



Endotoxin levels in house dust samples and juvenile inflammatory bowel disease — a case–control study[☆]

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Abstract

Background and aims: Animal contact may reduce the risk not only for allergic diseases but also for inflammatory bowel diseases (IBD). We aimed to clarify the association between endotoxin levels in the home environment and IBD.

Methods: A case–control study including 85 IBD cases (aged: 8–29 years) and 91 controls undergoing strabismus surgery (aged: 11–23 years) was conducted in Southern Germany. A questionnaire and a dust sampling sock were mailed to the parents (Response: 71% among cases, 58% among controls). Endotoxin levels were determined using Limulus Amoebocyte Lysate assay tests. Logistic regression models adjusting for age, sex, high parental school education, family history of IBD, environmental factors in the first year of life (urban place of living, farm animal or pet contact), and presence of cats or dogs in the room were performed.

Results: Geometric mean levels of endotoxin were lower among cases (50.67 Endotoxin Units (EU)/mg; geometric standard deviation (GSD); 2.32) than controls (60.25 EU/mg; GSD: 2.22). Endotoxin levels were inversely, but not statistically significantly, related to case status in the multivariate logistic regression analysis (OR for the interquartile range increase: 0.70; 95%CI: 0.46–1.04).

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Conclusion: In our study sample, high endotoxin levels were inversely related to case status. Whether endotoxin is a marker of hygiene or causal needs further investigation.
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1. Introduction

Inflammatory bowel diseases (IBD) – with its main types Crohn's Disease (CD) and Ulcerative Colitis (UC) – are on the rise on a global level.¹ While pediatric-onset of UC remains stable, incidence of CD has significantly increased within the last decades.¹ In Germany are 300,000 people estimated to suffer from IBD, with 20% children and adolescents.²

Clearly, genetic background is important for the pathogenesis of IBD.^{3,4} The increasing incidences of IBD mainly in industrialized countries with Western life style,⁵ however, suggest a strong role of environmental factors.^{6,7} "Westernized" societies are characterized by a modern lifestyle, including less crowded living, better sanitation, vaccination, smaller family size, refrigeration, and lower level of microbiological burden.

The hygiene hypothesis has recently been associated with the increasing incidence of immune-mediated diseases, including Crohn's Disease.⁸ The hygiene hypothesis originating from asthma and allergy studies⁹ states that exposure to microbes¹⁰ has a stimulating effect on the immune system and is inversely related to the development of autoimmune diseases.^{11,12} Endotoxins are parts of the outer membrane of gram-negative bacteria and a marker of microbial compounds.¹⁰ Endotoxin levels can be increased by e.g. the presence of animals.^{13,14} In this context, farm animal contact was shown to be inversely related to allergies¹⁵ and IBD.¹⁶

The cause of the association between endotoxin levels and asthma and allergy has not yet been clarified,^{17–19} but results from the International Study on Asthma and Allergies in

childhood (ISAAC) study using cross-country samples suggest that the burden of endotoxin level is inversely related to asthma.²⁰

To our knowledge, no study has examined the relationship between microbial compounds and IBD using objective measures. The aim of this study was to report our findings on the association between burden of endotoxin and IBD in children and young adults aged 8 to 29 years.

2. Material and methods

We re-contacted 331 patients (147 IBD cases, 184 controls) previously participating in a multi-center case-control study on animal contact and IBD.¹⁶ Details of this study have been described previously.¹⁶ In brief, cases and controls of one of the study centers (Munich) who participated in the original study in 2006 and who then agreed to be re-contacted were invited to participate in the current study (Fig. 1).

In the original study, IBD cases were drawn from the gastroenterology unit of the Dr. Hauner Children's Hospital, University Hospital of Munich (LMU). Diagnosis of IBD was based on established diagnostic criteria for pediatric patients such as radiological, upper gastrointestinal endoscopy and investigations.²¹

Controls underwent strabismus surgery at the ophthalmic center of the University Hospital of Munich (LMU). Patients were included irrespective of age at diagnosis (cases) or date of strabismus surgery (controls). Both studies were approved

