

The role of the changing human microbiome in the asthma pandemic



Timothy C. Borbet, MSc,^a Xiaozhou Zhang, MSc,^b Anne Müller, PhD,^b and Martin J. Blaser, MD^c *New York, NY, Zurich, Switzerland, and Piscataway, NJ*

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Activity Objectives:

1. To understand the biology of *Helicobacter pylori* colonization in the human stomach.
2. To learn how *H. pylori* hijacks the immune response to further its colonization and how this affects immunity in the lung.
3. To gain an understanding of how the microbiome develops during early life in human subjects.
4. To consider how modern health practices might impair the early-life microbiota.

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Asthma and allergy incidence continue to increase globally. We have made significant strides in treating disease, but it is becoming more apparent that we need to advance our knowledge into the origins of asthmatic disease. Much recent work has indicated that microbiome composition influences immune regulation and that multiple health care factors have driven a loss in microbiome diversity in modern human populations. Evidence is growing of microbiota-driven influences on immune development, asthma susceptibility, and asthma pathogenesis. The focus of this review is to highlight the strides the field has made in characterizing the constituents of the human gastrointestinal microbiota, such as *Helicobacter pylori*, other members of the neonatal intestinal microbiota, and microbial peptides and metabolites that influence host immunity

and immune response to allergens. As we delve further into this field of research, the goal will be to find actionable and clinical interventions to identify at-risk populations earlier to prevent disease onset. Manipulation of the host microbial community during infancy might be an especially promising approach. (J Allergy Clin Immunol 2019;144:1457-66.)

Key words: Asthma, allergy, microbiome, *Helicobacter pylori*

Asthma is a chronic inflammatory condition of the lungs that occurs worldwide. Asthmatic symptoms result from airway obstruction caused by inflammatory responses to certain triggers, most commonly environmental antigens.¹ It is estimated that

From ^athe Department of Pathology, New York University School of Medicine; ^bthe Institute of Molecular Cancer Research, Zurich; and ^cthe Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway.

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Corresponding authors: Anne Müller, PhD, Universität Zürich Institut für Molekulare Krebsforschung, Winterthurerstrasse 190, CH 8057 Zürich, Switzerland. E-mail: mueller@imcr.uzh.ch. Or: Martin J. Blaser, MD, Center for Advanced Biotechnology and Medicine, Rutgers University, 679 Hoes Lane West, Room 106A, Piscataway, NJ 08854. E-mail: martin.blaser@cabm.rutgers.edu.

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Abbreviations used

DC: Dendritic cell
 pTreg: Peripherally induced regulatory T
 TLR: Toll-like receptor
 Treg: Regulatory T
 VacA: Vacuolating cytotoxin

more than 300 million persons have asthma, with a burden that has increased substantially over the past several decades.¹ Several risk factors have been implicated in asthma susceptibility, including host genetics, specific environmental exposures, obesity, and respiratory tract infections during early life. Attention is now turning to the role of the human microbiome in asthma pathogenesis and protection.

The microbiome, a community of organisms, including bacteria, archaea, fungi, and viruses, that live in and on us is now recognized as an important contributor of host homeostasis through physiologic, immunologic, and metabolic regulation. With improved analytic tools, there has been increasing focus on elucidating the mechanisms by which the constituent microbiota affects both homeostasis and disease susceptibility. It has become clear that environmental factors, such as antibiotic use, birth mode, and formula feeding, play important roles in shaping the composition of the early-life microbiota.² This highlights consideration of how generational losses in microbial diversity in the human microbiota might contribute to immune-regulated disease susceptibility.

An important frontier is to identify potential therapies to improve human health, prevent the onset of microbiota-related diseases, and identify appropriate therapies. Although these topics are now being studied in relation to obesity, diabetes, inflammatory bowel disease, and neoplasia, in this review we will focus on asthma and related allergic disorders. We will consider the overall biology of the microbiome, its role in immunologic development, the contributions of reduced microbial diversity, and how perturbations are affecting the asthmatic diathesis. Finally, we will consider how the emerging knowledge can be harnessed for improved prevention and therapies.

BIOLOGICAL CHARACTERISTICS OF THE HUMAN MICROBIOME

Understanding the biology of the microbiome is a starting point for considering its relationship to asthma. There are 7 critical characteristics. First, the human microbiome is niche specific. In each anatomic locale that harbors a microbiome, microbial populations differ. These populations are both unique and overlapping, and there is evidence for interactions among them.³ Even niches that once were considered “sterile” are being shown to contain resident microbial populations, including the esophagus, stomach, and lower respiratory tract.⁴

Second, in all niches there are both persistent and transient organisms. Although the definitions and boundaries vary and are biologically relevant, the emphasis of investigation has been on understanding the persistent microbiota, the organisms that can endure for years, decades, or life.

Third, across subjects, microbiota are taxonomically highly diverse. This diversity has been best studied in relation to

bacterial populations but also is present for fungi and viruses. At a functional level, the overall outlines are more conserved because, for example, all bacteria use stereotypic mechanisms to process energy, build cell walls, reproduce, and move, but nevertheless, there is enormous variation as well.

