

# Infectious Burden as a Determinant of Atopy – A Comparison between Adults in Finnish and Russian Karelia

Leena C. von Hertzen<sup>a</sup> Tiina Laatikainen<sup>c</sup> Mika J. Mäkelä<sup>a</sup>  
Pekka Jousilahti<sup>c</sup> Timo U. Kosunen<sup>d</sup> Tuula Petäys<sup>a, c</sup> Pirkko J. Pussinen<sup>b, e</sup>  
Tari Haahtela<sup>a</sup> Erkki Vartiainen<sup>c</sup>

<sup>a</sup>Skin and Allergy Hospital and <sup>b</sup>Department of Oral and Maxillofacial Diseases, Helsinki University Central Hospital, <sup>c</sup>Department of Epidemiology and Health Promotion, National Public Health Institute, and <sup>d</sup>Haartman Institute and <sup>e</sup>Institute of Dentistry, University of Helsinki, Helsinki, Finland

## Key Words

Atopy · Finland · *Helicobacter pylori* · Hepatitis A · Hygiene hypothesis · Infections · Karelia · Russia

## Abstract

**Background:** Evidence of the influence of pathogen exposure on the development of atopy and atopic disease is not unequivocal. We investigated the association between markers of infections and occurrence of atopy among adults in eastern Finland and western Russia, two adjacent areas with profound differences in living conditions and lifestyles. **Methods:** Randomly selected adults aged 25–54 years from Finland (n = 790) and from Russia (n = 387) participated in the study. Skin prick tests were performed to 11 common airborne allergens, and at least one positive prick reaction was considered to indicate atopy. Antibodies to different pathogens including hepatitis A virus (HAV), *Helicobacter pylori*, *Toxoplasma gondii*, herpes simplex virus (HSV), *Chlamydia pneumoniae* and the periodontal pathogens *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* were measured. **Results:** In Finland 34.3% and in Russia 23.3% of the study population was atopic (p < 0.001). Sero-

prevalences to all these pathogens were significantly higher among the Russians. In multivariate logistic regression analysis, only *H. pylori* was inversely associated with atopy in Russia. A further stepwise analysis revealed that *H. pylori* alone can explain 32% of the difference in atopy between the countries, and *T. gondii*, *A. actinomycetemcomitans*, HSV and *C. pneumoniae* had a slightly additive effect, whereas, unexpectedly, seropositivity to HAV and, to a lesser extent, *P. gingivalis* had an opposite effect. The net result of the stepwise analysis showed that 44% of the difference in atopy between the countries could be explained by seropositivity to these seven pathogens. **Conclusions:** Seropositivity to select pathogens, particularly to *H. pylori*, could explain a substantial part of the difference in atopy prevalence between Finland and Russia. Exposure to HAV was not associated with protection against atopy in this adult population.

Copyright © 2006 S. Karger AG, Basel

This research has been funded by the Academy of Finland (grants Nos. 201346 and 37590) and by grants from the University of Helsinki (Nos. 2303 and 5201).

## Introduction

Since the original findings of an inverse association between exposure to certain pathogens and atopy at the end of the 1990s [1–3], numerous studies have been published to examine this association. The results of these studies have, however, been controversial. There is circumstantial evidence that pathogens that are spread by the orofecal route, such as hepatitis A virus (HAV), *Helicobacter pylori* and *Toxoplasma gondii*, can confer protection against atopy and atopic disease [4–7]. On the other hand, at least as many studies have not found any protective role for HAV or other such pathogens [8–11]. One study found an inverse association between HAV and atopy, but this association disappeared after adjusting for age [12]. With one exception [11], all of these studies have been performed in western countries and have comprised mostly young adults.