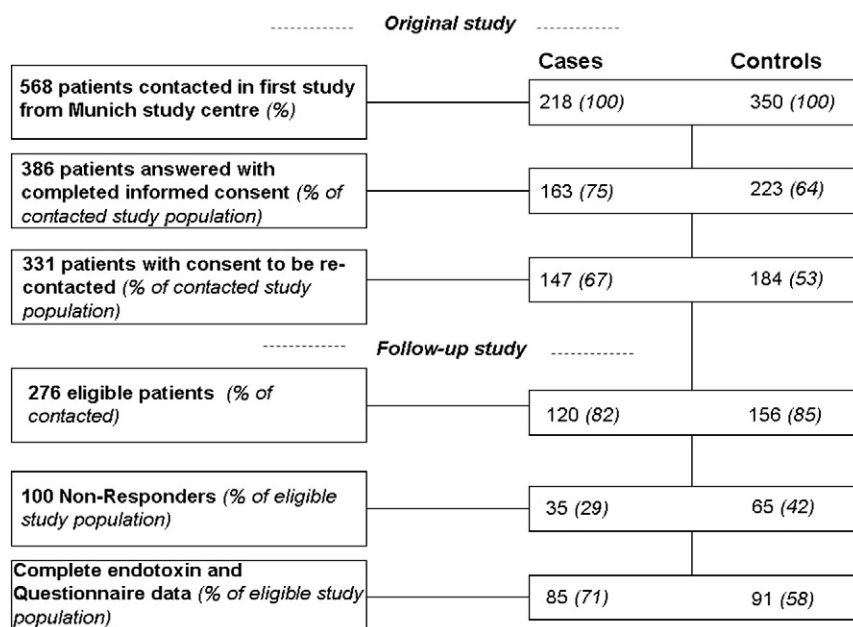


Figure 1 Recruitment of the study population (%) from an IBD case-control study.

by the ethics committee of the LMU. Parental or patient's written informed consent was obtained to merge the data of the original and the follow-up study.

Parents of the 147 case subjects and 184 control subjects were contacted between September 2009 and January 2010, by using a postal parental questionnaire including a dust sampling sock (Allied Filter Fabrics Pty. Ltd., Sydney, Australia). Ten cases and 20 controls were contacted during a pilot study to test the feasibility of the approach. Up to two reminders were set to all non-responders within three weeks after the questionnaire mailing. Additionally, non-responders were contacted via telephone up to five weeks after the first mailing. Those patients who could not be contacted via telephone were contacted again by mail. Fifty-five subjects (27 cases, 28 controls) were not eligible. The majority of the non-eligible patients (69%, $n=38$) had moved since the previous study. Furthermore, subjects born outside Germany ($n=2$), controls with self-reported IBD ($n=2$) or cases not reporting to have an IBD in the questionnaire ($n=3$) were excluded. Response was 71% among cases and 58% among controls (Fig. 1). Only cases and controls with complete questionnaire data and dust analyses ($n=176$ of $N=180$) were used for all subsequent analyses.

2.1. Exposure assessment

2.1.1. Questionnaire

Most of the items of the follow-up parental questionnaire were taken from preexisting validated questionnaire instruments (the International Study of Asthma and Allergies in Childhood²² or the Allergy and Endotoxin study²³). The self-reported questionnaire included questions about sociodemographic characteristics; patient's early and present environmental exposures (e.g. place of living, smoking exposure, pet and animal contact, number of siblings, day care attendance, and breastfeeding).

2.1.2. Dust sampling and measurement of endotoxin levels

Additionally, a dust sampling sock (Allied Filter Fabrics Pty. Ltd., Sydney, Australia) was sent to each participant. This method was cost-effective and practical as patients lived up to 150 km from the referral clinic. Parents were asked to take a dust sample with their residential vacuum cleaner from the living room. Fine dust was separated from large particles and fibers within three days after reception and kept at room temperature according to a standardized protocol.²⁴

All dust samples were processed and analyzed for their endotoxin content at the Institute and Outpatient Clinic for Occupational, Social-, and Environmental Medicine, University Hospital of Munich (LMU). Endotoxin levels were determined by Limulus Amoebocyte Lysate assay (LAL) (kinetic-QCL, Lonza Inc., Walkersville, MD, USA).²⁴ Endotoxin levels were expressed as endotoxin units (EU)/mg of dust.

2.1.3. Potential confounders

Age (in quartiles: 8–≤16 years/<16–≤19.4/19.4–≤21.2/years) of both cases and controls, sex, family history of IBD (at least one of the parents, siblings or grandfathers with IBD), high parental education (at least one of the parents with A-level degree or university degree vs. both parents

10 years or less of schooling), urban place of living¹⁶ regular farm animal (cattle, horses, pigs, goats, sheep, or poultry), pet (dog, cat, or rabbit) contact in the first year of life,¹⁶ and present dog (yes/no) or cat in the room (yes/no)^{14,25} were included as potential confounders.

