

Original article

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

Background: This study assessed the hypothesis that a decreased exposure to childhood infectious diseases is associated with signs and symptoms of 'atopic' hypersensitivity diseases.

Methods: A cross-sectional survey was performed among 1368 Spanish and German first-year university students between 1997 and 1999, including self-administered questionnaire data and serological tests.

Results: No association between the disease outcomes and hepatitis A, *Helicobacter pylori* infection and herpes simplex infection was observed in logistic regression analyses, adjusting for potential confounders (centre, sex, smoking, parental education). In contrast, vaccination against hepatitis B was associated with a decreased risk for the outcomes allergic rhinitis (OR 0.63, 95% CI: 0.42–0.95) and total serum IgE above 100 U/ml (OR 0.56, 95% CI: 0.39–0.81). Conversely, seropositivity to HBc antigen was significantly associated with high total serum IgE (OR 2.04, 95% CI: 1.34–3.06).

Conclusions: Our study partly confirmed and partly contradicted previous evidence and hypotheses, respectively, concerning the role of the infections considered. The observation of a decreased risk in persons vaccinated against hepatitis B warrants further, prospective investigation.

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Key words: atopic diseases; cross-sectional study; *Helicobacter pylori*; hepatitis A; hepatitis B; herpes simplex; immunoglobulin E; serology; vaccination.

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Considerable variation in the prevalence of 'atopic' diseases (allergic bronchial asthma, allergic rhinoconjunctivitis and atopic eczema/dermatitis syndrome) world-wide has been observed (1). These differences have been attributed at least partly to regional differences in childhood exposure to various infections (2). In addition, the increase in prevalence of atopic diseases observed in virtually all highly developed countries is considered to be because of decreased childhood exposure to infections in these countries (2). According to this concept termed 'hygiene hypothesis' [reviewed, e.g., in Ref. (3), recently refined by the 'anti-inflammatory network' theory (4)], at least some of these early infections prime the immune system in a process of maturation towards a more cell-mediated immunity (Th1 type response). However, there is conflicting evidence concerning the role of specific infections contracted during childhood. This motivated a multifactorial analysis of bi-national survey data studying the association between serological markers of infection and atopic diseases and sensitization in a sample of

healthy young adults. Infectious agents considered here include hepatitis A virus (HAV), hepatitis B virus (HBV), herpes simplex virus type I (HSV-1) and *Helicobacter pylori*, as well as serological markers of vaccination against hepatitis B.

Methods

Study populations

Students from three universities in Germany and Spain were enrolled in the survey, which included several other topics as well. German students at the University of Bielefeld (Universität Bielefeld, UB) were participants in a longitudinal health study and were invited for a follow-up by mail between December 1997 and January 1998. They were offered a financial incentive (EUR 20) for filling out a standardized questionnaire and for visiting the laboratory for venipuncture. The response rate was 48%. The sample consisted of 195 students and 188 blood samples were collected and analysed.

In Spain, the study population comprised 662 students at the Navarra Public University (Universidad Publica de Navarra, UPNA) and 508 students from the University of Navarra (Universidad de Navarra, UN), both located in Pamplona, who were recruited by advertisement at these campuses for a general students' health study between January and March 1999. The study targeted first-year students and 28% of all first-year students at these universities were recruited. A restaurant coupon for a sandwich and soft drink was offered as incentive for visiting the laboratory and filling out the questionnaire, followed by a medical examination, including venipuncture. Serum samples of 616 students from UPNA and 452 students from UN were analysed for the study. Participants gave their informed consent to data collection. All procedures were designed to protect students' privacy and allowed for anonymous participation. An anonymous feedback on the results of the laboratory tests was given at all study sites by providing the participants with individual anonymous codes with which their personal test results could be asked for.

Questionnaire

The self-administered questionnaire was developed and pilot-tested in German and then translated into Spanish. This was performed in duplicate by two independent translators, and the results were checked for inconsistencies. Then, the Spanish version was pilot-tested on Spanish students. The questionnaire addressed demographic information (age, sex, marital status, living situation, educational status of parents), self-reported health information, and a broad range of health behaviours including sexual behaviour. Using a standardized illness list (5), students were asked if they had the following atopic diseases in the last 12 months: atopic eczema/dermatitis syndrome, allergic asthma, allergic rhinitis and 'house dust or animal dander allergy'. Furthermore, participants were asked whether they had consulted a medical doctor because of these diseases. Smoking was assessed in three categories (never, occasionally, daily). Since in a previous study with university students only daily smokers had levels of urinary cotinine above detection threshold (5), daily smoking was contrasted to occasionally smoking, with 'never smoking' as reference category, in later analyses.

