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Development of the Infant Intestinal Microbiome: A Bird's Eye View of a Complex Process

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Abstract

Infants undergo profound shifts in colonizing intestinal microorganisms during their first year, especially during and after birth and during weaning. Microbiota are passed to infants through the placenta, during the vaginal birth process, and from early diet and other environmental exposures. These microbiota play an active role in the development of healthy infant metabolic and immunologic systems; profound shifts in microbiotal populations can be persistent, are associated with immediate alterations in gene expression, metabolic, immunologic, and neurologic function, and with downstream metabolic and immunologic consequences such as obesity, allergies, asthma, autoimmune diseases, and potentially neurologic conditions. Many modern exposures, including Cesarean section, formula feeding, and antibiotics, have been associated with microbiome shifts, and also with downstream diseases; while many published studies considered exposures individually, a more comprehensive understanding of their interaction and impact will consider the entirety of the infant's environment. It is not possible, nor desirable, to return to a world without toilets, sewers, tap water, delivery room antisepsis, Cesarean sections, antibiotics, immunizations, and refrigerators; our other alternative is to better understand these complex changes in infant developmental and molecular physiology. Protecting and repairing the developmental processes of the healthy infant microbiome is the modern medical frontier.

Keywords

anti-bacterial agents; child; drug resistance; microbiome; infant

"It's never the changes we want that change everything."

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—Junot Díaz, The Brief Wondrous Life of Oscar Wao

Introduction

The term microbiome refers to the population of more than 1 trillion microscopic organisms living in and on us through our lifetime—10× the number of our own cells (Palmer et al., 2007; Johnson and Versalovic, 2012). We have co-evolved with our human archetypal microbiota, and, similar to our genetic code, our "heirloom" microbiota composition has been passed down as part of our shared human ancestry (Dethlefsen et al., 2008; Sansonetti, 2011; Funkhouser and Bordenstein, 2013; Aagaard et al., 2014; Abrahamsson et al., 2015; Backhed et al., 2015; Frese and Mills, 2015). It has become increasingly evident that our relationship with these organisms is symbiotic, that we depend on them for digesting our food, training our immune and metabolic systems, and crowding out and serving as a barrier to more pathogenic organisms (Putignani et al., 2014) Molecular characterization methods have revealed that colonization is a complex dynamic process, influenced by the infants' cells and environmental influences, during a critical time period of infant development. Perturbation of this prototype population structure, termed *dysbiosis*, at any point along its development or equilibrium, is increasingly shown to be associated with altered microbial interactions and population dynamics, selective gene expression, epigenetic modification, and subsequent immune and metabolic system dysfunction, linked to a host of downstream related illnesses.

Antibiotic use, rocketing from none to virtually universal exposure over the past 75 years, has been blamed for the increasing prevalence of health conditions linked to aberrant metabolism and immune recognition, such as allergies, asthma, and obesity; however today's antibiotics are not encountering a pristine environment. Our native ancestral microbiome is now a vanished ideal; other modern practices including antiseptics, water and sewage systems, refrigeration, preoperative antisepsis, immunizations, insect and rodent control, Cesarean section (C-section), infant formula feeding, agricultural methods, grocery stores, and general germ aversion have all transformed our modern microbiota, compared to that of our ancestors (Frese and Mills, 2015).

While much antibiotic prescribing remains unnecessary, and up to 80% of antibiotics used in the U.S. are for growth promotion in food animals (Hollis and Ahmed, 2013), campaigns to improve judicious antibiotic use have decreased much unneeded prescribing, and many countries, including the U.S., are putting restrictions on agricultural use. Antibiotics will continue to be used for lifesaving treatment and prophylaxis; other modern phenomena, such as C-section and antisepsis, can be used more judiciously, but are also unlikely to be completely eliminated.

This article will consider the infant intestinal microbiome as a mediating influence between manifold environmental exposures and the developing infant metabolic and immunologic systems. First it will review factors thought to cause dysbiosis and next, will explore associated downstream adverse health outcomes. While the infant's respiratory microbiota are developing in parallel, the focus here will be on the intestinal environment. A recurrent theme, when considering the published literature, is that while exposures are often

considered in isolation, infants can be exposed simultaneously to many concurrent individual factors, although they are not always measured or reported; it is often difficult to tease out the contributions of each individual factor. A more comprehensive approach, considering a more complete conceptual model of microbiota function and dysfunction, is critically needed if we are to prevent or reverse developmental damage, and avoid or minimize downstream metabolic and immunologic disease.