Fourth, microbiota in aggregate are numerous. Current estimates are that the number of bacterial and human cells in our bodies are roughly equivalent to residential viruses, perhaps 10-fold greater. At a genetic level, the differences are greater. In comparison with the current view of 23,000 human genes, each of us carries from 2 to 20 million unique bacterial genes, all subject to regulatory pathways.³

Fifth, the microbiome is ancient. All animals have their own characteristic microbiomes. Among vertebrates, the phylogenies of the microbiota parallel those of their hosts. This provides strong evidence for the overall vertical transmission of the microbiome and also supports the concept of coevolution of host and microbial populations.⁵ In studies of primates, the congruent phylogenies at the aggregate level, and for particular marker taxa, are consistent with the overall view and provide a milestone of at least 8 million years of congruence.⁶

Sixth, the essential structure of the microbiome is acquired early in life. In the womb human life begins in a mostly sterile environment; there is no reproducible evidence for an *in utero* persistent microbiome. Exposure to microbes begins when the water breaks, the first step in the introduction and transfer of maternal microbiota to the infant. There are multiple parallel and redundant routes involving colonization of the gastrointestinal tract, mouth, and skin. The population structure of the infantile microbiome develops gradually and progressively and, by age 3 years, in the gastrointestinal tract at least, has taken on the major characteristics of the adult microbiota.⁷

Seventh, the microbiota is interactive with host physiology. Studies focusing on immunity, metabolism, and cognition all provide evidence for strong microbiota effects on all these characteristics, with reciprocal properties as well.^{8,9} It is this property, in the context of the above characteristics, that requires attention in relation to asthma and allergic disorders.

THE HUMAN MICROBIOME IS CHANGING

With advancements in economic and social development over the past 2 centuries have come new pressures on the human microbiome, accelerating in the past 50 years. Clean water, with all of its important benefits, limits the interpersonal spread of commensal organisms. Cesarean sections, now occurring in more than half of pregnancies in some populations and 25% to 33% in many others, bypasses the original microbial seeding that occurs during natural delivery. Formula feeding, substituting for human breast milk, does not contain the micronutrients that evolved over the eons to nourish ancestral microbes. The use of antibacterial agents in foods and in topical applications affects the developing microbiome. Most importantly, human children all over the world are receiving multiple courses of potent antibiotics, often at high doses, over the early years of life. All of these practices have been predicted to affect microbial composition and thus host physiology.^{10,11} Based on the largely vertical transmission of the microbiota, it was predicted that that loss of diversity would be cumulative across human generations.¹²

Unfortunately, there is increasing evidence that these predictions were correct. Studies of human populations at



FIG 1. Ecologic effects of antibiotic exposures. Antibiotic resistance has long been recognized as an ecologic consequence of antibiotic exposure. However, an additional hypothesis is that, like the proverbial iceberg, the disruption of the microbiome leading to clinical consequences is the “inapparent” or larger part. Even transient antibiotic exposures in early life or before adventitious infections can lead to long-term consequences.^{2,17-19} Exposures of women before the birth of their children can lead to consequences in the next generation,¹⁷ and exposures of adults can enhance the risk of both metabolic and neoplastic diseases.²⁰

differing levels of socioeconomic advancement have shown the greatest microbiota diversity in populations with limited access to modernization and the lowest diversity in industrialized countries.^{13,14} These trends are irrespective of continent of origin, diet, and ethnicity but point to modernization/urbanization and all of its accompanying trends as the key factor. Recent studies of immigrants to the United States from developing countries provide evidence of loss of diversity in real time.¹⁵

These trends have been encapsulated in the theory of the disappearing microbiota,^{10,11} which has 2 major tenets: (1) changed human ecology has altered transmission and maintenance of ancestral microbes, which affects the composition of the microbiota, and (2) the microbes, both good and bad, usually acquired early in life are especially important because they affect a developmentally critical stage. Because the interaction of the microbiota with human physiology is so profound, this theory predicts that the changed microbiota affects host physiologic functions,¹⁶ with immunity being particularly relevant to asthma.

Although the potential for antibiotics to lead to important ecologic effects has been well recognized, most of the focus has been on development of antibiotic resistance. Although resistance is quite important, it probably represents only the tip of the iceberg (Fig 1),^{2,17-20} in which the larger mass, currently hidden from plain view, is the effect on microbiome composition and the subsequent physiologic and clinical consequences. These views concern exposure to and acquisition of human-specific ancestral microbiota and can be contrasted with a more general construct, the hygiene hypothesis, which largely focuses on environmental exposures, such as having a pet in the home or exposure to farm animals.²¹

HELICOBACTER PYLORI AS AN INDICATOR ORGANISM AND PROTAGONIST

Initially identified in the 19th century, the organisms that now are called *H pylori* were first isolated from human gastric biopsy specimens in 1983.²² There is considerable evidence that *H pylori* is ancient,²³ having colonized our ancestors at least 100,000 years ago.²⁴ Based on its very long history, its presence in all human populations in which it has been studied, and its acquisition in early life and life-long persistence, *H pylori* clearly can be considered a member of the human gastric microbiota and, when present, is usually the dominant member.²⁵ However, it is progressively disappearing from human populations, reflecting many of the trends mentioned above. As such, it is possible to associate its presence or absence with human diseases.