Laboratory methods

Anti-hepatitis A (HAV) antibodies were analysed with a commercially available qualitative ELISA test kit (DRG Instruments, Marburg, Germany). Antibodies against the core antigen (anti-HBc) of HBV were measured using a qualitative enzyme immunoassay (DRG Instruments, Marburg, Germany) and antibodies against the surface antigen (anti-HBs) were measured using a quantitative enzyme immunoassay from the same manufacturer with a cut-off of 10 U/ml. Samples positive for anti-HBc and negative for anti-HBs were tested for the presence of HBs antigen by a qualitative immunoassay (DRG Instruments, Marburg, Germany). As all tests for HBs antigens were negative, anti-HBc positive cases showed no indication of chronic HBV infection or carrier status. IgG antibodies against HSV-1 were analysed by a quantitative enzyme immunoassay (DRG Instruments) with a cut-off at 10 U/ml. Anti-*Helicobacter* IgG or IgA antibodies were analysed with a qualitative enzyme immunoassay (recomWell, Mikrogen, Martinsried, Germany). Total serum IgE was determined by a quantitative ELISA (DRG Instruments, Marburg, Germany). A high level of IgE was defined as >100 IU/ml. All laboratory tests were done in the laboratory at UB using a standardized protocol.

Statistical analysis

Data analysis included descriptive and bivariate analyses. Furthermore, to address the independent influence of potential risk factors, controlling for the potentially confounding effect of other factors, a logistic regression analysis was performed. As dichotomous outcomes, the self-reported presence of one of the three clinical diseases (with additional more restrictive case definitions requiring that the disease needed current treatment), a 'high' IgE level (>100 IU/ml), and reported sensitization against house dust mite or animal dander were chosen in the respective models. As explanatory factor, the presence of the respective serological marker was analysed; 'indeterminate' levels were considered positive in this analysis. As potential confounders sex, the university, socio-economic status ('high' approximated by 'at least high school degree' of father or mother as opposed to 'low' otherwise) and current smoking status ('daily', 'occasionally', reference: 'never smoking') were considered. Statistical analyses were performed with the SAS program package (version 8.2, SAS Institute, Cary, NC, USA).

Results

Sample characteristics

Altogether 1368 participants completed the study. The median age was 19, the upper and lower quartiles 18 and 20, respectively, and the 5% and 95% percentiles 18 and 24, respectively. The proportion of females was 64.7%, with little variation between the three universities (range: 62.5–67.2%). Altogether 30.6% of the student participants admitted to be daily smokers, 17.3% reported they would smoke occasionally and 52.1% stated that they never smoked. Higher education of at least one parent was reported by 49.0%.

Prevalence of atopic diseases and serological infection markers

The 1-year prevalence of 'atopic' diseases is shown in Fig. 1, including combinations of disease manifestations. Total serum IgE displayed a typical skewed distribution

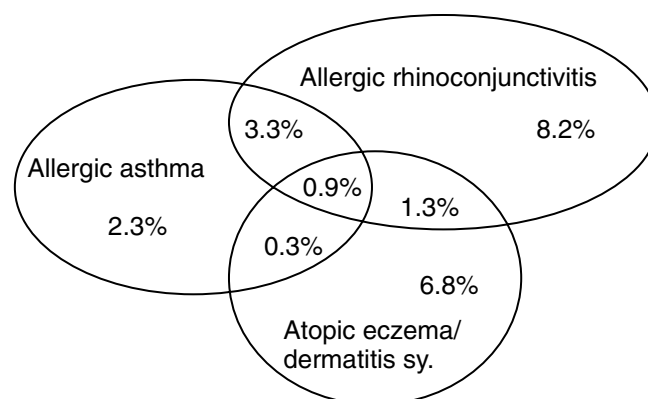


Figure 1. One-year prevalences of atopic diseases, including their combinations. None of the diseases was present in 76.9% of the participants.

