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ALLERGIES AND THE ENVIRONMENT (MICHELLE HERNANDEZ, SECTION EDITOR)

Birth Mode, Breastfeeding, Pet Exposure, and Antibiotic Use: Associations With the Gut Microbiome and Sensitization in Children

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Abstract

Purpose of Review The infant gut microbiota has become a focus of multiple epidemiologic and cohort studies. This microbiome is derived from the mother (via the vaginal canal, maternal skin contact, breastfeeding, and possibly in utero microbial transfer) and is likely influenced by multiple external factors. It is now believed by some experts that colonization and formation of the newborn and alterations of gut microbiota in children are dependent on earlier alterations of the microbiota of mothers during or perhaps even before pregnancy. This review will focus on specific factors (pet keeping, breastfeeding, antibiotic use, and mode of delivery) that influence the infant gut microbiome and atopy.

Recent Findings This is a review of recent literature describing how pet keeping, breastfeeding, antibiotic use, and mode of delivery influences and changes the infant gut microbiome and atopy. General trends in gut microbiota differences have emerged in different birth cohorts when each external factor is analyzed, but consistency between studies is difficult to replicate. The aforementioned factors do not seem to confer an overwhelming risk for development of atopy alone.

Summary This review provides a comprehensive review of early life environmental factors and their influence on the infant gut microbiome and atopy.

Keywords Gut microbiome · Early life · Atopy · Breastfeeding · Antibiotics · Pets · C-section

Introduction

There is increasing evidence that implicates the gastrointestinal microbiota as a critical player in the development of diseases in children, including atopic conditions such as asthma and allergies [1, 2]. Multiple epidemiological studies suggest that early life exposure can alter the gut and that differential microbial exposure and colonization may influence allergic and atopic diseases [3, 4]. While the first major microbial colonization of newborns happens at birth when newborns are "seeded" with their mothers' microbiota [5••], it is now believed by some experts that colonization and formation of the newborn microbiota may begin during *in utero* development [6–8], whereby adequate alterations of gut microbiota in children may depend on earlier alterations of the microbiota of mothers during pregnancy or even before pregnancy [9–11].

The microbiome is broad and this review will focus on a few key early life environmental factors that are believed to influence both gut microbiome and allergic sensitization.

Household Pets

Association Between Pet Keeping and the Microbiome

Emerging data indicate that pets contribute to the microbial content of the home environment and also alter the maturing gut microbiome during infancy—a process known to shape the developing immune system including the development of atopy and the overall production of IgE [12••, 13, 14]. Indoor household pets have been consistently linked to an altered home microbial environment through assessments of the microbial content in vacuumed house dust or on surfaces in the

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home, which may have an impact on children's microbiological or immune development. Reported microbial alterations have been related to pet-keeping in general [15, 16], and specifically with dogs [1, 17–19] and cats [19]. The impact of indoor furred pets on environmental microbes has been demonstrated by assessment of community metrics such as microbial richness and diversity and/or by compositional analysis comparing the relative abundance of specific types of bacteria and fungi. It appears that once a dog is introduced into a home, new rare taxa become rapidly detectable in house dust with large relative abundance shifts in microbial composition seen within a year [20].

Perhaps more relevant to a child's immune development and likelihood of developing atopy, several reports detail an effect of pet keeping on the gut microbiome during infancy. A limited number of studies have assessed the effect of early life pets on the gut microbiome but associations have been reported. For instance, pets were found to contribute to neonatal gut microbial composition independently of other factors in a multivariable analysis among 130 fecal samples collected at approximately 1 month of age from the Detroit WHEALS birth cohort [21••]. Although household cats and dogs were associated with 3–6-month fecal microbial composition in univariate analyses in the Vitamin D Antenatal Asthma Reduction Trial, neither was confirmed as an independent contributor in multivariable models [22].