2.2. Statistical analyses

The geometric mean (GM) and geometric standard deviation (GSD) of the endotoxin levels were calculated as data were not normally distributed. Cross-tabulation was used to describe bivariate distributions of covariates. Odds Ratios with 95% confidence interval for a change in endotoxin exposure corresponding to the interquartile range (IQR)²⁰ were calculated adjusting for potential confounders. The IQR, i.e. the difference between the third and first quartile, was 0.82 among the study population. We chose the IQR as it uses the middle 50% of the sample and because it is not affected by outliers. Unconditional analyses were used as cases and controls were frequency matched. All analyses were conducted using the SAS 9.2 software (SAS, Inc, Cary, North Carolina).

3. Results

Overall, 85 cases (57 Crohn's disease, 23 UC, 5 cases with indeterminate colitis) and 91 controls were included in the study (Fig. 1). On average, cases and controls were 19 years old (range among cases: 8–29, range among controls: 11–23 years). Cases were diagnosed on average at 11 years (Standard Deviation (SD): 3.88) and had been diagnosed with IBD on average for 8 years (SD: 3.76) (data not shown).

Cases were more likely to be male (61%, P^2_{χ} -test=0.09) and to report a family history of IBD (P^2_{χ} -test=0.07) than controls (Table 1).

3.1. Endotoxin results

On average, sampling time was 6 min (1–40 min) (data not shown). The amount of dust collected from the living room ranged from 38.30 to 120.10 mg, and was on average 93.5 mg among cases and 91.3 mg among controls, respectively (Table 1).

Endotoxin was detected in all samples (range: 0.7 EU/mg–3756.0 EU/mg) (data not shown). On average, among cases, geometric mean (GM) levels of endotoxin of 50.67 EU/mg dust (GSD: 2.31) were found, whereas dust samples from controls were 66.25 EU/mg dust (GSD: 2.22). MC patients had slightly lower endotoxin levels (Geometric mean: 48.91; GSD: 2.58) in comparison to UC patients (Geometric mean: 55.33; GSD: 1.76).

Results of the multivariate logistic regression analysis adjusting for potential confounders showed that endotoxin levels were borderline significant and inversely related to case status (adjusted Odds Ratio (aOR): 0.70; 95% Confidence Interval (CI): 0.46–1.04). Male sex (aOR: 2.17; 95%CI: 1.08–4.37) was significantly associated with case status. High parental school education (aOR: 1.63; 95% CI: 0.78–3.41), and a family history of IBD (aOR: 6.50; 95% CI: 0.74–57.08) were positively associated with IBD, albeit none of these factors were statistically significant associated (Table 2).

Table 1 Sociodemographic characteristics of the study population, bi- and multivariate logistic regression models (+95% confidence intervals).

Characteristic	Cases (n=85)	Controls (n=91)
Endotoxin measurements		
Initial weight (mean, standard deviation)	93.52 (14.96)	91.43 (17.4)
Endotoxin level, LAL (EU/mg) (geometric mean) (geometric standard deviation)	50.67 (2.32)	66.25 (2.22)
Age (years), n (%)		
8.2–≤16 (Reference)	22 (25.88)	22 (24.18)
<16.0–≤19.4	19 (22.35)	26 (28.57)
19.4–≤21.2	17 (20.00)	25 (27.47)
>21.2	27 (31.76)	18 (19.78)
Male, n (%)	52 (61.18)	44 (48.35)
Self-reported asthma	3 (3.53)	7 (7.87)
Urban place of living, n (%)	36 (42.35)	35 (38.46)
High parental school education, n (%)	40 (47.01)	39 (42.86)
At least one family member with IBD, n (%)	7 (8.24)	1 (1.10)
Regular (≥ once a week), n (%)		
farm animal contact ^a in the first year of life	9 (10.59)	12 (13.19)
pet contact ^b in the first year of life	23 (27.06)	34 (37.36)
Present in the room once a week, n (%)		
Dog	17 (20.48)	15 (18.52)
Cat	22 (27.5)	36 (43.37)

LAL, Limulus amebocyte lysate, NA not applicable.

^a Cattle, horses, pigs, goats, sheep, or poultry.^b Dog, cat, or rabbit.

4. Discussion

We carried out the present case–control study to examine the association between burden of endotoxin levels in the home environment and IBD in children and young adults. Our results suggest that endotoxin levels might be inversely related to IBD

and thus back up our hypothesis that similar environmental factors might be associated with both asthma and IBD.

To date, the final pathomechanisms on how endotoxin levels may decrease the occurrence of asthma and IBD remain unclear. It is suggested that asthma and IBD, being both chronic inflammatory diseases, share the same pathogenesis

Table 2 Bi- and multivariate logistic regression models (+95% confidence intervals).