Exposure to orofecal pathogens is associated with poor hygiene, crowding and non-affluence [7, 13], factors which are frequently interrelated. In western societies, such exposure has become uncommon. In a recent survey, for example, the seroprevalence to HAV was only <5% and to *H. pylori* <7% among school children in Finland [Pekkarinen et al., unpubl.]. We have performed a population-based study in the Karelian area, in eastern Finland and western Russia, to examine the occurrence of atopy and atopic disease among adults, and found significantly higher rates of allergic symptoms, positive skin prick tests and specific IgE levels in Finland compared with Russia [14]. The aim of this study was to clarify the influence of exposure to pathogens, particularly of those spread by the orofecal route, on the development of atopy among adults in the same area. In a preliminary univariate analysis, we assessed the relationship between atopy and seropositivity to 22 pathogens, including also pathogens such as respiratory syncytial virus, parainfluenza virus, influenza A and B virus, adenovirus, *Mycoplasma pneumoniae*, measles virus, cytomegalovirus, varicella virus, enterovirus, rotavirus, *Campylobacter jejuni*, *Salmonella enteritidis*, *Salmonella typhi*, and *Yersinia enterocolitica*, but could not find an association for any of these 15 pathogens. We report here the results of the remaining seven pathogens, HAV, HSV, *H. pylori*, *T. gondii*, *Chlamydia pneumoniae*, *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*, which were significantly associated with atopy in the univariate analysis.

## Material and Methods

### Subjects

This study was part of the National FINRISK Study performed since 1972 every 5th year to monitor risk factors for chronic diseases in North Karelia, Finland, and since 1992 the surveys have been conducted also in the Republic of Karelia in Russia. Study subjects, aged 25–54 years, were randomly drawn from the population registers of both countries, and in 1997, 71% (1,070) of those recruited in Finland and 75% (560) of those in Russia participated in the survey. These subjects were invited to take part in a further allergy study in 1998, and 790 Finns and 387 Russians participated in this study. Occurrence of atopy and atopic diseases was investigated using questionnaires and by performing skin prick tests (SPT) as well as specific and total IgE measurements. The results have been published earlier [14]. Here, the association between serological markers of the above-mentioned pathogens and the occurrence of atopy is assessed. The study protocol was approved by the Ethics Committee of the National Public Health Institute, and informed consent was given by all participants. The subject and living characteristics of the participants are presented in table 1. Both populations are of Caucasian ethnicity. Irrespective of the proximity of the study areas and the similarity of the geo-climatic and vegetative conditions, the lifestyles and living conditions are, due to the huge economic gap between the areas, fundamentally different even today.

### Questionnaire

A standardized, self-administered questionnaire including items on demographic factors and home/living characteristics (building material and heating of current dwelling, presence of visible mold or mold odor in current dwelling, smoking habits, having a dog and/or cat) was completed before the study visit.

### Serum Antibody Measurements

Antibodies to HAV and HSV were measured with commercially available assay kits (Enzygnost anti-HAV No. OQEC115 and Enzygnost anti-HSV/IgG No. OWMX155; Dade Behring, Marburg, Germany). The tests were performed and the results interpreted according to the manufacturer's instructions.

Complement fixation was used to measure antibodies against *C. pneumoniae* and *T. gondii*. The antigens used were from Dade Behring (*C. pneumoniae*) and from Virion (Rüschlikon, Switzerland; *T. gondii*). The complement used was from Rubion Oy (Helsinki, Finland), and an in-house hemolytic system was applied. The sera were first screened in dilutions from 1:8 to 1:64 and further titrated up to 1:5,096 if needed. The sera which were regarded as anticomplementary in the screening were not studied further. A titer of 1:8 was defined as the limit of positivity for antibodies detected by the complement fixation test.

*Helicobacter* antibodies were determined by an EIA test (Pylo-ri-set® EIA-G, Orion Diagnostica, Espoo, Finland) according to manufacturer's instructions. Titers  $\geq 25.0$  were considered positive for *H. pylori* IgG antibodies.

IgG antibodies against periodontal pathogens *A. actinomycetemcomitans* and *P. gingivalis* were determined by multisero-type ELISA. Formaldehyde-killed whole cells of six serotypes of *A. actinomycetemcomitans* and three serotypes of *P. gingivalis* were used as antigens [15], and levels higher than 5.0 EU were considered positive.

