

The maternal gut microbiome during pregnancy and offspring allergy and asthma



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Environmental exposures during pregnancy that alter both the maternal gut microbiome and the infant's risk of allergic disease and asthma include a traditional farm environment and consumption of unpasteurized cow's milk, antibiotic use, dietary fiber, and psychosocial stress. Multiple mechanisms acting in concert may underpin these associations and prime the infant to acquire immune competence and homeostasis following exposure to the extrauterine environment. Cellular

and metabolic products of the maternal gut microbiome can promote the expression of microbial pattern recognition receptors, as well as thymic and bone marrow hematopoiesis relevant to regulatory immunity. At birth, transmission of maternally derived bacteria likely leverages this *in utero* programming to accelerate postnatal transition from a T_H2- to T_H1- and T_H17-dominant immune phenotype and maturation of regulatory immune mechanisms, which in turn reduce the child's risk of allergic disease and asthma. Although our understanding of these phenomena is rapidly evolving, the field is relatively nascent, and we are yet to translate existing knowledge into interventions that substantially reduce disease risk in humans. Here, we review evidence that the maternal gut microbiome impacts the offspring's risk of allergic disease and asthma, discuss challenges and future directions for the field, and propose the hypothesis that maternal carriage of *Prevotella copri* during pregnancy decreases the offspring's risk of allergic disease via production of succinate, which in turn promotes bone marrow myelopoiesis of dendritic cell precursors in the fetus. (J Allergy Clin Immunol 2021;148:669-78.)

Key words: Gut microbiome, fetal immunity, allergy, asthma

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The modern environment, lifestyle, and diet have altered the composition and function of the human gut microbiome,¹⁻¹¹ which in turn plays a central role in early-life immune development.^{12,13} Although most research regarding the gut microbiome and allergic disease has focused on the postnatal period,¹³⁻¹⁵ the maternal gut microbiome during pregnancy promotes fetal immune development,^{16,17} and appears to influence the expression of allergic disease in the offspring.^{18,19} A range of mouse and human studies support the putative construct shown in Fig 1: In a traditional environment, adequate exposure to environmental microbes, the absence of antibiotics, adequate dietary fiber, and lower levels of maternal psychological stress and mental illness decrease the risk of offspring allergic disease and asthma via their impacts on the mother's gut microbiome and its products. The potential underlying pathways include the alignment between maternal and infant immunity; transplacental passage of microbial metabolites and components; and possible seeding of a fetoplacental microbiome. Collectively, these result in increased expression of microbe-associated molecular pattern (MAMP) recognition receptors, promotion of thymic regulatory T (Treg) cells, and the establishment

Abbreviations used

DC: Dendritic cell
 EV: Extracellular vesicle
 SCFA: Short-chain fatty acid
 TLR: Toll-like receptor
 Treg: Regulatory T

of dendritic cell (DC) networks in the gut, lung, and other tissues. Inoculation of the infant gut with the maternal microbiota during and after birth leverages the antenatal programming to accelerate the transition from T_H2 to T_H1, interferon regulatory transcription factor 7, and T_H17-dominant immunity required for pathogen clearance, and promotes DC induction of Treg cells in the gut and lung required to calibrate inflammatory responses.

There are however fundamental gaps in current knowledge. Few human studies have evaluated direct microbial transfer between mother and child, nor directly examined the relationship between the maternal gut microbiome and offspring allergic disease and asthma.^{18,19} No such studies have incorporated metagenomic measures; nor have human studies demonstrated mediation pathways linking environmental factors, via maternal microbiome, and infant immunity to clinical outcomes. Relatively little is known about the multitude of factors driving fetal immune development, few human studies have shown that microbiome-related interventions during pregnancy alter offspring immunity, and overall, findings regarding probiotics supplementation during pregnancy are limited and conflicting. In this context, there is a clear need for improved evidence to progress and translate the potential of manipulating the maternal microbiome to prevent offspring allergic disease and asthma.