The presence of specific gut microbial taxa during infancy have also been associated with pet keeping. Fecal samples from 1-month-old infants were significantly more likely to contain an animal-derived *Bifidobacterium pseudolongum* strain if living with furred pets versus in a pet-free home [23]. The relative abundance of specific fecal microbial taxa was assessed in a small subsample ($n = 24$) of infants from the Canadian Healthy Infant Longitudinal Development Study (CHILD) cohort. When compared to other infants, those living with pets had under-representation of *Bifidobacteriaceae* and over-representation of *Peptostreptococcaceae* in fecal samples collected at 4 months [24]. A subsequent report assessing a larger sample ($n = 746$) from the same cohort, but analyzing 3-month fecal samples, found that pet exposure was associated with significant enrichment of *Oscillospira* and *Ruminococcus* [25]. In 114 slightly older children in the Danish SKOT 1 cohort, infants with furred pets had lower fecal diversity in the *Firmicutes* phylum at 9 months and higher abundance of *Cronobacter* at 18 months of age, but pets were not associated with overall gut bacterial diversity or richness at either time [26]. Two previous published studies also failed to find associations between pets and the gut microbiome, although these reports were limited; in that, microbial assessments were done only on culturable bacteria [27] or by culture independent 16S profiling limited to *Bifidobacteria*, *Escherichia coli*, *Clostridium difficile*, *Bacteroides fragilis* group, and *Lactobacilli* [28].

Association Between Pet Keeping and Atopy

Epidemiological studies have often focused on the presence of "furred indoor pets" including household cats and dogs, during the first years of life, and assessed whether these exposures are associated with an altered future risk of atopy. Although not a universally reported finding, multiple epidemiological studies describe lower rates of atopy (IgE sensitization to common allergens) among children having early-life exposure to household pets.

Some cross-sectional, retrospective studies reporting associations between indoor furry pet exposure during the first 1–2 years of life and atopy estimate approximately 20 to 25% lower likelihood of atopy (typically defined as having at least one positive skin test or allergen-specific IgE tests to multiple common allergens) in school age children [29, 30]. Some similarly designed studies report significant risk reduction specific only for dogs or cats [31, 32]. One of these reports assessing dog exposure indicated lower allergic sensitization rates that persisted into adulthood, even after adjusting for parental allergy, sibling allergy, and adult pet ownership [32]. However, a large cross-sectional study failed to confirm associations of atopy with early-life dog or cat exposure other than a lower rate of cat skin prick test positivity in school age children among those cat-exposed during the first year of life [33].

Prospective cohort studies [34–37] also support an effect of early life household pets on reduced atopy with reported risk reductions up to more than 50% in some studies and perhaps a larger risk reduction with dog versus cat exposure [36, 37]. Similar to results in cross-sectional studies, there are prospective cohorts failing to confirm such associations [38, 39].

Potential reasons for the discrepancies between these studies may be related to nuances in data collection and analysis. For instance, one study found that only children exposed to multiple dogs or cats in the first year of life had a statistically significant reduction in allergen sensitization at age 6 or 7 years [36]. Another potentially important consideration is that the association may wane over time. In the same cohort described above, the strength of the association of pet keeping with lower atopy lessened and was not statistically significant approximately a decade later, although lower pet-specific sensitization rates (i.e., lower dog sensitization among dog-exposed infants) remained significant [40]. Furthermore, when assessing the relationships between pet keeping and atopy, the importance of adjusting for covariate exposures that may also impact the microbiome, such as mode of delivery, has also been recently highlighted [41]. Finally, some concerns have been raised regarding reverse causation, as atopic families may choose to not have household pets. However, we and others have not found evidence of selection bias for pet ownership based on family history of atopy [40, 42].

To summarize, a substantial volume of data supports the hypotheses that early furred pet exposure lowers the risk of

atopy and emerging data also supports the theory that the effect may be mediated through a microbial mechanism. Although inclusion of a detailed description of the data is beyond the space limitations of this review, it is tempting to note that increased microbial exposure attributed to keeping livestock is considered a key exposure explaining low rates of atopy among children growing up in farming environments [43, 44]. Research focused on identifying the specific microbes and mechanisms responsible for the association between animal exposure and low rates of atopy hold promise for the development of potential intervention strategies targeting the prevention and treatment of atopic disorders.

Breastfeeding

Association Between Breastfeeding and the Microbiome

Human breast milk is a complex fluid that contains an array of bioactive components, including immunoglobulins, fatty acids, hormones, and cytokines [45]. Though historically believed to be a sterile fluid, studies over the years have shown that human milk is both prebiotic and probiotic in nature, as it contains human milk oligosaccharides (HMOs) as well as microbes [46]. Therefore, human milk is capable of altering infant gut microbiota composition both indirectly (i.e., through the transfer of prebiotics which exert selective pressures by promoting the growth of specific bacteria) and directly (i.e., vertical transmission of bacteria which provides pioneering species). Previous studies have indicated that though the breast milk microbiota has high inter-individual variation, it is often composed of *Streptococcus*, *Staphylococcus*, *Propionibacteria*, lactic acid bacteria, and *Bifidobacterium* [47–49].