**Table 1.** Subject and living characteristics of the Finns and the Russians

|                                                   | Finns<br>n = 790 | Russians<br>n = 387 | p value |
|---------------------------------------------------|------------------|---------------------|---------|
| Mean age, years (SD)                              | 40.8 (8.3)       | 39.7 (8.3)          | 0.025   |
| Males                                             | 344 (43.5)       | 139 (35.9)          | 0.013   |
| Occupation in agriculture and/or forestry         | 79 (10.1)        | 22 (5.7)            | 0.011   |
| Building material of the current dwelling         |                  |                     |         |
| Wood                                              | 452 (57.7)       | 135 (35.1)          | <0.001  |
| Stone                                             | 237 (30.3)       | 151 (39.2)          |         |
| Elements                                          | 70 (8.9)         | 99 (25.7)           |         |
| Heating of the current dwelling                   |                  |                     |         |
| Wood                                              | 57 (7.3)         | 134 (34.7)          | <0.001  |
| Central                                           | 356 (45.4)       | 242 (62.7)          |         |
| Electricity                                       | 93 (11.9)        | 2 (0.5)             |         |
| Visible mold or mold odor in the current dwelling | 68 (8.6)         | 124 (32.2)          | <0.001  |
| Cat currently                                     | 193 (24.5)       | 224 (58.3)          | <0.001  |
| Dog currently                                     | 267 (33.8)       | 152 (40.0)          | 0.054   |
| Cat and/or dog currently                          | 376 (47.6)       | 277 (71.6)          | <0.001  |
| Smokers                                           |                  |                     |         |
| Current                                           | 178 (22.6)       | 145 (37.5)          | <0.001  |
| Former                                            | 170 (21.6)       | 26 (6.7)            |         |
| Never                                             | 439 (55.8)       | 216 (55.8)          |         |

Current smokers: daily regular smoking. Figures in parentheses are percentages except for age.

The results of exposure to pathogens were given as occurrences of positive serology, irrespective of the actual titer value.

#### Skin Prick Tests

Skin prick testing with a panel of 11 common airborne allergens (birch, timothy grass, mugwort, cat, dog, horse, cow, *Alternaria alternata*, *Cladosporium herbarum*, *Dermatophagoides pteronyssinus* and cockroach) was carried out using Soluprick solutions (ALK and Vivodiagnostic, Denmark) with positive (histamine dihydrochloride) and negative (solvent) controls throughout the study, as described earlier in detail [14]. A person was regarded as atopic if he/she had at least one positive test result with a wheal diameter  $\geq 3$  mm.

#### Statistical Analyses

Statistical analyses were performed by the SAS program, version 6.12 (SAS Institute, Cary, N.C., USA) using the  $\chi^2$  test for categorical data, t test for normally distributed data and analysis of variance adjusted for age and sex to assess the differences in seropositivities. The multivariate logistic regression model was used to identify explanatory variables for atopy, and a stepwise regression model to evaluate the magnitude of the fraction of each seropositivity to explain the difference in atopy prevalence between the countries. Explanatory power of seropositivity to the studied pathogens was tested by the reduction of explained variance of atopy prevalence that country alone provided. All regression models were adjusted for age and sex. A two-tailed p value  $<0.05$  was considered significant.

## Results

Atopy (at least one positive prick test result) occurred in 34.3% of the Finns and in 23.3% of the Russians (adjusted for age and sex,  $p < 0.001$ ), and the adjusted risk for atopy was 1.8-fold in Finland compared with Russia (odds ratio, OR, 1.78, 95% confidence interval, CI, 1.34–2.35). Seropositivity to all the seven pathogens was significantly higher in Russia than in Finland, particularly the differences for HAV and *H. pylori* seroprevalences were substantial (7.8-fold and 3.5-fold, respectively; table 2).

Multivariate logistic regression analysis to identify factors that are independently associated with atopy revealed that in this adult population, only exposure to *H. pylori* was inversely associated with atopy in Russia (OR 0.33, 95% CI 0.14–0.76). In addition, a weak inverse association of borderline significance ( $p = 0.065$ ; table 3; albeit significant in univariate regression analysis,  $p = 0.037$ ) was found for *C. pneumoniae* in Finland. Age was inversely associated with atopy in both countries, but female gender only in Russia (table 3). To examine the strength of each seropositivity to explain (reduce) the difference in atopy prevalence between the countries, a stepwise explanatory fraction analysis was performed. It re-