EXPOSURES IMPACTING BOTH MATERNAL GUT MICROBIOTA AND OFFSPRING ALLERGY

Farming environment

Studies in Europe, Asia, the United States, and Africa have shown that early-life exposure to a traditional farm environment is associated with reduced risk of allergic disease and asthma.²⁰⁻²⁶ Although prenatal and postnatal exposures were highly correlated in each of these studies, the Prevention of Allergy Risk factors for Sensitization In children related to Farming and Anthroposophic Lifestyle (PARSIFAL) cohort was large enough to show that prenatal farm exposure may be more protective than postnatal exposure.²⁰

Although the “farm effect” appears to relate to exposure to livestock^{20,27,28} and a diverse range of environmental microbes,²⁹⁻³² several studies have shown that maternal consumption of unprocessed milk is also relevant.^{27,33,34} Building on this, a recent study in mice showed that ingestion of raw versus pasteurized cow’s milk increased butyrate-producing *Clostridiales* in maternal fecal samples, decreased *Proteobacteria*, and conferred protection against IgE-mediated food allergy in the offspring.³⁵ The risk of *Listeria monocytogenes* infection associated with ingestion of raw milk is an important consideration, particularly in pregnancy; thus, specific understanding of the underlying pathways is required to inform more targeted and safe interventions. Although traditional farming is becoming uncommon in Westernized populations, the same protection from allergic disorders has been observed in rural living environments, where extended biodiversity³⁶ has been suggested as a mediator.

Farm children demonstrate prenatal “priming” characterized in functional studies of cord blood by higher expression of the microbial pattern recognition Toll-like receptor (TLR)7 and TLR8 receptors,³⁷ equipping them to respond to viral-based signals during early infancy, as well as increased Treg-cell number and function, required to control inflammatory responses to pathogens and environmental stimuli.³⁸ Numerous studies have shown that both increased T_H1/IFN- γ responses,^{39,40} increased Treg cells, and enhanced levels of neutrophils³⁷ at birth predict decreased risk of allergic disease and asthma.⁴¹⁻⁴⁴ Consistent with prenatal priming, farm children demonstrate accelerated progression from T_H2/eosinophil-skewed immunity at birth, which is associated with susceptibility to both infections and atopy, to a more balanced and competent immune state characterized by robust antimicrobial responses to pathogens, and enhanced Treg-cell function required for maintenance of immune homeostasis and control of excessive inflammation.³⁷ Further studies are required to evaluate the contribution of the maternal microbiome to the “farm effect.”

Antibiotics

Antibiotics have profound and lasting effects on the gut microbiome.⁴⁵ Maternal treatment with antibiotics during pregnancy has been linked to increased eczema,⁴⁶ food allergy,⁴⁶⁻⁴⁸ and asthma⁴⁹⁻⁵¹ in offspring. Evidence of a dose-response between maternal treatment with antibiotics and offspring asthma in human birth cohort studies⁵² and experimental models⁵³ strengthens the case for causality. Antibiotic treatment during pregnancy may influence both the maternal and infant gut microbiome in mice,⁵⁴ so it is difficult to delineate the relative importance of the prenatal and postnatal periods. It is also impossible to exclude confounding by indication, in particular antibiotic treatment of maternal respiratory infections, which may relate to maternal asthma, and thus shared maternal/infant genetic risk. From large register-based studies, where it is also possible to evaluate associations with antibiotic exposure to the mother around the pregnancy period⁵⁵ and by applying siblings design,⁵⁶ evidence of antibiotic use in pregnancy as a causal link to later disease disappears. Instead, these studies suggest that a susceptibility to infections may be the factor inherited, because the mother’s use of antibiotics outside the pregnancy period was just as important a risk factor for asthma in the child, and a higher risk of disease was also found in siblings unexposed to maternal antibiotics during pregnancy.

