

Infection with *Mycobacterium tuberculosis* Is Inversely Associated with Childhood Asthma

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Early-life exposures to microbes, coupled with genetically determined susceptibility, have an impact on the natural history of childhood asthma. We hypothesized that childhood infection with *Mycobacteria tuberculosis*, a bacterium that has infected *Homo sapiens* for close to millennia, may be relevant to the risk of asthma development. The aim of this study was to evaluate any associations between tuberculosis infection with asthma and allergies using the cross-sectional and the U.S. nationally representative 1999–2000 National Health and Nutrition Examination Survey. Adjusted odds ratios (ORs) for having a history of asthma, allergic rhinitis, or atopic allergic symptoms were compared to evidence of tuberculosis infection as determined by the presence of tuberculin skin test results of 10 mm or greater. Children <20 years of age infected with tuberculosis were significantly less likely to have a history of asthma [OR 0.2; 95% confidence interval (CI): 0.0–0.9] or symptoms of asthma over the prior year (OR 0.1; 95% CI: 0.0–0.5) than children not infected with tuberculosis. This finding was not confounded by bacille Calmette-Guerin vaccination. Tuberculosis infection is associated with a substantially decreased risk of asthma in children. This finding is consistent with the hygiene hypothesis, which suggests that microbes that co-evolved with humans may influence developing immune systems in ways that have a protective effect against the development of asthma.

Introduction

ASTHMA AFFECTS OVER 300 million people worldwide¹ and is the most common chronic disease in childhood. The increasing prevalence of asthma over the last few decades represents a major public health concern.^{2–4} Both genetic and environmental factors contribute to asthma development. For many with allergic asthma, the disease begins in early childhood, and researchers have suggested that the lack of exposure to bacteria and parasites has led to a dysregulated immune response in individuals affected by allergies and asthma.^{5–8} This theory, called the “hygiene hypothesis,” is supported by observations that people in resource-rich regions have more allergies and autoimmune diseases than individuals in resource-poor regions.^{9,10}

According to the hygiene hypothesis, microbial exposures of the respiratory and gastrointestinal tracts early in life prompt immune maturation and may inhibit the development of allergic diseases and asthma.^{6,11,12} Subsequently, children raised in modern metropolitan life styles, relatively reduced in the natural microbial burden, may have unbalanced immune systems in infancy, thereby allowing for an allergic march—a pattern of pro-allergic immune development and disorders that occurs in early life. Studies have shown the use of antibiotics during infancy correlated with an

increased risk of developing asthma, suggesting that bacterial infections early in life may help to inhibit asthma development.^{13,14} There also exists epidemiologic evidence connecting vaginal versus cesarean delivery with a decreased risk of atopy compared and earlier development of a pro-tolerant gut microbiota.¹⁵ Likewise, inverse relationships were observed between the presence of endogenous bacteria, *Helicobacter pylori*, and both asthma and atopic conditions in children.^{16,17}

Mycobacteria tuberculosis (M. tb) infects one third of the world's population. New models are in a broad agreement that human tuberculosis (TB) originated at least 35,000 years ago,¹⁸ and phylogenetic evidence from molecular studies of the entire M. tb complex indicates an African origin, perhaps more than 2.5 million years ago.¹⁹ According to the hygiene hypothesis, microbes that co-evolved with humans influenced the evolution of their immune systems.²⁰ Among individuals infected with M. tb, only about 5%–10% progress to active disease, and the rest of infected individuals remain asymptomatic. Progression generally occurs in individuals with less-effective immune systems such as the very young, old, or those who suffer from malnourishment or other diseases such as HIV infection. The high prevalence of asymptomatic infection indicates a possible commensalism or an evolved dependence between human host and bacterial pathogen.^{21,22}

As *M. tb* is transmitted through respiratory aerosols and disease manifestations usually involve the pulmonary system, it is intriguing to apply the hygiene hypothesis to the local respiratory system. It is possible that infection with *M. tb* in developing immune systems, such as in children, may immunize against an allergic march toward asthma. We postulated an inverse relationship between *M. tb* infection and either asthma or atopic conditions in children. Using data from the National Health and Nutrition Examination Survey (NHANES) 1999–2000, we tested the hypothesis.