Influences on Microbiota

PLACENTAL BACTERIA

While the prenatal environment was traditionally thought to be sterile, (Fanaro et al., 2003; Biasucci et al., 2010; Dominguez-Bello et al., 2010), the human placenta contains a low abundance of mostly nonpathogenic species of organisms, notably from the Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes, and Fusobacteria phyla. Prenatal exposure to small numbers of microorganisms may begin to prepare the immune system for the deluge of bacteria experienced after delivery (Roger and McCartney, 2010; Aagaard et al., 2014; Avershina et al., 2014; Endo et al., 2014; Abrahamsson et al., 2015). Aagaard et al. (2014) showed that placental species were most similar to those of the human mouth and were best explained by hematogenous spread of bacteria from the mouth to the placenta, rather than ascending from the lower genital tract. Colonization patterns were associated with preterm birth, which is intriguing, given the known association between maternal periodontal disease and premature delivery (Mysorekar and Cao, 2014). Supporting this link, Doyle et al. (2014) demonstrated distinct patterns of placental colonization between infants born at preterm versus at term, but there were not significant differences between infants born vaginally versus by Cesarean section (C-section). Aagaard et al. (2014) found an association between placental microbiome composition and a history of maternal prenatal infection; it was not possible to ascertain a possible role of antibiotics, as they did not have access to maternal treatment records.

MODE OF DELIVERY

Starting from birth, the newborn intestinal microbiome undergoes a series of successive profound changes over the first year of life. The specific bacterial species differ between studies, potentially due to between-study differences in locale, maternal colonization, environmental exposures, antibiotic prophylaxis policies, study inclusion criteria, infant and maternal diet, study timing, bacterial characterization techniques, and a host of other yet unknown factors (Arrieta et al., 2014). Understanding what constitutes “normal” baseline microbiota colonization is key to defining dysbiosis, and key to understanding how to avoid and even reverse the related downstream effects; although it may not be possible to define an ideal microbiome in modern times.

Studies assessing the role of delivery mode or diet in bacterial dysbiosis often address a single type of exposure and do not always consider other potential confounding factors. In particular, antibiotics given to mothers during delivery are not always recognized as neonatal exposures; many mothers likely receive prophylaxis for group B Streptococcus and/or antibiotic prophylaxis before C-section delivery.

Bacteria encountered by the infant with vaginal deliveries are very different than those experienced with C-section deliveries (Table 1). C-sections became increasingly common in developed countries since the 1950's, and currently account for around one third of U.S. births (Martin et al., 2011). Many studies have shown different bacterial microbiota patterns depending on mode of delivery; the source of transmission is not entirely clear. On day 3 of life, Biasucci et al. (2010) found that vaginally delivered infants had a much larger number of *Bifidobacterium* species, compared with those delivered by C-section, and *Bacteriodes* was found only in vaginally delivered infants. All infants were exclusively breastfed; the authors remarked that the relatively delayed lactation experienced by many of the C-section mothers may have played a role in colonization patterns. Dominguez-Bello et al. (2010) found that at <5 min of age, bacterial colonization profiles of vaginally delivered infants resembled their mother's predelivery vaginal profiles (primarily *Lactobacillus*, *Prevotella*, or *Sneathia* species), while those of C-section infants' resembled mothers' skin bacteria (primarily *Staphylococcus*, *Corynebacterium*, and *Propionibacterium*). Their implication is that the maternal vaginal community is vertically transmitted to the infant during delivery. Others identified strain-specific links between the early infant microbiome and the fecal microbiome of the mother in vaginally born infants, suggesting that vaginal delivery promotes fecal-oral transmission of maternal bacteria, especially microbes ultimately key to immune regulation, such as *Bifidobacterium longum*, whereas those born by C-section were more likely to be colonized with skin and oral species, and also bacteria from the surrounding delivery environment (Makino et al., 2013; Backhed et al., 2015; Frese and Mills, 2015). Avershina et al. (2014) found that infant colonization with maternal fecal organisms was delayed until after the first week of life, and suggested that important mechanisms of maternal infant transmission remain unknown.

Although reports differ, initial colonizing bacteria in vaginally delivered infants are typically organisms such as *Enterobacteriaceae, Staphylococci, Escherichia/Shigella*, and *Streptococcus*, which are eventually replaced after the first several days, after oxygen depletion, by anaerobes, typically *Bifidobacterium, Lactobacillus, Clostridia*, and *Bacteroides* (Matamoros et al., 2013; Backhed et al., 2015; Barrett et al., 2015).