First discovered as a pathogen in the 1980s, the presence of *H pylori* is clearly linked to the risk of peptic ulcer disease and adenocarcinoma of the stomach.²⁶ However, its absence has been linked to esophageal diseases, including gastroesophageal reflux disease, Barrett esophagus, and adenocarcinoma of the esophagus and adjacent gastroesophageal junction.²⁷ Similarly, its absence has been linked in multiple large, blind epidemiologic studies, with the risk of childhood-onset asthma.²⁸⁻³⁰ *H pylori* strains can be divided into those that do and do not possess the *cag* island with host-interactive genes.³¹ The *cag*⁺ strains are those that are most strongly related to the risk of disease (ulcers and cancer), as well as protection from disease (esophageal diseases and asthma), which is consistent because this is the subset of strains with the strongest host interactions.³¹ Epidemiologic studies also found parallel relationships with hay fever and cutaneous allergies, 2 disorders independently linked to asthma.²⁸

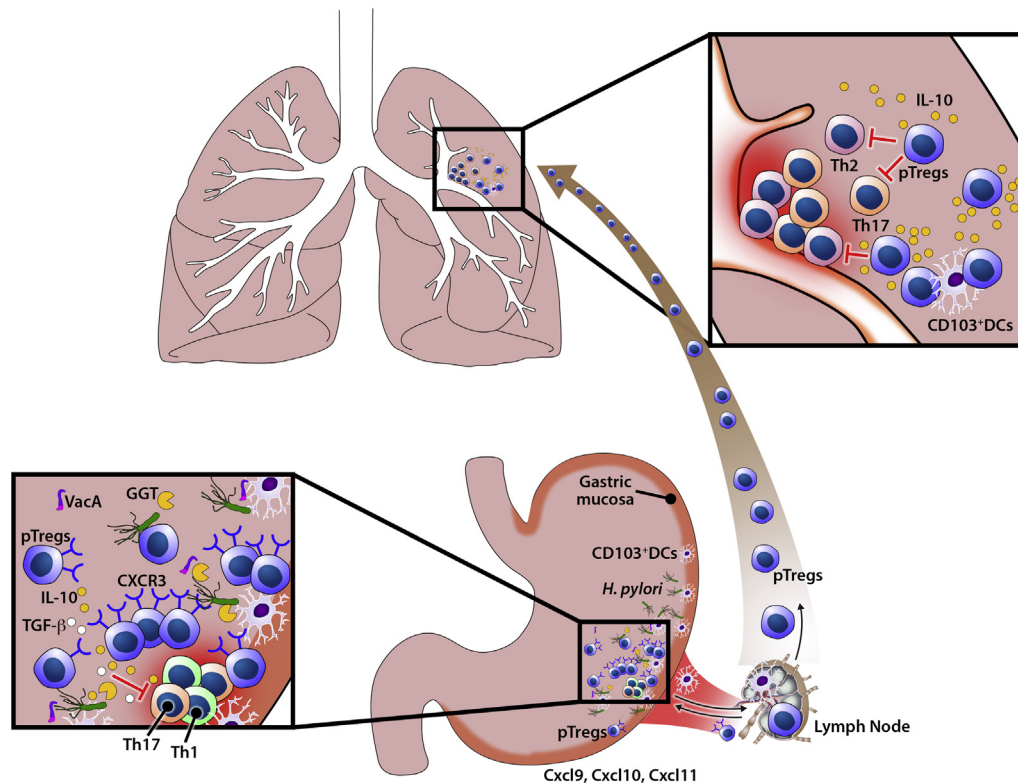


FIG 2. *H pylori* infection is strictly local but has systemic effects at distant sites. In experimental models *H pylori* recruits cells of myeloid origin into the gastric lamina propria, which are known to interact with live bacteria or their products, presumably through intraepithelial protrusions. Antigen-presenting cells and in particular CD103⁺ DCs migrate to the draining gastric and mesenteric lymph nodes, where they prime effector T-cell (mostly T_H1 and T_H17) and Treg cell responses. *H pylori*-induced T_H1 and Treg cells substantially express the chemokine receptor CXCR3 on their surfaces, which allows for their trafficking to the stomach along a gradient of the CXCR3 ligands CXCL9 and CXCL10. ROR_γt- and T-bet-expressing pTreg cells in the gastric mucosa suppress pathogen-specific T_H1 responses and promote persistent *H pylori* colonization. Importantly, pTreg cells with the same profile also traffic to and accumulate in the lungs of infected mice, where they are likely involved in suppression of allergen-specific T_H2 and T_H17 responses. pTreg cells strongly produce TGF-β and IL-10. GGT, Gamma-glutamyl transferase.