Diet

Although the relationship between maternal diet and offspring allergic disease remains controversial, recent work has added to the evidence that a maternal diet high in vegetables is associated with a reduced risk of offspring allergic disease in humans.⁵⁷ Diet has an important impact on gut microbiota composition and metabolic function.⁸ Ecological studies have consistently shown associations between a preagrarian or hunter-gatherer diet and lifestyle, and increased diversity of the gut microbiota, and increased production of specific microbial metabolites, in particular, short-chain fatty acids (SCFAs).^{1,2,9} In each of these studies, the hunter-gatherer communities had a diet high in plant-derived fibers,⁵⁸ which have a central role in shaping the composition and metabolic activity of the gut microbiome.¹⁰ A maternal diet high

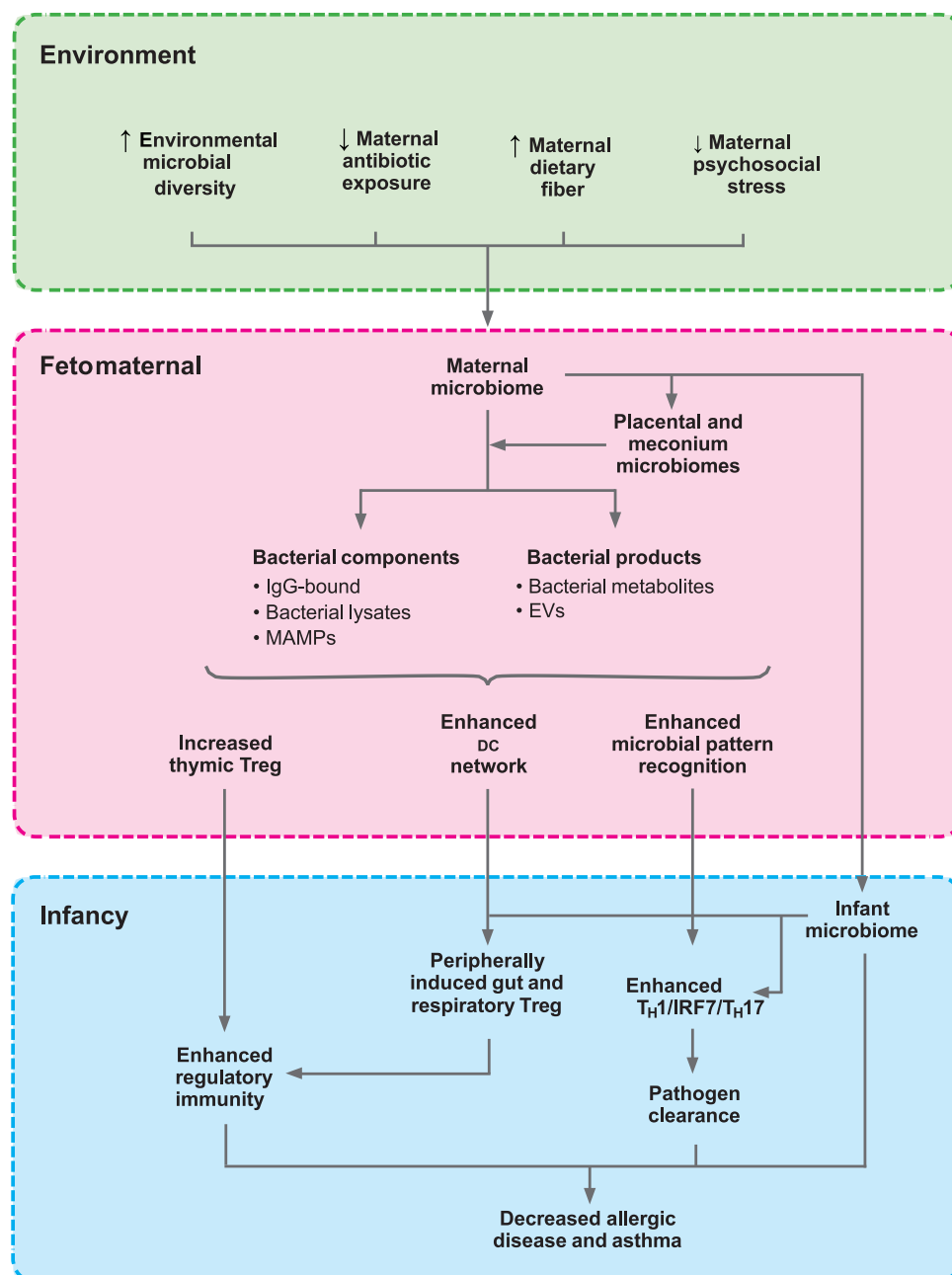


FIG 1. Putative pathways linking environmental factors, the maternal gut microbiome during pregnancy, early-life immune development, and protection against allergic disease and asthma. IRF7, *Interferon regulatory transcription factor 7*; MAMPs, *microbe-associated molecular patterns*.

