Intestinal archaea inversely associated with childhood asthma

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although to a greatly reduced extent compared with WT B_{mem} cells (see Fig E6, *A-C*, in this article's Online Repository at www.jacionline.org). Consistent with this, the few B_{mem} cells present in STK4-deficient patients were capable of differentiating into ASCs *in vitro*, although at reduced levels (see Fig E6, *D*). Interestingly, despite the impaired specific antibody secretion in mice with $Stk4^{Y88del/Y88del}$ B cells, STK4 deficiency did not quantitatively affect the ability of these SW_{HEL} B cells to generate plasma cells *in vivo* (Fig 2, *I-K*). Thus, similar to B cells from STK4-deficient patients, $Stk4^{Y88del/Y88del}$ B cells are able to differentiate into plasma cells, but these plasma cells do not secrete adequate amounts of specific antibody.

STK4 is a multifunctional kinase that phosphorylates multiple cellular proteins, including those in the Hippo signaling pathway.[E8,E9] Many of these substrates are also phosphorylated by its paralog, STK3, suggesting STK3 might functionally compensate for STK4 deficiency. Indeed, B-cell defects are more readily observed in *Stk3/Stk4* double-knockout mice.[E5] Interestingly, STK4 has been shown to phosphorylate FOXO1 and promote its nuclear localization,[E10] and FOXO1 was recently shown to be required for dark zone formation and GC maintenance.[E11-E13] However, although FOXO1 levels were decreased in $Stk4^{Y88del/Y88del}$ and $Stk4^{-/-}$ GC B cells, we could not rescue the GC defect through retroviral overexpression of *Foxo1* (see Fig E7 in this article's Online Repository at www.jacionline.org), suggesting that other mechanisms might also be involved.

Another limitation of our study is the small number of patients involved, which prevents any firm conclusion, especially regarding differences in serum immunoglobulin levels in our cohort of 9 patients and the previously reported 14 patients. Nevertheless, our data establish a B cell–intrinsic requirement for STK4 in humoral immunity in mice and human subjects.

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Intestinal archaea inversely associated with childhood asthma

To the Editor:

Both laboratory and epidemiologic studies have demonstrated the importance of the gut microbiota in immunologic development, childhood asthma, and allergic disease pathophysiology.[1] Although most studies have focused on bacteria, the methanogenic archaea that live alongside them are relatively understudied. Archaea are prokaryotic microorganisms belonging to their own domain of life distinct from bacteria and Eukaryota.

Methanobrevibacter smithii and *Methanosphaera stadtmanae* are the most abundant archaeal species in the gut.[2] Bang et al[3] and Blais-Lecours et al[4] demonstrated that *M stadtmanae* is particularly immunogenic in human cells and mice. Accordingly, we hypothesized that archaea, like bacteria, might play a role in childhood asthma or allergic disease risk, and in this letter we present the first epidemiologic evidence linking an archaeal species, *M stadtmanae*, with lower asthma risk.

We conducted cross-sectional analyses using fecal samples available at 6 to 10 years of age for a subset of children participating in the KOALA birth cohort study in The

TABLE I. Results for the presence of archaea (yes/no)

Exposure	Outcome	Sample size	OR (95% CI), crude*	OR (95% CI), adjusted*
M stadtmanae	Asthma	472	0.38 (0.10-1.10)	**0.32 (0.08-0.98)**
	Eczema	472	0.72 (0.34-1.46)	0.68 (0.30-1.42)
	Aeroallergen sensitized	355	1.04 (0.45-2.30)	0.83 (0.32-2.02)
	Food allergen sensitized	354	0.82 (0.27-2.09)	0.69 (0.21-1.95)
M smithii	Asthma	472	1.17 (0.63-2.27)	1.09 (0.56-2.20)
	Eczema	472	1.38 (0.86-2.25)	1.32 (0.81-2.18)
	Aeroallergen sensitized	355	0.96 (0.57-1.63)	1.05 (0.59-1.89)
	Food allergen sensitized	354	1.03 (0.56-1.94)	1.30 (0.68-2.59)

Boldface text indicates which results reached statistical significance, $P < .05$.