Characteristic	Crude odds ratio (95% confidence interval)	Adjusted* odds ratio (95% confidence interval)
Endotoxin level (interquartile range increase in endotoxin exposure)	0.75 (0.54–1.05)	0.70 (0.46–1.04)
Age (years)		
8.2–≤16 (Reference)	1	1
<16.0–≤19.4	0.73 (0.32–1.69)	0.63 (0.25–1.63)
19.4–≤21.2	0.68 (0.29–1.60)	0.57 (0.21–1.56)
>21.2	1.5 (0.65–3.47)	1.37 (0.53–3.54)
Male	1.68 (0.92–3.07)	2.17 (1.08–4.37)
Urban place of living	1.18 (0.64–2.15)	1.06 (0.49–2.27)
High parental school education	1.19 (0.65–2.15)	1.63 (0.78–3.41)
At least one family member with IBD	8.08 (0.97–67.14)	6.50 (0.74–57.08)
Regular (≥ once a week)		
farm animal contact ^a in the first year of life	0.78 (0.31–1.96)	1.49 (0.47–4.79)
pet contact ^b in the first year of life	0.62 (0.32–1.18)	0.66 (0.28–1.52)
Present in the room once a week		
Dog	1.13 (0.52–2.46)	1.33 (0.50–3.53)
Cat	0.50 (0.28–0.95)	0.51 (0.24–1.11)

LAL, Limulus amebocyte lysate, *mutually adjusted for all other variables presented in the table.

^a Cattle, horses, pigs, goats, sheep, or poultry.^b Dog, cat, or rabbit.

or genetic background.^{26,27} Epidemiological data from asthma studies suggest that a decreased exposure to pathogenic infectious agents early or later in life could lead to an inappropriate inflammatory process, such as asthma or IBD.^{9,11,17} Endotoxin exposure might be one marker of such agents.

We could only assess current endotoxin exposure. This is a general problem studying IBD as the disease is too rare to do prospective cohort studies. However, our results may reflect long-term exposure.¹⁷ About 88% of the patients still lived with their parents and two thirds of the parents reported that they still lived in the same place as when the child was born (data not shown).

Another weakness of our results is that we measured endotoxin levels only once. As shown previously, one measurement of endotoxins might be imprecise.²⁸ However, in an epidemiological study like this it does not seem to be feasible to take more than one dust measurement without losing too many participants. On the other hand, endotoxin levels are independent from weather and climate conditions.²⁹ In order to increase reliability and validity of the endotoxin results, several dust samples should be taken. Therefore, we decided to take only one dust sample as in previous studies.^{17,30}

A limitation of our study is that we could only include one study region which represents about 20% of IBD patients of the formerly conducted multi-center study.¹⁶ Therefore, our sample size is – compared to other studies^{31,32} – relatively small which could explain why our results did not reach statistical significance. However, our results were sufficient to show a borderline inverse association between levels of endotoxin and IBD.

It could be argued that the onset of IBD could have changed daily living (e.g. dietary habits or cleaning). But, as patients are usually not aware of the level of endotoxin exposure in the home environment a potential change in exposure over time can be assumed to result in non-differential misclassification. Such a misclassification usually results in a bias towards the null. Therefore, we feel that the difference between cases and controls shown in our study can neither be attributed to exposure misclassification nor to change of exposure habits.

The patients of our study are hospital-based. Our results might not be representative for the general population or cases with less severe IBD. However, our study population, i.e. both cases and controls, were drawn from sources within the same area and controls can be assumed to reflect the underlying population of the cases.

In our study the study participants were invited to take the dust samples by themselves. It could be argued that this could have led to a potential sampling bias. As shown by Shram-Bijerk and colleagues,³³ the dust sampling done by parents for microbial agent exposure is as valid as done by field workers. Yet, we do feel that self sampling might have resulted in a low response of study participants.

In this case-control study we found an inverse, albeit not statistically significant association between endotoxin levels and the presence of inflammatory bowel disease in children and young adults. This gives further evidence that the hygiene hypothesis could be also relevant for IBD. Whether endotoxin is a marker of hygiene or causal has to be evaluated in future, large-scale studies.

5. Statement of authorship

KR, RS, and MK conceived of the study, and participated in its design and coordination. KR, SK, RVK, and MK revised the manuscript critically for important intellectual content. CH, AB, coordinated the study. AB performed statistical analysis and drafted the manuscript. All authors read and approved the final manuscript.

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