Patients and Methods

The NHANES is a series of cross-sectional, nationally representative, health examination survey performed in the United States. The National Center for Health Statistics–Centers for Disease Control and Prevention periodically conducts the survey. Beginning in 1999, the NHANES became a continuous annual survey of 5,000 people rather than a periodic survey.²³ The survey protocol was approved by the institutional review board of the Centers for Disease Control and Prevention. All the participants gave a written informed consent.

The NHANES 1999–2000 uses a stratified, multistage, probability sample of the civilian noninstitutionalized U.S. population. NHANES 1999–2000 oversampled low-income persons, adolescents, persons aged 60 years and older, African Americans, and Mexican Americans to support separate analyses.²³ Among the 12,160 people selected, a total of 9,965 (81.9%) underwent structured interview and 9,282 (76.3%) completed physical examination. Data from NHANES 1999–2000 include medical condition information on asthma and allergic symptoms as well as participant characteristics such as age, sex, race, ethnicity, birthplace, and the presence of a bacille Calmette-Guerin (BCG) vaccination scar. NHANES medical examiners were trained to recognize BCG scars and differentiate them from smallpox vaccination scars. Visible scars evaluated by examiners as BCG scars were recorded and regarded as received BCG in our study.

Tuberculin skin test and definition of M. tb infected

Trained phlebotomists injected 5 tuberculin units (0.1 mL) of purified protein derivative (PPD) intradermally to the volar surface of the forearm using the Mantoux method. Participants were asked to return at 48–72 h after tuberculin skin test (TST) placement for measurement of reactions by trained NHANES TST readers who had no knowledge of the participants' TB-related history. Two separated and trained readers measured the in-duration in mm and recorded reactions. A positive PPD reaction was defined as an in-duration of >10 mm.

Definition of asthma

A history of asthma was based on the participant's response to the question: "Has a doctor or other health professional ever told you that you have asthma?" Those who responded in the affirmative were considered as having asthma. Those who did not answer this question were excluded from the analysis. Subjects with a history of asthma were grouped into 2 categories: those with a recent asthma attack in the previous 12 months and those reporting a last

asthma attack over a year ago. Secondary outcome variables included dermatologic and other respiratory symptoms such as symptoms of wheezing, dermatitis, and hay fever.

Statistical analysis

To account for the complex survey design and to incorporate sample weights, all analyses were performed with the SAS Version 9.2 survey procedure (SAS Institute). Additionally, we used the recommended 2-year sample weight (variable name: wtsmec2y) to adjust for nonresponse bias and the oversampling of NHANES. Distribution of potential confounders was compared between those with and without physician-diagnosed asthma using chi-square statistics. We estimated associations of odds ratios (ORs) with logistic regression. The ORs were adjusted for age, gender, race, country of birth, and smoking status when comparing the *M. tb* infected and BCG association to asthma, allergies, and atopic dermatitis. Multiplicative interaction between age and the PPD result with respect to the risk of asthma, wheezing, or atopic allergy in the past year was tested using the cross-product term in a logistic regression model. A *P*-value of 0.05 was considered significant.

Results

M. tb infection in the study population

A total of 9,965 individuals, aged 1 year or older, participated in NHANES 1999–2000. The median age in the study population was 25 years. Among these participants, 7,613 (74.1% of total) reported no serious prior positive reaction to a TST and had undergone a tuberculin skin test with PPD. Only 7,386 (83.63%) had valid test results and were included in this analysis.

In total, 407 (4.1%) participants had a positive skin test. Skin test positivity varied in relation to demographic factors, consistent with a well-recognized trend observed in a prior study.²⁴ The participants more likely to be infected with *M. tb* were older men, cigarette smokers, and born outside the United States. For children aged <20 years, 71(1%) had a positive skin test reaction (Table 1). Skin test positivity in children and adolescents was associated with older age, being a Mexican-American, born outside the United States, and prior BCG vaccination.