*The crude model is adjusted only for study design–related variables: fecal sampling round and recruitment group. The fully adjusted model also included birth mode and place, antibiotic exposure, farm animal exposure, cat/dog exposure, body mass index z score, maternal age at delivery, maternal education, parental atopy, sibling atopy, sex, organic dairy consumption, and abundance of the other archaeal species.

TABLE II. *M stadtmanae* abundance and asthma risk (sample size = 472)

M stadtmanae abundance	No.	Asthma prevalence (%)	OR (95% CI), crude	OR (95% CI), adjusted
Absent	433	17.3	1.00 (reference)	1.00 (reference)
Low abundance	23	13.0	0.37 (0.08-1.35)	0.43 (0.08-1.73)
High abundance	16	6.2	0.40 (0.02-2.03)	0.20 (0.01-1.18)
P value for linear association with interval-scale abundance categories			.096	**.036**

Boldface text indicates which results reached statistical significance, $P < .05$.

Netherlands, as shown in Fig E1 in this article's Online Repository at www.jacionline.org. The KOALA study, which is described in detail elsewhere,[5] consists primarily of children (n = 2359) whose mothers were recruited as healthy pregnant women from an ongoing prospective cohort study on pregnancy-related pelvic girdle pain. Additionally, an "alternative recruitment group" of 496 women was recruited through advertisements in organic food shops, anthroposophic doctors and midwives, Steiner schools, and dedicated magazines. The KOALA study was approved by the Medical Ethical Committee of the Maastricht University/University Hospital of Maastricht and the National Ethical Committee for Medical Research. All parents signed informed consent forms.

We requested childhood fecal samples from 1432 families, targeting those who had already provided other biosamples, such as maternal and child blood samples. We also oversampled for 27 asthmatic patients from within the KOALA cohort to increase statistical power. We received 672 samples and excluded samples with transport times of greater than 4 days, leaving 472 available for analysis. We quantified *M smithii* and *M stadtmanae* in these samples by using quantitative PCR and targeting regions of the 16S rRNA genes specific for these species.[5]

All 472 children had data on asthma and eczema diagnoses, symptoms, and medication use from up to 4 parentally completed questionnaires spanning 6 to 10 years of age. Three hundred fifty-five children had total and specific serum IgE measurements (with 1 missing for food allergens), and 337 had spirometric lung function data. These blood samples and spirometric readings were taken during home visits at 6 to 10 years of age by trained research nurses. Serum specific IgE levels defined aeroallergen and food allergen sensitization outcomes, and the normalized FEV_1/forced vital capacity ratio was used as a continuous lung function outcome. Asthma diagnoses were based on modified International Study of Asthma and Allergies in Childhood questionnaires requiring a reported physician's diagnosis, as well as reported recent symptoms or medication use. Further details of data collection protocols and outcome definitions are included in the Methods section in this article's Online Repository at www.jacionline.org.

Asthma and eczema are overrepresented in the study population relative to the whole KOALA cohort, as was expected from the oversampling of asthmatic children (16.7% vs 10.4% and 34.1% vs 28.5%, see Table E1 in this article's Online Repository at www.jacionline.org). A larger proportion of the study population (24.8%) are from the alternative recruitment group than in the overall KOALA cohort (17.4%). This was also expected because the alternative recruits are more likely than the conventional recruits to have early-life biosamples, such as infant blood, because KOALA's alternative recruitment program began at approximately the same time as the biosampling program. Early-life biosample availability was a criterion for inclusion in the childhood fecal sampling program.

Further description of the study population can be found in Table E3 in this article's Online Repository at www.jacionline.org.

M stadtmanae and *M smithii* were detectable in 8.3% and 78.2% of samples, respectively.[6] Because abundance distributions of both *M stadtmanae* and *M smithii* followed an apparent trimodal pattern (see Fig E2 in this article's Online Repository at www.jacionline.org), we also classified participants into 3 abundance categories.