Results of these epidemiologic studies generate the hypothesis that gastric *H pylori* colonization is protective against asthma and related disorders and that the increase in asthma is at least in part fueled by loss of this ancestral gastric colonizer and loss of protective immunologic functions. Indeed, there are substantial experimental data from mouse models to provide evidence for causality. The gold standard mouse models of allergen-induced airway inflammation and hyperresponsiveness entail the sensitization and subsequent challenge (both ideally performed intranasally or intratracheally to mimic the dominant route of exposure in human subjects) of mice with potent allergens, such as ovalbumin or house dust mite extract. In such experimental models persistent infection with *H pylori* efficiently reduces the markers of allergic asthma, including (1) excessive pulmonary T_H2 responses and associated high levels of the T_H2 cytokines IL-5 and IL-13, (2) aberrantly high systemic levels of allergen-specific IgE, (3) bronchoalveolar eosinophilia, and (4) goblet cell hyperplasia and the associated excessive mucus production.³² Lung function is restored as well in *H pylori*-infected mice, as determined by using a methacholine challenge assay.³² In line with observations in human subjects, which highlighted particularly strong inverse associations of

H pylori with early-onset asthma,^{12,33} the experimental data suggest that neonatal infection with *H pylori*, but not infection of adult mice, results in protection against allergic asthma signs and severity.³²

Several prerequisites of protection have been identified in addition to the early-life window of opportunity, which might be considered the “neonatal tolerance window” (discussed in more detail below).^{34,35} In particular, an immunomodulatory molecule that all *H pylori* strains produce, the so-called vacuolating cytotoxin (VacA), has been implicated in *H pylori*'s asthma-protective effects.^{36–38} VacA-deficient isogenic mutants do not protect against allergic asthma in neonatal infection models,³⁸ and VacA purified from culture supernatants of *H pylori* is potent at suppressing allergic asthma in prophylactic settings.³⁷ More recent tracing experiments have shown that VacA targets different myeloid cells in the gastric mucosa, generating a tolerogenic environment characterized by high levels of immunoregulatory cytokines, such as IL-10 and TGF-β (Fig 2).³⁶ Because VacA is not only required for the protective effects of live infection but also sufficient to prevent allergy on its own, it deserves to be investigated further and developed for possible interventional application in human subjects.

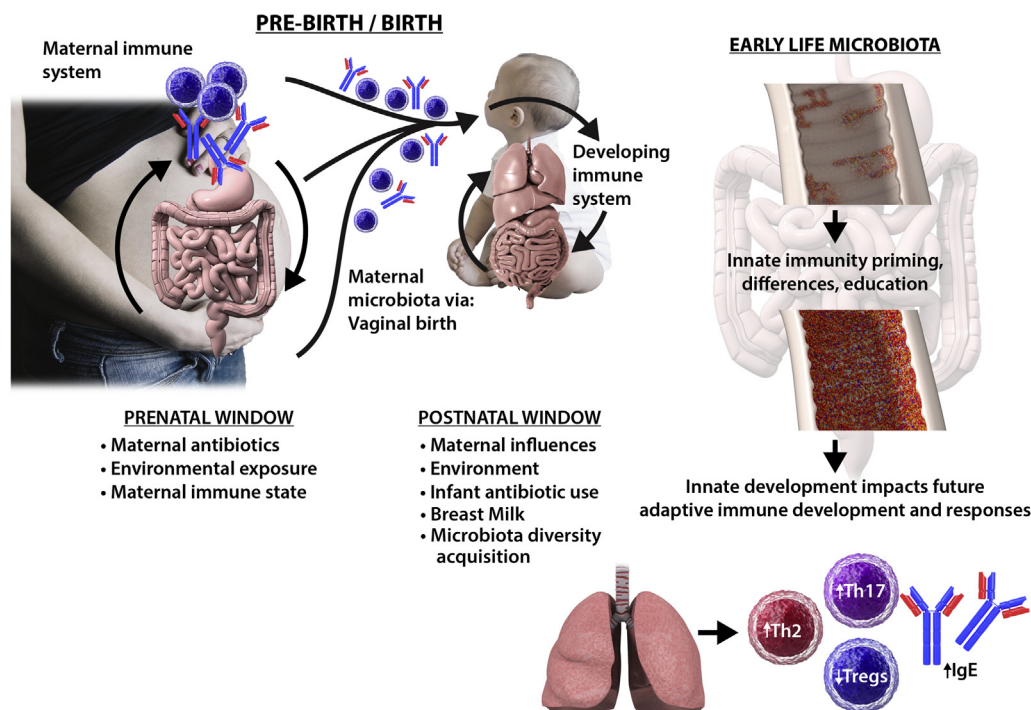


FIG 3. Early-life influences on the developing microbiome affect “immunologic tone.” The maternal microbiome interacts with the mother’s immune system, which then further shapes the microbiome; it is a continuous cycle. At birth, the maternal microbiome is transferred to the baby, as well as immunologic molecules (including antibodies, cytokines, and specific metabolites). Together, these interact with innate immunity to set the immunologic tone, which then affects the development of adaptive immunity. Many aspects of modern health care can perturb these relationships.

H pylori has not only been inversely linked to allergic asthma but also appears to have strong immunomodulatory and beneficial effects in other settings of allergic and chronic inflammation and possibly autoimmune disorders. In particular, patients with either of the 2 major forms of inflammatory bowel disease, Crohn disease and ulcerative colitis, harbor *H pylori* at much lower rates than the general population.³⁹ In mouse models of inflammatory bowel disease, *H pylori* infection and regular administration of *H pylori* extracts protect against severe disease, as determined by using histologic analysis and other readouts.⁴⁰

Among allergic disorders, food allergy to ovalbumin allergen and peanut extract has been shown to be alleviated by live *H pylori*, its extract, and purified VacA.⁴¹ One study has shown an inverse association of *H pylori* infection with multiple sclerosis and has provided experimental evidence in the mouse model of multiple sclerosis, experimental autoimmune encephalomyelitis, for protective effects of *H pylori* on the central nervous system findings associated with this disease.⁴² Atopic dermatitis is another allergic disease manifestation to which *H pylori* has been inversely linked in human subjects,^{43,44} as well as skin sensitivity to a panel of allergens,²⁶ but experimental data to support protective effects of the bacteria in the skin are not available. In yet another study, the presence of *H pylori* was inversely related to the diagnosis of celiac disease,⁴⁵ but the direction of causality has not been confirmed.