in fiber is associated with increased diversity and richness of gut microbiota during pregnancy, with increases in specific taxa including *Holdemania*, *Roseburia*, and *Lachnospira* and *Coprococcus*.⁵⁹⁻⁶¹ Studies in mice have shown that a diet low in fiber leads to intergenerational depletion in the diversity of gut microbiota, which only partially recovers following reinstitution of a high-fiber diet.⁶² This suggests that the low-fiber diet, which is now endemic in many parts of the world, is contributing to the loss of our “ancestral” gut microbes. Anaerobic bacteria in the gut ferment fiber to produce SCFAs, which have potent local and systemic immune effects⁶³ and can cross the placenta. In mice, a maternal diet high in fiber attenuates inflammatory airway

responses to ovalbumin challenge in the offspring.⁶⁴ Although the underlying mechanisms are not fully characterized, SCFA induction of Treg cells may be relevant,⁶⁵ and human studies investigating maternal diet-by-microbiome pathways to enhanced infant immune competence are required.

Psychosocial stress and mental illness

Maternal psychosocial stress and mental illness during pregnancy are associated with increased risk of allergy and asthma in the offspring.⁶⁶⁻⁶⁸ Stress-induced release of glucocorticoids has been suggested to mediate these effects,⁶⁹ but the impact of

psychological stress and anxiety on the maternal gut microbiome may also be relevant.^{11,70-72} In support of this, animal studies have demonstrated that maternal psychosocial stress before and during pregnancy may alter both the mothers' and infants' intestinal microbiota, in part mediating the effects of maternal stress on immune development of the offspring.⁷³ Related studies are needed in humans.

POTENTIAL MECHANISMS

Maternal-fetal immune alignment

Preparing the fetal immune system *in utero* for the extrauterine environment is germane for survival, with maternally conferred immune protection against environmental pathogens representing a key defense mechanism. In this context, maternal transfer of humoral immunity, specifically of maternal IgG through an active mechanism enabled through neonatal Fc receptors, has been well established.⁷⁴ Through this process serum IgG levels of the term newborn are equal to or even exceed maternal levels. Of note, maternal IgG transfer of gut microbial components to the offspring during gestation also plays a crucial role in fetal innate immune development in mice.¹⁷ Postpartum, transfer of maternal immunoglobulins continues through lactation, but these antibodies confer mucosal immunity only.⁷⁵

In contrast, for cellular immunity it has been a long-held view that the fetus holds an immune privileged status, keeping the fetal and maternal immune cells separate, allowing physiologic development of the semiallogenic fetus without triggering inflammatory responses.⁷⁶ Immune tolerance toward the fetus is upheld by a series of highly effective mechanisms, with the placenta providing a barrier preventing immune rejection and/or trafficking of immune cells across the maternal-fetal interface.⁷⁷ The latter concept has been challenged by studies providing compelling evidence that traces of maternal cells can be found in fetal organs including the thymus⁷⁸ and vice versa.⁷⁹ This early exchange seems to shape the offspring's immune response and can in part explain the better acceptance of maternal versus paternal organ transplants.⁸⁰

There is also evidence for alignment of maternal and fetal lymphocytes, specifically of Treg cells. This alignment is likely linked to soluble factors that cross the placenta from the mother to the fetus and effectively influence the size of the fetal Treg-cell pool^{81,82} and metabolites of the maternal gut microbiome are likely candidates.⁸³

