8-year storage at −20°C [20]. Their data on 91 additional sera tested with the two methods confirmed also the reproducibility of the quantitative measurements of IgE antibodies over the 8-year interval. Linneberg et al. also used the Pharmacia CAP System to test a subset of 38 sera with an 8-year interval and found similar positivity for birch-specific IgE in 100%, and for house dust mite in 95.8% of them [5]. Further, although indirect, evidence for the stability of serum IgE at −20°C was reported by Henderson et al. [21]. The median serum total IgE levels of the samples collected for neurological studies from women and stored at −20°C for 32–37 years were equal to or higher than those of the newer, age-matched samples collected for preeclampsia studies and stored at −70°C for 5 years. Serum sodium concentrations studied indicated that

Table 2. Prevalence of IgE antibodies (>0.35 IU/mL) against four inhalant allergens in 165 females and 161 males in 1973, and in 167 females and 152 males in 1994 in Vammala, Finland. Values given are numbers (%) of participants unless stated otherwise

Allergen	Sex	IgE antibody prevalence (%)		Relative increase Odds ratio (CI 95%)	*P*-value
		1973	1994		
Birch					
	Female	3 (1.8)	9 (5.4)	3.08 (0.82–11.6)	0.1388
	Male	6 (3.7)	18 (11.8)	3.47 (1.34–9.00)	0.0069
Timothy					
	Female	9 (5.5)	13 (7.8)	1.46 (0.61–3.52)	0.3953
	Male	11 (6.8)	24 (15.8)	2.56 (1.21–5.42)	0.0121
Cat					
	Female	5 (3.0)	6 (3.6)	1.19 (0.36–3.99)	0.7292
	Male	5 (3.1)	17 (11.2)	3.93 (1.41–10.9)	0.0051
Dog					
	Female	11 (6.7)	10 (6.1)	0.89 (0.37–2.16)	0.7690
	Male	5 (3.1)	19 (12.5)	4.46 (1.62–12.3)	0.0017

Table 3. Prevalence of IgE antibodies (>0.35 IU/mL) against at least one of the four inhalant allergens tested in subjects with and without antibodies against *Helicobacter pylori* in different age groups in Vammala, Finland in 1973 and 1994. Values given are numbers (%) of participants unless stated otherwise

Age group (Years)	IgE antibodies	*H. pylori* antibodies	IgE antibody prevalence (%)		Relative increase Odds ratio (CI 95%)*	*P*-value
			1973	1994		
15–24	Yes	Yes	6/39 (15.4)	1/5 (20.0)	1.38 (0.13–14.5)	0.7912
25–34	Yes	Yes	6/40 (15.0)	0/7 (0)	nc	nc
35–44	Yes	Yes	2/33 (6.1)	1/18 (5.6)	0.91 (0.08–10.8)	0.9416
45–54	Yes	Yes	2/35 (5.7)	1/29 (3.4)	0.59 (0.05–6.85)	0.6726
All	Yes	Yes	16/147 (10.9)	3/59 (5.1)	0.63 (0.17–2.40)*	0.6285*
15–24	Yes	No	5/63 (7.9)	25/63 (39.7)	7.63 (2.69–21.7)	0.0001
25–34	Yes	No	13/57 (22.8)	15/71 (21.1)	0.91 (0.39–2.10)	0.8193
35–44	Yes	No	1/34 (2.9)	10/66 (15.2)	5.89 (0.72–48.1)	0.0979
45–54	Yes	No	1/25 (4.0)	4/60 (6.7)	1.71 (0.18–16.1)	0.6376
All	Yes	No	20/179 (11.2)	54/260 (20.8)	2.59 (1.43–4.39)*	0.0013*

nc=not calculable. *=age-adjusted.1973 yes-yes vs. yes-no: $P=0.9340$, OR 1.03, CI 95% 0.51–2.07.1994 yes-yes vs. yes-no: $P=0.0095$, OR 4.89, CI 95% 1.47–16.2.

dehydration during storage was insignificant [21]. Thus it is very unlikely that long-term storage of the sera would significantly influence IgE findings. Dehydration, a conceivable source of error, would weaken the trend we observed – lower IgE concentrations in 1973 than in 1994. Note that not only the IgE levels but also the distribution of concentrations were different in the two samples taken 21 years apart (Fig. 2).

The prevalence rate of specific IgE antibodies to the four most common inhalant allergens causing allergic reactions in Finland had increased significantly – 3.5-fold – in 15–24 years-old population from 1973 to 1994. In other age groups the trend was essentially similar. Not only did the prevalence of positive IgE findings increase, but also the proportion of high-positive (>6 IU/mL) findings increased.

Our results are direct, IgE-based evidence of an increase in IgE-mediated allergy. Such evidence has been scarce [3–5] and, as in our study, limited to inhalant allergens. In Grabs, Switzerland, a significant increase from 1983 to 1991 in the prevalence rate of IgE antibodies specific to at least one of the four allergens tested (an increase from 22% to 33% to birch, timothy, cat and house mite) and to timothy alone (from 15% to 27%) was reported in 15-year-old children (in 1983 $n=78$, in 1991 $n=67$) [3]. In northern Japan an increase in the IgE responses in cohorts of 13–14-year-old girls over a 13-year period from 1978 ($n=140$) to 1991 ($n=94$) was found [4]. The prevalence rate of IgE antibodies specific to one or more of the 16 allergens tested increased from 21% to 39%; the most significant increases were against timothy, sweet vernal grass, house dust and Japanese cedar. According to a recent cross-sectional study in a 15–41-year-old Danish population the prevalence of specific IgE to at least one of the six allergens tested (birch, grass, mugwort, dog, cat and house dust mite) increased from 26.5% (81/306 in 1990) to 33.9% (155/457 in 1998) within eight years [5]. They found also a corresponding increase in the prevalence of allergic rhinitis symptoms associated with specific IgE positivity.