Over the first year of life, microbiota of vaginally delivered and C-section infants become more similar, however long-term differences can persist: at four months of age in the Canadian CHILD study, at twelve months in Swedish children, and even at seven years of age in a Finnish cohort (Salminen et al., 2004; Azad et al., 2013; Backhed et al., 2015).

DIET

Type of feeding, breast milk versus formula, has a large influence on microbiome composition (Table 1). In breastfed infants, microbiota undergo multiple large shifts over the first year. Breast milk is colonized with *Bacteroides* and *Clostridia*, is rich in complex nondigestible human milk oligosaccharides (HMOs), and includes maternal antibodies (IgA) that inhibit colonization with competing pathogens. By serving as nutritional substrates, HMOs promote colonization with *Bifidobacterium*, generating lactate and fatty acids, which in turn cause a more acidic environment, preventing invasion of competing pathogens (Arrieta et al., 2014). *Bifidobacteria* species rapidly become predominant and remain so

until weaning, when *Clostridia* and *Bacteroides* species become more prevalent (Matamoros et al., 2013). In a cohort of Swedish infants, *Lactobacillus* colonization reached a peak at 6 months, was more prevalent in breast than formula fed infants, and was not associated with delivery mode (Ahrne et al., 2005). Formula fed infants have a more complex pattern of colonizing flora, they may have delayed and less prominent colonization with *Bifidobacteria*, although not all studies agree, and they tend to have higher proportions of *Bacteroides, Clostridia* species, and *Enterobacteriaceae* than breast fed infants (Roger and McCartney, 2010; Guarino et al., 2012; Barrett et al., 2015).

After mode of delivery, cessation of breastfeeding is the most significant factor pushing microbiota toward a more adult composition (Fallani et al., 2011). In a large Danish cohort, after weaning, a microbiota dominated by *Lactobacilli, Bifidobacteria* and *Enterobacteriaceae* was replaced by a population dominated by *Clostridium*, and *Bacteroides* species (Bergstrom et al., 2014). Later during the first year, microbiota are enriched in microorganisms capable of degrading more complex sugars and starch typical of the older infant diet (Backhed, et al., 2015). By 18 months of age, the proportion of organisms that produce short-chain-fatty acids is positively correlated with an increase in body mass index (Bergstrom et al., 2014). By 36 months of age, microbiota composition approaches that of adults (Arrieta et al., 2014; Avershina et al., 2014; Endo et al., 2014). In a mouse model, during weaning and similar periods of microbiota transition, toll-like receptors and interleukin 1 receptor signaling play important roles in regulating intestinal gene expression across virtually all aspects of intestinal physiology (Rakoff-Nahoum et al., 2015).

GEOGRAPHY

The Environmental Determinants in the Young (TEDDY) study, following 90 infants at high risk of Type 1 Diabetes, 15 each from Finland, Sweden, Germany and the U.S., showed large differences in infant microbiota depending on geographic location (Kemppainen et al., 2015). Intestinal bacterial diversity was relatively lower in Finland and Colorado, and infants in Sweden and Washington had higher early proportions of *Bifidobacterium*. Other studies in children and adults also show differences across geographic locations (Brandt et al., 2012; Yatsunenko et al., 2012).

ANTIBIOTICS

Antibacterial drugs were developed in the early 1900s; by World War II, penicillin had revolutionized our ability to treat infectious disease. By 1990, U.S. children had virtually universal antibiotic exposure, with an average of 1.4 yearly antibiotic prescriptions per child <5 years of age (McCaig et al., 2002). Following public health efforts to curb unnecessary antibiotic use beginning in 1993, and the 2000 introduction of pneumococcal conjugate vaccine, U.S. antibiotic prescribing rates decreased, and then plateaued by 2010; children <2 years of age still receive, on average one antibiotic course per child per year (Vaz et al., 2014), and broad spectrum antibiotic use has doubled over the past decade, accounting for 34% of pediatric antibiotic prescriptions in 2010 (Lee et al., 2014). Much attention to bacterial dysbiosis has focused on overuse of antibiotics, however not all antibiotic use is unnecessary.