**Table 2.** Occurrence of IgG seropositivity (%) to different pathogens among the Finns and the Russians, without and with stratification by atopy

|                                 | Finns<br>n = 775 | Russians<br>n = 387 | p value | Finns             |                       |         | Russians         |                       |         |
|---------------------------------|------------------|---------------------|---------|-------------------|-----------------------|---------|------------------|-----------------------|---------|
|                                 |                  |                     |         | atopic<br>n = 268 | non-atopic<br>n = 507 | p value | atopic<br>n = 90 | non-atopic<br>n = 297 | p value |
| <i>T. gondii</i>                | 7.0              | 20.1                | <0.001  | 5.6               | 7.8                   | 0.296   | 18.0             | 20.8                  | 0.573   |
| HSV                             | 65.2             | 97.9                | <0.001  | 62.7              | 66.5                  | 0.623   | 100.0            | 97.2                  | 0.092   |
| HAV                             | 10.4             | 81.6                | <0.001  | 7.5               | 11.9                  | 0.411   | 85.1             | 80.5                  | 0.089   |
| <i>H. pylori</i>                | 26.2             | 92.5                | <0.001  | 23.1              | 27.8                  | 0.526   | 86.5             | 94.3                  | 0.011   |
| <i>A. actinomycetemcomitans</i> | 40.9             | 64.6                | <0.001  | 36.9              | 43.0                  | 0.165   | 65.6             | 64.3                  | 0.317   |
| <i>P. gingivalis</i>            | 28.3             | 68.5                | <0.001  | 25.8              | 29.7                  | 0.637   | 71.1             | 67.9                  | 0.210   |
| <i>C. pneumoniae</i>            | 5.2              | 10.7                | <0.001  | 3.0               | 6.4                   | 0.032   | 10.1             | 10.9                  | 0.859   |

p values are adjusted for age and sex.

**Table 3.** Explanatory factors for the occurrence of atopy in Finland and Russia derived from multivariate logistic regression analysis adjusted for age and sex

| Variable                        | Finns            |         | Russians         |         |
|---------------------------------|------------------|---------|------------------|---------|
|                                 | OR (95% CI)      | p value | OR (95% CI)      | p value |
| Age                             | 0.97 (0.95–1.00) | 0.015   | 0.95 (0.92–0.99) | 0.008   |
| Sex                             | 1.04 (0.75–1.45) | 0.818   | 0.35 (0.18–0.67) | 0.002   |
| Seropositivity to               |                  |         |                  |         |
| <i>H. pylori</i>                | 0.84 (0.58–1.24) | 0.383   | 0.33 (0.14–0.76) | 0.010   |
| HAV                             | 0.78 (0.43–1.42) | 0.411   | 1.92 (0.91–4.05) | 0.087   |
| HSV                             | 0.90 (0.64–1.27) | 0.548   | –                | –       |
| <i>A. actinomycetemcomitans</i> | 0.76 (0.54–1.06) | 0.101   | 1.22 (0.69–2.13) | 0.497   |
| <i>P. gingivalis</i>            | 0.95 (0.65–1.39) | 0.798   | 1.44 (0.79–2.64) | 0.238   |
| <i>T. gondii</i>                | 0.88 (0.46–1.68) | 0.706   | 0.88 (0.46–1.68) | 0.702   |
| <i>C. pneumoniae</i>            | 0.46 (0.20–1.05) | 0.065   | 0.96 (0.41–2.24) | 0.925   |
| Mold in the current dwelling    | 0.77 (0.42–1.40) | 0.392   | 0.89 (0.50–1.58) | 0.694   |
| Cat currently                   | 0.84 (0.57–1.24) | 0.378   | 1.90 (1.10–3.28) | 0.021   |
| Dog currently                   | 0.97 (0.68–1.37) | 0.848   | 0.97 (0.55–1.72) | 0.921   |
| Wood heating                    | 1.01 (0.71–1.43) | 0.955   | 1.19 (0.66–2.15) | 0.561   |
| Smoking ever                    | 1.10 (0.79–1.54) | 0.575   | 0.58 (0.30–1.11) | 0.100   |