One of the earliest studies examining how infant gut microbiota composition differed between breastfed and formula-fed infants used quantitative polymerase chain reaction (qPCR) to characterize the infant gut microbiota of 1032 children from a Dutch birth cohort at 1 month of age; they found that breastfed infants had a lower colonization rate of *E. coli*, *Clostridium difficile*, *Bacteroides*, and lactobacilli, compared with formula-fed infants [28]. A later study using fluorescent in situ hybridization (FISH) combined with flow cytometry to quantify the 6-week intestinal microbiota of European infants found that *Bifidobacteria* dominated the microbiota of breastfed infants, whereas formula-fed infants had significantly higher proportions of *Bacteroides*, *Clostridium coccoides*, and *Lactobacillus* [50]. More recently, Bäckhed et al. performed metagenomic shotgun sequencing on the stools of 98 Swedish infants and found that the cessation of breastfeeding shifted the microbial ecology toward an adult-like composition, whereas the gut microbiome of breastfed infants at 12 months was still dominated by *Bifidobacterium*, *Lactobacillus*, *Collinsella*, *Megasphaera*, and *Veillonella* [5••]. In a US birth cohort, the gut microbiota at 1 and 6 months of age was examined in 298 children using 16S rRNA sequencing; they found that breastfeeding was associated with decreased diversity and compositional differences at both time points, and that breastfeeding independently explained compositional differences in multivariate models. At 1 month of age, breastfeeding was associated with an increased abundance of *Streptococcus*, *Staphylococcus*, and *Prevotella*; at 6 months of age, it was associated with an increased abundance of *Lactobacillus* and *Bifidobacterium* [21••].

A large international study ($n = 684$) that used 16S rRNA sequencing to examine the gut microbiota up to 6 months of age found that breastfed children had lower diversity as well as lower abundances of *Bacteroidetes* and *Firmicutes*, and that breastfed children had less mature microbial compositions [51]. Most recently, a case-control study designed to examine type I diabetes in children reported on the development of the gut microbiome from 3 and 46 months of age in 903 children, using both 16S rRNA sequencing and metagenomic sequencing [52]. They found that breastfeeding was the most significant factor associated with microbiota composition, with breastfeeding being associated with lower diversity and higher levels of *Bifidobacterium* species, and the cessation of breastfeeding resulting in the maturation of gut microbiota, with increases in the abundance of *Firmicutes*.

Though the findings of these studies are not identical likely due to differences in technology, collection and processing protocols, timing of sample collection, and populations under study, they consistently indicate that breastfed children have lower bacterial diversity compared to formula-fed children, that breastfeeding is a large predictor of infant gut microbiota composition, and that the cessation of breastfeeding is associated with a shift to a more mature, adult-like microbiome. However, the specific taxa associated with breastfeeding across studies did differ somewhat, but generally indicate an increased abundance of *Bifidobacterium* and a decreased abundance of *Firmicutes* and *Bacteroides* in breastfed children.

Association Between Breastfeeding and Atopy

The World Health Organization (WHO) recommends that infants should be exclusively breastfed for the first 6 months of life, with continued breastfeeding up to 2 years of age or beyond [53]. Although breastfeeding is considered the gold standard of infant nutrition and is recommended for overall health and development, the results of studies investigating its role in allergy prevention have not been conclusive [54, 55]. For studies specifically examining allergic sensitization defined by specific IgE (sIgE) or skin prick tests (SPTs), many studies failed to find a significant association with breastfeeding. For example, a large cluster randomized trial ($n = 13{,}889$) with a breastfeeding promotion intervention found that the experimental group had no reduction in risks of inhalant sensitization (defined by + SPTs) at 6.5 years of

age [56••]. A large birth cohort in the United Kingdom (n = 9166) also failed to find a significant association between breastfeeding and inhalant sensitization (defined by + SPTs) at 7 years [57]. More recently, a large birth cohort in the Netherlands (n = 5828) failed to find an association between breastfeeding and food and inhalant sensitization (defined by + SPTs) at age 10 [58]. A smaller analysis of 335 high-risk children (maternal history of asthma) from a Danish birth cohort also failed to find an association between breastfeeding and development of sensitization (defined by both sIgE and SPT to foods and inhalants) over the first 6 years of life [59].