Asthma status in the study population

A total of 946 (13.6%) individuals reported a history of asthma. Asthma prevalence was associated with the following factors: younger age, being non-Hispanic black, and birth in the United States. Among children <20 years of age, 535 (17.3%) had a history of asthma. On average, the children and adolescents more likely to have asthma were older, male, and born in the United States.

Association between M. tb infection and asthma

In all participants, a trend suggesting an inverse relationship between *M. tb* infection and those of ever having asthma or having asthma attack in the past year exists, but was not statistically significant (Table 2). However, this association was stronger and significant in participants <20 years. Children and adolescents infected with *M. tb* were less likely to have ever been diagnosed with asthma, compared

TABLE 1. DEMOGRAPHIC CHARACTERISTICS AND LIFESTYLE FACTORS OF PARTICIPANTS <20 YEARS, ACCORDING TO THE TUBERCULIN SKIN TEST STATUS AND ASTHMA HISTORY AMONG PARTICIPANTS IN THE NATIONAL HEALTH AND NUTRITION SURVEY, 1999–2000

Variance	TST+ (n=71)	TST- (n=3470)	P	+ Asthma (n=535)	No asthma (n=3,006)	P value
Gender						
Male	55.3	53.8	0.9	64.4	51.6	<0.01
Female	44.7	46.2		35.6	48.4	
Age	11.3	10.4	<0.01	10.6	10.4	<0.01
Race						
Non-Hispanic White	45.2	60.2	<0.01	60.2	60.0	0.12
Non-Hispanic Black	15.4	15.4		16.9	15.1	
Mexican American	33.3	11.4		7.0	12.6	
Other	6.1	13.0		15.9	12.3	
Country of birth						
US	31.2	93.1	<0.01	95.6	91.8	<0.01
Mexico	23.1	1.7		0.6	2.2	
Elsewhere	45.7	5.2		3.8	6.0	
BCG						
Yes	3.6	0.8	<0.01	0.8	0.9	0.88
No	96.4	99.2		99.2	99.1	

Data are percentage of participants.

TST, tuberculin skin test; BCG, bacille Calmette-Guerin.

with those not infected with *M. tb*. Thus, infection with *M. tb* was inversely associated to ever having a diagnosis of asthma in children and adolescents [OR 0.2; 95% confidence interval (CI): 0.0–0.9] (Table 2). Additionally, children and adolescents infected with *M. tb* were significantly less likely to report any episodes of wheezing in their chest over the past year (OR 0.1; 95% CI: 0.0–0.5) compared to children and adolescents not infected with *M. tb*. The sign of whistling or wheezing in the chest and association with *M. tb* were significantly different between age groups $p=0.005$, most likely highlighting the fact that wheezing in adults, unlike in children, often signifies etiologies other than asthma. The participants <20 years who reported having a recent asthma

attack over the previous year were inversely related to having infection with *M. tb* (OR 0.5; 95% CI: 0.0–4.3), although the estimate was not significant.

Infection with M. tb and association with atopic dermatitis and allergic rhinitis

In all participants, symptoms of atopic dermatitis in the previous 12 months were not associated with *M. tb* infection (OR 0.8; 95% CI: 0.3–2.0). Stratifying by age, participants <20 years were less likely to have atopic dermatitis if infected with *M. tb* (OR 0.1; 95% CI: 0.0–1.2), although this finding was not statistically significant. Symptoms of allergic rhinitis

TABLE 2. ASSOCIATION OF *MYCOBACTERIA TUBERCULOSIS* INFECTION WITH ASTHMA, WHEEZING, ATOPIC DERMATITIS, AND ALLERGIC RHINITIS BY AGE