vealed that seropositivity to *H. pylori* alone explained 32% of the difference in atopy between Finland and Russia. When *A. actinomycetemcomitans*, *T. gondii*, HSV and *C. pneumoniae* were included into the model, they further decreased the difference in atopy between the countries by 8, 7, 2 and 4%, respectively, whereas seropositivity to HAV and *P. gingivalis* increased the difference by 6 and 3%, respectively. The net cumulative effect of adding all seropositivities into the model explained 44% of the difference in atopy prevalence between the

countries (table 4). Except having currently a cat, which was positively associated with atopy in these Russian adults, no other associations for various current housing and living characteristics, including exposure to molds, having a dog, wood heating or smoking ever, were found (table 3). Even if current smokers (defined as daily regular smoking) and former smokers were analyzed separately, no association with atopy was found (data not shown).

**Table 4.** Reduction in OR for country (adjusted for age and sex) predicting atopy by stepwise addition of seropositivity to different pathogens into the model

|                                           | OR   | 95% CI    | Change in OR, % |
|-------------------------------------------|------|-----------|-----------------|
| No antibodies in the model (only country) | 1.78 | 1.34–2.35 | 100             |
| Stepwise addition of seropositivities     |      |           |                 |
| <i>H. pylori</i>                          | 1.53 | 1.07–2.21 | –32             |
| <i>A. actinomycetemcomitans</i>           | 1.47 | 1.01–2.13 | –40             |
| <i>T. gondii</i>                          | 1.41 | 0.97–2.05 | –47             |
| HSV                                       | 1.40 | 0.96–2.06 | –49             |
| <i>C. pneumoniae</i>                      | 1.37 | 0.92–2.02 | –53             |
| <i>P. gingivalis</i>                      | 1.39 | 0.93–2.07 | –50             |
| HAV                                       | 1.43 | 0.87–2.37 | –44             |

## Discussion

This study provided further evidence that exposure to pathogens may reduce the risk of atopy. A significant proportion of the difference in atopy prevalence between the countries here could be explained by exposure to pathogens mostly indicating low socioeconomic status and poor hygiene. Seropositivity to *H. pylori* alone explained most of this difference in atopy prevalence, although *T. gondii*, *A. actinomycetemcomitans*, HSV and *C. pneumoniae* could all decrease the difference further in the stepwise model. By contrast, HAV and, to a lesser extent, *P. gingivalis* affected to the opposite direction. Seropositivity to HAV showed also a tendency to a positive association with atopy in Russia in multiple regression analysis.

Our finding concerning HAV is in sharp contrast with previous results from Denmark, Italy and USA [4–6], albeit in line with those reported from a German-Spanish study [9] and two recent studies from England [8] and Spain [12]. This tendency to a positive association between atopy and HAV in Russia is either a chance finding or it has a true biological basis. The importance of timing of exposure in the gene-environment interactions and the development of atopy has increasingly been recognized. An association between the TIM-1 gene and atopy has recently been reported [16]. This gene is also the receptor for HAV, and a further analysis has shown that individuals with a certain variant of TIM-1 are protected against atopy but only if HAV infection has occurred before the development of atopy [13]. Even among adults in Russia, HAV seropositivity increased along with age (data not

shown), and the infection appears mostly to be acquired in adolescence and adulthood [Pekkarinen et al., unpubl.].

Seropositivity to *H. pylori* was inversely associated with atopy in Russia, where this pathogen still occurs commonly; nearly all Russians were seropositive to *H. pylori*, whereas this was true for only a quarter of the adults in Finland. Results obtained from schoolchildren have provided evidence that *H. pylori* is mostly acquired in childhood [Pekkarinen et al., unpubl.]. This pathogen also readily establishes chronic infection, contrary to HAV [17]. The chronic nature of *H. pylori* infection may be one major factor in its ability to reduce the risk of atopy. Increased IL-10 production appears to be a characteristic feature of many chronic infections [18, 19]. IL-10 has been proposed to be the necessary differentiation factor for the development of inducible regulatory T cells [19, 20], which in turn appear to be crucial for the development and maintenance of immunological homeostasis [20, 21]. Data obtained from murine models also support the view that *Helicobacter* is able to induce regulatory T cells [22]. This finding that *H. pylori* is inversely associated with atopy is in line with many [4–7], albeit not all [8–10, 23] of the earlier results. The very high explanatory fraction of 32% for *H. pylori* alone, as compared with other pathogens here, points to the importance of this microorganism as an immunomodulator in reducing the risk of atopy in Russia. Irrespective of its well-established role in gastric pathology, 85–90% of all *H. pylori*-infected persons remain asymptomatic [24]. This microorganisms may have been part of the indigenous gut microflora of humans for millions of years, but is being gradually eliminated due to profound changes in our modern life [25].