However, some studies have indicated a protective effect of breastfeeding on sensitization, while others have associated it with increased risk. An early analysis of 2187 Australian children in a prospective birth cohort found a protective effect of breastfeeding on inhalant sensitization (defined by + SPTs) at age 6 [60]. More recently, a Taiwanese birth cohort of 258 children found that breastfeeding was associated with a decreased risk of cow's milk sensitization (by sIgE) up to 2 years of age, but did not find an association with the other examined allergens [61]. On the other hand, a study in New Zealand of 1037 children found that breastfeeding was associated with an increased risk of inhalant sensitization (defined by + SPTs) at 13 years of age [62]. Similarly, an analysis of 405 children from a Detroit area birth cohort found that breastfeeding was associated with an increased risk of inhalant sensitization (defined by + SPTs) at 6–7 years of age [63].

Researchers have recognized that there are many factors that likely contribute to the heterogeneity of these findings [54, 55]. In the above referenced studies, we generally refer to the exposure as "breastfeeding," but there are differences in these definitions that limit the ability to compare results from different studies; these complications also make meta-analyses particularly difficult to perform. There are also many challenges that arise due to the observational nature of the majority of studies. For example, confounding and effect modification must be carefully considered and evaluated. There is also potential for bias due to loss to follow-up (e.g., breastfeeding mothers may be more likely to participate), and reverse causation is also possible (e.g., mothers may prolong breastfeeding due to early symptoms of allergic disease). Additionally, comparing children who are breastfed versus formula fed may not adequately capture the variability intrinsic to these exposures, as human milk composition differs both within and between individuals [54] and the composition of infant formula also varies by brand and type [64].

Antibiotics

Association Between Antibiotic Use and Microbiome

It is scientifically logical to assume that courses of antimicrobials can disrupt the infant gut microbiome. Much interest has been generated to examine this possible association during the first few months of life given the plasticity of the infant microbiome. Several studies have investigated how antibiotics given in the immediate postpartum (to the infant) or antepartum (to the mother) window influence the gut microbiota of the infant.

In a small study of 15 preterm infants, short treatment (≤ 3 days) of antibiotics led to decreased infant gut *Bifidobacterium* immediately after treatment (p = 0.027) till postnatal week three (p = 0.028) [65]. Long treatment (≥ 5 days) caused *Bifidobacterium* abundance to remain decreased until postnatal week 6 (p = 0.009). In both short- and long-term treatment infants, *Enterococcus* became a dominant member of the microbial community in several infants. Although the total bacterial count (by qPCR) was not significantly decreased in infants who received both short- and long-term antibiotics, some of the infants had lower bacterial counts early in life suggesting a delay in colonization with *Bifidobacterium* due to antibiotic therapy. Infants who received only short-term antibiotics had gut microbiota that more closely resembled control infants, and their microbiotas appeared to eventually recover despite initial compositional shifts. When performing redundancy analysis, length of antibiotic use was the main factor that explained variation between samples. However, antibiotic use did not alter community richness and diversity. Over a decade prior, the KOALA study similarly demonstrated in 1032 infants that antibiotic use was associated with decreased numbers of *Bifidobacteria* and *Bacteroides* in infant stool samples obtained at age 1 month. After antifungals, there were also lower levels of *Bifidobacterium* [28].

Mothers are given intrapartum antibiotic prophylaxis (IAP) as standard treatment for prevention of vertical transmission of Group B *Streptococcus* (GBS) to neonates per ACOG guidelines and can be given every 4 h for the duration of labor [66]. This standard of care treatment is an opportune window to examine how antibiotics can affect the infant microbiome. The association IAP in the mother and infant gut microbiome was examined in a longitudinal prospective birth cohort of 83 mother-infant pairs, comparing infants born vaginally with no antibiotic exposure (n = 53), infants who were exposed to intrapartum antibiotic prophylaxis (IAP) for Group B *Streptococcus* (GBS; n = 14), and cesarean section (C-section) born infants (n = 7) [67••]. Early in life, the bacterial community of infants exposed to IAP for GBS differed from that of unexposed infants at 10 days and 6 weeks of age (p < 0.05) but these differences were not seen by 12 weeks. There was also a delay in colonization with *Actinobacteria* in both vaginally born infants exposed to IAP for GBS and those delivered via C-section. Regarding the length of time of exposure to antibiotics, for each hour of IAP for GBS administration during vaginal birth, there was a decrease of 7.2% in the abundance of *Bifidobacterium* and a positive effect on the abundance of *Clostridium*. A similar pattern of lower abundance of

Actinobacteria and *Bacteroidetes* as well as an overrepresentation of *Proteobacteria* in infants born to mothers who had received IAP was also observed by Aloisio et al. [68].