We tested for associations between the presence of each archaeal species and the risk of our primary outcome of asthma using logistic regression models adjusted for multiple potential confounders (Table I). We adjusted for recruitment group and fecal sampling round in all analyses. No significant interaction between recruitment group and archaeal exposure was found, and therefore pooling these groups was considered appropriate. Analyses involving serum IgE data were also adjusted for analytic batch numbers.

The vast majority of participants had no missing covariate data. Seven covariates had 5 or fewer missing data, which we imputed with the most common category or mean value. Organic dairy consumption was imputed for 19 children by using the categories predicted by using a regression model based on prior dietary and lifestyle information.

Our results indicate that the presence of *M stadtmanae* is associated with a lower risk of asthma at 6 to 10 years of age (adjusted odds ratio [OR], 0.32; 95% CI, 0.08–0.98; *P* = .045; Table I). Furthermore, the risk of asthma showed a monotonic decrease over the 3 categories of *M stadtmanae* abundance (adjusted *P* = .036 for linear trend over the *M stadtmanae* abundance categories, Table II). No significant interaction was found between *M stadtmanae* exposure and parental asthma history (*P* = .495), suggesting that the association does not significantly differ by parental asthma status.

For secondary outcomes, *M stadtmanae* also appeared to be associated with a slightly lower likelihood of eczema, aeroallergen sensitization, and food allergen sensitization, but these associations did not reach statistical significance (Table I). *M stadtmanae* was not associated with either of the continuous outcomes of total serum IgE or FEV_1/forced vital capacity *z* score (see Table E4 in this article's Online Repository at www.jacionline.org). We found no significant associations between *M smithii* and any outcome (Tables I and see Table E4). We also conducted a complete case analysis that produced similar results (see Table E5 in this article's Online Repository at www.jacionline.org).

Additionally, to check whether the oversampling of asthma cases in the second sampling round had introduced any bias, we repeated the analyses with only the 372 children sampled in the first round (see Table E6 in this article's Online Repository at www.jacionline.org). Similar ORs were observed in this subgroup (eg, *M stadtmanae* presence and asthma: OR, 0.39; 95% CI, 0.06-1.60). The lower precision observed was expected from the smaller sample size and fewer asthma cases, and this sensitivity analysis otherwise showed no evidence of any bias introduced by the oversampling.

Because of the low prevalence of *M stadtmanae* and cross-sectional study design, our results should be interpreted with caution. Although bacterial species not examined in our analysis might confound this association, we speculate that a mechanistic link between *M stadtmanae* and allergic disease appears plausible. Prior evidence demonstrates the airway and gut immunogenicity of *M stadtmanae* in mouse models and human cells.[3,7] Epidemiologic evidence also indicates a timing-dependent relationship between asthma risk and exposure to farm environments[8] where *M stadtmanae* is present.[9] However, farm exposure has also been associated with risk of atopy, which our study does not show.

Exposure to immunogenic bacterial species in infancy has been shown to be tolerogenic and reduce asthma risk.[1] Our study identifies an association between the presence of *M stadtmanae* at school age and lower asthma risk, leading us to speculate that early-life archaeal exposures might also be tolerogenic. Therefore we encourage further research into the immunologic roles of archaea.

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Prophylactic allergen immunotherapy with Der p 2 prevents murine asthma by regulating lung GM-CSF

To the Editor:

The prevalence of allergic diseases is increasing, urging new ways of prevention. Allergen immunotherapy (AIT) is currently the only clinical intervention that can alter the natural course of allergy and can offer long-term clinical benefit. Although AIT is traditionally used as a treatment in patients with established disease, the National Institutes of Health's Immune Tolerance Network has proposed that prophylactic AIT could also be used as a primary prevention for new sensitizations and allergic disease in high-risk children born to atopic parents and with a personal