Table 1. Serological results

	Positive	Indeterminate* (cutpoint $\pm$ 10%)	Negative	Missing or invalid result
Anti-HAV	109 (8.8%)	18 (1.4%)	1117 (89.8%)	124
Anti-HBc	157 (12.9%)	3 (0.3%)	1059 (86.9%)	149
Anti-HBs	842 (72.6%)	46 (4.0%)	273 (23.5%)	207
Anti- <i>Helicobacter pylori</i>	243 (19.7%)		990 (80.3%)	135
Anti-Herpes simplex type I	652 (53.8%)		559 (46.2%)	157
HBV vaccination (anti-HBs+/Anti-HBc-)	768 (66.3%)		391 (33.7%)	209

* Indeterminate results regarded as positive in further analyses.

with a median of 22.4, lower and upper quartiles of 6.6 and 64.5, respectively, and the 5% and 95% percentiles 0.1 and 311.3 IU/ml, respectively. In 131 cases total serum IgE had not been analysed. A 'high' level of total serum IgE was found in 16.8% of students. The prevalence of a high level of total serum IgE was significantly higher in German students (28.2%) than in Spanish students (14.8%) ($P < 0.0001$, Fisher's Exact Test).

Serological results concerning infections considered here are given in Table 1. There was virtually no association between hepatitis A and anti-HBs and anti-HBc serological status ($P > 0.05$, Fisher's Exact Test). Altogether 66.3% of persons had serological markers of vaccination against HBV, indicated by a positive anti-HBs and negative anti-HBc status. While 47.7% of persons who gave no history of herpes simplex (HSV) infection were seropositive to HSV, this proportion was 90.7% in those with a positive history.

Associations between markers of infection and atopic disease outcomes

Results in terms of the association between the different atopic disease outcomes and serological markers of (previous) infection and hepatitis B vaccination, respectively, are given in Table 2. Concerning HAV, *H. pylori* and HSV-1 infection there was no consistent pattern

indicative of increased or decreased risk. In contrast, HBV vaccination was associated with a decreased risk of most outcomes. These associations were significant for allergic rhinitis in the first case definition and total serum IgE above 100 U/ml. Conversely, a positive antibody status for anti-HBc was associated with a slightly, but consistently increased risk of disease manifestations, and a significant doubling of risk of having 'total serum IgE above 100 U/ml'.

Some confounding was noted in most of the 40 logistic regression models. Thus, these confounders were kept in the respective model.

In analyses stratified for country, considerable variation of the effect of HB vaccination was noted with regard to the risk of 'high total serum IgE' and 'allergic rhinitis', in that protective effects were found only in Spanish students. Similarly, anti-HBc seropositivity was a risk factor for 'high total serum IgE' in Spanish participants only.

Discussion

In our analysis of cross-sectional data from a sample of young adults, we found no evidence of an association between three of the infections analysed here and atopic diseases, namely, HAV, *H. pylori* and HSV-1 infections. In accordance with our findings, Scrivener et al. (6) and Bodner et al. (7) found that seropositivity to HAV was unrelated to atopic status; the latter study also showed no association with *H. pylori* infection. In contrast, Matricardi et al. found a lower prevalence of antibodies to hepatitis A virus (30 vs 16%, $P = 0.004$) and *H. pylori* (18 vs 15%, $P = 0.325$) in atopic study participants (a sample of male recruits) (8), even after adjustment for potential confounders (9). However, this study has also demonstrated a lack of association between atopy and HSV infection (9).

In contrast, previous vaccination against HBV with current immunity, i.e., anti-HBs titres above the threshold (10 U/ml), had a protective effect which was consis-

Table 2. Summary of results of 40 logistic regression models, analysing the association between infectious disease markers and 'atopic' diseases and findings, respectively, quantified with adjusted* OR with 95% confidence intervals