ROLE OF *H PYLORI* IN SHAPING THE GASTROINTESTINAL IMMUNE SYSTEM (EVIDENCE FROM HUMAN SUBJECTS AND EXPERIMENTAL MOUSE MODELS)

H pylori creates a tolerogenic environment in the gastric mucosa that is at least in part driven by the above-mentioned VacA and its interactions with the myeloid compartment.⁴⁶ Recent work has shown *H pylori* (expressing red fluorescent protein [RFP] and thereby allowing its tracking to specific immune compartments) to interact directly with various myeloid cell populations, which are recruited to the gastric mucosa along chemokine gradients.⁴⁶ At least 6 distinct myeloid populations with diverse functions appear in the mouse stomach on *H pylori* infection but are virtually absent in the steady-state stomach⁴⁶; of these, 3 are considered *bona fide* dendritic cells (DCs) because they express CD11c and depend on the growth factor FLT3 ligand for their differentiation from bone marrow precursors. The others are macrophages and monocytes expressing the respective lineage markers F4/80, CD64, and Ly6C among others. RFP⁺ bacteria are in direct contact with all macrophage and monocyte lineages and some but not all DC lineages in the gastric lamina propria and also encounter large numbers of eosinophils in their natural environment.^{46,47}

Some of these interactions have been investigated functionally using mouse strains deficient for the respective lineages. From this work, it is now clear that CD103⁺ DCs are required for T_H1-driven immunity on the one hand and for recruitment to infected tissues of peripherally induced regulatory T (pTreg) cells

on the other.⁴⁸ In the absence of CD103⁺ DCs (in *Batf3*^{-/-} mice, which lack the key transcription factor driving CD103⁺ DC differentiation⁴⁹), priming of T_H1 and pTreg cells in lymph nodes proceeds normally, but both T-cell subsets fail to upregulate expression of the chemokine receptor CXCR3 and therefore cannot traffic to the infected gastric mucosa along CXCL9 and CXCL10 chemokine gradients (Fig 2).⁴⁸ The defects of *Batf3*^{-/-} mice in both populations manifest in 2 ways: on the one hand, *Batf3*^{-/-} mice are severely hypercolonized because they cannot control *H pylori*,⁴⁸ and on the other hand, they are not protected against allergic asthma on neonatal infection because of their functional regulatory T (Treg) cell defect.³⁷

Several studies in human subjects and mice have pointed to the relative strength of effector T-cell and Treg cell responses to *H pylori* as a major determinant of disease risk and severity, both within the stomach and outside. Human subjects with a Treg cell–predominant anti-*H pylori* response are less likely to have gastric ulcers than those with a T_H1- or T_H2-polarized effector T-cell response to the organism, and the levels of circulating *H pylori*-specific Treg cells, especially those producing the regulatory cytokine IL-10, are inversely associated with serum IgE levels.^{42,50,51} In *H pylori*-positive children, gastric inflammation is typically much less severe than in adults and effectively controlled by a dominant Treg cell response.⁵²

These data are all consistent with the beneficial effects of *H pylori* in childhood and adolescence but not later in life and implicate *H pylori*-specific Treg cells as mediators of asthma protection. Several lines of experimental evidence from mouse models support this idea: (1) neonatally infected mice have much less severe gastritis on *H pylori* infection than mice exposed as adults and never exhibit evidence of preneoplastic lesions, which are common in animals infected as adults⁵³; (2) in asthma models protection is limited to mice infected as neonates; (3) depletion of Treg cells abrogates protection in the neonatal infection model; and (4) adoptive transfer of forkhead box P3–positive Treg cells is sufficient to confer protection in naive recipients.³² The protective population of Treg cells has yet to be characterized in detail using functional approaches but appears to be pTreg cells rather than thymus-derived cells and expresses the transcription factors retinoic acid–related orphan receptor γ t and T-bet.^{36,48} *H pylori*-induced pTreg cells are enriched in the infected mouse gastric mucosa but traffic to and accumulate in the lungs (Fig 2). This (mis) localization is specific to neonatally infected mice and believed to underlie the protective effects of neonatal infection on pulmonary manifestations of allergic diseases.^{36,48} In another murine model with experimental *H pylori* challenge, colonization led to changes in gastric gene expression, immunologic function, and hormone secretion, and effects on the intestinal microbiota, as well as changes in pulmonary gene expression and T-cell populations, support the linkage with extragastric pathophysiology.⁵⁴

ROLE OF OTHER CONSTITUENTS OF THE MICROBIOME IN SHAPING THE GASTROINTESTINAL AND SKIN IMMUNE SYSTEMS (EVIDENCE IN HUMAN SUBJECTS AND IN MODEL SYSTEMS)

