Revisiting the hygiene hypothesis for allergy and asthma

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The hygiene hypothesis, which describes the protective influence of microbial exposures in early life on the development of allergy and asthma, has continued its swell of academic interest, investigation, and evolution. This article is focused on studies published in the past 3 years that have furthered the substance and shape of hygiene theory, primarily as it relates to allergic airways and asthma. Recent investigations have furthered an overarching "microbiome hypothesis" to home features, medical practices, and cleanliness behaviors that are suspects in the hygiene effect. Relatively crude markers of the protective microbial environment have been supplanted by culture-independent microbiome science, distinguishing the characteristics of potentially protective microbiomes from pathologic features. Understanding how the microbiome is shaped and affects healthful versus harmful outcomes in the human host is relatively nascent. Good clues are emerging that give mechanistic substance to the theory and could help guide microbe-based therapeutics to fill the allergy and asthma management gap in prevention and disease modification. (J Allergy Clin Immunol 2015;136:860-5.)

Key words: *Asthma, hygiene hypothesis, asthma prevention*

Abbreviations used
TLR: Toll-like receptor
Treg: Regulatory T
URECA: Urban Environment and Childhood Asthma Study

Of microbes and humans: the sequel. With the discovery of microbial pathogens in the mid- to late-1800s, a successful war against these microscopic invaders with a broad-based campaign of public and personal hygiene measures and therapeutic and preventive interventions was a victory for human health. But did this antimicrobial war, with other aspects of modern sanitized living, disrupt an immune balance between microbes and human subjects with an abruptness that did not allow for evolutionary adaptation, leading to the unintended consequence of the allergic march underlying the allergy and asthma epidemic of the past century?

Since its academic re-emergence in the past few decades, the hygiene hypothesis (reviewed by Liu[1]) has been broadly investigated. Some have interpreted this growing body of evidence to have consistency and potential to serve the unmet need of preventive measures for allergy and asthma by mimicking nature's ways. Others have been doubtful because a wide range of microbes (eg, respiratory viruses, bacteria, and molds) have a well-established provocative effect on asthma symptoms. How could microbial exposures also have a paradoxical preventive effect on allergies and asthma? The seeds of this paradox were already apparent in the beginnings of allergy's discovery, with Dr Charles Blackley's experimental observations that the grass pollen he used for conjunctival challenges to demonstrate pollen's causal role in "hay-fever or hay-asthma" was obtained from farmers who were nonallergic.[2] In keeping with the current theme, let us begin by revisiting Blackley's farmer collaborators through a recent documentary about allergy and asthma in Amish farmers in America.

THE AMISH IN AMERICA: A CASE STUDY

Life on rural European farms has been a rich resource of naturally occurring evidence that microbial exposures from proximal living with domestic animals beginning in early life might protect against the development of allergies and asthma.[3,4] This has provided a compelling body of evidence in support of hygiene theory. Could these farming ways indicate environments and lifestyles that are allergy and asthma protective today and were even more so in earlier times before the global increase in allergy and asthma?

The Amish, a sect of Anabaptists, adhere to a lifestyle of approximately 150 years back in time, adhering to simple living, plain dress, and a traditional lifestyle eschewing modern conveniences and technology, which is fundamental to their faith. Dairy farming is a desirable lifestyle and avocation for Amish families. The Amish in America emigrated from the Swiss region of the European farm studies noted above. An allergist serving an Amish community in Indiana, Dr Mark Holbreich, in collaboration with rural European farm investigators Drs Erika von Mutius and Charlotte Braun-Fahrlander and their colleagues, determined that Amish children in Indiana have a very low prevalence of allergy, asthma, and aeroallergen sensitization when compared with both Swiss nonfarm and farm children. Results in Amish versus Swiss farm vs Swiss nonfarm children were as follows: asthma, 5.2% vs 6.8% vs 11.2%; hay fever, 0.6% vs 3.1% vs 11.6%; and atopic eczema, 1.3% vs 7.6% vs 12.1%, respectively. Also notable as an objective measure, aeroallergen sensitization was as follows: 7.2% vs 25.2% vs 44.2%, respectively.[5]

This Amish case study is a provocation to the notion that allergy and asthma were uncommon in a past time and influenced by environments and lifestyles still retained by European dairy farmers today. Hygiene theory implicates human subjects' microbial environment in early life as essential

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to proper immune development, both toward protective antimicrobial and regulatory immune responses to environmental antigens and away from atopy and asthma. In addition to differences in the microbial environment, there is much to lifestyle differences from modern metropolitan living that are characteristic of Amish farming families. For example, in the study by Holbreich et al,[5] the Amish cohort averaged 6 children per family, which was double the numbers seen in the Swiss farmers and nonfarmers. Could the low prevalence of allergy and asthma in these Amish be related to large family size and independent of microbial environment? This study was not large enough to discern lifestyle differences, although it is believed that these children have more physical outdoor play and are needed to participate in farm chores as soon as they are able. This hygiene anecdote is intriguing, even if it is conjecture.