An anecdotal finding here was the weak inverse association between *C. pneumoniae* and atopy, only found in Finland. Our results are in line with those reported recently by Normann et al. [26]. They found that exposure to *C. pneumoniae*, demonstrated by PCR in throat swabs, provided protection against allergy (defined as symptoms of hypersensitivity to fur or pollen) during the 4-year follow-up among Swedish children. The effect was most apparent among children with atopic heredity [26]. *C. pneumoniae* is an intracellular pathogen that, similar to *H. pylori*, has the tendency to establish chronic infections difficult to eradicate [27]. A totally different issue is its ability to aggravate adult asthma, being mostly non-atopic [28]. Similar to the case of HAV, the effect of *C. pneumoniae* on atopy is obviously timing dependent with respect to sensitization. This probably also applies

for HSV and *T. gondii*, which are similarly known to readily establish chronic infection (*T. gondii*) or latency with the potential for subsequent reactivations (HSV) [29]. Another recent Swedish study [30] showed that acquisition of the Epstein-Barr virus during the first 2 years of life is associated with a reduced risk of atopy, and this effect was enhanced by cytomegalovirus coinfection. Both Epstein-Barr virus and cytomegalovirus belong to the family of Herpesviridae [29].

The present study is among the first to examine the association between periodontal pathogens and atopy. *A. actinomycetemcomitans* and *P. gingivalis* showed opposite effects in the stepwise regression model; seropositivity to the former decreased the (explained) difference in atopy prevalence between the countries whereas seropositivity to the latter increased it, similarly to seropositivity to HAV. This might be partly explained by the notion that *A. actinomycetemcomitans* is involved in fulminant periodontal diseases developing particularly at young age whereas *P. gingivalis* is the major periodontal pathogen among middle-aged or elderly people. However, both bacteria may also colonize at early age and can be found at low proportions in periodontally healthy people, raising the possibility that they are also members of the normal oral flora [31].

The strengths of this study include the unique setting; two neighboring study areas with an economic gap between that is considered as one of the widest in the world. This setting provided us an opportunity to compare the impact of factors associated with two different lifestyles, in this case markers of infections, on the occurrence of atopy. In addition, we screened seropositivity to a wide range of pathogens, including respiratory as well as orofecally and person-to-person spread pathogens, to identify those microorganisms that would have the greatest im-

pact on conferring protection against atopy. Some limitations should also be acknowledged. No information of the time point of infection/exposure was available. We either had no data on the magnitude of the exposure as the titer values were expressed using only the dichotomous positive/negative scale. In addition, whether the exposure was chronic or recurrent in nature, or an acute infection in the past, has not been determined. Nonetheless, we have assessed serological markers of exposure and atopy using objective, validated methods, which allowed us to reliably compare these two populations in areas with similar geoclimatic and vegetative conditions but leading fundamentally different lifestyles.

Although saprophytes in the environment and commensals in the gut appear to play a decisive role in the immunoregulatory network [32, 33], involvement of certain pathogens may not be ignored either. A common feature for all microorganisms that were able to explain the difference in atopy prevalence between the countries here is their tendency to establish chronic infections, but is not alone a sufficient factor. One important determinant appears to be the timing of exposure in relation to the development of atopy. Other factors may include dose and route of exposure and the concomitant overall microbial load the subject is exposed to [34, 35].

In conclusion, a large part of the significantly lower occurrence of atopy in Russia as compared with Finland could be explained by seropositivity to certain pathogens, particularly to *H. pylori*, in this adult population.