Outcome	<20 years			>20 years			P^b
	No Tb	LTBI	OR ^a (95%CI)	No Tb	LTBI	OR ^a (95%CI)	
Asthma history							
None	2939	67	1	3110	312	1	
Ever	531	4	0.2 (0.0–0.9)	387	24	0.8 (0.3–2.3)	0.12
In past year	182	1	0.5 (0.0–4.3)	131	9	0.5 (0.1–4.4)	0.8
Wheezing or whistling in chest in past year							
No	3007	69	1	3016	297	1	
Yes	454	2	0.1 (0.0–0.5)	478	38	1.0 (0.5–2.0)	0.005
History of atopic dermatitis							
None	2502	65	1	3066	312	1	
Ever	260	1	0.1 (0.0–1.2)	427	23	0.9 (0.4–2.2)	0.17
Allergic rhinitis in past year							
No	3118	65	1	N/A	N/A	N/A	N/A
Yes	329	5	1.36 (0.3–6.5)	N/A	N/A	N/A	N/A

N/A, data not available from NHANES 1999–2000.

Data not reported in 13, 718, and 24 participants for wheezing status, dermatitis history, and allergic rhinitis, respectively.

^aOR adjusted for country of birth, race-ethnicity, BCG history, gender, and smoking status.

^bData indicate the interaction between age groups and having a positive TST test result on the risk of ever having asthma, wheezing, or atopic dermatitis in the past year.

OR, odds ratio; Tb, tuberculosis; LTBI, latent tuberculosis infection; NHANES, National Health and Nutrition Examination Survey.

over the past year were not associated with *M. tb* infection in children or adolescents.

BCG association between asthma, atopic dermatitis, and allergic rhinitis

In all subjects, having a history of BCG vaccination was not related to having a history of asthma. This observation was consistent between age groups while adjusting for other factors, with an OR of 1.4 (95% CI: 0.98–2.05) and 1.5 (95% CI: 0.97–2.07) in adults and participants <20 years, respectively. Similarly, there was no association between the BCG status and atopic dermatitis for adults and participants <20 years; 1.2 (95% CI: 0.82–1.53) and 1.3, respectively (95% CI: 0.83–1.55).

Discussion

Using the NHANES database, we observed that the children infected with *M. tb* were significantly less likely to have a history of asthma or symptoms of asthma over the prior year than children not infected with *M. tb*. This finding was not secondary to BCG vaccination, a common cause of TST cross reactivity, as no associations between BCG status and asthma were observed. Although the United States is considered a region of low TB prevalence, two thirds of the TST+ children are foreign-born, and possible exposure to microbes early in life, such as *M. tb*, may have had a protective effect against development of asthma.

In this way, there is evidence to support the hygiene hypothesis theory; however, timing and the primary site of infection appear to be somewhat important in determining local immune regulation. Inverse associations between *M. tb* infection and asthma were significantly pronounced only in children and adolescents and not in adults surveyed in the NHANES. Similar age-dependent findings were observed by the International Study of Asthma and Allergies in Childhood, an ecological study in over 700,000 children aged 6–7 years and adolescent aged 13–14 years from 56 countries.^{25,26} The younger group of children, aged 6–7 years, was significantly ($P < 0.0001$) less likely to have wheezing in the prior 12 months in regions where TB notification rates and WHO TB incidence rates were high, whereas this inverse association was not significant ($P = 0.263$) in the older group of children, aged 13–14 years, surveyed.²⁵ It is possible that any protective-effect *M. tb* infection may induce in the respiratory system against asthma may be most effective at a critical time, as the immune system is developing and being stimulated from self and nonself, that is, microbe and allergen antigens.

Prior studies performed in TB-endemic regions investigated associations between BCG vaccination and asthma. Reports varied between no-associations^{27–30} or weak-inverse associations.³¹ A systemic review and meta-analysis of epidemiological studies found that BCG vaccination indicated a modest protective effect against the development of childhood asthma with an overall-pooled OR of 0.86 (95% CI: 0.79–0.93).³² Several studies report inverse relationships between TST reactions and atopic disorders.^{33,34} A cross-sectional analysis on 23 studies examining mycobacterial infection and atopy reports a significant inverse correlation (OR 0.63; 95% CI: 0.51–0.79).³⁵ We found no association between BCG vaccination and asthma or atopic symptoms in all participants, including children and adolescents.