Severity†		Anti-HAV+	Anti-HBs+/anti-HBc-	Anti-HBc+	Anti-HSV+	Anti- <i>H. pylori</i> +
Bronchial asthma	1	1.14 (0.59–2.21)	0.88 (0.54–1.48)	1.38 (0.76–2.38)	0.93 (0.60–1.45)	0.99 (0.57–1.64)
	2	1.03 (0.35–2.43)	0.86 (0.45–1.75)	1.13 (0.45–2.43)	0.75 (0.41–1.39)	0.77 (0.33–1.58)
Allergic rhinitis	1	1.26 (0.74–2.07)	0.63 (0.42–0.95)	1.47 (0.90–2.33)	1.18 (0.83–1.67)	0.96 (0.62–1.44)
	2	1.27 (0.64–2.34)	0.61 (0.37–1.04)	1.26 (0.65–2.28)	1.29 (0.82–2.06)	0.84 (0.46–1.45)
Atopic eczema	1	0.61 (0.27–1.22)	1.08 (0.67–1.77)	1.24 (0.68–2.13)	1.02 (0.68–1.54)	1.05 (0.63–1.70)
	2	0.99 (0.33–2.34)	0.63 (0.31–1.33)	1.66 (0.70–3.53)	0.96 (0.52–1.79)	0.97 (0.41–2.02)
House dust or animal dander allergy		1.46 (0.86–2.34)	0.83 (0.55–1.26)	1.10 (0.65–1.77)	1.09 (0.77–1.54)	0.82 (0.52–1.25)
Total serum IgE >100 IU/ml		1.05 (0.63–1.68)	0.56 (0.39–0.81)	2.04 (1.34–3.06)	0.99 (0.72–1.35)	0.88 (0.58–1.30)

* Adjusted for centre (university), education of parents, sex, smoking status.

† Severity: 1 = reported disease, 2 = reported disease currently requiring treatment.

tent in all outcomes considered, albeit significant only for allergic rhinitis (outcome 1) and increased total serum IgE level. Persistent immunity is observed in about 85% of previously vaccinated persons 6.5 years later (10), a period relevant for our sample. Thus, a certain proportion of participants may have been vaccinated, but lost their immunity until examination. It is not unlikely that these 'intermediate' cases may have diluted the effect estimate. To our knowledge, there are no other studies analysing the association between HBV vaccination and atopy. Although our analysis included important confounders, the possibility of residual confounding cannot be dismissed: it is entirely possible that children who have been vaccinated differ from nonvaccinated children with regard to factors relevant to atopy, namely, the number of siblings or attendance of day care institutions (11) – factors we were unable to take into account in this survey.

Conversely, seropositivity to anti-HBc indicating, in view of negative HBs antigen in all participants, previous, resolved HBV infection, was associated with a nonsignificantly, but consistently increased risk of atopic diseases. One of the most pronounced findings of our analyses is a striking increase in the risk of having a high level of total IgE in persons with markers of HBV infection.

One possible explanation for a failure to detect a (significant) effect of infections on the risk of atopic diseases in our study might be the fact that we examined young adults, and not children, and our approach, using serological markers, cannot rule out that infection took place only recently. However, programming of Th-cell memory against allergens commonly occurs during early childhood (12); the critical time period during which immunomodulation with long-lasting effects is considered most successful is probably even within the first 2 years of life (13). Thus, some proportion of infections occurring later during childhood, or in adulthood, in our sample may have shifted risk estimates towards no effect, i.e., could have masked potentially protective effects. Besides, different average timing of individual vaccination against HBV in the two countries may have contributed to differential effects in our analysis.

Unfortunately, relatively little is known about the age of acquisition of the infections considered in our study. For *H. pylori* infection, this question has been investigated in a follow-up study in the US. According to this report, the prevalence was only 8% in 1–3-year-old children (with significant ethnic differences), whereas the prevalence of infection was 24.5% at the age 18–23, i.e., roughly the age of our sample (which had 19.7% seroprevalence) (14). Thus, the bulk of *H. pylori* infections are not acquired in the first few years of life in a developed country but at older ages, and may thus be less important than the early infections.

Furthermore, the set of infections considered here may be less important than other, e.g., bacterial, infections which are (repeatedly) contracted during early childhood, in particular if older siblings are in the family – it is well known that the risk of atopic disease declines with the number of older siblings (11).

In conclusion, our data add further evidence to feed the ongoing debate on the role of infections and vaccinations for the development of atopy. The finding that vaccination with HBs antigen possibly has a protective effect warrants further investigation in well-adjusted longitudinal analyses.

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