Bacterial metabolites

The SCFAs butyrate, propionate, and acetate are by-products of dietary fiber fermentation by gut bacteria.⁵⁸ In mouse models, maternal SCFAs attenuate inflammatory airway response in offspring.⁶⁴ Although the underlying mechanism is unknown, SCFAs have potent immunomodulatory properties including induction of Treg cells from naive T cells via dendritic cells (DCs) either through epigenetic changes by inhibiting histone deacetylases^{64,84,85} or through the activation of specific G-protein-coupled receptor (GPR)43.⁸⁶ Recently, acetate has been identified as an important factor in fetal thymic development, linked to the expression of the autoimmune regulator, which is known to contribute to Treg generation.⁸⁷ Succinate is another immune-active microbial metabolite that is increased in animals fed a high-fiber and high-fat diet.⁸⁸ Succinate binds GPR91,

stimulating DCs and enhancing DC-mediated T-cell induction and cytokine production.⁸⁹ Further studies are needed to investigate the impact of these metabolites of the maternal microbiome on fetal immune development and offspring allergic disease.

Bacterial components

In mice, repeated administration of the bacterial lysate OM-85 during pregnancy attenuates aeroallergen-induced asthma-like responses in the offspring.⁹⁰ This finding is consistent with earlier work showing that maternal oral intake of *Acinetobacter lwoffii* F78, derived from farm (barn) dust, during pregnancy attenuated aeroallergen-induced inflammation in the offspring, and that this process was TLR-dependent.⁹¹ The offspring of mothers treated with OM-85 during pregnancy demonstrate increased capacity for expansion and functional activation of Treg cells in the airway mucosa in response to aeroallergen challenge.⁹⁰ This enhanced regulatory capacity is underpinned by a dramatic expansion of bone marrow myeloid progenitors during late pregnancy, which in turn supply the establishment of tissue DC networks that mediate local immune surveillance and homeostasis.⁹⁰ Subsequent studies have shown that maternal OM-85 treatment is associated with transcriptional activation of cellular cholesterol biosynthesis pathways (mediated via the mevalonate pathway) and expansion of myeloid progenitors (myelopoiesis) and downstream conventional DCs displaying enhanced functional maturation within fetal bone marrow.⁹² The establishment of DC networks in late gestation can be considered a form of immune training—a process in which innate immune cells exhibit a sustained state of enhanced functionality. Recently, it has also been demonstrated that treatment of pregnant mice with OM-85 during pregnancy protects offspring against mouse-adapted rhinovirus, reducing cellular inflammation and clinical severity of lung disease.⁹³ Studies are needed to determine whether maternal gut microbiota and their products promote fetal immune development via the same pathways.

Bacterial extracellular vesicles

Like other cells, gut bacteria produce extracellular vesicles (EVs), which are blebs of plasma membrane carrying a cargo of intracellular products such as proteins, nucleic acids, and lipids.⁹⁴ There is growing interest in the role of EVs in mediating the immune effects of gut bacteria. For example, the activation of TLR2 by *Bacteroides fragilis* has been shown to be mediated by the release of EVs.⁹⁵ In postnatal mouse studies, the administration of *Lactobacillus plantarum* EVs prevented the development of atopic dermatitis,⁹⁶ whereas the administration of EVs from *Bifidobacterium longum* prevented food allergy.⁹⁷ Given that EVs are small structures that can be actively transported from the mother to the fetus,⁹⁸ studies are needed to evaluate whether EVs produced by maternal gut microbiota impact fetal immune development.

In utero bacterial colonization

The presence of bacteria in sites including placenta, amniotic fluid, and meconium was reported in 2011,^{99,100} and has since been the subject of intense debate. Subsequent studies have identified bacteria in placenta,¹⁰¹⁻¹⁰⁵ amniotic fluid,¹⁰⁶⁻¹¹¹ and meconium,^{103,104,112-114} and 1 study found that maternal consumption

of probiotics and contact with furry pets during pregnancy may each influence the microbiota present in meconium.¹¹⁵

Ascension and translocation through the choriodecidual barriers is one potential mechanism of *in utero* bacterial colonization.^{102,108,116,117} Alternatively, microbes derived from the maternal intestine and oral cavity may be translocated by hematogenous spread to the placenta during implantation,^{17,102} due to higher intercellular junctional permeability and/or DC transport.¹¹⁸ Translocation of bacteria to the maternal circulation, via the epithelial gaps of the intestine and the oral mucosa, is enhanced during pregnancy, and this enables the transfer of low numbers of bacteria to possibly seed the placenta.^{102,118-120}