Antibiotic resistance—Despite the declaration by Surgeon General William Stewart in 1946 that infectious disease was conquered, antibiotic resistance is now a global health crisis. By killing off colonizing organisms, antibiotics can promote overgrowth of competing antibiotic-resistant pathologic organisms (Jernberg et al., 2007; Seekatz and Young, 2014). Additionally, frequent and chronic low-dose antibiotic exposure promotes stress related bacterial mutations and transmission of resistance to other colonizing organisms, even different species, on transmissible genetic elements (Choffnes et al., 2010; Looft and Allen, 2012). Resistant infections are increasingly community-acquired, and young children, with their frequent antibiotic exposure, serve as reservoirs of community antibiotic resistance (Penders et al., 2013; You and Silbergeld, 2014).

Antibiotic-related dysbiosis—Antibiotic treatment is associated with abrupt profound changes in intestinal microbiota; there is increasing evidence that these changes can be persistent, can mediate malfunctions in infants' nascent developing immunologic, endocrine, and metabolic systems, and can increase risks of subsequent downstream chronic immunologic and metabolic disease (Looft and Allen, 2012; Vangay et al., 2015). Antibiotic treatment is associated with decreased intestinal bacterial counts and species diversity. (Dethlefsen et al., 2008; Tanaka et al., 2009; Jernberg et al., 2010; Dethlefsen and Relman, 2011; Russell et al., 2012). There is also evidence of rapid increases, or "blooms" of competing bacterial species, especially *Escherichia coli* (Looft and Allen, 2012). Certain enteric colonizers have been shown to inhibit *Enterobacteriaceae* species, and in vitro, *Lactobacilli* secrete mucins that inhibit adhesion of enteropathogenic organisms (Ferreira et al., 2011; Looft and Allen, 2012).

Previously, antibiotic-related dysbiosis was believed to be transient (Welling et al., 1991; Cavallaro et al., 1992; Toltzis et al., 2007), but recent studies using molecular methods, most in mice or adults, have revealed that antibiotic-associated changes in microbiota composition can be persistent. For example, microbiota differences persisted for up to 2 years in clindamycin-exposed adults, compared with unexposed patients (Jernberg et al., 2007; Jernberg et al., 2010). Three adults treated with antibiotics for *Helicobacter pylori* manifested alterations in gut microbiota for at least 4 years, compared with three not receiving antibiotics (Jakobsson et al., 2010). Dethlefsen et al. (2008) followed three adults over 10 months including two courses of ciprofloxacin. They found a rapid profound loss of diversity and altered composition of the intestinal bacterial community within the first 3 to 4 days after antibiotic exposure. Within 1 week, there was some return of communities toward their baseline state, but the recovery was incomplete in all subjects at ten months (Dethlefsen et al., 2008; Dethlefsen and Relman, 2011). Mouse and human adult studies of amoxicillin ± clavulanic acid and mouse models of vancomycin exposure (Jernberg et al., 2010; Robinson and Young, 2010) have not shown as persistent effects (Jernberg et al., 2010; Fouhy et al., 2012).

So far, there are fewer child data; Fouhy et al. (2012) compared nine newborn infants who received parenteral ampicillin and gentamicin within 48 h of birth with control infants who received no antibiotics. At 4 weeks, treated infants had higher proportions of *Proteobacteria*, and lower proportions of *Actinobacteria, Bifiobacterium*, and *Lactobacillus*, compared with controls. By 8 weeks, the *Actinobacteria, Bifodobacterium*, and *Lactobacillus* total numbers

had recovered and were no different than those of controls; however, the number of different *Bifidobacterium* species remained reduced in the antibiotic-treated infants. Five of nine infants in the antibiotic group were born by C-section and four were exclusively breast-fed; of the nine control infants, all were born vaginally and three were exclusively breast-fed. Tanaka et al. (2009) followed 18 vaginally delivered infants; five received oral broad spectrum antibiotic treatment during the first 5 days of life and all received both breast milk and formula. During the first week, antibiotic treated infants showed lower fecal bacterial diversity, attenuated *Bifidobacterium* colonization, and overgrowth of *Enterococcus*, compared with the unexposed infants. Differences in fecal microbiota composition between the groups persisted after 1 month.

Conditions Associated with Dysbiosis

IMMUNE SYSTEM

There is growing evidence to support a causal role for dysbiosis in immune dysregulation. In 1958, Strachan (1989) proposed the Hygiene Hypothesis, positing that decreased exposure to infectious agents has resulted in inadequate stimulation of our developing immune systems, leading to the rising prevalence of modern diseases related to immune regulation. The more recent term: the “microflora hypothesis” acknowledges a mediating role for dysbiotic distortion of immunologic tolerance caused by antibiotics and/or other modern environmental influences (Noverr and Huffnagle, 2005; Walker, 2013).