## Acknowledgments

The authors are indebted to Prof. Tapani Hovi (National Public Health Institute, Helsinki) for stimulating discussions, and Dr. Tiina Viikuna-Rautiainen (Dental Surgeon; University of Hel-

## References

- 1 Shaheen SO, Aaby P, Hall AJ, Barker DJ, Heyes CB, Shiell AW, Goudiaby A: Measles and atopy in Guinea-Bissau. *Lancet* 1996;347:1792-1796.
- 2 Matricardi PM, Rosmini F, Ferrigno L, Nisini R, Rapicetta M, Chionne P, Stroffolini T, Pasquini P, D'Amelio R: Cross-sectional retrospective study of prevalence of atopy among Italian military students with antibodies against hepatitis A virus. *BMJ* 1997;314:999-1003.
- 3 Shirakawa T, Enomoto T, Shimazu S, Hopkin JM: The inverse association between tuberculin responses and atopic disorder. *Science* 1997;275:77-79.
- 4 Matricardi PM, Rosmini F, Riondino S, Fortini M, Ferrigno L, Rapicetta M, Bonini S: Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. *BMJ* 2000;320:412-417.
- 5 Matricardi PM, Rosmini F, Panetta V, Ferrigno L, Bonini S: Hay fever and asthma in relation to markers of infection. *J Allergy Clin Immunol* 2002;110:381-387.
- 6 Linneberg A, Ostergaard C, Tvede M, Andersen LP, Nielsen NH, Madsen F, Frolund L, Dirksen A, Jorgensen T: IgG antibodies against microorganisms and atopic disease in Danish adults: the Copenhagen Allergy Study. *J Allergy Clin Immunol* 2003;111:847-853.
- 7 Kosunen TU, Höök-Nikanne J, Salomaa A, Sarnas S, Aromaa A, Haahtela T: Increase of allergen-specific immunoglobulin E antibodies from 1973 to 1994 in a Finnish population and a possible relationship to *Helicobacter pylori* infections. *Clin Exp Allergy* 2002;32:373-378.