Environmental and microbial exposure in various mucosal sites is important for the development of the immune system in children.³⁶ In this respect, a fine balance between T cell subsets, including Th1, Th2, Th17, and regulatory T cells (Tregs), is central in determining a child's predilection toward asthma.¹⁰ Supporters of the hygiene hypothesis suggest that modern lifestyles cause an imbalance shift toward Th2 immune responses,^{37,38} which drive asthma development in susceptible individuals. Infection with *M. tb* not only increases the effector T cell responses^{39–41} but also has recently been shown to increase the frequency of Tregs in the airways.^{42,43} Tregs are known to play a role in controlling Th2-mediated inflammation in asthmatic patients,⁴⁴ and it was recently observed that the frequency and function of Tregs were reduced in bronchoalveolar lavage samples from asthmatic children.⁴⁵

An eventual hope for the treatment of allergic airway disease is to design therapies or vaccines that stimulate endogenous Tregs.⁴⁶ That *M. tb* could be protective against asthma is biologically plausible, through either recruitment or stimulation of pulmonary Tregs. Recent work performed in the murine model yielded convincing data supporting the concept that mycobacterial infection conferred protection, either prophylactically or therapeutically, against allergen-induced asthma. Several studies have shown that prophylaxis treatment with both live and heat-killed BCG leads to a polarized Th1 immune response in the lung and inhibits the development of allergen-induced Th2 responses.^{5,47–50} The application of live or dead mycobacteria inhibits the recruitment and expansion of Th2 cells homing into the lung, increases interferon- γ levels, and decreases the eosinophilia after ovalbumin airway challenge.^{51–55} A recent pilot study investigated the effect of intranasal delipidated acid-treated *Mycobacterium vaccae* on adults with asthma and found no significant difference between the treatment group and placebo.⁵⁶ The results from the *M. vaccae* study may not be surprising, for timing of microbe or allergen co-exposure appears to be relevant. As illustrated in the murine model of allergic airway disease, transfer of allergen-specific Tregs prevented, though did not reverse, airway-remodeling changes in a chronic challenge model,^{57,58} possibly illustrating that a developing immune system may hold greater potential for abating asthmatic inflammation.

Potential limitations of the present study include the use of a cross-sectional study design and self-reported health data. The use of self-reported data on asthma and atopic conditions may have led to recall bias, though prior studies have suggested that self-reported information on asthma has acceptable validity and reliability.^{59,60} Also, the TST remains as an imperfect diagnostic tool for *M. tb* infection, as false-positive reactions can be associated with nontuberculosis mycobacteria and BCG vaccination. That said, we did not observe any association between BCG-vaccinated subjects and asthma/atopy, and the United States is considered a low-prevalence region for nontuberculosis mycobacterial infections. Indeed, *M. tb* is an ancient member of the human microbiota, and its absence may not be correlated with the recent and on-going epidemic of asthma and related allergic disorders. As in any cross-sectional study, it is possible that the observed associations are casual and not causative, and many factors account for the current prevalence of asthma, of which the hygiene hypothesis is but one.

Conclusion

In this large study of a nationally representative population, we observed inverse associations between children infected with *M. tb* and asthmatic disease. The *M. tb* has plagued *Homo sapiens*, and probably our hominid ancestors, for millennia. Given our long co-evolutionary history, it is possible that *M. tb* has immunoregulatory properties and a lack of exposure to it early in life may predispose to asthmatic manifestation.

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Authors' Contributions

Study concept and design: J.L.-F. Acquisition of data: J.L.-F. and C.-H.P. Analysis and interpretation of data: J.L.-F. and C.-H.P. Drafting of the article: J.L.-F. Critical revision of the article for important intellectual content: J.L.-F. and C.-H.P. Statistical analysis: C.-H.P. Administrative, technical, and material support: J.L.-F. Study supervision: J.L.-F.

Author Disclosure Statement

The authors declare that they have no competing interests.

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