Two clever “natural experiments” described by Hesselmar et al. (2013) support this idea. Among 18 month-old Swedish children with a family history of allergy, those whose parents sucked their pacifiers to clean them were less likely to have asthma, eczema, or allergic sensitization, characterized by specific IgE against inhalant or food allergens, than children whose parents did not clean their pacifiers this way; this effect persisted at 36 months. Vaginal delivery showed independent and additive protective effects against eczema. In a questionnaire study by the same group, children from families who washed dishes by hand had reduced risk of developing allergy compared with children from families who used a dishwasher (Hesselmar et al., 2015). Infant antibiotic exposure was not reported in either of these studies and could have potentially been a confounding variable, if not measured and considered.

Numerous studies support the role of the early gut microbiota in regulating immune function. In piglets, development of a normal immune system requires intestinal bacterial colonization, and does not take place in germ-free animals (Schmidt et al., 2011).

Colonizing bacteria could serve several potential roles in the infant’s developing immune-modulatory system. First, commensal organisms could serve a barrier role, blocking access to gut epithelial cells by producing antibacterial substances, enhancing tight junctions, and/or stimulating IgA production (Kelly et al., 2007; Sansonetti, 2011; Li et al., 2014). Second, they may serve as gatekeepers, training the infant’s nascent immune system to distinguish between self versus symbiotic versus pathogenic bacteria at the gut-mucosal surface. Evidence from mouse studies suggests that large shifts in intestinal microbiota associated with weaning can promote up-regulated gene transcription, possibly involving

toll-like receptors and interleukin (IL)−1 signaling (Blander and Medzhitov, 2006; Kelly et al., 2007; Rakoff-Nahoum et al., 2015). They can also directly influence dendritic cells, regulatory T cells, B lymphocytes, and epithelial and stromal cell biology. Dysbiosis may interfere with immune regulatory processes; resulting chronic inflammation may underlie the etiology of metabolic dysfunction, and immune-regulated diseases, such as food and environmental allergy, eczema, asthma, inflammatory bowel disease, celiac disease, idiopathic arthritis, and Type 1 diabetes mellitus.

Allergy/asthma/eczema—The prevalence of allergic diseases increased worldwide over the past 40 years, especially in developed countries. In U.S. children, from 1997 to 2011, the prevalence of food allergy and eczema increased from 3.4% to 5.1% and from 7.4% to 12.5%, respectively (Jackson et al., 2013), and asthma prevalence has doubled for U.S. children over the later part of the past century, up to 12% in 2005 (Akinbami, 2006). In developed countries, including the U.S., up to 20 to 32% of children have some kind of allergic airway disease (Bisgaard and Szefler, 2007).

There is great interest in elucidating the role of specific colonizing organisms in immune regulatory development, but the interactions between organisms, their hosts, each other, and environmental exposures are complex and often not considered concurrently (Table 2). Vebo et al. (2011) showed that among children at risk for future allergic disease, *Enterococcus* was overrepresented in bowel microflora at 4 months of age, while *Bifidobacteria* were overrepresented at 1 year, compared with a control cohort; however, delivery mode, type of feeding and antibiotic exposure were not reported. Yap et al. (2014) found higher abundance of *Enterobacteriaceae* and *Clostridium perfrigens* in children who developed eczema before age 2 years, and lower abundance of *Bifidobacterium* in those with eczema at age 5.

Early gut profiles are related to subsequent immune response, and specific bacterial species seem to influence T helper cell subsets. Johansson et al. (2012) studied gut colonization at 2 months of age in thirty infants, in relation to mononuclear cell cytokine responses at age 2 years. Early *Staphylococcus aureus* colonization was associated with higher numbers of cells producing interleukin IL-4 and IL-10. Co-colonization with *S. aureus* and *Lactobacilli* was associated with suppression of IL-4, IL-10, and interferon (IFN)-ϒ producing cells. In vitro, *Lactobacillus rhamnosus* seemed to suppress *S. aureus*-induced cytokine responses.

Even before delivery, Benn et al. (2002) found that maternal vaginal colonization with *Ureaplasm urealyticum* and maternal antibiotic treatment were each associated with infant hospitalization for wheezing between 0 and 3 years of age, but not by 4 and 5 years of age. There was an increased risk of asthma at 4 to 5 years if mothers received antibiotics during pregnancy, but no related interaction with colonization patterns. Mode of delivery, infant antibiotic exposure, and infant feeding beyond the first few days of life were not reported.