Although some studies suggest that the presence of bacterial DNA in pregnancy tissues may relate to contamination of samples or reagents,^{104,105,121} it remains a possibility that a fetoplacental microbiome plays a role in fetal immune development. The meconium microbiota was found to reflect microbial composition of the amniotic fluid,¹²² suggesting that the fetal intestine could become colonized via ingestion of small quantities of amniotic fluid during the antenatal period and thus the meconium microbiome could be a proxy for the fetoplacental microbiome. Of relevance, a study from the Canadian Healthy Infant Longitudinal Development (CHILD) cohort suggests that the metabolic diversity of meconium collected at birth predicts both the infant's postnatal gut microbiota and the risk of allergic sensitization at 1 year.¹²³ Moreover, a recent study found live bacterial strains including *Staphylococcus* and *Lactobacillus* in fetal tissue, which induced *in vitro* activation of memory T cells in fetal mesenteric lymph nodes, supporting a role for microbial exposure in fetal immune programming.¹²⁴

Vertical transmission of gut microbiota at birth

In infants delivered vaginally, the maternal gut microbiome is the largest donor of the infant-acquired strains^{125,126} whereas the maternal vaginal transfer appears of minor importance.¹²⁷ Strain-level analyses of mother-infant pairs revealed that 50% of the microbial species in the infant gut on the day of delivery were transmitted from the mother's microbiome, and this fraction was relatively stable over the next 4 months.¹²⁵ The largest contribution at birth was from the mothers' gut, accounting for 22%, followed by the vagina (16%), the oral cavity (7%), and the skin (5%).¹²⁵ Moreover, the maternal gut microbial strains were more persistent in the infant's gut and more stably detected than those obtained from other maternal or environmental sources.^{125,126,128}

It is therefore unsurprising that mode of birth profoundly influences the infant's gut microbiome.¹²⁸⁻¹³³ Although the link between mode of birth and allergic disease remains controversial, a recent study found that increased risk of asthma following cesarean delivery was apparent in only those children who retained a "cesarean section signature" in the gut microbiome to age 1 year.¹³⁴ Strain-level analyses directly implicate the interruption of maternal-infant transmission in this effect.^{125,126,133,135} Interventions proposed to offset the alteration in gut microbiota induced by cesarean section include dietary supplementation with a mix of probiotics during pregnancy and to the infants,¹³⁶ and postnatally, orally delivered fecal microbiota transplantation from their mothers.¹³⁷ Both techniques appear to ameliorate birth mode-associated differences in infant intestinal microbiota, but

there is currently no evidence that either reduces the baby's risk of allergic disease or asthma.

DIRECT LINKS BETWEEN MATERNAL GUT MICROBIOTA AND OFFSPRING ALLERGY IN HUMANS

As far as we are aware, only 2 published studies have directly investigated the relationship between the maternal gut microbiota and offspring allergic disease in humans. A small study (n = 60) from 2012 used culture-based techniques to show that higher total aerobes and *Enterococci* in maternal fecal samples predicted increased risk of infant wheeze.¹⁹ More recently, we used 16S gene amplicon sequencing to demonstrate that maternal carriage of *Prevotella copri* during pregnancy predicts decreased IgE-mediated food allergy in the offspring.¹⁸ Importantly, maternal rather than infant carriage drove this association. *Prevotella* carriage is virtually ubiquitous in preagrarian, hunter-gatherer populations,^{1,2,9} and the genus is among the so-called ancestral microbes present in the human gut at least as far back as the Neanderthal period,^{7,138} and which are now disappearing in Westernized populations.¹⁻⁷ *P. copri* is the predominant *Prevotella* species within the human gut microbiome. Our study showed that reduced household size and the recent exposure to antibiotics were associated with decreased maternal carriage of *P. copri*.¹⁸ The link between household size and *P. copri* carriage is consistent with previous studies,¹³⁹ and intriguing given the well-known, yet still unexplained, association between larger household size and decreased allergic disease.¹⁴⁰ *P. copri* is an important producer of succinate,⁸⁸ which can stimulate function and migration of DCs.⁸⁹ Previous studies have shown that the metabolic products of the gut microbiome stimulate bone marrow hemopoiesis of DC precursors, which then migrate to peripheral tissues including the lung, attenuating the features of allergic airways disease.¹⁴¹ Considering the mounting evidence regarding the role of DC hemopoiesis in late pregnancy in prevention of allergic disease,⁹⁰⁻⁹² we hypothesize that maternal carriage of *P. copri*, via production of succinate, promotes the establishment of fetal DC networks, conveying protection against allergic disease and asthma (Fig 2).