In a study evaluating the impact of IAP in a cohort of 40 full-term vaginally born infants (18 of whose mothers received IAP), IAP infants displayed lower relative proportions of *Actinobacteria* and *Bacteroidetes* and increased levels of *Proteobacteria* and *Firmicutes* [69]. More specifically, in IAP infants, the level of *Firmicutes* increased from an initial 24% at 2 days of age to a 38% at 10 days and then stabilized. In contrast, *Firmicutes* levels were relatively stable in infants not exposed to IAP. Another study demonstrated that infants exposed to antibiotics after 3 months of life had significantly lower total bacterial counts, as well as lower counts of *Bifidobacterium* and *Staphylococcus* at 6 months, suggesting again that the first few months of life are indeed a critical window for influencing the gut microbiome [70].

Association Between Antibiotic Use and Atopy

A recent meta-analysis was unable to find any studies showing an association between antibiotic use during the first 2 years of life and allergic sensitization by specific IgE or skin testing (22 studies), although there were small but significant associations with clinical outcomes such as hay fever, eczema, and food allergy [71••]. The Manchester Asthma and Allergy Study examined multiple outcomes and exposures in their population-based birth cohort over 11 years. When examining antibiotic exposure (during first year of life, ever, and age when antibiotics were given) there was no association with sensitization to allergens [72].

The Avon Longitudinal Study of Parents and Children also was unable to show an association between antibiotic use and atopy (by skin testing to dust mite, cat, and grass) [73]. Mothers were asked about antibiotic exposure during the first 2 years of life (0–6 months, 6–15 months, and 15–24 months of life). Children were skin tested at 7.5 years of age to dust mite, cat, and grass. Although there was increased odds of asthma, there was no increased odds of sensitization even with four or more courses of antibiotics.

In a high-risk birth cohort, parents kept daily diaries for their child's health over 5 years (starting at the time of birth) and included information on antibiotics used [74]. The diaries were usually by the mother, and involved ticking the relevant boxes if their child had a fever, runny/blocked nose, cough, or wheeze, as well as the use of prescribed and over-the-counter medication. At age 5 years, total IgE was checked from venous blood samples and children were skin prick tested to a panel of allergens including fresh cow's milk, egg white, rye grass, *Alternaria*, *Aspergillus*, house dust mite, and cat dander. Similar to aforementioned studies, no association was found between antibiotic use throughout the first 12 months of life and skin prick test as well as total IgE.

On a larger scale, A New Zealand birth cohort ($n = 1005$) examined the association between antibiotics given between birth to 3 months of age, and skin prick testing at 15 months to *Dermatophagoides pteronyssinus*, cat, dog, rye grass, cow's milk, egg white, peanut, aspergillus, and cockroach [75]. There was a marginal association between antibiotic use and sensitization (OR 1.44, 95% CI 0.96–2.14) which became nonsignificant after adjustment for chest infections (OR 1.36, 95% CI 0.91–2.05).

Combined, these findings suggest that while antibiotics may alter the microbiome, there is a lack of evidence supporting their role in the development of allergic sensitization. Investigation of a causal pathway between antibiotics, the microbiome, and atopic sensitization may help to further clarify their relationships.

Delivery Mode

Association Between Delivery Mode and Microbiome

C-section is one of several factors that can lead to dysbiosis of the newborn gut microbiome [76]. It is thought that C-section can contribute to infant gut dysbiosis because of a lack of exposure to the maternal vaginal and fecal bacteria that results from term vaginal birth [77••, 78]. Numerous studies have reported differences in newborn/infant gut microbiome diversity and/or composition between vaginal and C-section delivered infants [5••, 6, 21••, 22, 28, 78–84]. Consistent among many of these studies are findings of lower overall microbial diversity and decreased abundance of *Bifidobacterium*, *Bacteroides*, and *Lactobacillus* in infants born by C-section compared to those vaginally born [21••, 22, 28, 77••, 78, 80–84]. Studies that examined planned versus emergency C-section found differences between these groups, suggesting that partial labor may somewhat skew the infant gut microbiome toward that of vaginally born infants [21••]. In contrast, some studies have found no difference in infant gut microbiome composition between C-section and vaginal birth [85] or have found higher diversity in the infant gut microbiome with C-section birth [22].