Mode and place of delivery are also associated with risk of allergic disease. Several studies have found that infants born by C-section are more at risk of eczema, food allergy, and asthma (Eggesbø et al., 2003; Laubereau et al., 2004; Negele et al., 2004; Thavagnanam, et al., 2008). Interestingly, among infants born by vaginal delivery, infants born at home were

less likely to develop eczema and asthma than infants born in hospital; this beneficial effect was only found in infants of parents with a history of eczema (van Nimwegen et al., 2011).

Infant diet also seems to play a role; Schwartz et al. (2012) confirmed very different intestinal bacteria phylogenetic profiles between breast- and formula-fed infants, and compared intestinal mRNA signatures, or transcriptomes, between the feeding groups. The closest associations to diet type were found for 11 genes related to the virulence characteristics of immunity and defense, potentially important to regulatory pathways; the authors surmised that through gene expression, human milk promotes the mutualistic relationship between the infant microbiota and the immune system at the intestinal mucosa (Schwartz et al., 2012).

Reduced organism diversity may be more important for the developing immune system than relative prevalence. Abrahamsson et al. (2012) showed that low intestinal bacterial diversity during the first month of life was associated with later development of atopic eczema, even excluding infants who received antibiotics; however, maternal antibiotics were not reported. Bisgaard et al. (2011) showed that risk for microbiota diversity during the first year of life was inversely associated with the risk of allergic disease at school age. In a Swedish cohort, during their first 2 years of life, infants born by C-section, but not exposed to antibiotics, compared with vaginally born infants, had lower intestinal microbiota diversity, and lower blood levels of the Th-1 associated chemokines CXCL10 and CXCL11, suggesting reduced Th1 responses. This implies that C-section could interfere with the development of balanced T-cell immune regulation, and have downstream impacts on immune mediated diseases (Jakobsson et al., 2014).

Antibiotics cause both decreased prevalence and diversity of microbiota. Antibiotic treatment in the neonatal period was found to be a risk factor for wheezing at 1 year of age, in a questionnaire study of 10,592 Swedish parents, although antibiotics received after the neonatal period but during the first year were not included in the multivariable model (Alm et al., 2008). Marra et al. (2009) reported that antibiotic exposure in the first year of life was associated with a small increased risk of developing asthma after 2 years of age, after adjusting for many covariates. There was a dose effect; the number of antibiotic courses received was associated with increased asthma risk with a maximum risk associated with more than four antibiotic courses.

Inflammatory bowel and celiac disease—The incidence of the inflammatory bowel diseases (IBD), Crohn’s disease, and ulcerative colitis, has been increasing worldwide, especially in countries experiencing more Westernized culture (Molodecky et al., 2012). A 2014 meta-analysis showed no difference in IBD risk for infants delivered by C-section versus vaginal delivery (Bruce et al., 2014). Kronman et al. (2012) used electronic health records for 1.1 million UK patients to show that childhood exposure to antianaerobic antibiotics was associated with the risk of developing IBD. Exposure before 1 year of age had the highest risk, and there was a dose effect, with higher risk of more than two doses compared with one to two doses (Kronman et al., 2012).

In the last several decades, the diagnosis of celiac disease (CD) has more than doubled in the U.S. and quadrupled in the U.K., however, it is unclear if this represents increased incidence of disease or merely diagnosis (Marild et al., 2014; West et al., 2014). Marild et al. (2014) used questionnaire data from the prospective All Babies in Southeast Sweden cohort study; 46 of 8729 children developed CD. They found no association between antibiotic exposure during pregnancy and the child's risk of CD, adjusted for breastfeeding, age of gluten introduction, and infections during the first year of life. This group also performed a population-based case control study linking administrative data to nationwide histopathology data (Marild et al., 2013). They compared antibiotic exposure between 2933 individuals with CD, 2118 with lower-grade inflammation, and 620 with normal mucosa but positive CD serology, with five matched controls per case individual. Antibiotics were associated with CD, inflammation, and positive serology. CD results were unchanged when individuals with antibiotics in the year before diagnosis or individuals with comorbidity were excluded. And finally, Olivares et al. (2015) studied 1-month-old infants who had at least one first-degree relative with CD. All were exclusively breastfed and vaginally delivered, and 11 were carriers of the human leukocyte antigen (HLA)-DQ2 genotype, considered at higher risk of developing CD. HLA-DQ2 carriers had quite different bacterial microbiota composition, compared with non-carriers. Their conclusion was that the composition of the early gut microbiome is influenced by HLA- DQ2haplotype.