CLEAN (AND NOT SO CLEAN) LIVING

Recent investigations have shed light on personal hygiene behaviors suspected of contributing to the hygiene effect. In a German longitudinal birth cohort study, personal cleanliness (eg, hand washing and showering) was associated with lower levels of endotoxin (ie, a bacterial marker) and muramic acid (ie, a fungal marker) in bedding and floor dust.[6] In comparison, household cleanliness (eg, cleaning floors and bathrooms, dusting, and changing towels) was associated with less dust but not with lower microbial marker levels. Endotoxin in infancy was associated with less allergic sensitization and less asthma when these children reached school age, whereas muramic acid exposure at school age, but not infancy, was associated with less school-age asthma and eczema. It might be surprising to some that neither personal nor home cleanliness activities were directly associated with atopy or asthma outcomes. This study points to the importance of early-life timing of actual microbial exposures and not cleanliness behaviors, with the influence of endotoxin exposure being in infancy.

Two behaviors that could affect exposure to oral microbes were recently associated with less allergy. In a Swedish study hand dishwashing, which is presumed to be less effective in eliminating microbes when compared with machine dishwashing, was associated with less eczema and less "total allergy" (ie, a combination of eczema, asthma, and/or allergic rhinoconjunctivitis).[7] The protective effect of hand dishwashing was stronger in children eating fermented foods, farm-bought foods, or both, raising the possibility of specific sources of food-borne microbes being transmitted through the presumably less hygienic method of hand dishwashing. The same research group reported on the parental behavior of infant pacifier "cleaning" by putting it in the parent's mouth before giving it back to the infant; 48% of the cohort reported this practice in the first 6 months of life.[8] Children with parents who orally "cleaned" their pacifiers were less likely to have allergic sensitization, eczema, and "asthma" by age 18 months, implicating parent-to-infant oral microbe transmission as atopy protective. If or how these behaviors might significantly alter microbial exposures in an atopy-protective manner or might be behavioral markers of other protective factors is not elucidated but gives hope to the notion that allergy and asthma prevention might be as simple and benign as these frequent oral microbial exposures beginning in early life.

SUBSTANTIATING PROTECTIVE HOME ENVIRONMENTS

Recent studies have substantiated prior observations attributed to hygiene theory. David Strachan, who first demonstrated the relationship of increased sibship with less atopy, extended these associations on a global scale in the International Study of Asthma and Allergies in Childhood cohort.[9] Globally, increasing numbers of siblings, especially older siblings, were negatively associated with hay fever and eczema. These associations were stronger in more affluent countries, which is consistent with the notion that less developed nations have additional significant contributors to a hygiene effect. The infant gut microbiome associated with having older siblings was recently shown in a birth cohort to have more *Bifidobacterium*, *Lactobacillus*, *Escherichia*, and *Bacteroides* genera and less Clostridia.[10] This is similar to the infant gut microbiome associated with vaginal delivery versus cesarean section exhibiting greater gut microbial diversity, earlier colonization with *Bacteroides*, less colonization with Clostridia, and subsequently higher levels of the T_H1-associated chemokines CXCL10 and CXCL11 in peripheral blood.[10,11] Vaginal delivery and older siblings appear to have additive favorable effects on the gut microbiome.

A recent study of ambient endotoxin exposure provides further credence for the paradoxical differences in exposure-health outcomes in adults compared with children. Dose-dependent endotoxin exposure has been associated with more asthma in adults[12] and paradoxically less atopy and allergic asthma in children.[3,6] In a recent nationally representative US study based on the US National Health and Nutrition Examination Survey 2005-2006, greater house dust endotoxin levels were associated with a lower risk of allergic sensitization to pets and pollens in children/adolescents; in contrast, the risk of aeroallergen sensitization was higher in adults.[13] This is consistent with the German longitudinal birth cohort study noted above: endotoxin concentration in mattress dust collected at age 3 months was associated with a lower likelihood of asthma and aeroallergen sensitization at age 5 years.[6] Possible explanations for this paradoxical difference include timing of exposure: preventing atopy in early life versus promoting atopy persistence in adulthood. There might also be significant differences in relevant coexposures in children compared with adults. For example, more adults might be exposed to tobacco smoke, which interacts with endotoxin to provoke asthma.[14] This duality of healthful versus harmful outcomes from microbial exposures and the importance of key determinants is well exemplified by endotoxin.[15,16]

THE PARADOX OF US INNER CITIES

Because there is a high burden of severe asthma in US inner cities, the National Institutes of Health/National Institute of Allergy and Infectious Diseases has sponsored an inner-city birth cohort study, Urban Environment and Childhood Asthma (URECA), to understand how US inner cities foster severe asthma development.[17] From a hygiene hypothesis perspective, inner-city environments could be suspected of being protective on allergy and asthma development, which is a paradox. Although this study is ongoing, recent investigation of URECA participants' first 3 years of life provides paradigmatic insights.[18]

First, house dust cockroach, mouse, and cat allergen exposure in the first year of life was negatively associated with recurrent