The microbiota is a known regulator of immune development, cell differentiation, and cell function. Increasing evidence over

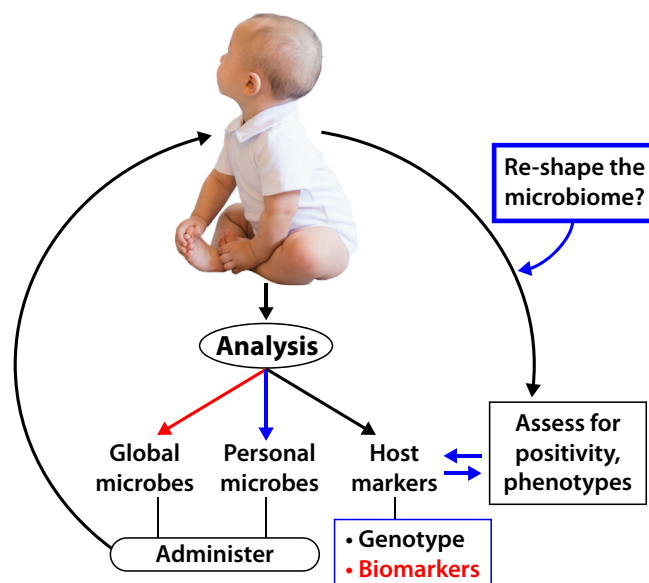


FIG 4. A prediction for the medicine of the future. For well-baby visits, future pediatricians will examine both babies and their diapers. They will determine whether that infant has the ideal microbiota for their genotype and other markers. If not, they will administer the “missing microbes” to optimize their health trajectory through life. Physicians will follow microbiological parameters during childhood, adjusting as necessary to continue optimization.

the past decade has shown that specific bacterial strains, as well as defined mixtures of bacteria, can elicit predicted immune phenotypes.^{9,55–57}

Intestinal

Specific members of the murine microbiome, some of which are shared with human subjects, have stereotypical interactions with immunologic effector cells. Specific clades of Clostridia species isolated from human stool samples can induce colonic Treg cells in a murine model.⁵⁵ Segmented filamentous bacteria (*Candidatus* Savagella) are members of murine microbiota that elicit T_H17 differentiation in the intestinal tract of mice.⁵⁶ *Helicobacter hepaticus* induces retinoic acid–related orphan receptor γ t⁺ Treg cells that suppress T_H17 cell function; in the absence of these induced Treg cells, *H hepaticus* potentiates T_H17-driven colitis.⁵⁸ A mixture of 11 human commensal bacterial strains has been identified that induced IFN- γ production by CD8⁺ T cells in the mouse colon; these had effector functions useful in antipathogen and antitumor responses.⁵⁷ The above taxa provide examples of how well-characterized bacterial commensals can drive immune differentiation and translate to actionable phenotypes within the intestinal environment.

Skin

Applied to the skin of mice, human cutaneous commensals elicited both cytokine and T-cell responses. Of these, *Staphylococcus epidermis* was unique in its ability to induce CD8⁺ T cells producing IL-17 at the inoculation site.⁵⁹ Commensal-specific T cells, both CD4⁺ T_H17 and CD8⁺ T_C17 cells, are transcriptionally programmed to maintain a type 17

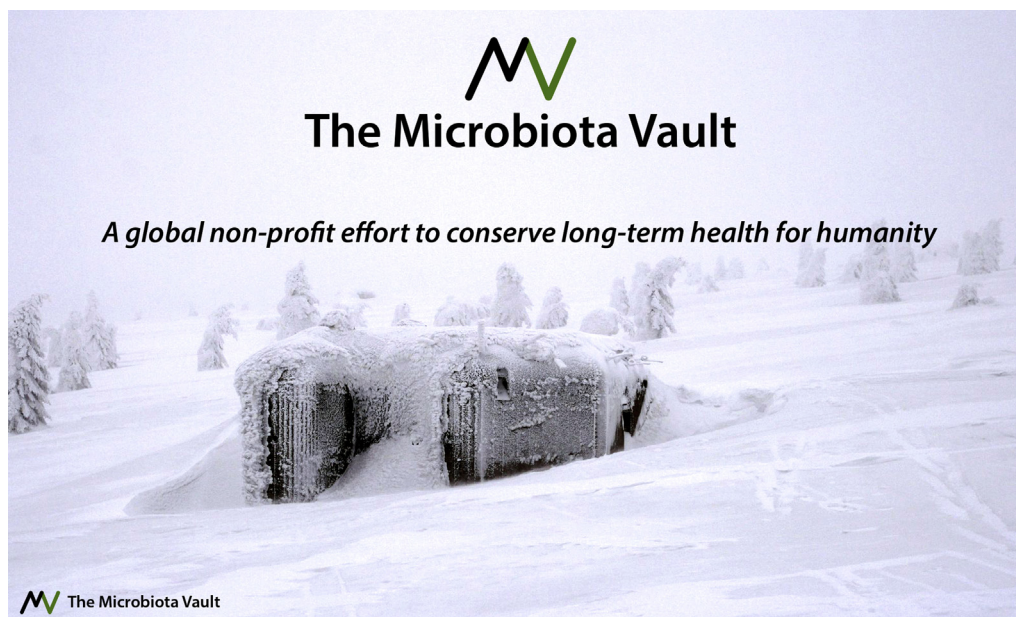


FIG 5. Creating a repository to preserve the ancestral microbiota. A nonprofit foundation (the Microbiota Vault; www.microbiotavault.org) has been established to develop a global repository that can be used to facilitate microbiome restoration in future generations.⁷⁹

antimicrobial response and a type 2–poised response that is elicited in response to tissue inflammation, epithelial damage, and alarmins.⁶⁰ This work identifies how microbiota-driven immune development plays a role in maintaining physiologic homeostasis and adaptable immune responses at mucosal sites.