Diabetes mellitus type 1 (T1D) and juvenile idiopathic arthritis—The peak age of T1D incidence is around puberty, however antibodies against pancreatic islet cells typically appear years before onset of clinical disease. Ziegler et al. (2012) showed that in a cohort of 1650 children with heightened genetic risk of T1D, the peak time window for autoantibody development was between 9 months and 2 years of age. This implies an increased susceptibility of the infant immune system to autoimmunity and/or triggering exposure during this time period.

C-section and antibiotic exposure are individually associated with a risk of developing T1D, but there are few data considering both factors together. In a meta-analysis of 20 studies, Cardwell et al. (2008) identified 25% increased odds of developing type 1 diabetes (T1D) in children born by C-section versus by vaginal birth, in a model adjusted for gestational age, birth weight, maternal age, birth order, breast feeding, and maternal diabetes, but antibiotic exposure was not discussed.

Boursi et al. (2015) demonstrated a higher risk for T1D in UK primary care patients who had received two to five courses of penicillin, cephalosporins, macrolides and quinolones >1 year before diabetes diagnosis; the risk increased with the number of antibiotic courses but was not found with exposure to antiviral or antifungal medications. Mode of delivery was not considered.

Endesfelder et al. (2014) suggested that rather than considering bacterial types individually, a disturbed connectivity network might be the common pathway to immune dysfunction. Among 44 German infants with a first-degree relative with T1D, they found no differences in intestinal bacterial diversity, community composition, or abundance of specific genera between islet cell positive versus islet cell negative children at 3 to 36 months of age,

however, the groups had differences in their correlation-based interaction networks; antibody-positive infants had more isolated nodes with less interaction.

Children with newly diagnosed juvenile idiopathic arthritis (JIA) were more likely to have been previously exposed to antibiotics; the relationship was dose-dependent and strongest for exposures within 1 year of diagnosis. This association may be confounded by antibiotic treatment for symptoms heralding the JIA diagnosis, but the authors felt this was not likely to be an important issue (Horton et al., 2015).

OBESITY

Worldwide obesity has more than doubled since 1980 (World Health Organization, 2015). In the mid 1960s, 4 to 5% of U.S. school age children were obese, by 2011 to 2012, this portion had increased to 18 to 20% (Schwartz et al., 2012). There is mounting belief that this trend in adiposity is as least in part related to the explosive growth of antibiotic use during this same time period. For decades, we have known that antibiotics, provided at subtherapeutic doses, promote weight gain in animals, both by increasing food intake and by increasing the efficiency of turning food into body mass (Blaser, 2012); 80% of the volume of antibiotics used in the U.S. are for agricultural purposes (Hollis and Ahmed, 2013; FDA Annual Report on Antimicrobials Sold or Distributed for Food-Producing Animals, 2013; Record-High Antibiotic Sales for Meat and Poultry Production, 2013). As virtually all children are exposed to antibiotics directly and/or from environmental exposure, this concept has considerable face validity.

As discussed above, many studies have described relationships between exposures, especially antibiotics, and changes in microbiota member numbers and diversity. There is some evidence that overweight/obesity at school age is related to colonization differences of the intestinal microbiota during infancy (Kalliomaki et al., 2008; Scheepers et al., 2015). Bäckhed et al showed that conventionally raised mice developed increased fat stores, compared with germ-free mice, despite reduced caloric intake (2004). Many studies showed epidemiologic associations between antibiotics and risks for future obesity, and even Type 2 diabetes (T2D) (Ajslev et al., 2011; Trasande et al., 2013; Bailey et al., 2014; Mikkelsen et al., 2015; Saari et al., 2015).

An elegant series of studies by Blaser (2012) has provided fascinating insights into the mediating factors between antibacterial-induced microbiota changes and clinical effects. Following 11,532 children in Avon, U.K., they found that antibiotic exposure during the first 6 months of life was associated with increased body mass at 10 to 38 months (Cho et al., 2012; Blustein et al., 2013; Cox and Blaser, 2015). Later exposures, from 6 to 23 months, did not have the same association (Trasande et al., 2013). In their mouse work, penicillin, given in pulse doses to simulate repeat antibiotic courses for acute illnesses at critical time windows early in development, disturbs microbiota, induces metabolic changes, affects ileal expression of genes involved in immunity, and increases adiposity, enhancing the effect of a high fat diet (Cox et al., 2014). Cox et al. (2014) showed that if altered microbiota from antibiotic-treated mice are transferred to germ-free mice, they develop the obese phenotype. The phenotype persists even if the antibiotic pulses are discontinued and the microbiome composition seems to return to “normal,” (Blaser, 2012; Cho et al., 2012; Cox et al., 2014).