PROBIOTIC SUPPLEMENTATION DURING PREGNANCY

Recent meta-analyses have found no consistent beneficial effects from probiotic supplementation (either prenatal or postnatal or both) for prevention of eczema¹⁴² or asthma.¹⁴³ These inconsistencies may be due to differences in the study populations, the probiotic strains, using a single strain rather than a complex mixture, and administration protocols. Although several trials found that combined maternal and infant probiotic supplementation with various strains of *Lactobacillus* reduced the risk of allergy,¹⁴⁴ others found no benefit^{145,146}; and the only trial of *Lactobacillus* in pregnancy alone was negative.¹⁴⁷ Potential benefits may be modified by mode of delivery. One trial randomized high-risk mothers to receive either a probiotic mixture (*L. rhamnosus* GG ATCC 53103 5, *L. rhamnosus* LC705 DSM 7061, *Bifidobacterium breve* Bb99 DSM 13692, and *Propionibacterium freudenreichii* ssp. shermanii JS DSM 7076) or placebo twice daily starting from 36 weeks of gestation.¹⁴⁸ Their infants then received the same probiotic combination mixed with galacto-

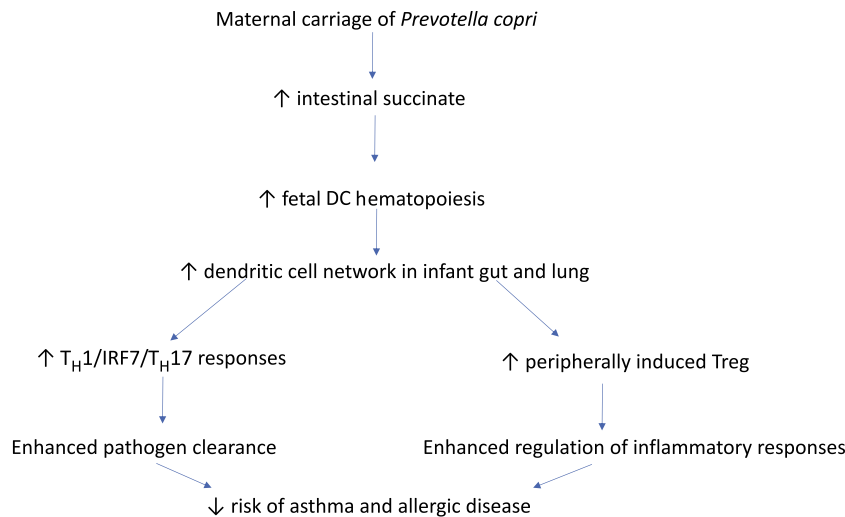


FIG 2. Hypothesized pathway by which maternal carriage of *Prevotella copri* trains fetal immunity, conveying protection against subsequent allergic disease and asthma. *IRF7*, Interferon regulatory transcription factor 7.

oligosaccharides once daily from birth until 6 months. Treatment with either probiotic mixture was associated with a reduced risk of allergen sensitization and eczema among children born via cesarean section but not among those born vaginally. Another trial found that maternal ingestion of probiotic milk containing *Lactobacillus acidophilus* LA-5, *Bifidobacterium lactis* Bb12, or *L. rhamnosus* in pregnancy was associated with a slightly reduced relative risk of atopic eczema at 6 months and allergic rhinitis between 18 and 36 months compared with no consumption during pregnancy.¹⁴⁹

Importantly, to date there have been no trials of supplementation with Gram-negative organisms, which may be relevant given that oral administration of the Gram-negative *Acinetobacter lwof-fii* F78 in mice resulted in decreased allergy in the offspring.⁹¹ This benefit was TLR-dependent, suggesting that LPS in the cell membrane of Gram-negative organisms may be important. Another consideration is that, in general, probiotic strains that do not colonize the bowel are specifically chosen; that is, they do not live permanently and multiply in the gastrointestinal tract. It is possible that active growth and multiplication is required to produce the metabolites that influence fetal and neonatal immune development, with subsequent reduction in offspring allergic disease.