- 8 Law M, Morris JK, Wald N, Luzynska C, Burney P: Changes in atopy over a quarter of century, based on cross sectional data at three time periods. *BMJ* 2005;330:1187–1188.
- 9 Uter W, Stock C, Pfahlberg A, Guillén-Grima F, Aguinaga-Ontoso I, Brun-Sandiumenge C, Krämer A: Association between infections and signs and symptoms of 'atopic' hypersensitivity – results of a cross-sectional survey among first-year university students in Germany and Spain. *Allergy* 2003;58:580–584.
- 10 Bodneer C, Anderson WJ, Reid TS, Godden DJ: Childhood exposure to infection and risk of adult onset wheeze and atopy. *Thorax* 2000;55:383–387.
- 11 Scrivener S, Yemaneberhan H, Zebeignus M, Tilahun D, Girma S, Ali S, McElroy P, Custovic A, Woodcock A, Pritchard D, Venn A, Britton J: Independent effects of intestinal parasite infection and domestic allergen exposure on risk of wheeze in Ethiopia: a nested case-control study. *Lancet* 2001;358:1493–1499.
- 12 Gonzalez-Quintela A, Gude F, Boquete O, Aguilera A, Rey J, Meijide LM, Fernandez-Merino MC, Vidal C: Association of hepatitis A virus infection with allergic sensitization in a population with high prevalence of hepatitis A virus exposure. *Allergy* 2005;60:98–103.
- 13 Umetsu DT, McIntire JJ, DeKruyff RH: TIM-1, hepatitis A virus and the hygiene theory of atopy: association of TIM-1 with atopy (review). *J Pediatr Gastroenterol Nutr* 2005;40(suppl 1):S43.
- 14 Vartiainen E, Petäys 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;109:643–648.
- 15 Pussinen PJ, Vilkkuna-Rautiainen T, Alftan G, Mattila K, Asikainen S: Multiserotype enzyme-linked immunosorbent assay as a diagnostic aid for periodontitis in large-scale studies. *J Clin Microbiol* 2002;40:512–518.
- 16 McIntire JJ, Umetsu SE, Macaubas C, Hoyte E, Cinniolu C, Cavalli-Sforza L, Barsh G, Hallmayer J, Underhill P, Risch N, Freeman G, DeKruyff R, Umetsu D: Hepatitis A virus link to atopic disease: association of TIM-1 with atopy. *Nature* 2003;425:576.
- 17 Swenson P: Hepatitis viruses; in Balows A, Hausler W Jr, Herrmann K, Isenberg H, Shadomy J (eds): *Manual of Clinical Microbiology*. Washington, American Society for Microbiology, 1991, pp 959–983.
- 18 Redpath S, Ghazal P, Gascoigne RJ: Hijacking and exploitation of IL-10 by intracellular pathogens. *Trends Microbiol* 2001;9:86–92.
- 19 Mills KH, McGuirk P: Antigen-specific regulatory T cells – their induction and role in infection. *Semin Immunol* 2004;16:107–117.
- 20 Hawrylowicz CM, O'Garra A: Potential role of interleukin-10-secreting regulatory T cells in allergy and asthma. *Nat Rev Immunol* 2005;5:271–283.
- 21 Umetsu DT, Akbari O, DeKruyff RH: Regulatory T cells control the development of allergic disease and asthma. *J Allergy Clin Immunol* 2003;112:480–487.
- 22 Kullberg MC, Jankovic D, Gorelick PL, Caspar P, Letterio JJ, Cheever AW, Sher A: Bacteria-triggered CD4<sup>+</sup> T regulatory cells suppress *Helicobacter hepaticus*-induced colitis. *J Exp Med* 2002;196:505–515.
- 23 Kolho ML, Haapaniemi A, Haahtela T, Rautelin H: *Helicobacter pylori* and specific immunoglobulin E antibodies to food allergens in children. *J Pediatr Gastroenterol Nutr* 2005;40:180–183.
- 24 Zevering Y: Vaccine against *Helicobacter pylori*? *Ann Med* 2001;33:156–166.
- 25 Blaser MJ: Helicobacters are indigenous to the human stomach: duodenal ulceration is due to changes in gastric microecology in the modern era. *Gut* 1998;43:721–727.
- 26 Normann E, Gnarp J, Gnarp H, Wettergren B: *Chlamydia pneumoniae* infection predicts a reduced risk for subsequent atopic disease. *Acta Paediatr* 2005;94:705–710.
- 27 von Hertzen LC: Role of persistent infection in the control and severity of asthma – focus on *Chlamydia pneumoniae* antibodies in adults. *Eur Respir J* 2002;19:546–556.
- 28 von Hertzen L, Töyrylä M, Ginishanov A, Bloigu A, Leinonen M, Saikku P, Haahtela T: The relationship between asthma, atopy and *Chlamydia pneumoniae* antibodies in adults. *Clin Exp Allergy* 1999;29:522–528.
- 29 Arvin A, Prober CG: Herpes simplex viruses; in Balows A, Hausler W Jr, Herrmann K, Isenberg H, Shadomy J (eds): *Manual of Clinical Microbiology*. Washington, American Society for Microbiology, 1991, pp 822–828.
- 30 Nilsson C, Linde A, Montgomery SM, Gustafsson L, Näsman P, Troye Blomberg M, Lilja G: Does early EBV infection protect against IgE sensitization? *J Allergy Clin Immunol* 2005;116:438–444.
- 31 Slots J: *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in periodontal disease: introduction. *Periodontology* 2000;1999;20:7–13.
- 32 Rook GA, Adams V, Hunt J, Palmer R, Martinelle R, Brunet LR: Mycobacteria and other environmental organisms as immunomodulators for immunoregulatory disorders. *Springer Semin Immunopathol* 2004;25:237–255.
- 33 Björkstén B: Effects of intestinal microflora and the environment on the development of asthma and allergy. *Springer Semin Immunopathol* 2004;25:257–270.
- 34 Herz U, Lacy P, Renz H, Erb K: The influence of infections on the development and severity of allergic disorders. *Curr Opin Immunol* 2000;12:632–640.
- 35 Liu AH: Endotoxin exposure in allergy and asthma: reconciling a paradox. *J Allergy Clin Immunol* 2002;109:379–392.