The Danish study [5] shows, like ours, that the increase of allergen-specific IgE antibodies in the population extends beyond childhood. They did not, however, report the distribution of prevalence rates of specific IgE antibodies by age within

the study groups. Our observation of a decreasing prevalence rate by age agrees with the data of Barbee et al. who in an 8-year follow-up found a clear tendency for total serum IgE levels to decrease with age in subjects less than 35 years of age [22]. The decline was greater in women, and after the age of 35 years the IgE levels in atopic male subjects were significantly higher than in atopic females. Our current data from the 1994 sample also show a higher prevalence rate of IgE antibodies in males than in females, particularly in the 15–24-year-old ones, which agrees with earlier Finnish data obtained by skin prick testing young adults [23].

In an earlier study we determined the prevalence of *H. pylori* antibodies in the sera now examined for IgE antibodies [16]. We found a substantial decrease from 1973 to 1994 in the rate with which *H. pylori* antibodies occurred. The decline was strongest in young adults, which indicated childhood as the main risk period for new infections [16]. This risk period has been further defined in a recent follow-up study of Finnish subjects; two thirds of those who became *H. pylori* antibody-positive until 20 years of age, did so before the age of 7 years [24].

The increase in the prevalence rate of IgE antibodies that occurred from 1973 to 1994 was limited mainly to the subjects with no *H. pylori* antibodies, and it was especially strong in the youngest 10-year subgroup. However, the number of *H. pylori*-positive young adults was small in 1994. Nevertheless, the results are in agreement with those of Matricardi et al. [13], who showed that helicobacter antibodies were less common in the atopic than in the the non-atopic subjects. This difference was not significant as such, but adding the helicobacter data to the cumulative lifetime exposure index strengthened the conclusion that high exposure to orofecal and foodborne microbes (hepatitis A, toxoplasmosis and *H. pylori*) may exert effects that prevent atopy.

It has been suggested that measles [9,14], hepatitis A [10], *Toxoplasma gondii* [13] and *Mycobacterium tuberculosis* [11] infections, and the development of delayed hypersensitivity to tuberculin [12] may prevent atopic diseases. Of the subjects participating in the present study only those born in 1973–79 have participated in the comprehensive, national measles vaccination program started in 1975. Toxoplasmosis has always been a rare disease in Finland. In Finnish cohorts born after 1958 the prevalence rate of hepatitis A antibodies had dropped to less than one percent [25]. The incidence of tuberculosis infections has been on the decline in Finland for nearly 100 years, and since 1951 practically every newborn has been vaccinated with BCG at the age of 3–5 days. BCG-vaccinated children have been reported to have lower IgE levels than non-vaccinated children in Japan [12], but Swedish data suggest that early vaccination does not affect the development of atopic diseases [26]. Thus the decreasing contacts with these microbes may have had a small effect on the observed rise in IgE levels. We are not aware of other non-disputed evidence on the effects of other infectious diseases or vaccinations on IgE levels or IgE-mediated allergy other than the recent report of Henderson and coworkers, which found no link between pertussis vaccination and wheezing illnesses in young children [27].

The thought that *H. pylori* infection may counteract atopy is still a hypothesis. In the 1973 population sample *H. pylori* infection did not indicate any apparent protection against atopy. This absence, however, may be explained by similar effects of other infections. As many infectious diseases have become rarer, the effects of the remaining chronic *H. pylori* infections may have become easier to distinguish in 1994 than in 1973. In Finland, the socioecomic status, as measured e.g. by educational level and per capita income, improved considerably over our study period according to the national statistics [17,18], and the population of Vammala experienced the same changes. In fact, we believe that the reduction in, amongst others, *H. pylori* infection rate is a result of improved socioecomic status. Instead of considering improved socioecomic level as a confounder, it reflects the actual change in Finnish society, and may thus be an underlying cause of the decrease of *H. pylori* infection rate.

Epidemiological studies on the association between atopy and infections can not directly prove that the relationship between the decrease of any single infection and the increase of atopy would be a causal one. The decline of exposure to a rather broad category of microbes may have contributed to the increasing tendency towards immunological reactivity promoting IgE production, observed particularly in younger cohorts. To test the hypothesis, further data on infections and atopy are needed.

Our main conclusion is, that in the period from 1973 to 1994 there was a rise of allergen-specific IgE antibodies to four common inhalant allergens in Vammala, Finland. At the same time childhood *H. pylori* infections became less common. The data are in agreement with the hypothesis that *H. pylori* could be one of the microbes counteracting atopy, since the increase in IgE antibodies was seen mainly in the subpopulation with no *H. pylori* antibodies.

Acknowledgements

Supported by grants from the Helsinki University Central Hospital and the University of Helsinki. The help of Fred Björkstén in preparing the manuscript is acknowledged.

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