AGE, MICROBIOTA, AND IMMUNITY

There is strong evidence that the age of the host is an important factor contributing to the host-immune axis. An important concept is that there is a critical window of development in which the microbiota regulate the immune education that cannot be meaningfully altered once that time period has elapsed (Fig 3).⁶¹ The acquisition of host-specific microbiota at birth differentially regulates intestinal immunity; mice raised with murine microbiota were more protected against gastrointestinal pathogens than mice raised with human microbiota.⁶² Several lines of evidence support the view that the composition of the microbiota during early life plays a critical role in immune phenotypes observed later in life. The maternal microbiota also plays a role in regulation of the offspring's innate immunity,⁶³ indicating that such development starts *in utero* and further points to the importance of postnatal events that regulate immune maturation.

In an animal model age at the time of first exposure is a major determinant of the outcome of the *H pylori*/host interaction. Whereas the neonatal tolerance window closes at 7 to 10 days after birth in mice, it might commence well before delivery. Evidence favoring this point comes from studies in which mice were exposed to *H pylori* extract *in utero* or transmaternally during lactation.⁴¹ Such offspring showed substantial protection against allergic disease manifestations independent of the protection status of the mother.⁴¹

These concepts support the importance of further characterizing early-life host-microbiota interactions and the

contributions of microbial diversity to immune regulation and, going forward, require focus on interventions that might have lasting clinical benefits.

THE MICROBIOME AS A MEDIATOR OF ASTHMATIC DISEASE

There is also evidence to support a role for both exogenous and endogenous microbiota contributing to childhood asthma development. For example, prenatal exposure to a rural farm environment correlated with protection against atopic sensitization in children.⁶⁴ In large epidemiologic studies antibiotic use during the first year of an infant's life correlated with increased asthma risk in young children.^{17,65} Similarly, in British Columbia a period of decreased antibiotic use during the first year of life significantly correlated with reduced asthma risk.⁶⁶ There is sufficient evidence to support that antibiotic use, mode of delivery, and diet can affect the composition of the human infant microbiota.² In support of the importance of microbial exposure and susceptibility to asthma, low doses of endotoxin were protective in a murine model of house dust mite–induced airway inflammation⁶⁷; in the same model germ-free mice had stronger immune responses.⁶⁸ Germ-free mice have greater serum IgE levels than select pathogen-free mice. As such, germ-free mice having increased susceptibility to anaphylaxis, a phenotype that can be ablated when mice are inoculated with a diverse microbiota before 6 weeks of age.⁶⁹ These data support the role of microbial protection from allergic diseases that is mediated by exposure during an early-life developmental period.

Given that microbial exposure, particularly during early life, has been identified as a factor in asthma development, the role of Toll-like receptors (TLRs) has been the focus of many investigations. TLRs are important receptors for activating innate immunity that have the ability to bind an array of microbially

derived ligands. Of significant interest is TLR4, which canonically binds the bacterial constituent LPS. TLR4 engagement is known to suppress T_H2 CD4⁺ T-cell differentiation and favor T_H1/T_H17 induction.⁷⁰ This is favorable because suppression of type 2 immunity is postulated to protect against asthma; supporting that claim, low doses of endotoxin were protective in a mouse model of asthma.⁶⁷ This evidence indicates that overall, increased exposure to particular types of bacteria or their products during early life correlates with protection from asthma and allergic diseases.

In the Canadian Healthy Infant Longitudinal Development cohort in Canada, children at risk for asthma were found to have reduced abundance of the genera *Lachnospira*, *Veillonella*, *Faecalibacterium*, and *Rothia*, as well as reduced fecal acetate levels. Transfer of fecal samples from at-risk children to germ-free mice and restoring the missing bacterial genera reduced the asthma phenotypes in an ovalbumin model of airway inflammation.⁷¹

In a Danish prospective cohort study on asthma, an association between the human fecal microbiota composition at 1 year and asthma risk at age 5 years was identified. Specifically, children born to asthmatic mothers and displaying altered microbial composition at age 1 year were 13 times more likely to have asthma at age 5 years than other children in the cohort.⁷²

In the United States children at the highest relative risk of atopy and asthma had reduced abundance of *Bifidobacterium*, *Akkermansia*, and *Faecalibacterium* species in the neonatal gut and enrichment of the linoleic acid 12,13-diHOME, a bacterial metabolite.⁷³ In a murine asthma model increasing intestinal concentrations of 12,13-diHOME led to reduced pulmonary Treg cell abundances and increased airway inflammation.⁷⁴ Overall, these studies have identified important and overlapping evidence of particular bacterial genera and metabolites that correlate with protection from asthma.