FUTURE DIRECTIONS

Correcting the absence of deep whole metagenome sequencing data from human prebirth cohorts incorporating robust allergy and asthma outcomes is a priority. In contrast to 16S rRNA gene amplicon, deep metagenomic sequencing provides sufficient resolution at the species and strain level to infer the functional capacity and nutrient interactions of a given bacterium.¹⁵⁰ Estimation of absolute abundances of microbiota may also provide more accurate information than currently used measures of relative abundance.¹⁵¹ In principle, the integration of metagenomics, downstream measures of microbiome activity including metabolomics, transcriptomics, proteomics, and lipidomics with various immune measures has significant promise. However, multiomic data generated using separate assays over the course of prenatal

and postnatal life are both multimodal and temporal in nature. As such, their analyses and interpretation pose formidable challenges, and methodological frameworks built specifically for modeling time-varying multiomic data are required.¹⁵² The compositional nature of microbiome data also has important implications.¹⁵³ This is generally addressed through various forms of preprocessing steps, the choice of which can substantially impact the results,¹⁵⁴ making quantitative metagenomics a preferable strategy, whenever possible.

Robust consideration of confounding bias and mediation pathways also poses an important, but neglected, challenge for the field. Microbiome data are highly dimensioned, and the determinants of specific components/metrics are largely unknown; thus, *a priori* identification of common causes of exposures and outcomes (confounders) is largely speculative. One approach to this dilemma is to apply the so-called disjunctive cause criterion,¹⁵⁵ which has an elegant proof and removes the need for previous knowledge of common causes. Others argue that a strictly *a priori*-defined approach to estimating causal effects using highly dimensioned data risks missing important signals and that one should therefore conduct multiple analyses aimed to build up layers of causal features and exclude noncausal explanations such as bias, chance, or reverse causation.¹⁵⁶

Whichever approach is taken to preprocessing and analysis of human multiomics data, it is crucial to formulate clear research questions, and to then choose methods that are appropriate. This is a highly complex task requiring clinical, biological, bioinformatic, biostatistical, and epidemiological input. Reporting from human studies should include effect sizes both with and without adjustment along with a clear description of the causal framework that underlies the model(s) that has been applied, and the sensitivity of the findings to key assumptions. Importantly, the risk of false discoveries in highly dimensioned data must be controlled for and replication is crucial.

Progressing from correlation in human microbiome studies to causation and effective interventions requires mechanistic insights.¹⁵⁷ Preclinical studies have generally involved the transplantation of bacteria from humans with and without disease into germ-free mice and comparative analysis of pathological

outcomes. Although this approach has merit, it is of concern that the vast majority of published studies using this approach have found that transplantation of a given human-derived microbe results in altered expression of the relevant disease phenotype in mice, which is implausible, and a more rigorous approach to inferring causality is required.¹⁵⁸

CONCLUSIONS

The maternal gut microbiome plays a fundamental role in priming the infant's developing immune system to acquire immune competence and regulation following exposure to the extrauterine environment. Evidence from a diverse range of studies suggests that loss of our ancestral gut microbes and altered metabolic activity of the maternal microbiome may be contributing to the high rate of allergic disease and asthma in the modern environment. Although rapid progress in omics technology has created tantalizing opportunities to progress current knowledge regarding interplay between the microbiome and early-life immunity, analysis and interpretation of such complex data brings formidable challenges. In this context, transdisciplinary collaboration is needed to generate strong and clearly defined research questions, fit-for-purpose analysis plans, and iterative human and animal research to identify mechanisms and confirm causality.

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