Mice given continuous low dose antibiotic treatment for 7 weeks after weaning had normal overall numbers of bacteria, but altered composition patterns; these mice developed an "obese" phenotype, and increased bone density. Exposed male mice developed elevated levels of a hormone that stimulates lipoprotein activity and is associated with obesity and elevated serum glucose; they also had altered metabolism of short-chain fatty acids, and showed a trend toward hyperglycemia (Cho et al., 2012). Blaser (2012) proposed that antibiotic exposure selects for microbiota with increased metabolic activity, organisms that are more efficient in extracting energy from the diet; this boosts short chain fatty acid concentration, which in turn induces increased hepatic lipogenesis.

NERVOUS SYSTEM AND THE BRAIN

Infant neurodevelopment could be influenced by exposure to placental microorganisms from the time of neural tube development (Borre et al., 2014). The enteric nervous system, consisting of 200 to 600 million neurons, allows for communication between the intestinal microbiota and the developing brain, through hormonal, immunologic or neural mechanisms (Douglas-Escobar et al., 2013; Clarke et al., 2014). Germ-free mice exhibited more stress behavior, memory problems, antisocial behavior, and elevated levels of stress hormones, and were deficient in specific brain proteins related to nerve-cell interactions (Cryan and O'Mahony, 2011). Administration of fecal matter from normal mice improved their behavior if given within an early time window, but not if delayed for several weeks. This implies that intestinal microbiota influence neurochemicals and proteins during early brain development, and exposures that cause dysbiosis, such as mode of delivery, mode of feeding, and antibiotics, could be associated with brain and neurologic system mal-development.

Antibiotic-treated mice showed changes in brain neurotransmitter levels and exhibited anxiety-like behaviors, but did not have associated levels of enteric neurotransmitters or evidence of gut inflammation. This could be related to the psychiatric symptoms experienced by patients with intestinal diseases, and the intestinal symptoms experienced by some individuals with autism spectrum disorders (Douglas-Escobar et al., 2013).

SUMMARY

Humans have co-evolved with a teeming universe of colonizing microorganisms. Passed vertically from maternal transmission and horizontally from the environment, infants' archetypal microbiota should be exquisitely suited for their key roles in the development and regulation of their metabolic and immunologic systems; however, the tradeoff for living in a contemporary world that includes sewers and soap is that, even in utero, infants inevitably experience exposures that are alien to this ancestral scheme. To prevent or repair the resulting microbiotal corruption, a combination of approaches will be needed. First we need to minimize unnecessary antibiotic prescribing and C-section deliveries, to phase out antibiotic use in healthy animals for growth promotion, and to discourage use of household antibacterial cleaning products. Second, considering the burgeoning number of microbiome studies, the most useful generalizable knowledge will consider exposures, and outcomes of interest within a comprehensive context of the infants' myriad other exposures and outcomes. Third, there is potential for probiotics to prevent or at least repair any developmental damage, but we currently have scant understanding of how this would be

accomplished; considerable further research is needed with great care that treatment recommendations are based on empirical evidence of effectiveness and safety (Angelakis et al., 2013). Minimizing the harmful effects of exposure-related dysbiosis is the modern medical frontier.

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TABLE 1

Intestinal Microbiome Flora Related to Various Perinatal Exposures

Exposure	Intestinal Flora
Vaginal delivery	*Bifidobacterium, Bacteroides, Lactobacillus, Prevotella*
Cesarean section	*Staphylococcus, Corynebacterium, Propionibacterium*
Breast milk	*Bifidobacterium, Bacteroides, Lactobacillus, Clostridia*
Formula	*Bacteroides, Clostridia, Enterobacteriacea*

TABLE 2

Factors Associated with an Increased Risk of Atopic Phenotypes

Exposures that have been linked epidemiologically to allergy, asthma and/or eczema
1. Maternal vaginal colonization with *Ureaplasm urealyticum*
2. Maternal antibiotic treatment
3. C section when compared to vaginal birth
4. Hospital birth compared to home delivery
5. Low intestinal bacterial diversity during the neonatal period
6. Neonatal antibiotic exposure
7. Antibiotic exposure in the first year of life
8. Using a dishwasher compared to hand washing dishes
9. Parent not sucking on infant pacifier to clean it