Beyond the gastrointestinal tract, the human lung bacterial community appears to be dominated by 6 genera during the first 2 years of life, at which point further diversification takes place. Colonization with pathogens predicts chronic wheeze in sensitized children. In addition, children with increased abundance of *Streptococcus*, *Hemophilus*, and *Moraxella* species in induced sputum samples had increased risk of chronic wheeze at age 5 years.⁷⁵ Asthmatic patients were also reported to have increased diversity in the lung microbiota but reduced biomass.⁷⁶ These findings support roles of the pulmonary microbiota in influencing susceptibility to asthma in addition to the influence of the gastrointestinal tract microbiota on asthmatic pathophysiology.

SOLUTIONS

Based on the increasing evidence of the microbiota's role in asthma, it is important to begin to consider specific solutions to both prevention and treatment. An obvious approach, which nevertheless must be stated, is to avoid further damage to the microbiome. Physicians and parents should juxtapose the physiologic costs of antibiotic courses, formula feeding, and cesarean section when considering their benefits in individual patients. A more accurate estimation of antibiotic benefit and risk would clearly reduce their overuse, as well as that for other medical interactions. The conversation should shift from "this might be helpful" to "is this necessary?"

A more specific approach is to attempt to restore particular disappeared organisms to either prevent or treat asthma. The development of such approaches will ultimately require clinical trials to assess benefit and risk. An intriguing possibility will be to give *H pylori* to children to restore this ancestral organism (Fig 4) and take advantage of its asthma- and reflux-reducing properties and then eliminate it with antibiotics in adulthood to reduce its potential to drive gastric cancer.

Clinical trials now aim to also exploit the prenatal window of opportunity. In one ongoing trial pregnant women and prospective mothers of at-risk offspring are being enrolled and given the prebiotic inulin to determine effects on the occurrence of atopic dermatitis as a primary end point and early predictive readout of allergy.⁷⁷ The results of this study are still pending.

A recent meta-analysis of 28 studies investigating the effects of probiotics administered prenatally and/or postnatally concluded that the risk of atopic dermatitis (as the earliest possible readout of atopy) could be reduced by starting probiotic treatment during gestation and continuing it through the first 6 months of the infant's life.⁷⁸ The available mouse data suggest that live *H pylori* or its extract might be equally or more efficient than prebiotics or probiotics at reducing allergy risk when given as early as possible in life.

In the future, microbes that have stereotypic interactions with particular arms of human immunity will be important candidates for trials. Alternatively, microbes or chemicals (prebiotics) that have no direct effect in immunity but that nourish or stabilize endogenous immunologically active populations might be useful. Recent studies that have identified particular taxa^{71,72} and bacterial metabolites⁷³ associated with asthma risk in human children might be especially helpful.

If the most beneficial organisms for asthma prevention have already largely disappeared from developed country populations, where will we obtain the organisms necessary for restoration? One solution will be to identify subjects and populations with little or no exposure to the modernizing practices and stockpile these specimens and purified cultures for future generations. The Microbiota Vault (microbiotavault.org, Fig 5),⁷⁹ a nongovernmental nonprofit foundation, has recently been established to facilitate this process in analogy to the Seed Vault, which is now in existence to preserve our precious patrimony of seeds for food cultivation.

CONCLUSIONS

A growing body of evidence is linking both the respiratory and gastrointestinal microbiome with the altered pathophysiology operant in asthmatic patients and patients with related allergic disorders. Such linkage is biologically plausible and ultimately actionable because tools are already in existence to reshape the microbiome in desired directions. However, much foundational work must be done to establish particular preventive and therapeutic modalities. In addition, a focus must be made to define protective organisms and the relevant periods of early-life development required to confer protection against asthma. Nevertheless, the promise is great for curtailing the pandemic of asthma by applying the knowledge learned about microbiome-immunologic interactions. Further explorations in this clinically important area will deepen our understanding of human immunology as well, with applications to other immune and autoimmune conditions.

What do we know?

- Lifestyle factors, such as exposure to antimicrobials, are leading to a loss in bacterial diversity in the human microbiome.
- Composition of the microbiota can affect immunologic development and phenotypes.
- We know candidate organisms identified in both the gastric mucosa (*H pylori*) and intestinal tract that can confer protection in murine asthma models and associate with protection in human subjects.
- We have begun to identify bacterial lung species, intestinal species, and metabolites that correlate with asthma or atopy.

What is still unknown?

- We need a better understanding of how generational losses in bacterial diversity contribute to asthma development and how much this contributes to the increased asthma burden observed globally.
- We need to better understand how to take actionable steps to modulate the microbiota and the windows of time at which these interventions are optimal in human hosts.
- We need a greater understanding of how candidate strains of bacteria or their metabolites interact with the rest of the microbiome. In the context of an ecosystem, we need to understand how one introduced member affects the whole.
- Although there is a growing body of evidence with respect to metabolites and bacterial strains on Treg cells in patients with asthmatic disease, we need to further advance our understanding of how bacteria-driven immune differentiation can alter immune responses to environmental antigens and allergens.

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