In support of the epidemiologic findings of differences in infant gut microbiome composition between C-section and vaginal birth, mice born by C-section were found to have differences in gut microbiome composition and maturation compared to those born vaginally [86, 87, 88••]. In addition, the C-section-born mice had alterations in their immune function, including a reduction in the proportion of regulatory T cells and downregulation of the regulatory markers *Foxp3*, *Il10*, and *Ctla4* [86, 88••].

Association Between Delivery Mode and Atopy

Birth by C-section has been associated with allergic outcomes in children in numerous studies, but the findings are variable

and conflicting. Several recent reviews [77••, 89, 90] summarize the findings of numerous studies reporting associations, or lack thereof, of C-section with various allergic outcomes in children, including atopy, atopic dermatitis, food allergy, and asthma. A meta-analysis of 20 studies of the association between C-section and childhood asthma reported in 2007 [91] found a statistically significant association with an odds ratio of 1.20 (95% CI 1.14, 12.6). A more recent (2015) meta-analysis of 26 studies (12 of which were also included in the 2007 meta-analysis and 14 of which were published since the 2007 meta-analysis) on the association between C-section and childhood asthma revealed an odds ratio of 1.16 (95% CI 1.14, 1.29) [92]. Bager et al. [93] published a meta-analysis of six allergic outcomes (food allergy/food atopy, inhalant atopy, eczema/atopic dermatitis, allergic rhinitis, asthma, and hospitalization for asthma) in association with C-section, including 26 studies through May 2007. They reported that C-section was associated with an increased summary odds ratio (OR) of food allergy/food atopy (OR 1.32, 95% CI 1.12–1.55; six studies), allergic rhinitis (OR 1.23, 95% CI 1.12–1.35; seven studies), asthma (OR 1.18, 95% CI 1.05–1.32; 13 studies), and hospitalization for asthma (OR 1.21, 95% CI 1.12–1.31; seven studies). In contrast, they found no association with inhalant atopy (OR 1.06, 95% CI 0.82–1.38; four studies) or eczema/atopic dermatitis (OR 1.03, 95% CI 0.98–1.09; six studies). For each significant association with an allergic outcome, only 1–4% of cases were attributable to C-section [93]. The consensus finding appears to be that C-section delivery is a modest, but real, risk factor for allergic outcomes in children and the degree of risk imposed by C-section likely varies by numerous biological and environmental factors related to the infant and its parents.

Several investigators have reported alterations in gut microbiome of infants that later developed allergic outcomes compared to infants that did not develop allergic outcomes. A decreased prevalence or abundance of *Bifidobacterium* or *Bacteroides* is a consistent finding, being reported among children with atopic dermatitis and/or at least one positive skin prick test result at 2 years of age [94], at least one positive skin prick reaction at 12 months [95], allergic symptoms and skin prick test positive through age 5 years [96], eczema at 5 years of age [97], IgE-associated eczema through 2 years of age [98], and multi-sensitized atopy at 2 years of age [13]. In contrast, both increased [3, 99] and decreased [96] *C. difficile* have been reported in infants who went on to develop allergic outcomes.

To summarize, the relationship between C-section and infant gut microbiome composition and allergic outcomes in children is not definitive and is likely confounded by numerous factors including reason for C-section, planned versus emergent C-section, maternal health and obesity, duration and exclusivity of breastfeeding, antibiotic use in mother and/or infant, tobacco smoke exposure, and socioeconomic status as well as and the timing and methodology of microbiome and allergic outcome determinations.

Conclusion

To date, no specific organism, or group of organisms, in the infant gut has been reported to be definitively protective or causative of allergic outcomes during childhood. This is perhaps in no small part related to the lack of understanding of what constitutes a "normal" or "healthy" gut microbiome. Longitudinal studies with multiple early and sequential stool samplings of infants into childhood and adolescence and monitoring of infants/children for development of allergic outcomes are necessary to continue to tease apart the various contributors to infant gut microbiome dysbiosis and childhood allergic outcomes.

Compliance with Ethical Standards

Conflict of Interest Drs. Zoratti and Kim report grants from NIAID, during the conduct of the study. Ms. Sitarik reports grants from NIH, during the conduct of the study. The other authors declare no conflicts of interest relevant to this manuscript.

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